UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Departamento de Enxeñaría Química

# Environmental sustainability of bioactive molecules from marine organisms

Memoria presentada por:

**Paula Pérez López**

Para optar ao grao de Doutor pola
Universidade de Santiago de Compostela

Santiago de Compostela, setembro de 2015

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D. **Gumersindo Feijo Costa** e Dna. **María Teresa Moreira Vilar**, Catedráticos de Enxeñaría Química da Universidade de Santiago de Compostela,

Informan:

Que a memoria titulada "Environmental sustainability of bioactive molecules from marine organisms" que, para optar ao grao de Doutor en Enxeñaría Química e Ambiental, presenta Dna. Paula Pérez López, realizouse baixo a nosa inmediata dirección no Departamento de Enxeñaría Química da Universidade de Santiago de Compostela.

E para que así conste, firman o presente informe en Santiago de Compostela, o 4 de setembro de 2015.

Fdo. G. Feijoo Fdo. M.T. Moreira Fdo. P. Pérez-López

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*"Et je goûtais, plutôt en curieux qu'en gourmet, tandis que le capitaine Nemo m'enchantait par ses invraisemblables récits.*

*– Mais cette mer, monsieur Aronnaux, me dit-il, cette nourrice prodigieuse, inépuisable, elle ne me nourrit pas seulement; elle me vêtit encore. Ces étoffes qui vous couvrent sont tissées avec byssus de certains coquillages; elles sont teintes avec la pourpre des anciens et nuancées de couleurs violettes que j'extrais des aplysis de la Méditerranée. Les parfums que vous vous trouverez sur la toilette de votre cabine sont le produit de la distillation des plantes marines . Votre lit est fait du plus doux zostère de l'océan. Votre plume sera un fanon de baleine, votre encre la liqueur sécrétée par la seiche ou l'encornet. Tout me vient maintenant de la mer comme tout lui retournera un jour!*

*– Vous aimez la mer, capitaine.*

*– Oui! Je l'aime! La mer est tout! Elle couvre les sept dixièmes du globe terrestre. Son souffle est pur et sain. C'est l'immense désert où l'homme n'est jamais seul, car il sent frémir la vie à ses côtes. La mer n'est que le véhicule d'une surnaturelle et prodigieuse existence; ell n'est que mouvement et amour; c'est l'infini vivant comme l'a dit un de vos poètes. Et en effet, monsieur le professeur, la nature s'y manifeste par ses trois règnes, minéral, végétal, animal. Ce dernier y est largement représenté par les quatre groupes des zoophytes, par trois classes des articulés, par cinq classes des mollusques, par trois classes des vertébrés, les mammifères, les reptiles et ces innombrables légions de poissons, ordre infini d'animaux 'ui compte plus de treize mille espèces, dont un dixième seulement appartient à l'eau douce. La mer est le vaste réservoir de la nature. C'est par la mer que le globe a pour ainsi dire commencé, et qui sait s'il ne finira pas par elle! Là est la suprême tranquillité. La mer n'appartient pas aux despotes. À sa surface, ils peuvent encore exercer des droits iniques, s'y battre, s'y dévorer, y transporter toutes les horreurs terrestres. Mais à trente pieds au-dessous de son niveau, leur pouvoir cesse, leur influence s'éteint, leur puissance disparaît! Ah! Monsieur, vivez, vivez au sein des mers! Là seulement est l'indépendance! Là je ne reconnais pas de maîtres! Là je suis libre!"*

Jules Verne, *20000 lieues sour les mers* (1869-1870)

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# *Abstract*

Marine and freshwater ecosystems have been widely exploited throughout history as vast sources of food and valuable natural products. The diversity of aquatic organisms has led to the isolation of more than 24000 bioactive compounds with applications in strategic industries such as pharmaceutical, nutraceutical and cosmetic sectors. Moreover, the biochemical composition and growth characteristics of microalgae and seaweeds suggest their capability to overcome some bottlenecks of the production of energy compared to terrestrial feedstocks. The potential of blue biotechnology, especially in the European context, is reflected in the increasing market and the numerous international programs and initiatives focused on the promotion of research and partnerships to empower technological advances in the field.

The development of sustainable processes for the balanced exploitation of marine resources requires the use of management tools to measure the performance of the systems according to environmental and socio-economic criteria. The standardized methodology of Life Cycle Assessment (LCA) is widely applied in the field of microalgal biofuels and has also been used to evaluate active ingredients.

Therefore, this doctoral thesis focuses on the application of LCA methodology to analyze the environmental performance of valuable bioactive molecules and commodity products throughout the whole production chain. Key social and economic indicators have been included in the evaluation of some processes to consider a holistic perspective that takes into account all the dimensions of sustainable development.

In the first stage, the LCA methodology is applied to obtain the complete life cycle inventories and perform the environmental impact assessment of the production of diverse high value added compounds obtained from microalgae, macroalgae, sponges, bacteria, chromists and fungi. Although LCA has been previously applied for the evaluation of microalgal biofuels, this thesis presents

the first environmental studies addressing the impacts of blue biotechnology and high value added biocompounds from marine origin. Moreover, the work includes a wide variety of organisms that had not been evaluated according to environmental criteria before, such as sponges, epiphytic bacteria or marine fungi.

A cradle-to-gate perspective is proposed, to take into account the environmental aspects of the production of the different inputs to the system, the cleaning and sterilization operations, the cultivation of marine organisms and the downstream processes of biomass harvesting and extraction stages. The conducted LCA studies were based on real operating facilities at different production scales (lab, semi-pilot and pilot systems).

The main hot spots (problematic stages of the process chain from an environmental point of view) are discussed for all the evaluated species and products. In several case studies, alternative scenarios and optimized techniques are proposed according to the identified hot spots and their potential benefits are quantified with respect to the impact of the original production systems. Additionally, LCA is used as a decision-making tool to compare the available technologies for certain cultivation and extraction steps and to select the most appropriate route in the design of novel processes.

The second part of the thesis is focused on key aspects of the performance of microalgal systems, due to the current importance of these processes in the framework of blue biotechnology and exploitation of marine resources. The environmental behavior of the main reactor configurations available for the cultivation of microalgae at pilot and large scale is analyzed in detail. A comparison of the environmental efficiency of the different systems is provided, and the possible consequences of changing operating conditions are also discussed.

The variability of environmental results due to the wide number of uncertain parameters and assumptions in microalgal simulation models is also addressed by applying the Monte Carlo simulation method. The uncertainty analysis is conducted for a multi-product microalgal scheme that combines the extraction and further conversion of the lipid fraction into renewable diesel with the separation of other valuable components to obtain protein fraction, and

fertilizers as well as energy recovery by anaerobic digestion. This tool is used not only to evaluate the range of possible environmental scenarios, but also to estimate the economic potential of the process based on the minimum selling price of the obtained diesel. The influencing factors are grouped into three categories, namely process parameters, characterization factors and economic parameters. The probability distributions for the three environmental categories and the economic indicator were obtained to estimate the global variability of the system including all the sources of uncertainty simultaneously. The correlations between indicators were also analyzed. The influence of each group of parameters was evaluated separately, and the effects of individual uncertain parameters were further analyzed for the group identified as the main cause of uncertainty.

The work developed in this thesis proved the suitability of LCA methodology as a valuable environmental management tool that provides strategic information for process design in innovative sectors. The identification of environmental bottlenecks and the comparative evaluation of improved scenarios suggest the potential achievement of sustainable production systems for the commercial exploitation of marine resources in the future.

**Keywords:** Environmental Life Cycle Assessment (LCA), blue biotechnology, sustainability dimensions, socio-economic indicators, marine resources, microalgae, seaweeds, sponges, epiphytic bacteria, marine fungi, microalgal process uncertainty.

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# Index

**SECTION II: HIGH VALUE ADDED MOLECULES FROM AQUATIC ORGANISMS**

**SECTION III: MICROALGAL BIOREFINERIES**

## ADDITIONAL CONTENTS

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# SECTION I

## INTRODUCTION TO THE STUDY

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# Chapter 1

# Living natural resources from aquatic ecosystems[1]


*Summary*

Water is an essential resource that covers 71% of Earth's surface. The large number of marine and freshwater ecosystems with diverse environmental conditions makes them the most promising habitats to explore due to their rich biological diversity. Aquatic ecosystems have been widely exploited throughout history and currently constitute a vast source of high value compounds with potential applications in food, pharmaceutical, nutraceutical, cosmetic and agrochemical industries. To date, more than 24000 natural products from aquatic organisms have been reported. Moreover, several algal species are producers of several energy sources such as biodiesel, biogas or biohydrogen, showing significant benefits compared to conventional terrestrial crops. Current research aims at developing integrated systems to obtain a combined set of co-products that contribute to the economic and environmental feasibility. The potential of blue biotechnology has led to the development of international programs and policies to empower technological advances by providing funding for research and development and promoting partnerships between academic and industrial stakeholders.


[1] **Pérez-López P**, Feijoo G, Moreira MT. Aplicación de la metodología de Análisis de Ciclo de Vida para la producción sostenible de ingredientes activos a partir de organismos marinos. Revista alimentaria 2014, 445:40-46 [In Spanish].

# Index

## 1.1. Oceans and rivers as reservoirs of natural resources

### 1.1.1. Biological diversity in aquatic ecosystems

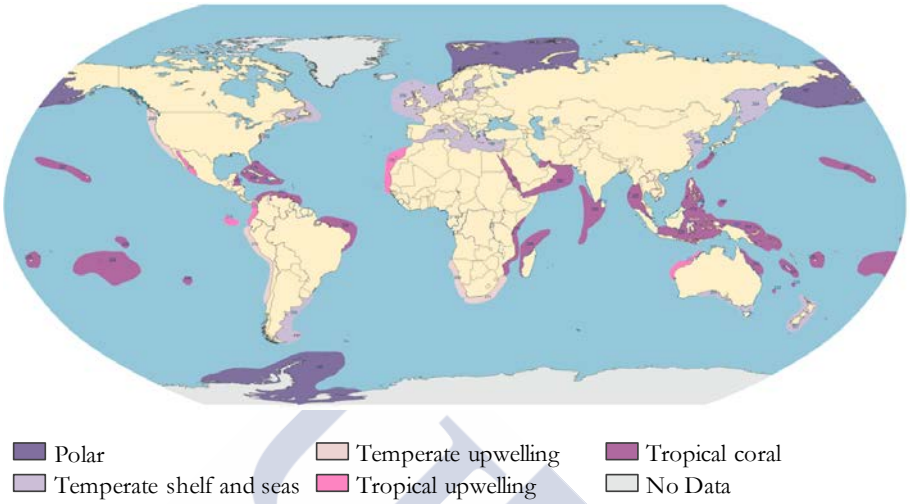
Water reservoirs cover 71% of the Earth's surface and are considered an essential resource for all forms of life in this planet (Hornberger et al., 2014; Shiklomanov and Rodda, 2003). This surface corresponds to 361 million km², with 43% of this area located in the Northern Hemisphere and 57% in the Southern Hemisphere (Shiklomanov and Rodda, 2003). Saltwater oceans constitute the largest fraction, accounting for 97% of the planet's water; whereas freshwater from lakes and rivers only stands for 0.01% (Hornberger et al., 2014; Shiklomanov and Rodda, 2003).

Besides the abundance of water sources, there are a wide variety of aquatic habitats with different chemical and physical characteristics, especially in the case of freshwater ecosystems (Hiscock and Bense, 2014). The diversity of aquatic ecosystems is reflected in the large number of ecosystem regions (also called ecoclimatic zones or ecoregions) that have been defined to classify these ecosystems based on geography, physiography, hydrology, climate and other environmental and ecological parameters (Wiersma, 2004).

According to World Wildlife Fund (WWF), aquatic systems can be classified in 43 marine ecoregions and 53 freshwater ecoregions, shown in **Figure 1.1**. The marine ecoregions are grouped in ten major marine habitat types, including polar seas, temperate shelfs and seas or tropical coral reefs, among others. The freshwater ecoregions are distributed in twelve freshwater habitat types, such as small or large rivers, lakes and river deltas (FEOW, 2015; WWF, 2015).

The large number of aquatic ecosystems with diverse environmental conditions makes them the most promising habitats to explore due to their rich biological diversity. Indeed, this biodiversity has been estimated to be higher than that of tropical rainforests (Larsen et al., 2005). Despite the potential, the majority of living species in aquatic environments remain still unexplored (Appeltans et al., 2012; Balian et al., 2010).

**a) Major marine habitat types**




**b) Major freshwater habitat types**

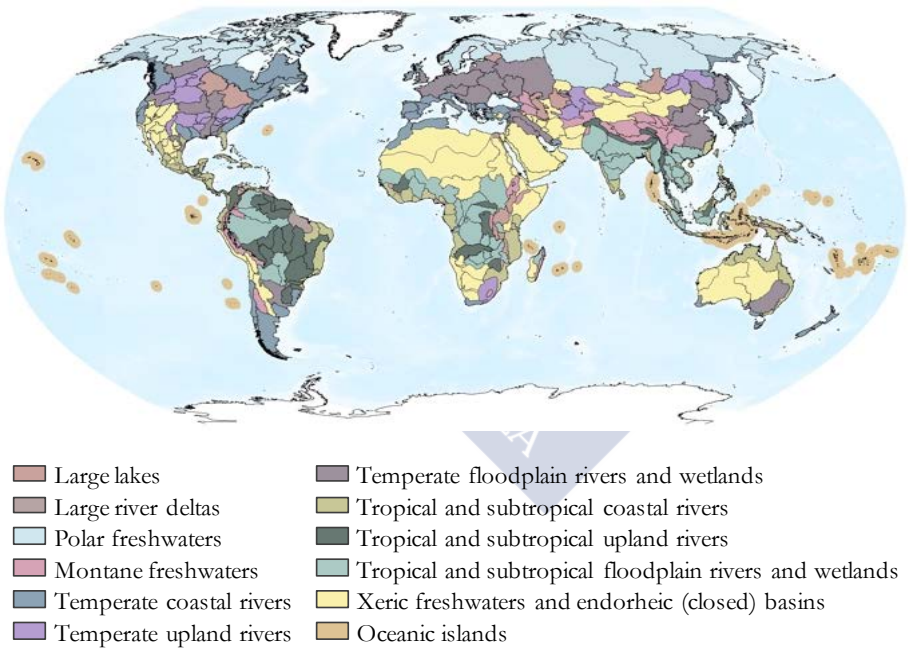



**Figure 1.1.** Geographical distribution of major habitats for a) marine ecosystems (excluding pelagic, abyssal and hadal) and b) freshwater ecosystems. Source: Adapted from WWF (2015) and FEOW (2015).

According to the open-access database World Register of Marine Species (WoRMS), a limited number of around 230,000 taxonomically accepted marine species have been described to date (**Figure 1.2**). Most of these species (nearly 200,000) are included in the Kingdom Animalia, with 20300 belonging to Chromista, 8800 to Plantae, 1400 to Fungi, 600 to Protozoa, 1700 to Bacteria and 120 to Archea (WoRMS Editorial Board, 2015).

In the case of freshwaters, the described species include approximately 126,000 in the Kingdom Animalia and 2600 in Plantae. Most of other groups from freshwater ecosystems are still remarkably understudied, although some rough estimates indicate about 3000 freshwater Fungi and 2400 Protozoa already recorded (Balian et al., 2010).

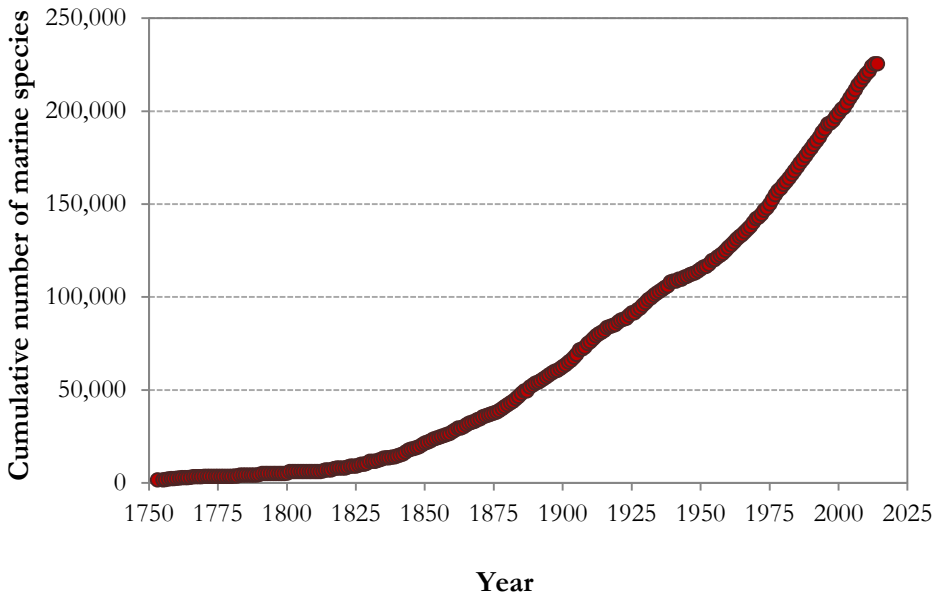



**Figure 1.2.** Evolution of the number of taxonomically accepted marine species. Source: Adapted from WoRMS Editorial Board (2015).

Current analyses suggest that the total existing organisms in oceans may vary between 300,000 and one million, while freshwater species can exceed 250,000 (Appeltans et al., 2012; Balian et al., 2010). Undiscovered species may not be uniformly distributed within the different taxonomic groups. Appeltans et al. (2012) claim that no new species from the best-known groups are expected to

be discovered. On the contrary, less than 20% of total species of other taxonomic groups have already been described. For example, more than 80% of marine vascular plants (including mangrove species and seagrasses) are known, whereas few microalgae and seaweeds have already been studied. Nevertheless, according to the high current rate of new species described per year, most species are likely to be discovered in this century (Appeltans et al., 2012).

### 1.1.2. Exploitation of aquatic resources through history

Archaeological evidence suggests that the earliest exploitation of marine resources by humans dates back to at least 164,000 years ago, during the Palaeolithic period (Marean et al., 2007; Walter et al., 2000). The first remains include fifteen categories of marine invertebrates from a coastal site located in South Africa. The finding of these organisms, jointly with several pigment pieces, confirms the introduction of shellfish as a food source for human diet, as well as a pigment source. The dietary expansion to marine sources would have led to the migration of hunter-gatherers to coastal sites within Africa but also in Southern Asia and the settlement of the first permanent communities (Marean et al., 2007).

Marine resources continued being the main protein source during the Mesolithic (20000-5000 BC). The consumption was remarkably reduced in the European Neolithic (10000-2000 BC) due to the appearance of farming and cattle raising, and resulting increase in meat consumption (Milner et al., 2004). Despite the decrease, the importance of fishing and shellfish collection was maintained continuously in some areas, including Baltic, Atlantic and Mediterranean coasts (Lidén et al., 2004; Ramos et al., 2011).

Both the North Pacific area, from Japan to Baja California, and the Andean Coast of South America are home to extensive kelp forests that support a rich variety of shellfish, fish, marine mammals and seaweeds. These ecosystems may have been an attractive site for early maritime communities and led to the establishment of migration routes from Asia to America about 16000 years ago. Kelp forests offered a large number of food resources, which could be harvested with relatively simple techniques, as well as reducing the wave energy and providing holdfasts for boats (Erlandson et al., 2007).

Pacific Asian civilizations have exploited aquatic organisms for thousands of years (Makino and Matsuda, 2005). Indeed, abundant shell remains found in the Pacific coast of Japan demonstrate that early fishing and shellfish gathering communities were settled there by 4500-2000 years ago. The found materials suggest that the settlements were specialized in different aquatic ecosystems, including freshwaters, brackish or shallow waters and inshore marine waters (Ruddle, 1987). During the next period (300 BC-300 AD), fisheries acquired a higher level of specialization and a larger scale (Makino and Matsuda, 2005; Ruddle, 1987). Besides catching fish from wild environment, aquaculture systems for fish farming were developed at least 3000 years ago in China (Castelló Orvay, 1993).

In addition to fish, the Chinese and Japanese have consumed algae for more than 1000 years, both for their dietary and medicinal value (Lobban and Wynne, 1981). Their therapeutic applications included the treatment of goiter and other glandular diseases, stomach and intestinal disorders and the use as antihelmentic agent to control worms in the digestive tract (Chennubhotla et al., 2013). This extensive use resulted in the establishment of algal cultivation systems by 960-1300 AD in China and by 1600 AD in Japan, long before the beginning of modern aquaculture industry (Blouin et al., 2011).

Prehistoric Polynesian native populations, established between 3000-1000 years ago, would also base their subsistence on marine and freshwater fauna such as large fish, mollusks (gastropods, cephalopods) and crustaceans (Anderson, 2008; Oliver, 1989). The area included a wide variety of ecosystems, ranging from lagoons and tropical coral reefs to subpolar zones (Anderson, 2008).

Fishing is considered to have played a key role in diet during both prehistoric (6000-3500 BC) and Dynastic Egypt (3100-330 BC) (Bard, 1999; Luff and Bailey, 2000). This assumption is supported by the finding of fish remains and fishing tools (including nets and hooks) and the depictions in tomb scenes showing fish given as wages and sales in markets (Bard, 1999). Other materials from aquatic origin such as marine and freshwater shells are commonly found in graves, as part of amulets, personal ornaments (e.g. necklaces, bracelets, rings) and utensils (Lucas and Harris, 2000). Similar objects were also used in ancient Mesopotamia, where mollusks were a source of food and shells (Moorey, 1994).

Marine mollusks would have provided pigments, such as porphyrins that Minoans (in Crete) and Phoenicians (in Tyre and Sidon) obtained by adding salt to the hypobranchial gland of the organisms and then boiling it in water (Cooksey, 2001; Reuben, 2006). According to Cooksey et al. (2001), the purple dye was generated from precursors rather than contained in the hypobranchial gland itself. The quantity of pigment in each organism was so scarce that about 12,000 mollusks had to be processed to obtain a gram of purple, which was required to dye one toga. Therefore, "Tyrian purple" was a very valuable product, only reserved for the robes of emperors and high priests (Reuben, 2006). Similar pigments from mollusks also have a long history in Central and South America (Cooksey, 2001).

**Figure 1.3.** *Plicopurpura pansa*, one of the mollusks found in Central American coasts from which purple pigment can be obtained.
Source: Natural History Museum, London (2015).

Fish consumption in the ancient Greece (8800-500 BC) has commonly been considered limited, linked to inefficient fishing and aquaculture practices (Marzano, 2013; Vika and Theodoropoulou, 2012). However, recent studies suggest that the presence of fish and shellfish in Greek diets may have been more frequent that previously thought in several regions (Vika and Theodoropoulou, 2012).

The use of marine resources showed a rapid increase in the Roman period (750 BC - 476 AD), as reflected by the rise of the number and size of facilities, besides well-organized labor and trade networks, and innovative technologies (Marzano, 2013). Moreover, classical Greek and Roman civilizations would have used marine organisms not only as food sources but also for dye production, personal hygiene preparations and even for their therapeutic properties (Marzano, 2013; Voultsiadou, 2010). The first contributions to the description and classification of marine biodiversity correspond to the work of Aristotle and Plinius. Aristotle also reported the existence of cultivation systems for oysters in Greece (Historia Animalium), whereas Plinius described similar systems for oysters and moray eels in Rome (Historia naturalis, liber IX) (Castelló Orvay, 1993; Coll et al., 2010).

**Figure 1.4.** Roman seafood mosaic from the House of the Faun in Pompeii (Italy), currently displayed in the Archaeological Museum of Naples.
Source: Sheila Terry, in FineArtAmerica (2015).

During the Middle Ages (500-1500 AD), Europe's consumption of fish and other aquatic organisms was mainly related to human feeding. However, other uses of marine resources have been reported, including the use of sponges as therapeutically valuable resources against some diseases (Müller et al., 2004). At the time, the term "fish" included all water-living organisms and its consumption was linked not only to nutritional but also to cultural and religious reasons, such as showing high social rank or obeying Christian precepts (Hoffmann, 2005). Fresh fish was obtained from wild catching but also from aquaculture systems, which were mainly found in monasteries and abbeys (Beveridge and Little, 2007; Castelló Orvay, 1993).

The following period (1500-1800 AD) supposed a remarkable rise in the commercialization of resources, which was even more pronounced after the beginning of the Industrial Revolution. However, it was not until the twentieth century when most market and trade evolved from a regional to a global context (Lotze et al., 2011). In contrast to the exploitation of other food resources (cultivation of crops and cattle raising), the use of marine resources is mainly based on the catching of wild fish. Therefore, the industrialization of fishing was more likely to affect the size of fish stocks and even lead the extinction of species. For this reason, fisheries science emerged in the nineteenth century linked to marine biology and oceanography to control the exploitation of resources (Higman, 2012). By the end of the century, the first controlled aquaculture systems were developed, although industrial-scale systems, based in ponds or sea pens, became widespread after 1980s, beginning with salmon farming (Castelló Orvay, 1993; Higman, 2012).

Despite the long history of exploitation of aquatic resources by humans, the systematic collection of samples for the study of marine biodiversity began in the seventeenth and eighteenth centuries. The first studies (~1700-1900 AD) consisted of European, North American and Russian exploration expeditions in several regions (e.g. South Africa, South and Caribbean America or Pacific Ocean) to gather samples. The collected specimens were mainly brought to the explorer's countries, where they were described and included in museum collections. In the early 1900s, the first institutions and research stations in developing countries were founded, allowing on-site descriptive studies. The

enhancement during this period was linked to the broader availability of research experts and material resources, and evolved to wide-scale laboratories in several countries since the 1950s. Finally, in the last three decades, marine research has adopted a large-scale multidisciplinary perspective, as a result of the development of innovative technologies and international research projects (Costello et al., 2010).

## 1.2. Blue biotechnology

### 1.2.1. Natural biologically active molecules from aquatic organisms

Due to the large biological diversity of aquatic ecosystems, oceans and freshwaters have proven to be a unique source of natural compounds (Haefner, 2003; Larsen et al., 2005). Biotechnology can be defined as *"any technological application that uses biological systems, living organisms, or derivates thereof, to make or modify products or processes for specific use"* (FAO, 2000). In particular, blue biotechnology is an emerging sector that aims at developing technological bioprocesses to provide goods and services from aquatic organisms and their derivatives (Freitas et al., 2012).

Although extracts from aquatic organisms have been used for centuries, systematical research on marine and freshwater resources with potential uses in biotechnology began in the middle of the twentieth century. In 1947, nine species of marine organisms with antibiotic activity were identified by Rosenfeld and ZoBell. By 1950s, Ross Nigrelli, involved in one of the first marine biotechnology projects in US, identified a toxin with antitumor activity in mice, named holothurin, which can be extracted from the sea cucumber *Actinopyga agassizi* (Colwell, 2002). In the same period, two nucleosides from the marine sponge *Tethya crypta* were discovered by the organic chemist Werner Bergmann and led to the synthesis of the first marine-derived anticancer compound cytosine arabinoside (Ara-C), which is currently used to treat leukemia and lymphoma (Beedessee et al., 2015; Mayer et al., 2010).

Nigrelli's and Bergmann's findings served as a starting point for the search of cytotoxic molecules of marine origin with potential uses as anticancer drugs. Moreover, Bergmann's research also resulted in the synthesis of the antiviral

compound adenine arabinoside (Ara-A). This nucleoside was approved by the US Food and Drug Administration in 1976 and used to treat keratoconjunctivitis and keratitis caused by herpes until 2001 (Mayer et al., 2010).

Despite the biotechnological potential of aquatic natural products, early research in the 1950-1960s entailed purely chemical studies that excluded tests on biological activities (Carté, 1996; Radjasa et al., 2011). Novel organic molecules were isolated and characterized, following the model of previously reported extracts from terrestrial plants (Ireland et al., 1993). The investigation of potential applications of aquatic organisms related to their biological activities gained importance throughout the 1970s, thanks to several symposia on food and drugs from the sea (funded by the Marine Technology Society) as well as strategic publications (Carté, 1996; Radjasa et al., 2011).

Since 1960s, more than 24000 natural products from aquatic organisms have been isolated (Blunt et al., 2015). These compounds have their origin in the strong need for survival strategies associated with the competitive nature of aquatic ecosystems (Simmons et al., 2005). For this reason, numerous species have developed metabolic pathways for the production of chemicals to protect themselves against external factors such as ultraviolet radiation or predators, defend against overgrowth by competing species or paralyze mobile preys for ingestion (Haefner, 2003; Kim, 2015; Simmons et al., 2005).

The synthesized metabolites include a high diversity of chemical classes, such as terpenoids, steroids, alkaloids, carotenoids, polyunsaturated fatty acids, peptides and polysaccharides, among others (Murray et al., 2013; Simmons et al., 2005). These metabolites show a wide range of functional properties, including antioxidant, antimicrobial, antiviral, antitumor, anticoagulant, antihypertensive, antielastase and antihyaluronidase activities (Kim, 2015; Murray et al., 2013).

Due to the variety of structure and chemical properties of the metabolites, aquatic organisms can provide not only pharmaceuticals, but also other valuable bioactive molecules for food, cosmetics, nutraceutical, animal feed and agrochemical industries (Leary et al., 2009; Murray et al., 2013; Pérez-López et al., 2014).

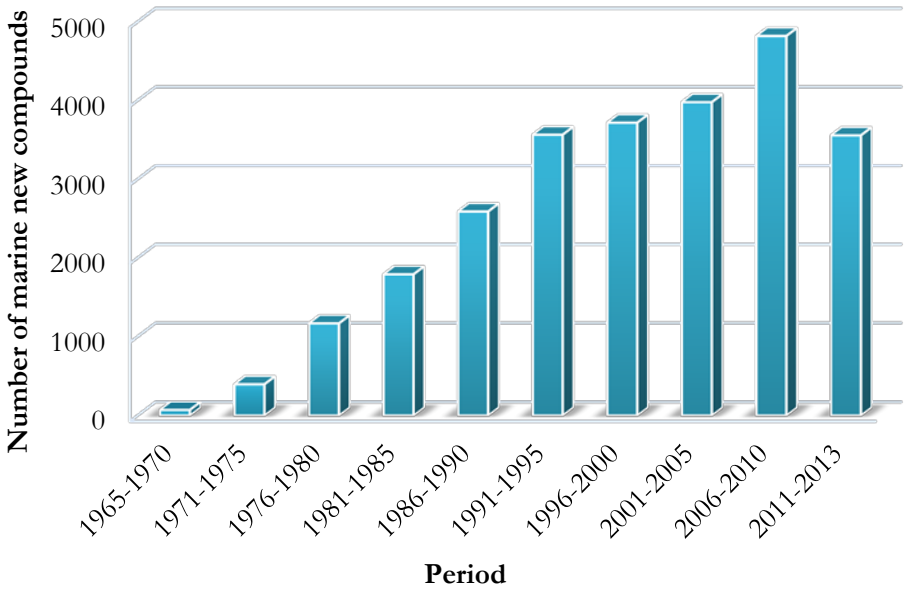



**Figure 1.5.** Number of marine natural products identified between 1965-2013. Source: Adapted from Blunt et al. (2007) and MarinLit database (2015).

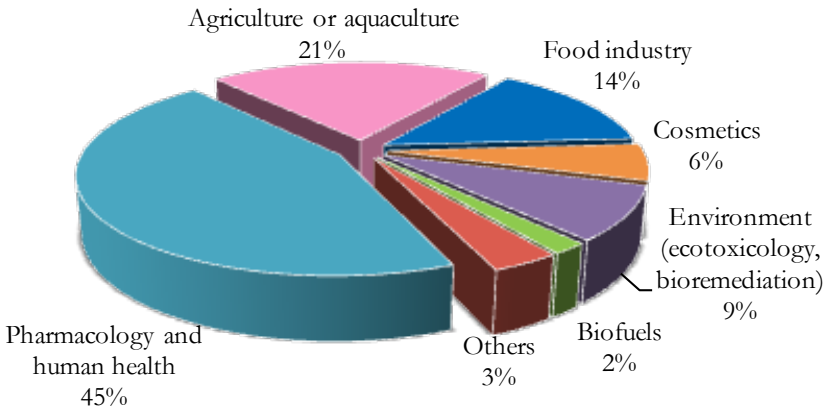



**Figure 1.6.** Percentage of patents related to marine genetic resources per sector (normalized to avoid double counting of resources with multiple applications). Source: Adapted from Arrieta et al. (2010).

- Pharmaceuticals

  Aquatic bioresources have a huge potential as human medicines. Since the approval of the first marine-derived bioactive compounds (antileukemia Ara-C and antiviral Ara-A) in 1970s, many natural products from aquatic organisms and their derivatives have been involved in preclinical studies and 25 are currently in clinical trials (Beedessee et al., 2015; Mayer, 2015).

  Nowadays, at least eight drugs from aquatic origin have been approved, most of them linked to the treatment of cancer (**Table 1.1**). This number of pharmaceuticals approximately corresponds to one therapeutic agent for every 3000 identified natural products (Mayer, 2015). The ratio reflects the high potential of blue biotechnology for pharmaceutical applications, compared to the global estimate of one approved drug for each 15000 tested compounds (Gerwick and Fenner, 2013).

- Food and nutraceuticals

  Aquaculture can provide fish, which is the most significant aquatic source of protein in human diet, together with other organisms that are consumed as food, such as algae or invertebrates (Kim, 2015).

  Moreover, several compounds from aquatic origin have applications in food industry. Among these biomolecules, polysaccharides (e.g. alginate, carrageenans and agar) are common in macroalgae and can be used as thickeners, stabilizers and gelling agents (Freitas et al., 2012; Pérez-López et al., 2014). Polyunsaturated fatty acids (e.g. eicosapentaenoic acid, docosahexaenoic acid) are accumulated in several microalgae and seaweeds and have potential uses as dietary supplements and ingredients for infant nutrition. Photosynthetic pigments, including carotenoids and phycobiliproteins, are mainly found in microalgae and serve to obtain nutraceuticals, food colorants and additives for animal feed. Enzymes produced by fungi and bacteria can be applied in food preservation, improvement of food taste and texture, dietetic food and cheese manufacturing, among others (Pérez-López et al., 2014).

**Table 1.1.** Currently approved pharmaceuticals from aquatic natural products (Biosyn Corporation, 2015; Gerwick and Fenner, 2013; Mayer, 2015; Mayer et al., 2010)

| Compound | Source organism | Chemical class | Year of approval | Trademark | Disease Area | Company |
|---|---|---|---|---|---|---|
| Ara-C | Sponge | Nucleoside | 1969 | Cytosar-U® | Leukemia | Bedford Laboratories |
| | | | | Depocyt® | Limphomatous meningitis | Enzon Pharmaceuticals |
| Ara-A | Sponge | Nucleoside | 1976 | Vira-A® | Keratoconjunctivitis Superficial and recurrent epithelial keratitis caused by herpes simplex virus | King Pharmaceuticals (now discounted) |
| Keyhole limpet hemocyanin | Mollusk | Oxygen-carrying protein | 1997* | Immucothel® | Bladder cancer | Biosyn Corporation |
| Ziconotide | Cone snail | Peptide | 2004 | Prialt® | Sever chronic pain in patients with cancer or AIDS | Jazz Pharmaceuticals |
| Omega-3-acid ethyl esters | Fish | Omega-3 fatty acids | 2004 | Lovaza® | Hypertriglyceridemia | GlaxoSmithKline |
| Ecteinascidin-743 | Tunicate | Alkaloid | 2007 | Yondelis® | Soft tissue sarcoma Ovarian cancer | Pharmamar |
| Erybulin mesylate | Sponge | Macrolide | 2010 | Halaven® | Metastatic breast cancer | Eisai Inc. |
| Brentuximab vedotin | Mollusk cyanobacterium | Antibody-drug conjugate | 2011 | Adcetris® | Anaplastic large cell and Hodgkin's lymphoma | Seattle Genetics |

* Only marketed in Argentina, Austria, South Korea and the Netherlands (Biosyn Corporation, 2015)

- Cosmetics and cosmeceuticals

  The development of cosmetics from aquatic origin is related to the research in other fields of application. In particular, the applications on personal care and beautifying products can arise as a parallel pathway when testing bioactive compounds for pharmaceutical purposes (Martins et al., 2014).

  Moreover, there is a recent trend toward combining cosmetic and pharmaceutical properties to obtain products that are referred to as cosmeceuticals (Kim et al., 2008; Martins et al., 2014). These products contain active ingredients that are beneficial for human health. Bioactive compounds such as vitamins, enzymes, antioxidants, phytochemicals and essential oils are applied as creams, lotions but also as ingestible liquids or pills. The sources of the biomolecules include microalgae, seaweeds, marine bacteria, fish, coral and crustaceans (Kim et al., 2008).

  Some examples of marine-derived cosmeceuticals recently launched by prestigious companies include Blue Therapy (Biotherm, Tours, France), Crème de la Mer (Estée Lauder, New York, NY, USA), and Elemis (The Steiner Group, London, UK) (Martins et al., 2014).

- Agrochemicals

  Agrochemical agents are a key factor involved in the increasing agricultural productivity over the last decades. Although conventional synthetic pesticides have been successfully used for years, there is a current need to find alternative pesticides for two main reasons: 1) insects, slugs and other pests are developing resistance to existing agrochemicals; and 2) synthetic chemical pesticides exhibit toxicological and environmental risks that are becoming a major concern for society (Peng et al., 2003).

  The wide range of potential applications of agrochemicals from aquatic origin still remains largely unexplored, compared to other sectors (e.g. pharmaceutical biocompounds). Nevertheless, there are already some examples of marine-derived pesticides in the market, mainly associated

with metabolites from marine sponges and microbes (Gerwick and Sparks, 2014). In addition, a significant number of active compounds from aquatic origin are being investigated due to their activities as insecticides, herbicides and fungicides (Peng et al., 2003).

Nereistoxin is a sulfur-containing biocide metabolite from a marine annelid that has led to the synthesis of the analogues bensultap, cartap and thiocyclam. These biopesticides are currently commercialized as insecticides (e.g. Padan®, Evisect®S). Among other compounds with insecticidal or herbicidal activities are polyhalogenated metabolites from red algae, phosphorylated derivatives from marine sponges and manzamine-derived alkaloids from marine sponges (El Sayed et al., 1997; Gerwick and Sparks, 2014; Peng et al., 2003).

- Animal feeding

The application of blue biotechnology in animal feeding is mainly associated with algae. Indeed, about 30% of the world algal production is sold for animal feeding. Most of this consumption is related to aquaculture and feed supplements for poultry (Becker, 2007). In addition, some seaweed-derived bacteria (particularly, *Bacillus* spp.) are currently being investigated for their potential as probiotics in pigs (Prieto et al., 2014).

Despite the diversity of applications, many bioactive compounds from aquatic organisms have a complex chemical structure, which results in a difficult and costly synthesis. Hence, the commercial implementation by developing chemical routes is limited to certain molecules and unfeasible for others. In addition, the bioactive metabolites are usually found in extremely low concentrations, so natural sources are rather scarce to base the production on wild harvest (Pérez-López et al., 2014). Therefore, the feasibility of aquatic-based compounds relies on the development of sustainable cultivation methods that allow a consistent supply (Murray et al., 2013).

### 1.2.2. Production systems for the different aquatic sources

The main sources of marine and freshwater natural products are algae, invertebrates and microorganisms (Larsen et al., 2005). In particular, 53% of aquatic natural compounds isolated to date have been extracted from invertebrates belonging to phyla *Cnidaria* (e.g. anemones, corals, jellyfish) and *Porifera* (i.e. sponges), 12% from *Rhodophyta* (i.e. red algae) and *Ochrophyta* (mostly brown algae and diatoms) and nearly 9% from *Ascomycota* (i.e. phylum of fungi) (Blunt et al., 2015). Since the detailed taxonomic classification of aquatic organisms is too complex for the scope of this thesis, the addressed producers of biologically active compounds are here grouped into the five categories described below:

- Microalgae

  This category includes a wide variety of unicellular and simple multi-cellular microscopic organisms that are primary producers of oxygen in aquatic ecosystems (Brennan and Owende, 2010; Kim, 2015). Virtually all microalgae are photosynthetic organisms capable of converting solar energy and carbon dioxide ($CO_2$) into biomass and oxygen. However, some microalgal species can also grow under heterotrophic and mixotrophic conditions. Heterotrophic growth consists in the use of organic compounds and nutrients for growth in absence of light and without fixing $CO_2$. Mixotrophic conditions corresponds to the simultaneous assimilation of $CO_2$ and organic carbon to perform respiratory and photosynthetic metabolism at the same time (Perez-Garcia et al., 2011).

  Microalgae are found as phytoplankton floating throughout the oceans and freshwaters, or as benthic or sediment-dwelling communities attached to the bottom or sides of aquatic habitats (Roesijadi et al., 2008). They can be both eukaryotic and prokaryotic organisms and exhibit diverse sizes, morphology and metabolism (Brennan and Owende, 2010; Khan et al., 2009; Kim, 2015). Microalgae are mainly classified according to their pigmentation, life cycle and cellular structure (Brennan and Owende, 2010). Among the different species, the most abundant are eukaryotic green algae (*Chlorophyceae*), golden

algae (*Chrysophyceae*) and diatoms (*Bacillariophyceae*); jointly with prokaryotic blue-green algae, also known as cyanobacteria (*Cyanophyceae*) (Khan et al., 2009; Kim, 2015).

The interest in microalgal cultivation relies on the diversity of valuable metabolic products that include polyunsaturated fatty acids, pigments, enzymes, polymers and toxins (Brennan and Owende, 2010; Perez-Garcia et al., 2011). In addition, microalgae can be directly used in animal feeding (especially for aquaculture) and as a tertiary wastewater treatment. Its high lipid content makes them an attractive alternative to conventional energy crops for biofuels production (Perez-Garcia et al., 2011).

Microalgae cultivation can be performed in two main types of reactors: open systems and closed photobioreactors (Brennan and Owende, 2010; Chisti, 2007). Open systems are exposed to the surrounding environment and include natural (lakes, lagoons) and artificial (circular ponds, raceway ponds) arrangements. Closed photobioreactors (PBRs) are used to overcome typical bottlenecks of open systems such as low biomass productivities or contamination risks, but have higher costs of infrastructure and operation. The most common closed PBRs are tubular, flat panel and column photobioreactors (Brennan and Owende, 2010). Open raceway ponds (ORPs) and tubular PBRs are currently considered as the two most feasible methods for large-scale cultivation of microalgae (Chisti, 2007).

- Seaweed

Macroalgae or seaweeds are macroscopic multicellular primary producers that, as microalgae, base their growth on photosynthesis (Kim, 2015; Singh et al., 2011). Algae are essential elements of the food chains of all aquatic ecosystems. As well as their nutritional interest as low calorie sources of vitamins, minerals and dietary fibers, seaweed can provide compounds with antioxidant, antiviral, antifungal and antimicrobial properties, among others.

Macroalgae are especially common in the euphotic region of lakes, rivers and seas (where light can penetrate up to 200 m deep), usually fixed to a solid substrate (e.g. rocky shore zones). They are classified according to their particular pigment composition and mainly found in three groups: red algae (*Rhodophyceae*), green algae (*Chlorophyceae*) and brown algae (*Phaeophyceae*) (Kim, 2015).

Nearly 90% of macroalgae for human consumption are obtained from cultivation, with the remaining 10% coming from wild harvest. (Roesijadi et al., 2008). Cultivation techniques can be based either on vegetative propagation or involving a reproductive cycle.

In the first case, small pieces of seaweeds are collected from the wild environment and tied to ropes or placed in nets on floating structures and periodically harvested. Alternatively, other species can be placed in land-based ponds and tanks (similar to microalgal ponds), which is economically less attractive, or in tidal flat farms (Fasahati et al., 2015; McHugh, 2003).

Regarding species that can only be grown through a reproductive cycle, cultivation from cuttings is not possible and systems involve the alternation of generations. This technique requires transitions from spores released by grown seaweed (sporophytes) to gametophytes, and from the gametes produced by gametophytes that join to form embryonic sporophytes, which can finally grow in the aforementioned vegetative systems and be harvested (McHugh, 2003). The transitions can take place in nursery tanks or ponds and in controlled photobioreactors (McHugh, 2003; Rorrer and Cheney, 2004). The main stages of production of seaweeds with growth based on a reproductive cycle are depicted in **Figure 1.7**.


**Figure 1.7.** Seaweed farming for reproductive cycle species.
Source: Adapted from Roesijadi et al. (2008).

❖ Sponges

Marine sponges have been the major source of aquatic natural products for the last two decades, with over 7000 new isolated molecules (Leal et al., 2012; Schippers et al., 2012). They are benthic and sessile organisms that base their communication and defense systems on the production of secondary metabolites. Sponges have a wide variety of applications ranging from pharmaceutical uses of their metabolites to the production of new biomaterials for nanotechnology (e.g. use of biosilica-producing ezymes) or fiber-optic communications (e.g. silicon skeleton of glass sponges with unique optical and mechanical properties) (Kulchin et al., 2009; Schippers et al., 2012).

The production strategy for the development of applications of sponges mainly depends on the concentration of the compound within the sponge biomass. Hence, for products that are found in a high concentration inside the sponge, or when the sponge is the product, cultivation of adult specimens is the most recommendable approach. It can be done in sea-based culture systems (*in situ*) by placing small explants on a substrate (e.g. ropes, nylon lines, nets) or in *ex situ* aquaria. On the contrary, when the interest relies on a secondary metabolite with a low concentration, *in vitro* cell cultivation may be more appropriate. In some cases, microbial symbionts are the real producers of bioactive compounds, rather than the sponge itself, so separate culture of the symbiont is possible. A combined co-culture of the sponge and the symbiont may be needed when the compound is partly produced by each of the two organisms (Schippers et al., 2012).

- Bacteria

Marine and freshwater bacteria are prokaryotic, mainly unicellular organisms isolated from aquatic organisms (Wagner-Döbler et al., 2002). They are plentiful in microbial communities of pelagic and benthic ecosystems (Kim, 2015). Among the large diversity of bacteria, the phyla *Actinobacteria* and *Proteobacteria* constitute significant sources of bioactive compounds, since they produce 4% of all the identified natural products from aquatic origin (Blunt et al., 2015).

Despite the capability of aquatic bacteria as producers of antimicrobial and antibiotic metabolites, research on bioreactor engineering and fermentation processes is still needed to develop feasible systems. Bioreactors for bacterial cultivation may operate in batch, fed-batch and continuous mode, depending on the type of microbe and target metabolite. Each species requires specific complex carbon sources as well as limited temperature and pressure ranges (Lang et al., 2005). In addition, a great number of bacteria coexist in symbiosis or commensalism with other marine organisms, so co-cultivation techniques may be required to increase the quantity and diversity of bioactive metabolites (Kim, 2015; Lang et al., 2005; Pettit, 2009).

❖ Fungi

The term "marine fungi" refers to an ecological category of fungi (unicellular and multicellular eukaryotic nonmotile organisms which grow by heterotrophic absorption of nutrients) that live in marine or estuarine habitats. "Obligate" marine fungi grow and sporulate exclusively in seawater, whereas "facultative" come from freshwater or terrestrial habitats but have developed physiological adaptations to grow and sporulate in marine environments (Kim, 2015).

Although fungi have received little attention compared to other aquatic sources, they have recently proved to be suitable producers of diverse bioactive metabolites, probably as a chemical defense and as a mean to compete for substrate (Bhadury et al., 2006; Kim, 2015). Some of these metabolites are already being produced through aquaculture, chemical synthesis or fermentation (including liquid and solid state fermentation) and tested in clinical trials. However, the commercial feasibility requires the development of large scale production systems. The most promising solution in the future may be the integration between combinatorial genetic and metabolic engineering (Bhadury et al., 2006).

### 1.2.3. Downstream processing

Regardless of the producer organism, the cultivated biomass has to be recovered and concentrated in sequential harvesting stages. Biomass recovery usually involves a first step of "bulk harvesting" to a total solid content between 2-7%, by technologies such as flocculation or gravity sedimentation, followed by a thickening step with techniques such as centrifugation or ultrafiltration and common efficiencies above 95% (Brennan and Owende, 2010).

The recovered biomass is subjected to an extraction and purification process to separate the target product. Chemical cell disruption, enzymatic hydrolysis, solvent extraction and mechanical disruption are some common conventional methods used for extraction. Their disadvantages include high costs and energy requirements, low yield, stability and safety concerns. In order to overcome the bottlenecks, novel techniques such as supercritical fluid extraction (e.g. using $CO_2$ as supercritical fluid), are currently being evaluated (Murray et al., 2013).

The complex nature of aquatic organisms and their interactions makes optimization and research on new culture and recovery methods necessary (OECD, 2013). In addition, due to the low content of high value molecules within the biomass, the economic viability will in many cases depend on the potential combined exploitation of co-products (Brennan and Owende, 2010).

## 1.3. Microalgal biorefineries

### 1.3.1. Biofuel conversion techniques

Microalgae are promising sources of different types of renewable biofuels that exhibit several advantages with respect to other bioenergy feedstocks. The main benefits are their higher photosynthetic efficiency, their ability to produce more biomass per unit area than terrestrial plants, and the possibility of cultivation in marginal land using fresh or saltwater (Brennan and Owende, 2010; Clarens et al., 2010). Moreover, microalgae do not compete directly with food crops and can be coupled to the treatment of waste streams such as nutrient-laden wastewater or flue gases enriched in $CO_2$ (Clarens et al., 2010).

Biofuels that can be obtained from microalgae include liquid fuels such as biodiesel or bioethanol, but also biogas or biohydrogen (Brennan and Owende, 2010; Chisti, 2007). Technologies for the conversion of microalgal biomass into biofuels can be grouped in three categories, namely chemical, thermochemical and biochemical processes, which are schematized in **Figure 1.8** .

The main example of chemical conversion is the transesterification reaction between an extracted crude oil containing triglycerides (TAGs) and alcohol, to give biodiesel and glycerol. Thermochemical processes (e.g. gasification, thermochemical liquefaction, pyrolysis) are based on the thermal decomposition of organic components within the microalgal biomass. Biochemical conversion refers to the production of energy by means of biological processes related to the metabolism of living organisms (e.g. anaerobic digestion, alcoholic fermentation). The selection of the conversion technique depends on the type and quantity of biomass, the economic parameters and the desired final form of energy (Brennan and Owende, 2010).

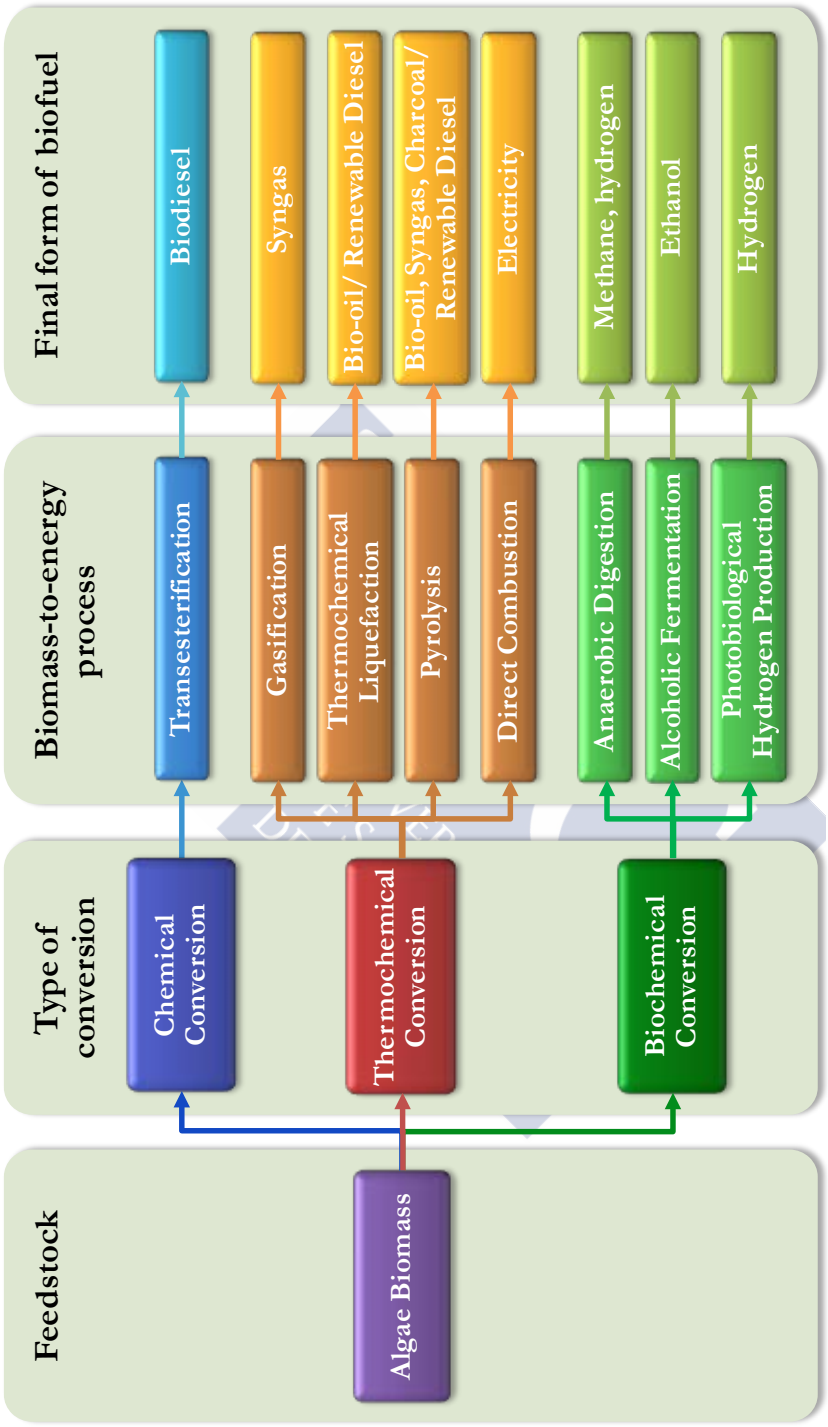



**Figure 1.8.** Microalgal biofuels conversion process.
Source: Adapted from Brennan and Owende (2010) and Demirbas (2011).

❖ Biodiesel and renewable diesel

Both fuels can be obtained from the algal lipid fraction separated from the biomass in the extraction stage (Brennan and Owende, 2010). The term "biodiesel" refers to a mixture of mono-alkyl esters of long-chain fatty acids (FAME) derived from renewable lipid feedstocks, such as vegetable and algal oils or animal fats (Brennan and Owende, 2010; Knothe, 2010). As aforementioned, the conversion is based on a chemical reaction between alcohol and crude oil rich in TAGs that results from the extraction stage, being glycerol a co-product (**Figure 1.9**). "Renewable diesel" is a petrodiesel-like fuel according to its composition, including a complex mixture of straight-chain alkanes (major constituents), branched alkanes and aromatic compounds. It can be produced by different catalytic processes applied to the TAGs fraction, which includes cracking or pyrolysis, hydrodeoxygenation, etc (Hoekman et al., 2012; Knothe, 2010).

**Table 1.2.** Typical properties of biodiesel and renewable diesel compared to petroleum diesel (Hoekman et al., 2012)

| **Property** | **Petroleum diesel** | **Biodiesel** | **Renewable diesel** |
|---|---|---|---|
| Specific Gravity | 0.85 | 0.88 | 0.78 |
| Energy content per mass unit (MJ/kg) | 43 | 39 | 44 |
| Energy content per volume unit (MJ/L) | 37 | 34 | 34 |
| Cetane number | 40-45 | 45-55 | 70-90 |
| Viscosity ($mm^2/s$, 40ºC) | 2-3 | 4-5 | 3-4 |
| Carbon (wt.%) | 86.8 | 76.2 | 84.9 |
| Hydrogen (wt.%) | 13.2 | 12.6 | 15.1 |
| Oxygen (wt.%) | 0.0 | 11.2 | 0.0 |
| Distillation temperature ($T_{90}$, ºC) | 300-330 | 330-360 | 290-300 |


**Figure 1.9.** Flowchart of algal lipid transformations to products of engine combustion.

Source: Adapted from (Knothe, 2010).

- Bioethanol

  Microalgae are gaining interest as an alternative source of biomass for alcoholic fermentation to produce bioethanol grouped under "third generation biofuels" (John et al., 2011). This is related to the substantial carbohydrate content of some species belonging to genera such as *Chlorella, Dunaliella, Scenedesmus or Spirulina* (10-30% dry weight), jointly with ethanol conversion efficiencies above 65% (Becker, 2007; Brennan and Owende, 2010; John et al., 2011).

  Carbohydrates can be extracted from the harvested biomass by mechanical means or by cell wall degrading enzymes. The resulting starch can be fermented with similar methods as other feedstocks, involving a saccharification in which the starch is hydrolyzed to simple sugars followed by the fermentation itself that is performed by yeasts (Brennan and Owende, 2010; John et al., 2011). Finally, the produced ethanol is purified by distillation and then condensed into liquid form to be blended with fossil fuels or directly used (John et al., 2011).

- Biogas

  Microalgal biomass can be subjected to anaerobic digestion (AD) to produce biogas, mainly composed of methane ($CH_4$, ca. 60-70%) and $CO_2$ (ca. 30-40%) (Brennan and Owende, 2010). The process occurs in four main stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. First, the complex compounds are broken down into soluble sugars (hydrolysis). The obtained simple sugars are converted by bacteria into volatile fatty acids (acidogenesis) that lead to the production of acetate, $CO_2$ and hydrogen (acetogenesis). Finally, acetate is metabolized into $CH_4$ and $CO_2$ (methanogenesis) (Brennan and Owende, 2010; Montingelli et al., 2015).

  Some estimates suggest that algal biomass could allow energy recovery comparable to that from the extraction of cell lipids, while producing a nutrient-rich effluent that could be recycled into the cultivation stage. However, there are several factors influencing the performance of the digester to be controlled (e.g. moisture content or C/N ratio). The

highest theoretical yields reported for AD of microalgae range between 0.4 and 1 L $CH_4$/g volatile solids (VS), although values obtained in practice hardly exceed 0.3 L $CH_4$/g VS (Mendez et al., 2015). These yields may increase by using optimized strategies, such as anaerobic co-digestion with appropriate co-substrates (Brennan and Owende, 2010).

❖ Hydrogen

Hydrogen molecule ($H_2$) is a clean and efficient energy carrier that can be produced by microalgae. Most current processes for the production of $H_2$ are thermochemical (e.g. steam reforming, coal gasification, partial oxidation or autothermal reforming of natural gas, petroleum refining). However, these processes require fossil fuels and produce $CO_2$. Hence, other technologies, such as water electrolysis or biological hydrogen production, seem more attractive from an environmental perspective. In particular, microalgae exhibit methabolic pathways for the production of $H_2$ including direct and indirect biophotolysis, as well as dark fermentation (Benemann, 2000; Pilon and Berberoğlu, 2014).

Direct biophotolysis involves the direct transfer of electrons from water to protons. Water splitting into hydrogen ions ($H^+$) and oxygen together with the reducing reactions of the electron carrier ferredoxin are coupled to a $H_2$ evolving hydrogenase enzyme (Benemann, 2000). However, photosynthetic oxygen causes inhibition to the hydrogenase enzyme, so the feasibility for industrial applications is very limited (Pilon and Berberoğlu, 2014).

An alternative to direct biophotolysis consists of a two-stage process. In this process, called indirect biophotolysis, electrons are first used to reduce $CO_2$ into organic compounds while oxygen is generated. In the next step, the accumulated organic compounds are separately used in absence of oxygen to generate $H_2$ (Benemann, 2000; Pilon and Berberoğlu, 2014).

In the case of dark fermentation, the organic substances are used as both energy and electron sources under anaerobic conditions, in the presence of hydrogenase enzyme. Since the microorganisms do not

need sunlight as the energy source and the system has no oxygen, $H_2$ production is continuously produced during day and night at higher rate and with no inhibition (Pilon and Berberoğlu, 2014).

Although microalgae can provide a diverse range of biofuels, techno-economic feasibility is essential for the industrial implementation. Viable biofuels must be competitive or cheaper than petroleum-based fuels, should require low land use, enable air quality improvement and minimize water use. Hence, exploitation of algal biofuels relies on the possibility to meet a sufficient production of net energy while providing environmental benefits (Brennan and Owende, 2010).

### 1.3.2. Novel integrated approaches: The need for co-products

Despite the advantages, there are still some concerns regarding the environmental and economic sustainability of microalgal feedstocks for biofuels (Lam and Lee, 2012). The implementation of large-scale systems is currently hindered by two main factors (Stephens et al., 2010). Firstly, the fractions that can be converted into biofuels are limited and the remaining biomass constitutes a significant quantity of waste in case no additional recovery steps are conducted (Soh et al., 2014). Furthermore, biofuels are a commodity of relatively low value, so the profitability of microalgal processes depends on the exploitation of co-products (Soh et al., 2014; Stephens et al., 2010; Wijffels et al., 2010).

Many authors agree that the extraction of more than one type of biofuel or additional co-products would allow increasing the value of the total harvested biomass, while offering benefits that can help to reduce the environmental impacts and improve the economics of the process (Jones and Mayfield, 2012; Soh et al., 2014; Stephens et al., 2010). Such approach corresponds to the "biorefinery" concept, according to which harvested microalgae should be processed in an integrated system to maximize the quantity and variety of products obtained, including not only biodiesel and other biofuels but also high value-added compounds such as organic pigments, omega-3 fatty acids or proteins for algal meals (Soh et al., 2014; Subhadra and Edwards, 2011). Thus, some rough estimates suggest that microalgal biodiesel may only be feasible for a biomass production lower than 0.40 €/kg and combined production of bulk products such as those detailed in **Figure 1.10** (Wijffels et al., 2010).

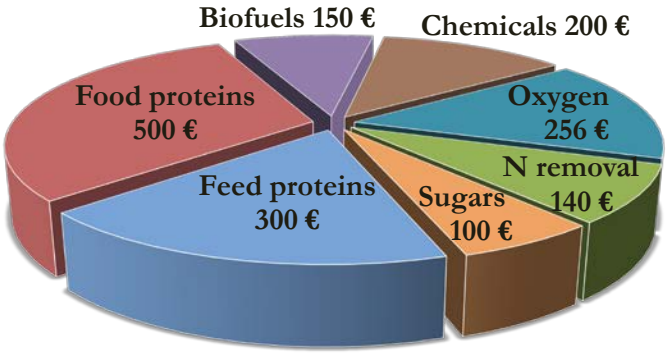



**Figure 1.10.** Approximate value of algal fractions per 1000 kg after a hypothetical biorefining scenario.
Source: Adapted from Wijffels et al. (2010).

Several hypothetical commercial algal biorefineries have already been simulated and analyzed in the literature. For example, Soh et al. (2014) proposed a system in which harvested biomass would be initially subjected to a lipid extraction stage to separate the oil for conversion into bio- or renewable diesel. The remaining biomass would be further processed to separate a protein fraction with applications as animal feed and the algal residue from this stage would be treated in an AD system to produce biogas and provide heat and electricity to the process. In addition, the liquid effluent from AD would contain nutrients that could be recycled to the cultivation stage. From the simulation, the authors conclude that the optimal environmental outcomes for microalgal processes correspond to equilibrium conditions in which lipid and non-lipid fractions are balanced to maximize the benefits of the whole produced biomass.

Another work evaluates the potential of two alternative biorefinery scenarios. In the first scenario, the conversion of microalgal oil to biodiesel would be combined with the production of glycerin (co-product in the transesterification process) and algal meal (alternative protein source to fish meal used for animal feeds). The second option includes an intermediate step that allows the recovery of omega-3 polyunsaturated fatty acids (PUFAs), mainly eicosapentaenoic and docosahexaenoic acids (EPA and DHA), which can be further refined to produce human nutraceuticals or increase the nutritional quality of algal animal feeds (Subhadra and Edwards, 2011).

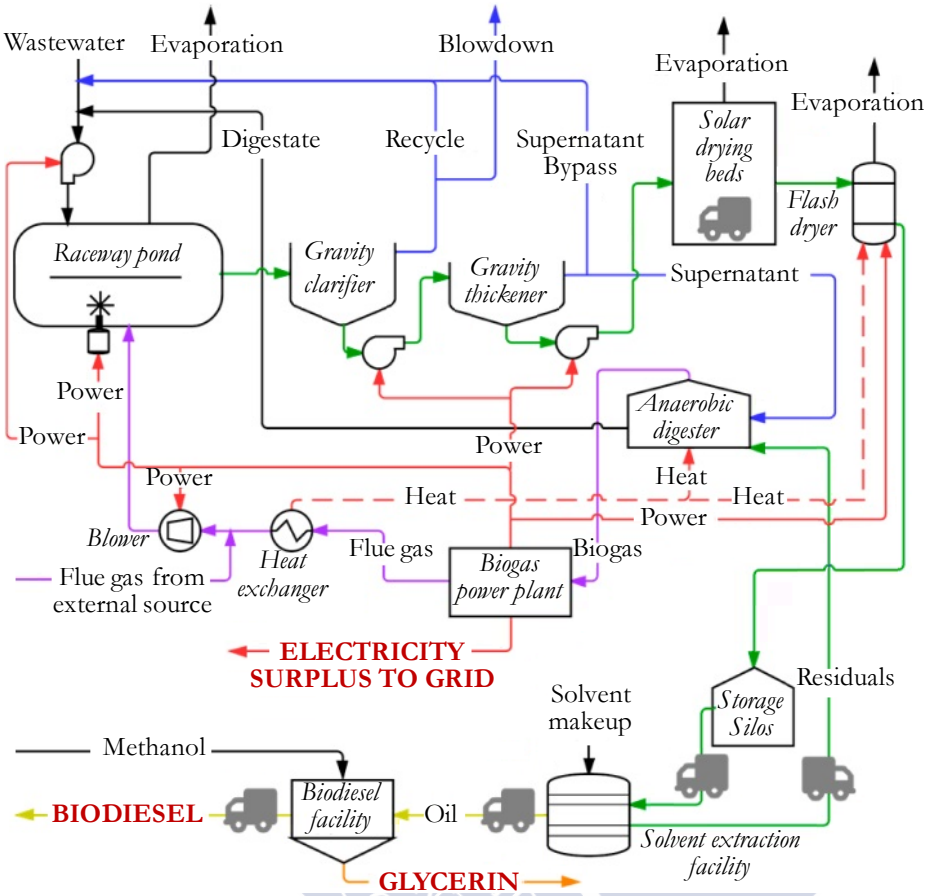



**Figure 1.11.** Process flowchart for a microalgal biorefinery with production of biodiesel and glycerin coupled to energy recovery and wastewater treatment. Source: Adapted from Woertz et al. (2014).

Despite the potential of co-product exploitation, the future development of commercial-scale systems for microalgal and other aquatic-origin products will also depend on the effectiveness of the mechanisms to drive investment in R&D and innovation strategies. In this context, combined initiatives and funding programs that empower international partnerships between scientific community and private sector are essential to attain global knowledge networks (Leary et al., 2009; OECD, 2013).

## 1.4. International market and policies towards Blue Growth

In last decades, blue biotechnology has arisen worldwide as a key emerging industry. The global market for this sector is expected to reach $4800 million by 2020 and exhibits a compound annual growth rate of approximately 5%. US leads the current market, with major companies such as CP Kelco, Cyanotech Corp. or Sea Run Holdings Inc (Global Industry Analysts, 2015). Although the Asian-Pacific area shows the highest market growth for the analyzed period, Europe still comprises a significant share of the global market (e.g. Aqua Bio Technology ASA in Norway, GlycoMar Ltd in Scotland, PharmaMar S.A in Spain). European market is expected to emerge due to the favorable geographic location as well as the large number of unexplored and underexploited aquatic habitats (Global Industry Analysts, 2015; Pérez-López et al., 2014).

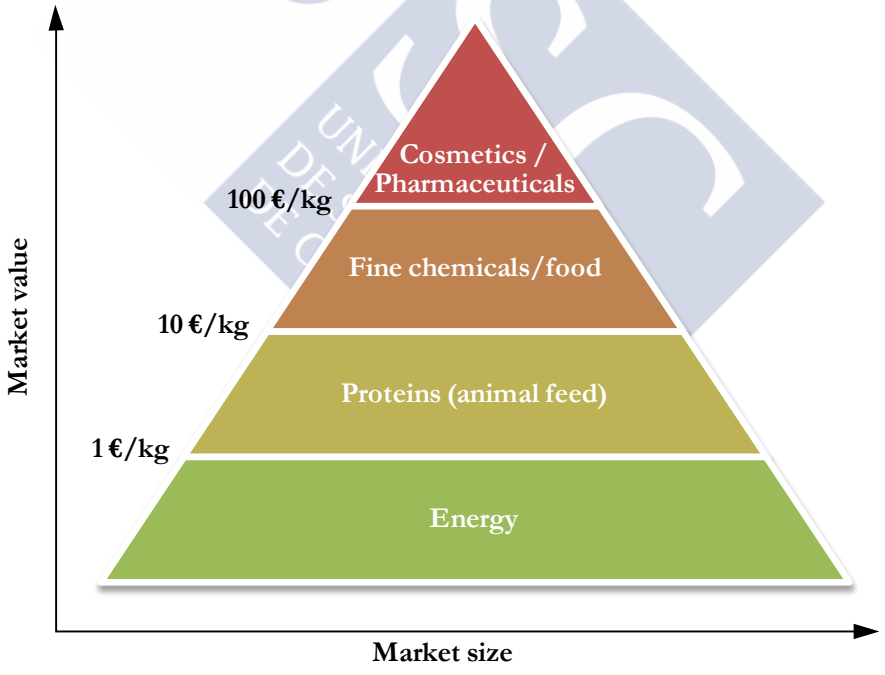



**Figure 1.12.** Estimated market value per mass unit of blue biotechnology products (Reference year: 2009).
Source: Adapted from OECD (2013).

Blue biotechnology is one of the key areas of the "Blue Economy", a recent term that designates all the economic activities that depend on the sea (e.g coastal tourism, maritime transport, offshore oil and gas, ocean renewable energies, etc.), excluding military activities. Blue economy is an essential group in the European Union (EU), due to the large number of jobs and gross added value associated (European Commission, 2012). For this reason, EU has adopted several programs in the last decade to promote an Integrated Maritime Policy and a sustainable "Blue Growth" in the marine and maritime sectors, aiming to maintain the balance between economic and environmental criteria (Ecorys, 2012; European Commission, 2012; Pérez-López et al., 2014).

Blue Growth initiative started in 2010 with a project that intended to provide policy-makers at EU with a comprehensive and detailed analysis of future options to support maritime activities. The analysis gave an insight into the state of the art within maritime sectors, as well as the main innovation and technological developments influencing these sectors, in order to identify the key economic activities within the blue economy and the potential effects of policy interventions (Ecorys, 2012). In the following years, EU has adopted an integrated European strategy towards a sustainable Blue Growth, related to the marine and maritime sectors in the long-term, which is in line with the global Europe 2020 targets. In particular, blue biotechnology has been identified as one of the five areas within this Blue Growth strategy.

According to EU's estimates, blue biotechnology may soon emerge as a niche market for health and cosmetic high-value compounds, as well as biomaterials. By 2020, the market is expected to expand as a source of primary products (e.g. lipids, sugars or proteins) with application in food, feed and chemical sectors, until it becomes a provider of bulk products in about 15 years (European Commission, 2012). In this context, the EU has developed different funding initiatives to promote key research and innovation in blue biotechnology (Ecorys, 2014; European Commission, 2012).

Between 2002 and 2006, the Sixth Framework Program (FP6) funded nine projects related to blue biotechnology, with a total budget of about 40 million € and a low involvement of industrial partners. Most of these projects focused on biodiversity of marine ecosystems, aquaculture and measures to stimulate the

sharing of "omic" (e.g. genomics, metabolomics) resources. Funding options increased for blue biotechnology with the Seventh Framework Program (FP7, 2007-2013), with a higher participation of small and medium enterprises (SMEs), which received 25% of the total budget (Ecorys, 2014). The program included calls with topics related to blue biotechnology such as the FP7 Knowledge-based Bio-Economy (KBBE) or the FP7 Ocean of Tomorrow, with about 82 and 90 million € respectively (Ecorys, 2014; European Commission, 2012,2015b). The most recent EU research program, Horizon2020, was launched in 2014 and will be ongoing until 2020. It is focused on three major priorities and includes one specific call for Blue Growth within one of the seven areas of the "Societal challenges" priority (European Commission, 2015a). The budget for this call accounts for 84 million € for the period 2014-2015, and is expected to achieve 160 million € for research into marine living resources and 500 million € for biotechnology (Ecorys, 2014).

While EU efforts are mainly related to aquatic biodiversity and high-value biotechnological products, most of R&D investments in the US are focused on the production of algae-derived energy (Global Industry Analysts, 2015). This strategy responds to the need for developing renewable fuels associated with the Renewable Fuel Standard (RFS) Program. The program was established by the Environmental Protection Agency (EPA) in collaboration with the US Department of Energy (DOE) and the US Department of Agriculture, in accordance with the Energy Policy Act approved by the US Congress in 2005 (Davis et al., 2012; EPA, 2015). In order to meet the RFS objectives, US public institutions are currently funding a wide range of activities to conduct research, development and demonstration towards the implementation of integrated biorefineries. As an example, four research consortia are now dealing with technical challenges of the large-scale production of algae, supported by a total federal budget of about $80 million (Davis et al., 2012).

The aforementioned initiatives play a key role for the development of partnerships and collaborations between researchers and industrial stakeholders. The development of integrated policies and investment in R&D may enable the creation of national wealth and global benefits while protecting the ecosystem health and sustainable use of aquatic resources (OECD, 2013).

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# Chapter 2

# Sustainability assessment tools and Life Cycle Thinking


***Summary***

The current population growth rate and technological advances put a real threat on the natural environment in the planet, which is already showing clear evidence of deterioration and resources depletion. The need to work towards a sustainable development is well recognized and considered a priority for governments and international organizations, but the achievement of this balance between progress and environmental protection requires objective measurement tools that allow monitoring the environmental and socio-economic dimensions of sustainability. Among the existing environmental management tools, which are briefly presented in this chapter, Life Cycle Assessment (LCA) is a worldwide accepted standardized methodology for assessing the environmental aspects and potential impacts of processes and activities. LCA is widely applied to the environmental assessment of marine-related processes, mainly in the field of microalgal biofuels, whereas several examples can also be found in the literature dealing with pharmaceutical and biotechnological products. In addition, the recent attempts for the application of Life Cycle Thinking to the evaluation of social and economic dimensions of sustainability are discussed. The goal and structure of this thesis are also summarized in Section 2.6.

# Index

## 2.1. Sustainable development

### 2.1.1. Origin and framework

The industrialization and globalization of the economy has led to a large number of changes in the world, especially related to the current human standard of living in developed countries (Ashford and Hall, 2011). The dramatic increase in population together with the development of new technologies and social structures are major issues affecting the surrounding natural environment (Ashford and Hall, 2011; Robinson, 2004). As a result, the planet seems to have reached a limit and is now showing evidence of deterioration and resources depletion (Ashford and Hall, 2011).

Over the last decades, the concept of sustainability has arisen as a major concern due to the growing awareness of a likely ecological crisis in the near future (Du Pisani, 2006). The first and most spread definition of the term was given by the World Commission on Environment and Development (WCED) in the Brundtland report (WCED, 1987). The report stated that the "sustainable development" referred to "***the development that meets the needs of present without compromising the ability of future generations to meet their own needs***".

Despite the recentness of the terminology, the underlying idea has a far longer history. Indeed, traditional wisdom and ancient indigenous beliefs were strongly linked to the importance of harmony of the society with nature (Mebratu, 1998). Moreover, environmental constraints can be observed as early as during Egyptian, Mesopotamian, Greek and Roman civilizations, with problems such as deforestation, salinization and loss of fertility of soil, or heavy metal pollution. However, whereas the first thoughts about progress date back to at least the classical Greco-Roman times, the concerns about the negative consequences related to the consumption of resources did not spread until the seventeenth and eighteenth centuries (Du Pisani, 2006; Mebratu, 1998).

The term "sustainability" was initially used in the eighteenth century by German experts on forestry to assert the need for a balance between harvesting and replacement of forest resources (Du Pisani, 2006). In the same period, Thomas Robert Malthus (1766-1834) was one of the first economists to highlight the

limits of the growth of population associated with the scarcity of agricultural land for a sufficient supply of food (Ashford and Hall, 2011; Malthus, 1798). During the nineteenth and first half of the twentieth century, different works started to focus on the possible exhaustion of non-renewable resources such as coal or oil, the disturbance of natural environment due to human intervention, and the need for a rational management of resources to ensure long-term use (Jevons, 1866; Marsh, 1864; Pinchot, 1910).

The environmental consequences of overexploitation and economic growth were further discussed after World War II, by authors such as H.F. Osborn (1948), K.W. Kapp (1950) and H.S. Brown (1956). In the next two decades, the awareness of people about the damage caused by humans to the total environment increased, to a large extent linked to the publication of books such as *Silent spring* (Carson, 1962), about environmental damages of the use of pesticides; *The population bomb* (Ehrlich, 1968), about negative effects of overpopulation; and *Small is beautiful* (Schumacher, 1973), about the need for adopting an appropriate scale for each activity rather than always focusing on the largest possible growth and scale.

This growing concern on environmental problems led to the establishment of the first public institutions, such as the United States Environmental Protection Agency (EPA), founded in 1970, and Environment Canada, in 1971 (Bowles, 2012). The first non-governmental organizations were also founded at that time, including Friends of the Earth in 1969 and Greenpeace in 1971 (Du Pisani, 2006). Moreover, the first United Nations Conference on the Human Environment took place in Stockholm (Sweden) in 1972. This was the first meeting in which a large number of countries (113 participants) discussed on the environmental consequences of human activities for the planet, due to problems such as industrialization and demographic growth, and the need for a global outlook for its preservation and enhancement (Najam and Cleveland, 2003; UNEP, 1972). Among the measures adopted after the conference, the creation of national environmental ministries around the world was stimulated and the United Nations Environment Programme (UNEP) was established (Baylis et al., 2014).

Ten years later in 1980s, the term "sustainable development" was introduced in several publications, including the *World Conservation Strategy: Living resource conservation for sustainable development* (IUCN, 1980), *Building a sustainable society* (Brown, 1981) and *Gaia: an atlas of planet management* (Myers, 1984). In 1983, the United Nations General Assembly established the WCED with the objectives of: i) formulating realistic proposals to deal with the critical environment and development issues, ii) proposing forms of international cooperation in order to influence policies towards needed changes, and iii) raising the levels of understanding and commitment to the action of the different stakeholders. The work of the WCED was summarized in 1987 with the release of the aforementioned Brundtland Report, so called by the name of the Norwegian Prime Minister and WCED's chairman, Gro Harlem Brundtland (WCED, 1987).


**Figure 2.1.** Prime Minister Gro Harlem Brundtland showing copies of Our common future.
Source: Norwegian Government (2007).

Besides defining the concept of sustainable development, the report provided a key policy framework and gave a major impulse to the adoption of global and regional environmental measures (Schubert and Láng, 2005). It also claimed to

promote a global equity in the future by redistributing resources to encourage the economic growth of poorer nations, in order to ensure the basic needs to all human beings. The international impact of the Brundtland Report was reinforced by its concurrence with several ecological disasters that included the leak from a pesticides factory, which killed more than 2000 people in 1984 in Bhopal, India; the explosion of several liquid petroleum gas tanks, which caused more than 1000 people in 1984 in Mexico City or the Chernobyl nuclear accident in 1986 in Ukraine (Du Pisani, 2006).

In the same decade, other initiatives related to environmental protection were adopted, including the Vienna Convention (1985), the Montreal Protocol (1987), the establishment of the Intergovernmental Panel on Climate Change, IPCC (1988) or the Basel Convention (1989). The Vienna Convention and later Montreal Protocol focused on the need for global efforts to protect the ozone layer and control the emission of substances (e.g. chlorofluorocarbons, hydrofluorocarbons, perfluorocarbons) that contributed to its depletion (Canan et al., 2015). The IPCC was founded by the UNEP and the World Meteorological Organization (WMO) to gather the available information and provide a global scientific view of the state of knowledge on climate change. Since then, IPCC has published periodical updates on the evidence of global warming, as well as the expected effects on the environment and human life (IPCC, 2015). The Basel Convention referred to the regulation of transboundary movements of hazardous wastes to avoid the disposal of toxic wastes from industrialized countries into developing areas as a cheap alternative to efficient management (UNEP, 2015).

The second global environmental conference organized by the United Nations (United Nations Conference on Environment and Development, UNCED, also known as the Rio Earth Summit) was held in 1992 in Rio de Janeiro, with the attendance of representatives from 178 countries (United Nations, 2012a). The vision and goals that resulted from the conference were built upon the previous Stockholm Declaration and synthesized in the Rio Declaration and the Agenda 21 (Handl, 2012; Najam and Cleveland, 2003). The Rio Declaration was composed of 27 basic principles with no legal binding purpose but aiming at settling expected future normative and international laws (Handl, 2012). Agenda

21 gave a detailed insight and a comprehensive plan of action at global, national and local scale of all the areas related to the integration of environmental and development issues (UNCED, 1992).

To commemorate the 10th and 20th anniversary of the 1992 Earth Summit, two more conferences were held: the World Summit on Sustainable Development, in 2002 in Johannesburg (South Africa), and the Rio Earth Summit 2012 (Rio+20), again in Rio de Janeiro (Brazil). At the Johannesburg Summit, the progress on Agenda 21 was revised and two main outputs were obtained: a political Declaration and a Plan of Implementation, less extensive than Agenda 21. In addition, the need for social criteria to achieve a sustainable development was introduced (Najam and Cleveland, 2003). Rio+20 led to a new document entitled *The future we want*, an agreement between the 193 Member States of the United Nations (United Nations, 2012a,b,c). Among the wide range of actions included in the document were:

- Establishing sustainable development goals.
- Empowering the UN Environment Programme by establishing a new intergovernmental political forum to discuss on the implementation of sustainable development.
- Describing the potential of green economy as a key tool for sustainability.
- Promoting measurement tools for corporate sustainability.
- Developing additional indicators to complement the gross domestic product (GDP) in the measure of well-being.
- Highlighting the importance of the participation of citizens and scientific community in decision making processes and policy development.
- Promoting sustainable development financing and sustainable consumption and production.
- Emphasizing the importance of freedom, peace and security, as well as the respect for all human rights, focusing on the rights to an adequate standard of living, to social equity and to gender equality.

In addition to these lines of action, voluntary commitments from governments, businesses, universities and other civil society groups provided a large source of funding to support different initiatives towards sustainable development (United Nations, 2012c).

Besides the Johannesburg Summit and Rio+20, other meetings of the United Nations have included environment protection and sustainable development as key issues. As an example, the United Nations Framework Convention on Climate Change was a decisive event that led to the adoption of the Kyoto Protocol. This international agreement was adopted in 1997 in Kyoto (Japan) and later signed by 84 countries in order to set binding reduction targets for greenhouse gas (GHG) emissions (UNFCCC, 2014).

Three years later, the 2000 Millennium Summit, which counted on the presence of 189 countries, resulted in the adoption of a set of eight goals referred to as the "Millennium Development Goals". These goals include both environmental sustainability and social improvements that are related to sustainable development (United Nations, 2006). Since the deadline for the proposed targets was 2015, a new summit will be held in September 2015 in New York to discuss on the adoption of post-2015 development agenda. This post-2015 agenda aims at providing an ambitious, action-oriented declaration under the general theme "*Transforming the world: realizing the post-2015 development agenda*" with the following proposed themes:

- Fighting poverty and hunger to achieve food security and prevent malnutrition.
- Promoting sustainable consumption and production, as well as dynamic and sustainable industrialization and innovation, in order to minimize environmental risks such as climate change and ensure the conservation and correct management of bioresources and ecosystems.
- Addressing inequalities between countries and ensuring access for all the population to education, health care and water, especially for vulnerable groups.
- Strengthening partnerships and institutions to achieve sustainability and inform all stakeholders and citizens of sustainable development goals.

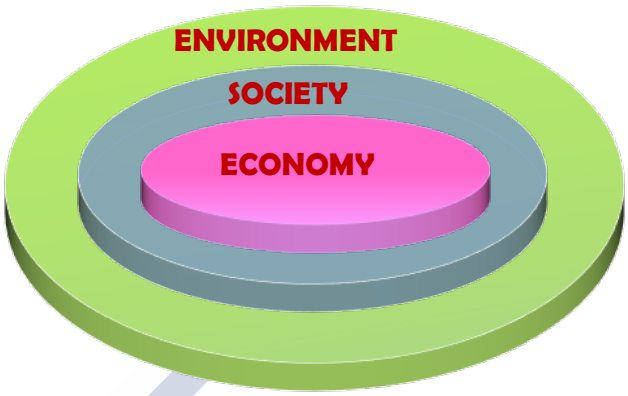
The declaration is expected to emphasize the novelty of the proposed agenda in terms of global applicability, transformative and cooperative nature and balanced integration of the different aspects (social, economic and environmental) related to sustainable development (United Nations, 2015).

### 2.1.2. Three pillars of sustainability

In agreement with the documents from the United Nations, most authors consider that sustainable development should integrate three main dimensions or pillars (Griggs et al., 2013; Lozano, 2008; Michel and Hudon, 2015; Murray et al., 2013). The idea dates back to 1980s, when the Brundtland Report highlighted the importance of achieving a future global equity that includes social equity, economic growth and environmental security (Du Pisani, 2006). These three dimensions should be addressed not only from an individual point of view but also in terms of the dynamic inter-relations between them, including the time perspective (Lozano, 2008; OECD, 2001). With this regard, Griggs et al. (2013) suggest to reformulate the definition of sustainable development from the Brundtland Report as: "*the development that meets the needs of present while safeguarding Earth's life-support system, on which the welfare of current and future generations depends*".

The interconnections between the three pillars can be deduced from graphical representations. The most common graphical depictions of sustainable development are: i) the diagram based on concentric circles and ii) the Venn diagram (**Figure 2.2**). In the first case, the largest circle depicts the natural environment, while society is identified as only a part of nature, being economy a part of society. However, the complex interconnections between the three pillars are poorly reflected. The other approach represents sustainability as the conjunction of the three aspects in the center of the diagram, whereas the integration of each pair of dimensions is considered a partial state of sustainability (Lozano, 2008).

a) Concentric circles




b) Venn diagram

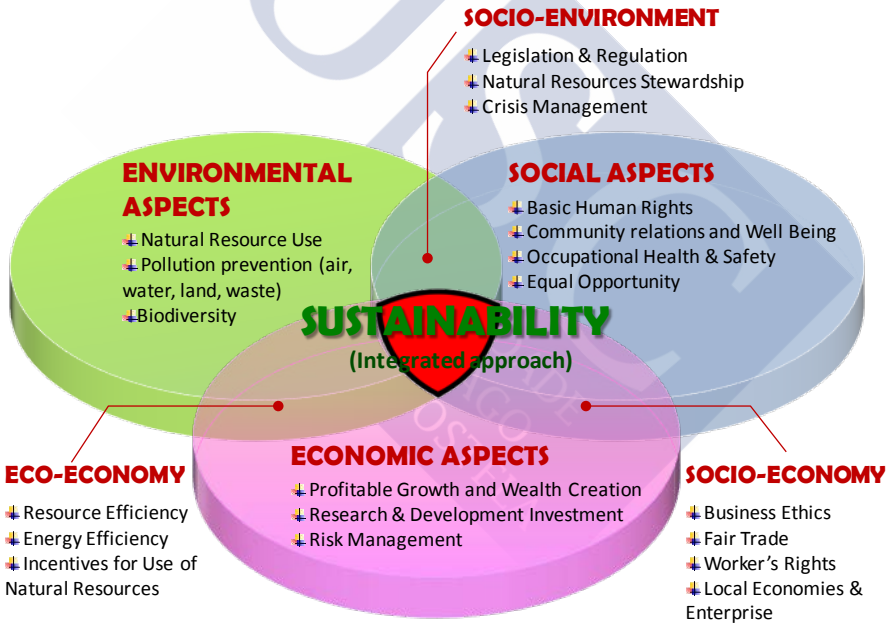



**Figure 2.2.** Three pillars or dimensions of sustainability, represented by: a) concentric circles and b) Venn diagram.
Source: Adapted from Lozano (2008), Murray et al. (2013) and Verify Sustainability (2008).

As shown in **Figure 2.2**, the integration of environmental and economic sustainability is commonly known as eco-economy (also ecological economics or eco-efficiency), and is linked to an efficient use of energy and resources. This situation can be achieved by combining the increase of product value with an optimization of the use of resources that results in a simultaneous reduction of environmental impacts and production costs.

Socio-economy is closely linked to the Corporate Social Responsibility of a company, and requires a set of actions including the development of ethics and security policies, social and community sponsorships, programs of employee training and development, among others (Verify Sustainability, 2008). Some of these actions are also related to the achievement of a socio-environmental balance.

The concurrent accomplishment of social and environmental objectives requires measures such as the establishment of a health and safety policy that allows avoiding environmental health problems or the guarantee of access to common goods (Spangenberg, 2004; Verify Sustainability, 2008). However, social and environmental pillars are considered opposed objectives, since social sustainability requires a minimum of economic growth whereas environmental sustainability sets an upper limit to this growth. For this reason, the adopted policies should focus on the enhancement of existing synergies and the development of balanced criteria to avoid overemphasizing one dimension with respect to the other (Spangenberg, 2004).

The combination of environmental, economic and social dimensions of sustainability is called *"The Triple Bottom Line"* in the business circles (Elkington, 1997). Managing the triple bottom line of profits, human beings and environment is currently becoming a need for strategic managers that are adopting corporate social responsibility as a strategic tool (McWilliams et al., in press). Thus, a holistic evaluation of the sustainability of a process must involve a comprehensive assessment that balances these three criteria by using appropriate tools and techniques (Bond et al., 2012; Murray et al., 2013).

## 2.2. Methodologies for sustainability measurement

### 2.2.1. General overview of environmental management tools

Due to the rapid deterioration of ecosystem goods and services as societies become wealthier, there is a strong need to quantify sustainability and improve public's ecological knowledge (Zhang et al., 2010). A growing number of tools and approaches have been proposed to measure the sustainability of processes (Bond et al., 2012; CML, 2011; Finnveden and Moberg, 2005).

These tools can be grouped according to different characteristics, such as the object of the study (e.g. policies and projects, organizations and companies, regions or nations, products and services), the technical or societal focus, the type of impact (e.g. used resources, environmental impacts, economic aspects), or the scope (i.e. descriptive or attributional in contrast with change-oriented or consequential) (Finnveden and Moberg, 2005). The available environmental management tools include:

- **Cost-Benefit Analysis (CBA)**

  CBA is a systematic tool for evaluating the strengths and weaknesses of investment projects or government policies from a social point of view by weighing the benefits and negative effects in economic terms (CML, 2011; Hanley and Barbier, 2009). The application of CBA methodology to environmental assessment is based on the monetary valuation or monetization of environmental goods and services and associated impacts (Baveye et al., 2013; Molinos-Senante et al., 2011).

- **Cumulative Energy Requirement Analysis (CERA)**

  CERA is an analytical tool to quantify the entire demand of primary energy required throughout the life cycle of a product or process, including production, use and disposal (CML, 2011). Primary energy refers to the energy contained in energy carriers before being subjected to any conversion (Ashley et al., 2004).

❖ **Design for the Environment (DfE)**

DfE (also known as eco-design) is a varied range of methods and approaches for the systematic integration of environmental, health and safety objectives in the design of existing products and unit processes (Kim et al., 2009a; Knight and Jenkins, 2009; Sanyé-Mengual et al., 2014). They are based on the philosophy of life cycle thinking (Müller, 2013). Beyond DfE, other methods such as Sustainable Product Design (SPD) aim at widening the scope by integrating ethical and social issues, functional requirements and innovation (Ioannou and Veshagh, 2011).

❖ **Eco-labelling**

Eco-labels are emerging environmental assessment instruments that provide comprehensible information to consumers based on particular criteria for the production of specific categories of products, such as food products, electronic devices, wood products, etc. The use of an eco-label by a company requires its products to be first environmentally verified (by means of eco-certifications) by a third party auditor. It contributes to consumer awareness of the use of improved environmental management practices to obtain a product, but its efficiency depends on the customers' willingness to pay higher prices for that product (Delmas and Grant, 2014).

❖ **Environmental Auditing (EA)**

EA is a qualitative assessment procedure applied by private companies and governmental organizations to include environmental aspects as well as resource use in order to improve their environmental performance. There are different available standards for the performance of EA, which include Eco Management and Audit Scheme (EMAS) and ISO 14001 (Finnveden and Moberg, 2005).

❖ **Footprint Analysis**

Footprints are a family of accounting tools that measure the human demand on ecosystem goods and services needed to satisfy certain level and type of consumption. They consider the life cycle of products and convert all the data into single-unit indicators, such as total water

consumption (Water Footprint, WF), total GHG emissions (Carbon Footprint, CF) or total land and ocean required (Ecological Footprint, EF) (EPA, 2015; Galli et al., 2012; Wackernagel et al., 1999).

- **Environmental Impact Assessment (EIA)**

  EIA groups all the systematic processes for identifying and evaluating the environmental consequences of an economic activity or production process. It is commonly used as a tool for decision making in large projects with several alternatives (e.g. for the choice between alternative locations) and when implementing policies and plans (CML, 2011; Finnveden and Moberg, 2005; Morgan, 2012).

- **Environmental Performance Evaluation (EPE)**

  EPE is a standardized internal management tool to support management decision of any type of organization according to environmental performance indicators that help to compliance with legal requirements, prevent pollution and maintain a continuous improvement strategy (ISO 14031, 2013).

- **Environmental Risk Assessment (ERA)**

  ERA estimates the potential or probability of damages or adverse effects on the ecosystem or human health that can occur as a result of management actions (CML, 2011; Galatchi, 2006). It involves a first step of hazard identification, followed by a dose-response assessment, an exposure assessment and a risk characterization (Galatchi, 2006).

- **Input-Output Analysis (IOA)**

  IOA is a quantitative tool for measuring interdependence between economy and material flows, including the connections between producers (industry) and consumers as well as the relations between the intermediate demand (inter-industry trading) and the final demand (households, governments, exports). This method provides a comprehensive analysis of the environmental problems and helps to develop policies to solve them (CML, 2011; Piaggio et al., 2015).

❖ **Life Cycle Assessment (LCA)**

Environmental LCA is an analytical method for assessing the environmental burdens associated with material and energy consumption throughout the entire life cycle of a product or process, according to a cradle-to-grave perspective (CML, 2011; Zhang et al., 2010). LCA is probably the most widespread approach to evaluate environmental impacts in a broad context and has involved an intense research over the last decades (Zhang et al., 2010).

After the introduction of the LCA methodology focused on environmental aspects, several specific tools have been developed to assess aspects of the three dimensions of sustainability, such as Life Cycle Screening (LCS), Life Cycle Costing (LCC) or Social Life Cycle Assessment (SLCA) (Benoît Norris, 2014; Gasafi and Weil, 2011; Swarr et al., 2011).

❖ **Material, Energy and Toxic analysis (MET)**

MET matrix is a tool that combines the product life cycle with a qualitative input-output model composed of three categories: materials cycle, energy use and toxic emissions. The accomplishment of the complete matrix is mainly based on the knowledge and experience of the team that performs the analysis and provides qualitative information on the environmental issues related to three stages of the cycle of a product: production, use and disposal (Knight and Jenkins, 2009; Van Berkel et al., 1997).

❖ **Material Flow Accounting (MFA)**

MFA is a family of methods focused on material flows, mainly referred to inputs, in order to match the use of resources and the emissions of wastes and pollutants (Finnveden and Moberg, 2005). They consist on accounting in physical units (usually mass units) of the extraction, transformation, consumption and recycling or disposal of materials in a region (CML, 2011). Two main approaches can be distinguished:

i) Bulk Material Flow Analysis (b-MFA) considers the flows of bulk materials (e.g. wood, steel, chemicals). It includes methods such as

Total Material Requirement (TMR) and Material Intensity Per Unit Service (MIPS). TMR calculates direct inputs and hidden flows of a nation or region, combining indicators of direct material inputs (DMI) with indirect materials extracted by economic activities but not used as input for domestic production or consumption (e.g. mining wastes). MIPS is similar, but focuses on products or services rather than a whole country or region (Bringezu et al., 2004; Finnveden and Moberg, 2005).

ii) Substance Flow Analysis (SFA) focuses on specific critical substances or groups of substances for the area or product under study. Typical examples are the studies of nitrogen and carbon flows or flows of specific heavy metals (CML, 2011; Finnveden and Moberg, 2005).

❖ **Material Intensity Analysis (MAIA)**

MAIA is an analytical tool to quantify the material inputs of a product from a life cycle perspective, including direct material inputs and all the materials that are not contained within the economic output but are necessary for any of the stages of its life cycle (CML, 2011; Giljum and Hubacek, 2001). Several authors consider MAIA as one of the calculation approaches that can be used to calculate indirect flows, linked to the application of aforementioned MFA methodologies and indicators, especially MIPS (Giljum, 2006; Liedtke et al., 2014).

❖ **Multi-Criteria Analysis (MCA)**

MCA (also called Multi-Criteria Decision Analysis, MCDA) includes a set of decision-making methods for addressing complex problems characterized by high uncertainty, opposite objectives, different sources of data and perspectives, and the accounting for variable biophysical and socio-economic systems (Wang et al., 2009). Due to the inherent characteristics, the proposed actions can be positive for some criteria but negative for others. Therefore, MCA does not provide a unique solution optimizing all the criteria but a set of compromise solutions among which the decision-maker has to choose (CML, 2011).

### 2.2.2. Life Cycle Thinking

Many of the environmental assessment methods are based on the philosophy of Life Cycle Thinking, which focuses on the integration of all the stages of the life cycle of a product to evaluate the environmental degradation from the extraction of raw materials, manufacturing, use and disposal stages. The described approach is referred to as "cradle-to-grave", or even "cradle-to-cradle", in case recycling and reclamation of degraded resources are included (Ulgiati et al., 2010).

This perspective is one of the five principles highlighted by the EU for the current implementation of an Integrated Product Policy (IPP) that leads to the minimization of environmental impacts while preventing individual stakeholders from shifting environmental burdens to different stages of the life cycle instead of reducing the global impact. The IPP communication also emphasizes environmental management tools as a key contributor that helps to increase the environmental awareness in companies and gives them a competitive edge with potential applications for marketing (European Commission, 2003).

The wide variety of methodologies requires a rational selection of appropriate approaches and the level of detail. The choice depends on several factors that include the overall scope of the study, the involved decision-makers or stakeholders and the context of the decision, the investigated dimensions of sustainability, the data quality and uncertainty, the scale and the complexity of the system. Moreover, the combination of two or more techniques may provide complementary information in some cases, and thus, a more comprehensive outcome (Finnveden and Moberg, 2005).

In this case, the LCA methodology has been selected as a suitable instrument to obtain detailed information on the different stages of the analyzed production processes, in line with the perspective and objectives of the Life Cycle Thinking and the EU IPP framework.

## 2.3. Life Cycle Assessment

The concept of LCA arose as an environmental tool in the late 1960s, when Harry E. Teasley Jr., from the Coca-Cola Company, brought to the Midwest Research Institute (MRI, Kansas City, Missouri) the idea to develop a study to quantify the materials and energy as well as environmental consequences of the production of a package, from the extraction of raw materials to disposal. Although the study was never published for confidentiality reasons, the MRI kept conducting LCA studies (called Resource Environmental Profile Analyses at that time) and working on the fundamentals of the methodology, which gained interest in some U.S. universities and the U.S. EPA. The current term "Life Cycle Assessment" was adopted 20 years later, in the first workshop on LCA from the Society of Environmental Toxicology and Chemistry (SETAC), held in August, 1990 (Hunt et al., 1996).

The first internationally accepted definition of LCA was given by the SETAC and stated that the methodology was "*an objective process to evaluate the environmental burdens associated with a product, process, or activity by identifying and quantifying energy and materials used and wastes released to the environment, and to evaluate and implement opportunities to affect environmental improvements. The assessment includes the entire life cycle of the product, process or activity, encompassing extracting and processing raw materials; manufacturing, transportation and distribution; use, re-use, maintenance; recycling and final disposal*" (Consoli et al., 1993).

The method was later standardized according to the ISO 14040 and 14044 standards, first published in 1997 and currently in force according to the 2006 version (ISO 14040, 2006; ISO 14044, 2006). These international standards define LCA as "*a technique for assessing the environmental aspects and potential impacts associated with a product by*

- *compiling an inventory of relevant inputs and outputs of a system;*
- *evaluating potential environmental impacts associated with those inputs and outputs; and*
- *interpreting the results of the inventory analysis and impact assessment in relation to the objectives of the study.*

*LCA studies environmental aspects and potential impacts through the product's life cycle (from cradle to grave), from raw material acquisition to production, use and final disposal*".

In practice, most LCAs omit certain stages of the life cycle and only assess the potential impacts of particular phases, using perspectives of cradle-to-gate, gate-to-gate, gate-to-cradle or gate-to-grave (Blengini, 2008).


**Figure 2.3.** Example of the entire life cycle of a product: stages of the production of bioactive high value molecules from marine origin. Source: Murray et al. (2013).

The ISO 14040 and 14044 establish the framework and the four required phases for the completion of a LCA study, which are depicted in **Figure 2.4** and include:

i) goal and scope definition,

ii) inventory analysis,

iii) impact assessment,

iv) interpretation.

LCA is an iterative method, so all the stages may involve changes as additional information is collected and more is learned about the system under study. The results are useful for a wide variety of applications, being the main of them:

- identifying problematic issues ("hot spots") and opportunities to improve the environmental performance of the analyzed products or processes,
- providing strategic information for decision-making by governmental and non-governmental organizations, and industrial stakeholders,
- selecting relevant environmental indicators,
- contributing to marketing purposes, by implementing tools such as eco-labeling, environmental claims or environmental product declarations.

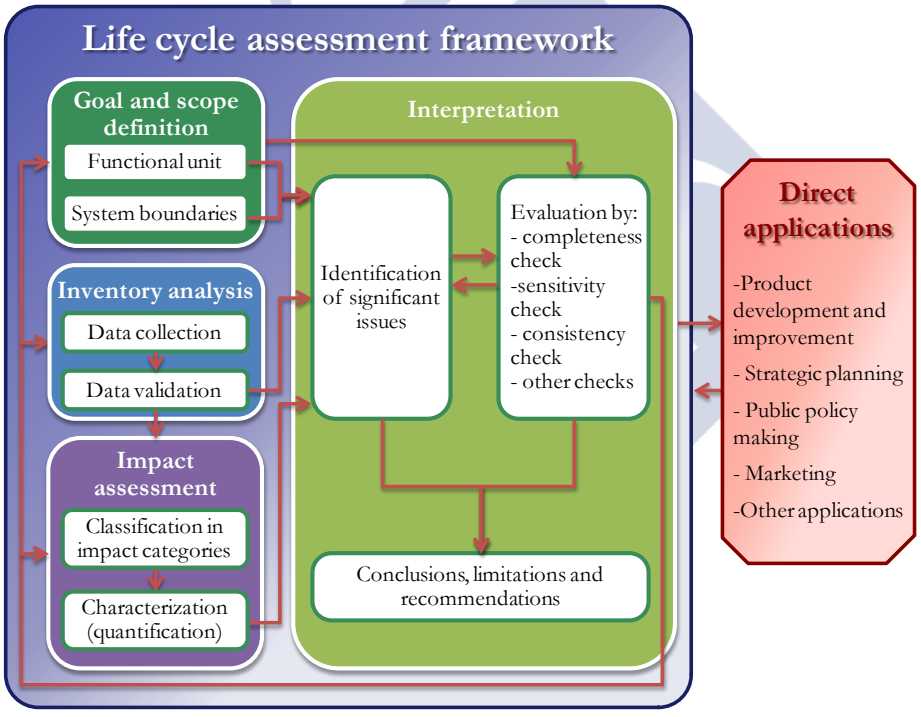



**Figure 2.4.** Framework for Life Cycle Assessment, including key elements of the different phases and their relationships.
Source: Adapted from ISO 14044 (2006).

### 2.3.1. Goal and scope definition

This is the first and probably most influencing stage of a LCA study. It requires a clear definition of the intended application, including not only the purpose and scope but also the main parameters and hypothesis affecting the following stages. Defining the goal of the study requires the specification of the reasons for conducting the work, the target audience (internal or public use of the outcomes) and the type of decisions that are expected as a result. Regarding the scope, a considerable number of issues need to be described. The most significant are:

- **Functional unit (FU)**

  It is a key parameter related to the scope, since it constitutes the basis upon which all inputs and outputs to the system are determined and the reference measurable unit for the quantification and comparison of the environmental impacts (ISO 14040, 2006). Although the FU is commonly expressed in terms of quantity of product, the real meaning requires it to represent appropriately the amount of material that fulfils the desired service (Rebitzer et al., 2004).

  The adequate selection of the FU is critical to obtain consistent results in line with the selected goal and scope, and ensure comparability of different alternatives. In the case of systems with several functions, the selection of the FU depends on the purpose of each particular study (ISO 14040, 2006).

- **System boundaries**

  The system boundaries determine the unit processes or stages that are taken into account in the LCA. The establishment of the system boundaries depends on several factors, including the application of the study, the considered assumptions, the level of detail as well as the cut-off criteria (to omit certain stages or unit processes of the system with very low significance), data and cost limitations. These factors have to be clearly identified and explained when defining the system boundaries (ISO 14040, 2006; ISO 14044, 2006).

The processes included within the system boundaries can be divided in two main groups: *background* and *foreground* systems. This classification is convenient for change-oriented LCA, in which LCA aims at reflecting the environmental consequences in the decision-making process. The *foreground system* is defined as the set of processes that are directly affected by the study, which means that the term refers to the collection of data for the sub-processes of the system on which measures may be taken concerning their selection of mode of operation as a result of decisions. Oppositely, the *background system* consists of all the processes that supply energy and materials to the foreground system, usually via a homogeneous market, so that individual plants and operations cannot be identified. It shall be remarked that the distinction between *foreground* and *background systems* is not related with the environmental importance of each category and the effects on environmental loads may be largest in either the foreground or the background processes (Tillman, 2000).

- **Data quality**

  Since the reliability and therefore the usefulness of the conclusions of a LCA depend on the quality of the original data that provide the required information for the assessment, data quality requirements must be specified to enable the goal and scope of the LCA to be met. The requirements should address the accepted period of time in which the data were gathered; the geographical area; the selected technology or mix of technologies; the level of precision or variability of data, completeness (percentage of flow measured or estimated) and representativeness with respect to the real situation; together with the consistency of the applied methodology; the origin of the data (primary or secondary sources); the reproducibility of the results and the acceptable level of uncertainty in the models and assumptions (ISO 14040, 2006; ISO 14044, 2006).

It should be noticed that, although the goal and scope must be stated at the beginning of the study, the iterative nature of LCA may involve modifications in the scope of the study associated with the collection of additional information and the outcomes from other phases.

### 2.3.2. Inventory analysis

The Life Cycle Inventory (LCI) analysis entails data collection and the procedures to calculate the input and output flows of the system under study. Although the goal and scope definition provides a starting point, the process of inventory data collection is iterative, so LCI may require changes in the collection and calculation procedures associated with new data requirements and constraints. The LCI is the most resource-intensive and time consuming step of the whole LCA due to the large quantity of information that needs to be collected. The recommended steps to undertake the LCI phase, according to the ISO 14044 (2006) and shown in **Figure 2.5**, are:

i) Data collection

To start the LCI analysis, quantitative and qualitative data must be gathered for all the unit processes within the system boundaries. The collected data can be "primary data", directly gathered on-site from a process through measurements or questionnaires; or "secondary data", estimated from other LCA studies, commercial databases or existing literature that must be correctly referenced (Kim and Overcash, 2003).

In order to obtain comprehensible details of the process to be modeled, ISO standards suggest building the process flow diagram of the system with all the unit processes and their interconnections, describing in detail each unit process and the factors affecting their inputs and outputs, and listing all the flows and associated data together with the units of measurement, collection and calculation procedures. These procedures and the considered assumptions must be explicitly described, and should be consistent throughout the study.

ii) Data validation

This step involves the verification of the data to ensure compliance with data quality requirements, by using methods such as mass and energy balances or comparative assessment of emission factors. If the validation step identifies any abnormality, the information must be completed with alternative data that meet the requirements indicated in the stage of goal and scope definition.

iii) Relating data to unit processes and functional unit

All the input and output flows for each unit process should be calculated, based on the flow chart, and referred to the selected functional unit. The level of aggregation of the inputs and outputs should be in accordance with the defined goal. Moreover, only data related to equivalent substances and analogous environmental impacts should be aggregated.

*Allocation procedures*

A specific group of calculation procedures are allocation rules. They are needed when dealing with systems that involve multiple products. Allocation procedures should be avoided whenever possible by dividing unit processes into sub-processes or expanding the product system to include the functions related to the co-products. If allocation cannot be avoided, the procedures must be clearly defined for each allocated input or outputs. The sum of allocated inputs and outputs must coincide with the original inputs and outputs obtained before allocation. The allocation procedures should be based on the partition of inputs and outputs between different products or functions, reflecting physical relationships between them, or an alternative relationship (e.g. economic value) in case physical criteria are not sufficient.

Allocation also applies to reuse and recycling of materials. In this case, changes in the properties of the material and recovery processes between the original and subsequent product systems must be carefully taken into account. The choice of the allocation procedure for reuse and recycling scenarios will follow the same criteria as those explained above for any other multi-product system.

iv) Adjusting the system boundaries

The established system boundaries should be revised in accordance with selected cut-off criteria and based on a sensitivity analysis to decide which life cycle stages, unit processes or inputs and outputs should be excluded due to lack of significance and which new processes may be significant.

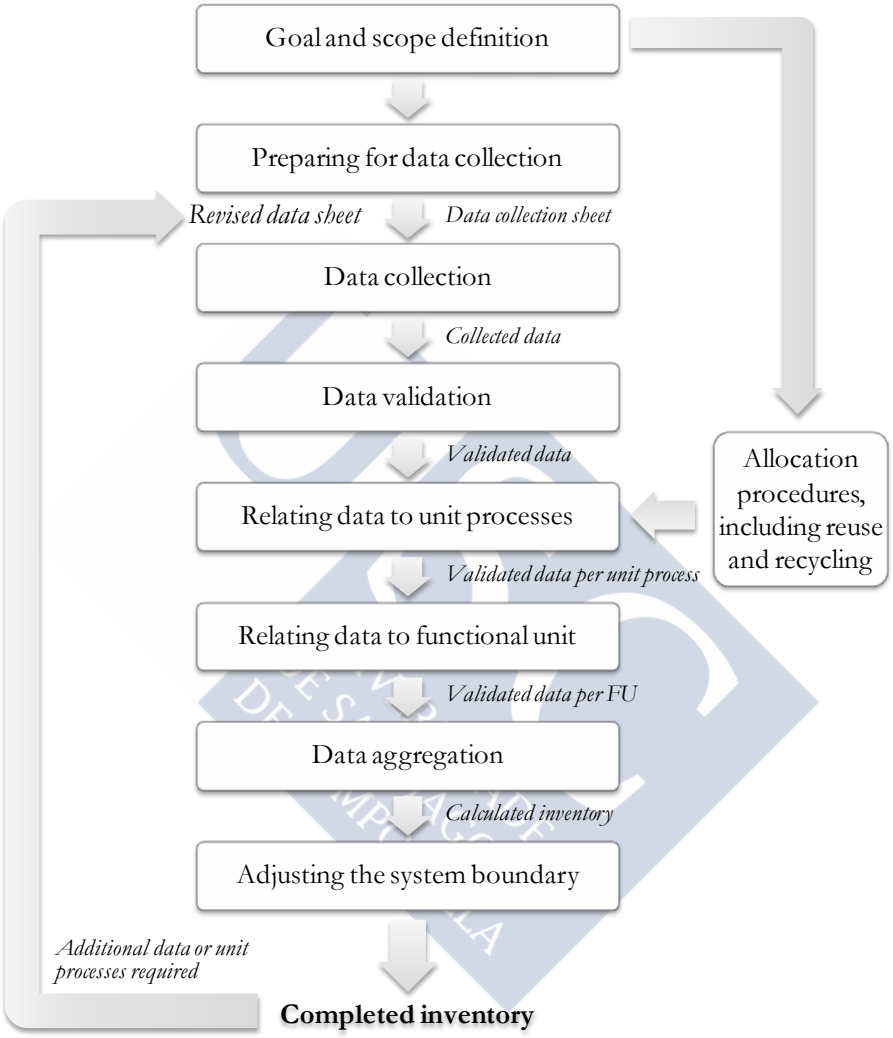



**Figure 2.5.** Recommended steps for inventory analysis.
Source: ISO 14044 (2006).

### 2.3.3. Life cycle impact assessment

The Life Cycle Impact Assessment (LCIA) stage aims at understanding and evaluating the magnitude and importance of the potential environmental impacts throughout the life cycle of the analyzed product. The accomplishment of this phase needs to be planned and coordinated with other stages in order to meet the goal and scope defined in the study. To do so, the previous decisions must be reviewed, including the defined system boundaries and cut-off criteria, the quality of LCI data and results, and the environmental relevance of the expected results in accordance with previously defined influencing factors such as FU, data aggregation and allocation.

The LCIA phase consists of three mandatory elements and several optional elements (**Figure 2.6**). The mandatory elements are:

i) Selection of impact categories, indicators and characterization models

Impact categories are the classes or groups that reflect relevant environmental issues to which inventory analysis results are assigned. These categories are quantitatively represented by category indicators, which are calculated from LCI results by using conversion factors (called characterization factors) to express these results in the specific unit of measurement of each indicator.

There are two main types of impact categories: midpoint and endpoint categories. Midpoint categories (e.g. global warming potential, acidification, eutrophication) reflect some point on the cause-effect chain between the environmental stressors and the endpoints. Endpoint categories (e.g. damage to human health, damage to ecosystems, damage to resources) refer to physical worthy elements that need to be protected according to the society (Bare and Gloria, 2008).

The selection of impact categories, as well as the related indicators and models, must be justified and linked to the goal and scope of the study. The chosen impact categories must provide a comprehensive and accurately named set of environmental aspects that are relevant to the studied process. Although ISO standards recommend the use of existing and internationally accepted impact categories, indicators and

models, and most LCA follow this principle, some special cases may require new categories to meet the goal and scope. In this case, the definition of the new elements must reflect a specific environmental mechanism, including the endpoint (i.e. final affected aspect of natural environment, human health or resources) of the impact category, the proposed category indicator, the LCI results assigned to the category, and the characterization model and factors.

ii) Classification

The classification step refers to the assignment of the LCI results to the category or categories in which they are involved. For substances from the system that affect more than one impact category, there is a need to distinguish between their involvement in parallel mechanisms (only a portion of the substance is assigned to each parallel category) and in serial mechanisms (the total quantity contributes to both categories).

iii) Characterization

The LCI results previously assigned to each impact category in the classification step are here converted into common units by using the characterization factors and the results (expressed in a uniform reference unit characteristic of each category) are aggregated to obtain a single value for each category indicator.

The significance of the results depends on the accuracy and quality of the LCI results and characterization models, which should be well documented in the study. The characterization results can be presented as a compilation of category indicators (LCIA profile), or a set of inventory data not assigned to impact categories, which may be expressed in terms of elementary flows or in an alternative manner.

Optional elements can be included in the assessment to provide additional information, depending on the goal and scope of the study. These elements include:

i) Normalization

This step refers to the calculation of the LCIA results with respect to some reference information, to obtain the relative magnitude. It helps in checking for inconsistencies and adds information on the relative significance of the results.

ii) Grouping

It involves the aggregation of impact categories into one or several sets, in order to list them with respect to specific characteristics (e.g. categories that refer to global, regional or local scales) or to rank their priority according to a hierarchy.

iii) Weighting

It consists on the aggregation of the calculated category indicators included in the LCIA profile by using numerical factors to obtained normalized results based on value-choices related to priority criteria. It may provide a final single impact score, although it is based on subjective value judgments rather than on scientific criteria. Thus, the data prior to weighting should be available to avoid loss of information.

iv) Data quality analysis

Several techniques may be applied to the LCIA results in order to identify significant changes that can occur due to uncertainties and methodological choices, negligible LCI elements and representative issues of the iterative LCA process. The techniques include i) gravity analyses to identify data with greatest contributions to the indicator results, ii) uncertainty analysis to evaluate the reliability of the results and iii) sensitivity analysis to determine the effect of changes in data and methodological choices.

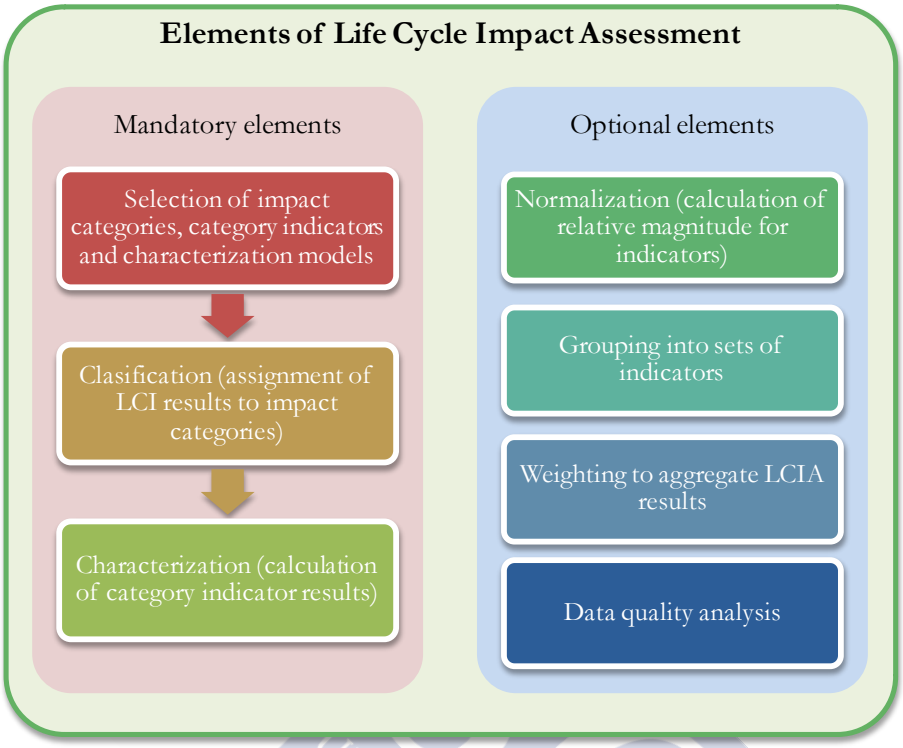



**Figure 2.6.** Elements of LCIA phase according to ISO standards.
Source: ISO 14040 (2006) and ISO 14044 (2006).

### 2.3.4. Interpretation of the results

The results from the LCI and LCIA phases need to be combined and interpreted in line with the goal and scope of the study. The interpretation allows the identification of significant issues including relevant inventory data such as energy, emissions and wastes, as well as critical contributions within the life cycle of the process to the different impact categories.

The stage involves a review of the scope and quality data of the LCA, as well as the considered assumptions and the consistency, completeness and sensitivity of the results. The interpretation of the results provides conclusions, limitations and recommendations related to the system that are relevant for the decision-making process.

### 2.3.5. Limitations of LCA

LCA methodology is one of the most common environmental management tools, internationally accepted and currently used by governmental and non-governmental institutions as well as industrial decision-makers (ISO 14040, 2006; Zhang et al., 2010). Its main strength is probably the holistic nature of the tool, which aims at quantifying all the possible environmental burdens from a global perspective, including the whole life cycle of the product or service (Udo de Haes et al., 2004). Thus, LCA is a consistent tool that allows a broad assessment of varied environmental issues rather than restricting the analysis to a single concern.

Despite its potential, LCA presents a certain number of drawbacks that have been extensively criticized. Most of them are related to the complexity and broad scope of the methodology, together with the vagueness of the ISO standards. Indeed, ISO 14040 and ISO 14044 provide a clear definition and a general framework but do not state specifically a single method for conducting the assessment. Curran (2014) proposes a classification of the main weaknesses in three categories and lists some examples that are summarized in **Table 2.1**.

**Table 2.1.** Examples of limitations of LCA methodology (Curran, 2014)

| Type of LCA limitation | Examples |
|---|---|
| i) Limitations that can be improved through further research | Missing impact data and models for LCIA.<br>Not readily-available life cycle inventories. |
| ii) Limitations that are inherent in the methodology | Data uncertainty of inventory data and impact assessment indicators.<br>Not always possible to find an optimal solution.<br>Steady-state and linear modeling approach. |
| iii) Limitations due to choices that affect the results | Inappropriate goal and scope definition.<br>Change in results due to co-product allocation procedures.<br>Different approaches to assign avoided burdens when expanding the system boundaries. |

## 2.4. Application of LCA to blue biotechnology and bioactive compounds

Biotechnology is a wide industrial sector that ranges from high value, low volume products such as pharmaceuticals to low value commodities such as biofuels. The main effort to date has focused on implementing processes effectively to meet the regulatory demands more than optimizing the operations or analyzing the sustainability, especially in the case of pharmaceuticals (Woodley, 2009).

In the last decades, there have been many attempts to develop methodologies for the measurement of bioprocess sustainability (Constable et al., 2002; Kim et al., 2009b). One of the available tools that has been proposed to analyze biotechnological systems holistically is LCA, although few examples of environmental assessments applied to bioactive compounds and pharmaceutical ingredients are found in literature (Jiménez-González and Woodley, 2010; Rubio Rodríguez et al., 2011).

In the specific case of marine biotechnology, the LCA studies conducted to date mainly analyzed the cultivation and extraction of fractions from microalgae and seaweed focusing on biofuels production (Aitken et al., 2014; Collet et al., 2015). Although different bioenergy sources have been evaluated, including renewable diesel, bioethanol or biogas, most of the work dealt with the production of biodiesel by transesterification (Collet et al., 2015).

Nevertheless, virtually no examples of LCA studies dealing with the production of fine chemicals and biologically active compounds from algae and other marine sources are available within the existing literature, except for the work that is presented in this thesis. In addition, due to the lack of commercial-scale facilities, the studies generally rely on extrapolations and simulation models rather than real field data from operating systems (Collet et al., 2015).

### 2.4.1. LCA of biologically active products

Many fine chemicals and specifically pharmaceutical compounds from human and animal use are more and more often found in the environment. Despite their probable harmful impact, few LCA studies evaluate the production and use of pharmaceuticals or related compounds from a life cycle point of view. The main reason for the low number of studies related to pharmaceutical ingredients and other fine chemicals is the scarcity of inventory data, since most production parameters are confidential and thus, not publicly available (Wernet et al., 2010). Other challenges that need to be faced are the multi-purpose nature of the processes, which usually share equipment and facilities for obtaining more than one product, and the lack of a coherent framework towards the characterization of toxicological impacts. Moreover, companies and decision-makers have to make a balance between complexity and practical usefulness of the performed LCAs (Cespi et al., 2015).

One of the most recent examples is the work by Cespi et al. (2015), who compared two different approaches for the environmental evaluation of the production of sildenafil citrate, the active pharmaceutical ingredient (API) of Viagra™, by two possible routes. The authors proposed a simplified green metrics approach based on a single indicator: the Process Mass Intensity (PMI), which refers to the ratio between the total quantity of raw materials and the quantity of product. In addition, the study also provided a LCA only focused on the synthesis stage, for which a combined set of data sources was used, including previous scientific articles, existing patents and estimating tools that relate molecular structures and key production and emissions parameters. As a result, the simplified tool allowed predicting the hot spots of the API in a practical manner, although LCA was needed to obtain a global evaluation with a more detailed set of environmental indicators.

Previous studies analyzed the production of both APIs and common solvents used in the synthesis of fine chemicals. Most of them based their LCA results in terms of mass of API as the functional unit (Jiménez-González et al., 2004; Ponder and Overcash, 2010), and detailed LCI data are only available in specific examples (Raymond et al., 2010; Wernet et al., 2010).

Jiménez-González et al. (2004) performed one of the first published LCA studies on the production of a complex pharmaceutical ingredient, an API by GlaxoSmithKline. The work included the LCI and LCIA stages of the standardized methodology following a cradle-to-gate perspective and analyzing the categories of eutrophication, acidification, GHG emissions, photochemical ozone creation, total organic carbon, energy requirements and total cradle mass (raw materials taken directly from earth). The main findings were the importance of solvents, with contributions between 50% and 80% in most categories, and the energy use, which was the main cause of resource depletion and GHG emissions.

Ponder and Overcash (2010) collected a detailed life cycle inventory to analyze the raw materials and energy consumption for the production of vancomycin hydrochloride, a glycopeptide antibiotic used to treat resistant infection, whereas Van der Vorst et al. (2011) conducted an exergy assessment to quantify mass and energy balances in the production of a precursor of galantamine, used in anti-Alzheimer treatments. Wernet et al. (2010) used several LCIA methods, including Eco-Indicator 99, TRACI, ReCiPe and IMPACT2002+, as well as cumulative energy demand (CED) and global warming potential (GWP). The results showed the large environmental impacts of pharmaceuticals with respect to common basic chemicals, with 20 times higher CED and 25 times higher GWP contributions, among others. The environmental impacts associated with the production of antioxidant fractions from natural sources were also determined by Rodríguez-Meizoso et al. (2012), who compared two innovative extraction techniques according to CML 2000 methodology categories.

Due to the large contribution of solvents to the life cycle emissions of APIs, the manufacturing process of ten common organic solvents used in pharmaceutical sector were evaluated by Raymond et al. (2010). In this study, the raw materials and water requirements, and the emissions to air, water and soil throughout the life cycle of each solvent were quantified. The GHG emissions and CED were also determined. Furthermore, the effect of solvent reduction on environmental impacts due to the implementation of greener processes was analyzed for three case studies of pharmaceuticals from different companies, with associated decreases up to 90% of total emissions.

### 2.4.2. LCA of products from aquatic origin

As previously mentioned, most of the published studies on LCA of products from aquatic organisms focus on the production of bioenergy from micro and macroalgae. Although most of these studies conclude that algal bioenergy show several environmental benefits compared to fossil fuels and other renewable sources, the specific results have a large variability, due to the different approaches and assumptions considered by the authors (Collet et al., 2015).

One of the most crucial choices of an LCA study is the selection of the FU. Although the FU is clearly defined in almost all the existing papers dealing with algal bioenergy, the approaches significantly vary between the different reports. Thus, some studies refer the analysis to the produced biomass and express it in as weight of algae produced (Jorquera et al., 2010), whereas others use the obtained energy as the FU, either in terms of energy (MJ of bioenergy source), mass (kg of biofuel) or alternative unit (e.g. vehicle kilometer travelled) (Brentner et al., 2011; Clarens et al., 2011; Soh et al., 2014). Regardless of the approach, the main assumptions for the conversions between mass and energy units should be explicitly stated in the assessment (Collet et al., 2015).

The selection of the system boundaries is also a controversial step that may contribute to widen the range and uncertainty of final results (Sills et al., 2013). Most LCAs divide the algal processes into five stages, consisting of: i) production of raw materials and energy required for the cultivation, ii) cultivation, iii) harvesting and dewatering, iv) transformation into energy carrier and v) use of the produced energy (Clarens et al., 2011; Lardon et al., 2009; Stephenson et al., 2010). However, some studies exclude the last usage step (Sills et al., 2013; Soh et al., 2014), whereas few of them only consider the production of inputs and cultivation itself (Jorquera et al., 2010).

Regarding the LCI stage, the selected cultivation system is a key factor that influences the input requirements. The open raceway pond is the most common reactor configuration, whereas less than one third of the publications consider tubular photobioreactors (PBR). The reactor configuration not only affects the contribution of infrastructures (included in 50% of LCAs dealing with open ponds and 75% of studies with PBRs), but also the water consumption, growth and operating parameters, as well as the energy consumption of the system.

For the downstream processing, a wide variety of technologies can be used in the harvesting and dewatering stage, with different energy requirements and efficiencies. Their selection mainly depends on the subsequent transformation processes. For example, a low dry matter content is needed for anaerobic digestion of microalgal biomass, whereas biodiesel production can be performed through wet or dry extraction, and direct combustion needs a high dry matter content (Collet et al., 2015). There are four possible products after the transformation stage: electricity, gasoline, diesel or biogas. According to Clarens et al. (2011), electricity production by biomass drying and co-combustion with coal is the alternative with the lowest impact and energy consumption. Gasoline pathway is rarely considered and seems to have higher climate change impact and energy consumption than biodiesel. Biodiesel is the energy carrier considered in 75% of the studies and is often considered jointly with the production of biogas by anaerobic digestion of the remaining biomass (Clarens et al., 2011; Collet et al., 2015; Soh et al., 2014).

The differences between the LCIA results of the available studies are linked to the selected impact categories and the method for dealing with co-products. All the published LCAs on algal biofuels evaluate the category of climate change, and most include an indicator of the energy consumption (either net energy ratio or cumulative energy demand). However, less than 50% of the references include other categories such as land use change, eutrophication, acidification or toxicity. In particular, Collet et al. (2015) recommended the incorporation of water balance as an essential environmental indicator, since it is one of the main drawbacks of first generation biofuels that algal biofuels may solve.

Some recent studies suggest that the combined production of biofuels with other valuable fractions may lead to significant environmental improvements of algal systems (Soh et al., 2014). To evaluate these benefits, appropriate co-product management approaches must be selected. These approaches can be based on allocation, in which environmental burdens are distributed between the co-products, or substitution, which adds the co-product to the functional unit by considering the concept of "avoided product", which involves the reduction of environmental impacts by considering co-product credits (Collet et al., 2015; Soh et al., 2014).

## 2.5. Social and economic dimensions

According to the principles of sustainable development, measuring sustainability for supply chain decision-making requires the integration of social and economic dimensions together with environmental aspects (Griggs et al., 2013; Hutchins and Sutherland, 2008). Although the incorporation of socioeconomic criteria is more recent than environmental LCA, the development of an appropriate methodology has been largely discussed over the last two decades (Benoît et al., 2010). The first proposals for the socio-economic assessment included works from Casado-Cañeque (2002), Norris (2003) and O'Brien (1996). Currently, an integrated framework for Life Cycle Sustainability Analysis (LCSA) has been proposed, combining conventional LCA with social LCA (SLCA) and LCC (Sala et al., 2013; Swarr et al., 2011).

### 2.5.1. Social Life Cycle Assessment

Concerning social aspects, there have already been many attempts to standardize and provide practical tools for SLCA (Benoît Norris, 2014; Jørgensen, 2013). Some examples are the publications of Benoît et al. (2010), Dreyer et al. (2006), Grießhammer et al. (2006), Hutchins and Sutherland (2008) and Jørgensen et al. (2008). However, it was not until 2009 that the UNEP-SETAC Guidelines for Social Life Cycle Assessment of Products were published (UNEP-SETAC, 2009). The guidelines define SLCA as a "*technique that aims to assess the social and socio-economic aspects of products and their potential positive and negative impacts along their life cycle encompassing extraction and processing of raw materials; manufacturing; distribution; use; re-use; maintenance; recycling; and final disposal*".

As in environmental LCA, the application of SLCA requires a method to quantify the impacts. For this purpose, the UNEP-SETAC guidelines recommend an analogous method within the framework of the ISO 14044:2006, including the four stages of goal and scope definition, life cycle inventory, life cycle impact assessment and interpretation of results (ISO 14044, 2006; UNEP-SETAC, 2009). The guidelines define social impacts as "*consequences of positive or negative pressures on social endpoints*" and propose the use of parameters in three levels to evaluate these impacts: i) inventory indicators, ii) subcategories and iii) impact categories. The hierarchy of the parameters is presented in **Figure 2.7**.

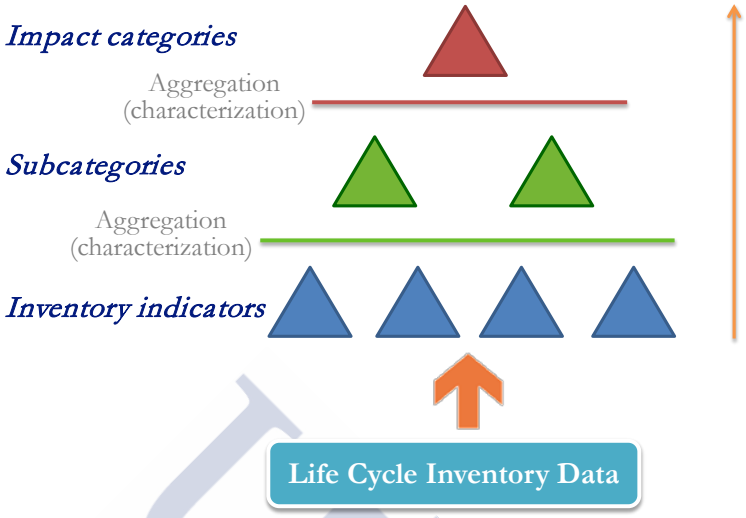



**Figure 2.7.** Hierarchy of SLCA parameters inspired by ISO14044:2006.
Source: Adapted from UNEP-SETAC guidelines (UNEP-SETAC, 2009).

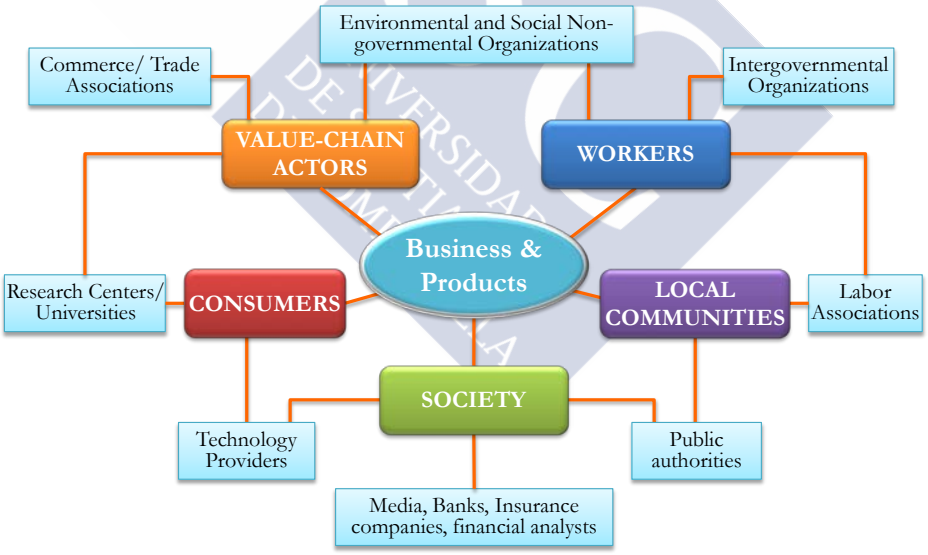



**Figure 2.8.** Hub and spoke stakeholder diagram.
Source: Adapted from UNEP-SETAC guidelines (UNEP-SETAC, 2009).

The impact categories are groups of social issues such as human rights, cultural heritage or governance, which affect a set of stakeholders (i.e. groups of people with common interests due to similar relationships with respect to the analyzed product system, depicted in **Figure 2.8**). The social impacts are assessed according to different subcategories by an aggregation step. Among the subcategories that reflect social impacts are fair salary, health and safety or equal opportunities. The subcategories are evaluated with a selection of qualitative and quantitative indicators such as annual salary, working hours per week, number of accidents or women-to-men ratio of employees (UNEP-SETAC, 2009).

In addition to the development of the methodological aspects, several authors have already published SLCA studies to assess different social impacts. These studies include the work of Jørgensen et al. (2010), who analyze the relationship between the "incidence of child labor" indicator and the impacts on the well-being. Dreyer et al. (2010) compared the performance of six companies from different countries, by obtaining a global company risk score (single-value) according to a multi-criteria model including forced labour, discrimination, restrictions of freedom of association and child labor. Feschet et al. (2013) built a pathway that correlated the effect of changes on a key economic activity of a country with low per capita income with the health status of its population. Other examples include the work of Ekener-Petersen and Finnveden (2013), who evaluate the social hot spots of a laptop computer by focusing on a simplified list of materials and a set of indicators of each relevant stakeholder category; and the study of Benoît-Norris et al. (2011), who developed a global model to facilitate data collection and applied it to the production of orange juice in the U.S.

Despite the sharp increase in the efforts to develop a consolidated methodology, most authors agree that SLCA is still in its early days, and further work is required in order to achieve a mature framework. With this regard, Jørgensen (2013) claims that the two main challenges for the future will be the improvement of the data availability and the role of SLCA as a tool for decision support.

### 2.5.2. Life Cycle Costing

The economic perspective of sustainability can be addressed by applying the methodology of Life Cycle Costing (LCC). Conventional LCC has been applied for decades (as early as in 1930s) and is defined in the ISO 15686 standards as a "*methodology for the systematic economic evaluation of life-cycle costs over a period of analysis, as defined in the agreed scope"* (ISO 15686, 2011; Schau et al., 2011).

More recently, an environmental approach for the application of LCC in sustainability assessment was proposed. Hunkeler et al. (2008) define environmental LCC as "*an assessment of all costs associated with the life cycle of a product that are directly covered by any one or more of the actors in the product life (supplier, producer, user/consumer, end-of-life actor), with complimentary inclusion of externalities that are anticipated to be internalized in the decision-relevant future*" .

Although LCC has already been recognized as a valuable tool for sustainability assessments, its application is relatively recent and few attempts to integrate the concept with the environmental LCA methodology have been conducted to date. With this regard, SETAC published in 2011 a code of practice based on the LCSA conceptual framework (Schau et al., 2011; Swarr et al., 2011).

The code of practice bases the application of the environmental LCC on the principles and the four-phase structure of the conventional LCA. Thus, goal and scope and LCI steps in a LCC tackle similar challenges than environmental LCA, such as the importance of clearly defining the product system and cut-off criteria, the need for appropriate allocation methods or the uncertainty of economic data. The main difference is the required monetization of environmental aspects and impacts to account for the decision-relevant costs. These costs must be expressed in terms of real monetary flows, which can be obtained by internalizing the costs (e.g. according to the polluter pays principle). Since all inventory data in LCC are expressed in a single unit of measure (currency), no characterization or weighting steps, typical of the LCIA step, are needed. The interpretation of results follows analogous procedures to those for the environmental LCA (Swarr et al., 2011).

Despite its usefulness, some authors argue that LCC presents two limitations. Firstly, it mainly focuses on the costs from an individual perspective rather than taking into account a global view that is inherent to sustainable development. In addition, the monetization of costs may lead to neglect capitals that are relevant to the sustainability of the analyzed system (Sala et al., 2013). With this regard, a LCC should include not only the cost of a product but also the economic benefit or value added to the society (Schau et al., 2011).

The implementation of LCC methodology for the evaluation of several case studies can already be found in the literature. For example, Dobon et al. (2011) conducted a LCC for the comparison of different food packaging scenarios. The study took into account two types of costs, depending on the degree of responsibility of the company. Internal costs included conventional costs (direct costs related to manufacturing, e.g. raw materials, electricity, etc.), hidden costs (general costs associated with license expenses, waste management costs, etc.) and less tangible costs (such as expenses on marketing). External costs referred to the social costs that are not restricted to the company but have an effect on the whole society in the long term (e.g. depletion of natural resources, impact on human health, etc.).

Schau et al. (2011) proposed another approach, analyzing the life cycle costs of automotive electrical generators according to two different perspectives: the manufacturer perspective and the user perspective. Hence, the considered costs for the LCC according to the manufacturer perspective include direct costs of manufacturing stages, as well as maintenance costs, indirect overhead costs, costs for warranties, depreciation and insurance. On the contrary, the LCC from the user perspective includes acquisition costs, fuel and maintenance costs, as well as insurances, licenses, fees and disposal costs.

Other authors use the concept of cost-benefit analysis, according to which the costs and benefits are measured over time in terms of willingness to pay, and then discounted to their present value according to specific rates of interest or discount. The net profit is then obtained from the difference between total estimated benefits and costs. Several examples apply this procedure for the assessment of energy efficiency measures or the feasibility of alternative water resources (Molinos-Senante et al., 2011; Morrissey and Horne, 2011).

## 2.6. Thesis outline: Goal and structure

The purpose of this doctoral thesis is to analyze the environmental performance of the different high value products and commodities that can be obtained from diverse aquatic organism, according to a life cycle perspective that allows a holistic evaluation. The document is divided in four sections, as shown in **Figure 2.9**: i) Introduction to the study, divided in two chapters; ii) High value products from aquatic organisms, composed of five chapters; iii) Microalgal biorefineries, presented in two chapters and iv) General conclusions.

- **Section I. Introduction to the study** aims at contextualizing the thesis and includes Chapter 1 and Chapter 2. Chapter 1 focuses on the potential of and current status of products from aquatic organisms, including the diversity of sources and compounds, as well as the economic significance and existing policies to promote the blue biotechnology. Chapter 2 introduces the fundamentals and available tools related to the sustainability assessment, with a special focus on the Life Cycle Assessment methodology, which is applied throughout the thesis.

- **Section II. High value added molecules from aquatic organisms** provides the environmental LCA of a wide range of aquatic organism, including detailed life cycle inventories and impact assessments.

  Chapter 3 evaluates the environmental performance of two microalgal sources of bioactive compounds, including an omega-3 fatty acid producer, *Phaeodatylum tricornutum*, and a multi-product system based on the cultivation of *Tetraselmis suecica*.

  Chapter 4 presents the detailed holistic evaluation of the sustainability of a typical process in the field of marine biotechnology, including the implementation of a microalgal system from lab to pilot scale, including the evolution of the environmental profile throughout the different stages of the scale-up process (corresponding to the production of a carotenoid pigment by *Haematococcus pluvialis*) and includes a socio-economic evaluation of the final pilot-scale facility.

Production and extraction processes for valuable compounds contained in macroalgae are evaluated in Chapter 5, whereas Chapter 6 analyzes the environmental response of alternative cultivation strategies for biologically active molecules contained in sponges. Chapter 7 presents the detailed inventories and LCA results of other aquatic producers of biocompounds.

- **Section III. Microalgal biorefineries** presents key environmental and economic issues related to the commercial implementation of multi-product microalgal systems.

  Chapter 8 includes a comparison between different types of real pilot-scale reactor configurations operated under variable surrounding conditions.

  Chapter 9 presents an integrated assessment of environmental and economic criteria of commercial algal systems including the influence of co-products and the uncertainty of key parameters in the global performance.

- **Section IV. Conclusions** summarizes in Chapter 10 the findings and achievements reached in the thesis, including the main conclusions and final recommendations.

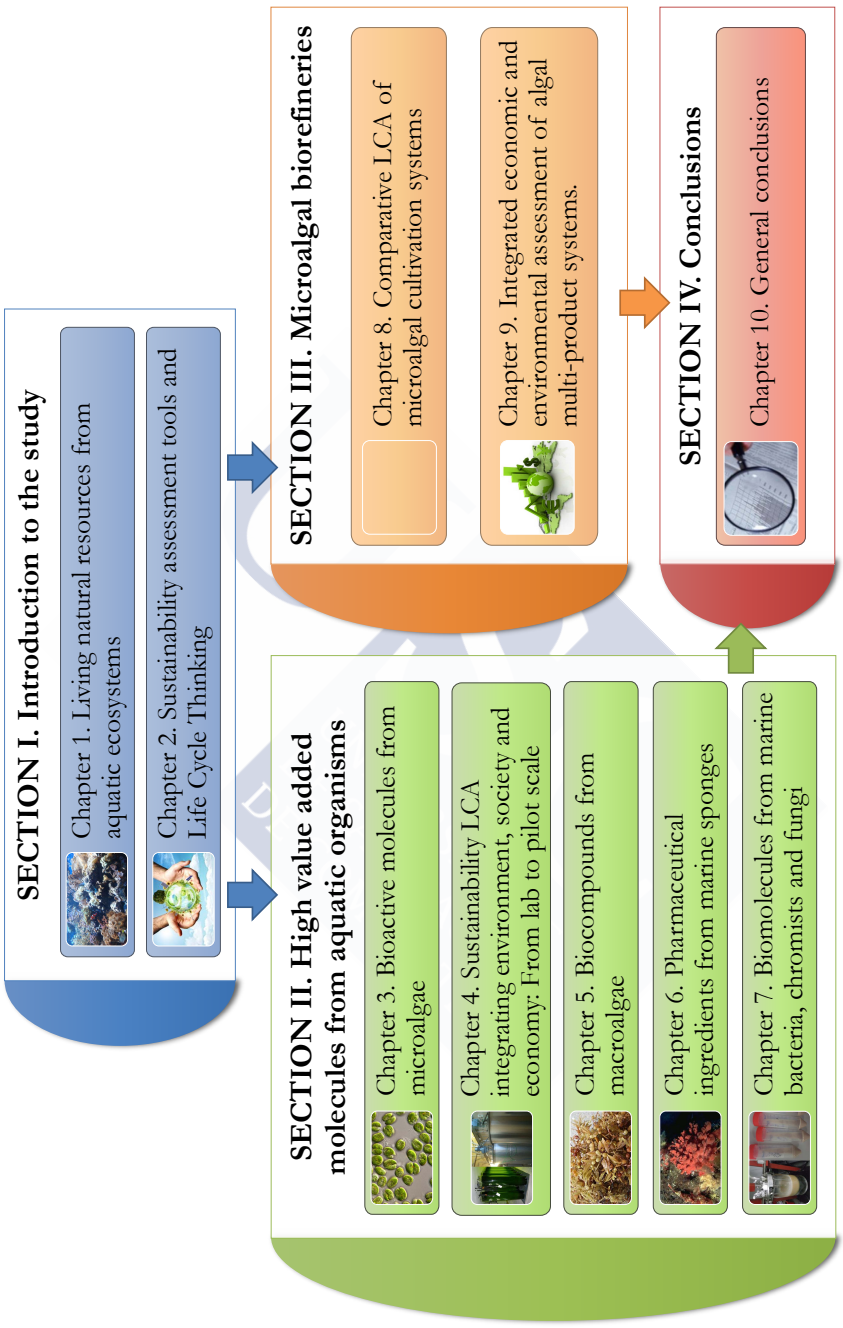



**Figure 2.9.** Schematic representation of the structure of the thesis.

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# SECTION II

## HIGH VALUE ADDED MOLECULES FROM AQUATIC ORGANISMS

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# Chapter 3

# Bioactive molecules from microalgae[1,2]


***Summary***

The marine microalgae *Phaeodactylum tricornutum* and *Tetraselmis suecica* are two promising sources of different biologically active compounds with potential applications in human health, cosmetics and food industries. These compounds include polyunsaturated fatty acids (PUFAs), pigments and vitamin precursors, such as eicosapentaenoic acid (EPA), β-carotene or α-tocopherol. Current efforts focus on the development of sustainable processes to ensure the continuous supply of these products on a commercial scale. In order to identify the main bottlenecks and alternative scenarios for a more efficient production of microalgal biocompounds, the environmental assessment of some of these novel processes is conducted at different levels of production (lab and pilot scale). The results revealed the strong influence of the formulation of the culture medium and the performance of the cultivation stages, which were dependent on large quantities of nutrient sources and electricity. Additional analyses suggest that the use of alternative nitrogen sources and optimized reactor configurations may allow significant improvements in the environmental performance of microalgal processes.


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[1] **Pérez-López P**, González-García S, Allewaert, C, Verween A, Murray P, Feijoo G, Moreira MT. Environmental evaluation of eicosapentaeoic acid production by *Phaeodactylum tricornutum*. Science of the Total Environment 2014, 466-467:991-1002.

[2] **Pérez-López P**, González-García S, Ulloa RG, Sineiro J, Feijoo G, Moreira MT. Life cycle assessment of the production of bioactive compounds from *Tetraselmis suecica* at pilot scale. Journal of Cleaner Production 2014, 64:323-331.

# Index

## 3.1. Benefits and limitations of microalgal processes

In recent years, increasing attention has been paid to microalgal processes due to the recognized potential of these organisms for the production of a wide variety of molecules. Microalgae are the primary producers of organic matter in aquatic environments due to their photosynthetic activity, which allows them $CO_2$ fixation (Suh et al., 2006). Moreover, they can grow under very different conditions (even in high salinity water) and accumulate large quantities of lipids (20-90% oil content depending on species and conditions) (Chisti, 2007; Kadam, 2002; Lam and Lee, 2012; Suh et al., 2006). Under certain cultivation conditions (e.g. nutrient deprivation, light limitation), microalgae can become stressed, causing them to overproduce some compounds of interest such as lipids or carotenoids (Aflalo et al., 2007; García-Malea et al., 2009; Shahid et al., 2013).

As cell factories for the production of high-value biomolecules, microalgae present numerous advantages such as i) the possibility of cultivation on non-arable and marginal land; ii) the possibility of using waste streams derived from industrial processes such as wastewaters or flue gases rich in $CO_2$ (anthropogenic $CO_2$) as nutrient source and, iii) the ability to modify the biochemical composition of the algal cells by varying growth conditions (Brentner et al., 2011; Clarens et al., 2010; Collet et al., 2011; Munir et al., 2012; Soh et al., 2014). Other advantages are their relatively fast growth rate and their higher productivity per unit area compared to terrestrial plants (Brentner et al., 2011; Clarens et al., 2010; Lam and Lee, 2012).

Concerning the mechanisms for microalgal cultivation, current technologies focus on the improvement of productivity and yield (Brentner et al., 2011; Stephenson et al., 2010). The cultivation systems can be classified in two main groups: i) open raceway ponds (ORPs) and ii) closed photobioreactors (PBRs). ORPs present higher losses by evaporation, larger water requirements, higher risks of contamination, lower volumetric productivity, poor mixing and reduced temperature control than PBRs. In contrast, they are less energy-intensive, which render into lower levels of greenhouse gas (GHG) emissions (Brentner et al., 2011; Jorquera et al., 2010). With regard to PBRs, they are closed systems with higher biomass yield but they are more expensive to build and operate than ORPs (Brentner et al., 2011; Jorquera et al., 2010; Stephenson et al., 2010).

Once the microalgae culture is grown, it is harvested. Depending on the product to be recovered, the next step typically entails reducing the water content of the microalgal biomass since low water content enhances the recovery of lipid soluble components and carotenoids (Brentner et al., 2011; Lardon et al., 2009). Different methods can be considered for this purpose: flocculation and settling, centrifugation, filtration or air flotation. The selection of the harvesting method will depend on factors such as energy requirement as well as microalgae cell characteristics: size and density (Beach et al., 2012; Brentner et al., 2011; Olaizola, 2003). After harvesting, the compounds of interest are extracted by different methods. Commonly, organic solvents such as hexane, ethanol or methanol (Brentner et al., 2011; Kobayashi and Sakamoto, 1999; Stephenson et al., 2010) as well as supercritical fluids (Brentner et al., 2011) are used for extraction. Enzymatic extraction, still under development, could be a very interesting alternative to be established in the future, specifically if the final product is intended for human consumption (Mercer and Armenta, 2011).

Numerous studies can be found in the literature concerning the cultivation of microalgae for energy purposes (Antoni et al., 2007; Chisti, 2007; Clarens et al., 2011; Collet et al., 2011; Lardon et al., 2009; Li et al., 2007). At present, microalgae are also considered a potential raw material for the production of a wide range of high value added bioactive compounds such as pigments, foodstuffs, chemicals, pharmaceuticals and nutraceuticals (Cerón García et al., 2006; Cerón García et al., 2005; Molina Grima et al., 2003).

Despite the potential, several limitations of the implementation of microalgal processes are related to the need for optimization of different parameters affecting growth conditions such as $CO_2$ supply, light or nutrients; as well as harvesting and extraction processes (Mata et al., 2010). Among the environmental concerns that have been identified in previous studies, the thermodynamic perspective related to the energy balance is one of the key issues that needs to be improved in order to increase the sustainability of products from microalgae (Lam and Lee, 2012). With this regard, environmental assessments constitute an essential tool to deal with bottlenecks and propose alternatives for more efficient processes.

## 3.2. Omega-3 polyunsaturated fatty acids from *Phaeodactyum tricornutum*

Polyunsaturated fatty acids (PUFAs) have been in the spotlight over the last years due to their potential applications in pharmaceutical, nutraceutical and cosmetic industries (Belarbi et al., 2000; Murray et al., 2013). They are considered essential nutrients due to their high physiological and therapeutic significance for humans. According to the level of bioactivity, the two long chain PUFAs eicosapentaenoic acid (EPA, C20:5ω3) and docosahexaenoic acid (DHA, C22:6ω3) are the PUFAs with higher potential in the health food market (Yen et al., 2013).

The ω3-PUFAs have proven to be effective for the prevention and treatment of coronary heart disease, tumors, blood platelet aggregation, increased cholesterol levels, inflammatory diseases and are even considered to enhance brain functioning (Belarbi et al., 2000; Deckelbaum and Torrejon, 2012). In Western diets, unbalanced intakes of ω3 and ω6 fatty acids are common and result in an increase in cardiovascular disease occurrence, higher risks of heart attack and mental illness (Khozin-Goldberg et al., 2011). For this reason, the Food Standards Agency recommends a consumption of 0.9 g of PUFAs per day (Gebauer et al., 2006).

At present, PUFAs are almost exclusively sourced from marine fish oil, typically obtained from species such as sardine, tuna, anchovy, bass, mackerel or salmon (Domingo et al., 2007; Patil et al., 2007). The use of fish oils is held by fluctuations on the prices. Although the global aquaculture production has annually increased by 9%, prices keep increasing with the growing demand (Patil et al., 2007). Moreover, there is a current concern related to the exposure of fish sources of PUFAs to chemical pollutants that are accumulated in the specimens and may lead to the intake of these contaminants by humans (Domingo et al., 2007; Patil et al., 2007). Given the possible decline of commercial fish stocks, the increased prices and the risk of intake of contaminants, the search for alternative and sustainable sources of PUFAs is continuously increasing (Belarbi et al., 2000; Patil et al., 2007). With this regard, microalgae are currently considered the real source of ω3-PUFAs that goes into the food chain via

zooplankton, finally reaching fish (Patil et al., 2007; Yongmanitchai and Ward, 1991). Therefore, they have been incorporated as one of the main nutritional sources in aquaculture systems (Patil et al., 2007).

Photosynthetic microalgae are the primary producers of PUFAs in aquatic environments and their production can be enhanced under stressful conditions (Cerón García et al., 2005; Molina Grima et al., 2003). Furthermore, the ratio of EPA to DHA can be altered by changing the cultivation conditions. Although the commercial exploitation of photosynthetic microalgae is currently performed in both outdoor open ponds and closed photobioreactors, the open systems are considered unfeasible for the production of high value metabolites such as EPA. This is due to several factors, including the high risks of contamination, poor mixing and $CO_2$ utilization, low illumination surface area and reduced degree of control on growth parameters that lead to low productivities and cell concentrations (Brennan and Owende, 2010; Cerón García et al., 2005). The development of different cultivation technologies and conditions to improve productivity and yield of microalgal processes have been studied by several authors (Cerón García et al., 2005; Mata et al., 2010; Olaizola, 2003; Pulz, 2001).

The selection of promising strains is one of the key factors to achieve the best yields (Brennan and Owende, 2010; Mata et al., 2010). In the case of EPA, the widely distributed marine diatom *Phaeodactylum tricornutum* (Bohlin, 1897) is one of the promising candidates (Cerón García et al., 2006; Ramírez Fajardo et al., 2007). According to Ramírez Fajardo et al. (2007), *P. tricornutum* is able to accumulate up to 30-45% PUFAs, among which EPA can account for up to 40% of the total fatty acids.

Regardless of the interest of producing microalgal PUFAs, the environmental impacts associated with the process have not been identified and evaluated before. Previous microalgae LCA have focused on biodiesel production (Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2010; Lardon et al., 2009; Sander and Murthy, 2010), biogas production (Collet et al., 2011) and co-firing microalgae with coal (Kadam, 2002). According to these studies, microalgae were demonstrated as promising sources of energy due to the higher lipid content in comparison with other potential terrestrial plants (Lardon et al.,

2009) and their ability to convert sunlight energy into usable energy carriers (Clarens et al., 2011). Some bottlenecks related to the production of microalgal biofuels are the high concentrations of nutrients needed for their growth, the energy demand required for the cultivation and harvesting, and the downstream processing (Brentner et al., 2011; Clarens et al., 2010; Lardon et al., 2009).

In order to verify the applicability of these results to other products from microalgae, the aim of this section of Chapter 3 is the performance of a detailed inventory analysis and environmental assessment of PUFA production by *P. tricornutum*, according to the Life Cycle Assessment (LCA) methodology. The results will highlight the most important elements that contribute to the environmental impact at both lab and pilot scales and point out where future development should pay special attention to develop a sustainable process for the production of PUFAs.

### 3.2.1. Goal and scope

The main objective is to assess the environmental impacts associated with the production of 1 kg (dry biomass) of EPA, which corresponds to 36% of total PUFAs accumulated by *P. tricornutum*. The production process was assessed under two production schemes: lab scale and pilot scale.

The lab scale study was performed for *P. tricornutum* cultured in a bubble column photobioreactor at the Laboratory of Protistology and Aquatic Ecology, Ghent University (Belgium) and allowed to identify the potential environmental burdens associated with the production of EPA.

Afterwards, the production at pilot scale was simulated in a hypothetical scenario. In this case, a sensitivity analysis was carried out to assess the influence of different alternatives on the final environmental performance. In both production schemes, the inventory data were collected from a cradle to gate perspective including: i) cleaning and sterilization, ii) preparation of the inoculum and culture medium, iii) cultivation of algal biomass, iv) harvesting and v) PUFAs extraction. The scenarios under assessment are following described:

### ❖ Lab-scale production scenario

**Figure 3.1** shows the different stages and processes performed at lab scale which were subsequently extrapolated to a large scale.

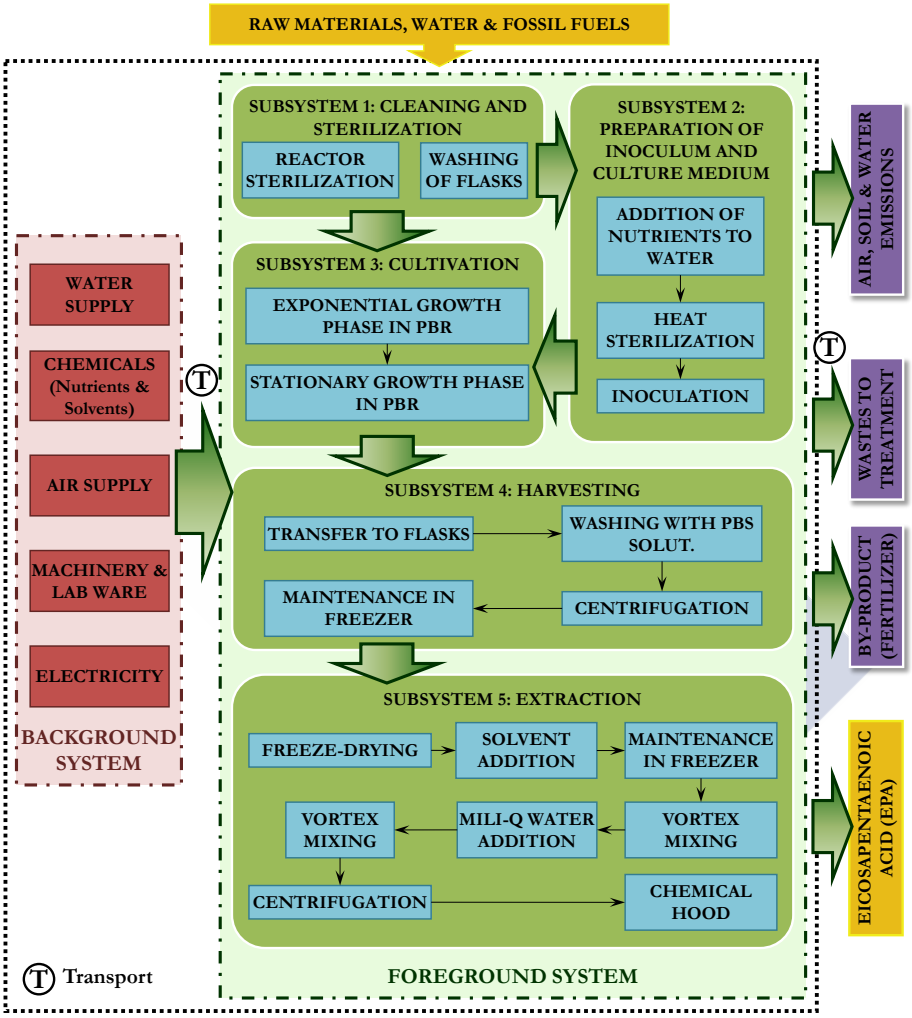



**Figure 3.1.** Process chain and system boundaries of the production of EPA by *P. tricornutum* at lab scale.

The PUFAs production line, from the cultivation of biomass to the extraction of EPA at lab scale, consisted of five main steps:

i) S1. Cleaning and sterilization: Before cultivation, 20 L plastic carboys were autoclaved for 45 min and 20 psi at 121ºC. The flasks for the preparation of the inoculum (S2) were rinsed with the laboratory detergent Mucasol and further washed in a dishwasher, with the addition of a chlorine tablet (containing sodium hypochlorite, NaClO). For the inoculation in the carboys at the beginning of the cultivation step (S3), the lab bench was disinfected with Norvanol.

ii) S2. Preparation of inoculum and culture medium: A slant of *P. tricornutum* CCAP 1055/1 was inoculated into 100 mL of culture medium in a 500 mL Erlenmeyer flask. The flasks were incubated at 23°C for several days and bubbled with a mixture of oxygen and $CO_2$.

Natural seawater enriched with F/2 medium was used, containing 0.075 $g \cdot L^{-1}$ sodium nitrate ($NaNO_3$), 0.0056 $g \cdot L^{-1}$ sodium phosphate monobasic ($NaH_2PO_4$), 4.36 $mg \cdot L^{-1}$ ethylendiaminetetraacetic acid disodium salt dihydrate (EDTA-$Na_2 \cdot 2H_2O$), 9.8 $mg \cdot L^{-1}$ copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), 6.3 $mg \cdot L^{-1}$ sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$), 22.0 $mg \cdot L^{-1}$ $ZnSO_4 \cdot 7H_2O$ (zinc sulfate heptahydrate), 0.18 $mg \cdot L^{-1}$ manganese (II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$), as well as 0.0001 $mg \cdot L^{-1}$ vitamin B1, 0.5 $mg \cdot L^{-1}$ vitamin H and 0.5 $mg \cdot L^{-1}$ vitamin B12 (Guillard, 1975). The preparation of the medium required the addition of nutrients in the specified amounts to filtered seawater, followed by the sterilization of the culture medium in an autoclave.

iii) S3. Cultivation step: Under sterile conditions, the content of the flasks from S2 (1 L of inoculum) was transferred into the 20 L carboys, previously filled with 11 L culture medium. The culture was incubated at 23°C for approximately 14 days. The carboys were fitted with a three stem apparatus (air inlet, air outlet and sampling stem), connected to filters (sterile aeration). Cool white fluorescent lamps were set in a 24 hour light regime of 60 µmol photons$\cdot m^{2} \cdot s^{-1}$.

iv) S4. Harvesting step: The culture medium (12 L) was harvested during both exponential (6 L culture harvested) and stationary (6 L culture harvested) stages, with biomass concentrations of 1-2 g dry weight per liter ($g_{DW} \cdot L^{-1}$) and 2-2.5 $g_{DW} \cdot L^{-1}$, respectively. The culture was first centrifuged at 3000 g for 15 min after washing out the salts twice with a phosphate buffered saline (PBS) solution. The algal paste was collected in Falcon tubes (50 mL) and placed in a freezer at -80ºC overnight.

v) S5. Extraction step: Samples were freeze-dried, crushed manually in a mortar and weighed. An amount of 100 mg of the dry algal biomass was transferred into a Sarstedt polypropylene tube (30 mL) and 10 mL of chloroform:methanol (2:1) solution was added and kept at 4°C in a refrigerator for 16 h. Later, 2.5 g of glass beads (425-600 μm) were added and mixed for 5-10 min. A new addition of chloroform:methanol (2:1) solution was carried out for complete extraction.

   The complete extraction took around 45-60 min and Milli-Q water was added to remove water soluble impurities. Thereafter, the sample was centrifuged for 2 min. The lipid layer (theoretically containing the total amount of PUFAs) was collected, transferred into a Sarstedt tube and mixed with sodium sulfate crystals. An additional centrifugation was performed for 2 min and the organic phase was transferred into an amber tube. Finally, the extract was kept for drying in a chemical hood overnight at room temperature.

   The crude extract generated allowed the quantitative determination of the total lipid content (gravimetrically), however, the EPA was not purified in this process. The EPA concentration was determined by gas chromatography-mass spectrometry after methylation, hydrolysis and extraction of the fatty acids from the crude extract.

❖ **Pilot-scale production scenario**

The system boundaries for the pilot scale system are displayed in **Figure 3.2**. The cultivation of *P. tricornutum* at pilot scale was simulated in an indoor PBR consisting of a vertical bubble column, with a working volume of 80 L (150 cm × 60 cm × 10 cm) based on real data for the production of other microalgae (in

this case, *Tetraselmis suecica*) and completed with available literature (Kadam, 2002; Khoo et al., 2011; Langlois et al., 2012). The described configuration is proposed as a compact, low cost and aseptic PBR (Fernández Sevilla et al., 2004). Information concerning algae growth rates, harvesting and final extraction of PUFAs was taken from Ulloa et al. (2012):

i) S1. Cleaning and sterilization: Firstly, the bioreactor was chemically sterilized with NaClO (5 $mL \cdot L^{-1}$) for 24 h. After that, air and sodium thiosulfate, $Na_2S_2O_3$ (0.1 $g \cdot L^{-1}$) were added for 2 h in order to remove residual chlorine. The reactor was washed twice with sterile distilled water previously to the addition of the inoculum and the culture medium.

ii) S2. Preparation of inoculum and culture medium: The inoculum (8 L, corresponding to 10% of the pilot-scale reactor volume) was grown indoors under artificial light. Detailed information on the culture medium can be found in Ulloa et al. (2012).

iii) S3. Cultivation step: The reactor was inoculated with 8 L *P. tricornutum* culture to start the operation with a biomass concentration of 0.155 $g_{DW} \cdot L^{-1}$ in the PBR, which was operated in a semi-continuous mode. An enriched $CO_2$ air flow (4.5 $L \cdot min^{-1}$) from a gaseous stream was considered. The bioreactor was initially filled with an extra volume of 72 L of natural seawater (to give a total culture volume of 80 L) supplemented with nutrients. The culture was daily supplemented with 32 L of seawater. Losses of seawater were occasionally satisfied but excluded from the assessment.

   Temperature in the reactor was maintained at 20°C by circulating thermostated water and pH was maintained at 7-8. Alternative 12 h periods of darkness and artificial light supply were considered (596 µmol photon $\cdot m^{-2} \cdot s^{-1}$). The semi-continuous regime was held for 2 months with a daily productivity of 0.788 $g \cdot L^{-1} \cdot d^{-1}$.

iv) S4. Harvesting step: The biomass was settled at 4°C for 24 h with a final recovery of 80%, which was later centrifuged for 10 min and frozen at -20 °C before freeze drying.

v) S5. Extraction step: In this process, lipid extraction from the freeze dried algal paste using hexane as extraction solvent was considered, due to its highly efficient extraction capacities and its low cost (Demirbas, 2009). Nearly all the solvent was recovered by means of evaporation after lipid extraction. The crude extract generated after solvent extraction contained 45% of PUFAs of which 36% corresponded to EPA. It is important to note that the EPA extract was not purified.

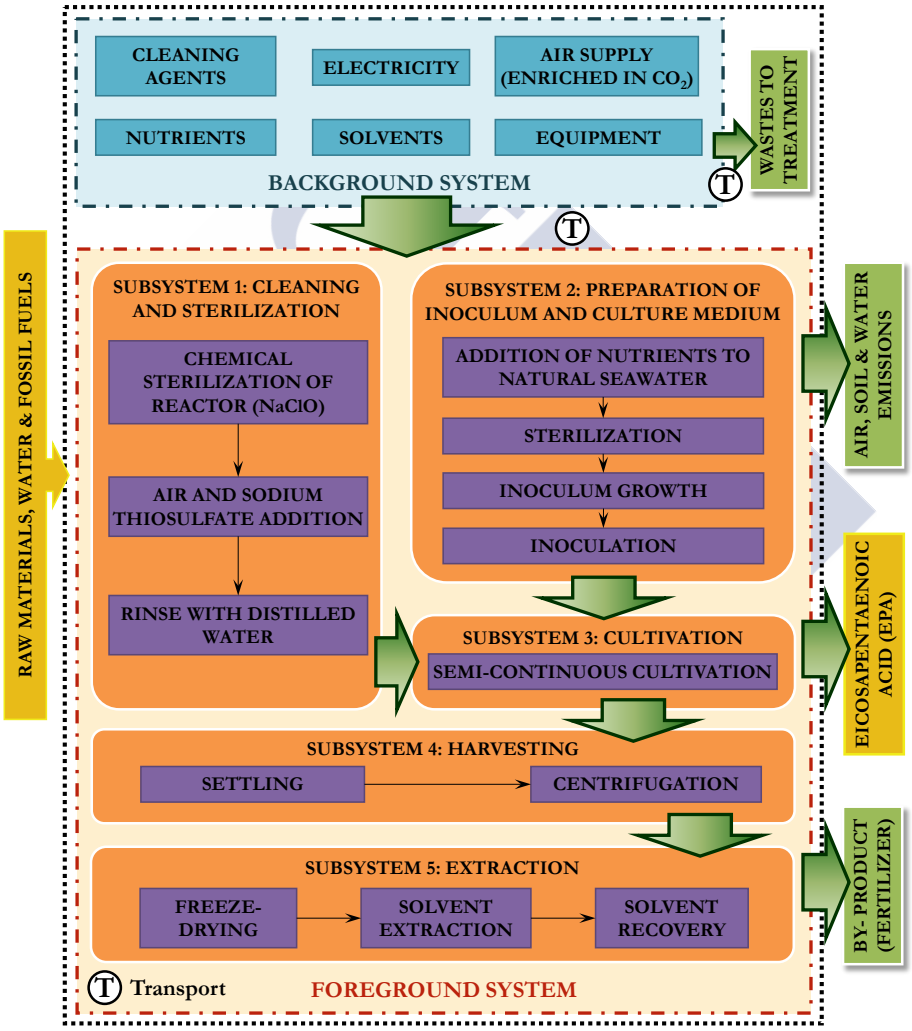



**Figure 3.2.** System boundaries of the production of EPA by *P. tricornutum* at pilot scale.

### 3.2.2. Life cycle inventory, data quality and assumptions

The life cycle inventory (LCI) data for the foreground system at lab and pilot scales comprising chemicals (nutrients, solvents, washing agents), lab ware (beads, glasses, etc.), equipment (bioreactors), lighting system, electricity consumption as well as transport distances consisted of average data obtained by on-site measurements (**Tables 3.1** and **3.2**). In the pilot scale process, materials for the different equipment were estimated according to other analyzed microalgal processes, reported by Pérez-López et al. (2014a; 2014b). Regarding water emissions from the different stages, they were calculated assuming that the non-depleted fraction of the nutrients was directly discharged as wastewater. An identical assumption was taken for air emissions.

Concerning the background system, inventory data for the production of the different chemicals required, the lab ware (polypropylene, polycarbonate resin and glasses), the bioreactor material, the electricity requirements, the waste treatment (sanitary landfills, inert material landfills and municipal incineration) and finally the transport of the different inputs were taken from the Ecoinvent database (Frischknecht et al., 2007). The cleaning agents required for washing the materials at lab scale were not specifically included in the Ecoinvent database. As Norvanol, used for the lab bench disinfection, is mainly made of ethanol (83%), the equivalent amount of ethanol was considered. Concerning Mucasol, a lab washing powder, the equivalent amount of a generic soap was considered. Finally, both calcium hypochlorite (CaClO) and NaClO can be used. In this case, the second one was considered. For the washing stage at pilot scale, the cleaning agents used were NaClO and $Na_2S_2O_3$ and inventory data were taken from the Ecoinvent database (Althaus et al., 2007). The corresponding foreground inventory data for the production of natural $NaNO_3$ were taken from the process developed by the Chilean nitrate industry (Pokorny et al., 2000), and completed with reports from Ecoinvent database for the background data. A detailed description of the literature considered for the background processes is presented in **Table 3.3**.

**Table 3.1.** Inventory data for the lab-scale production of EPA by *P. tricornutum* (FU=1 g EPA, 36% of total PUFAs)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Cleaning and sterilization* | | | |
| Tap water | 97.14 L | Mucasol (soap) | 37.36 mL |
| Norvanol (83% ethanol) | 18.68 mL | Chlorine tablet (NaClO) | 0.26 kg |
| *S2. Preparation of inoculum and culture medium* | | | |
| Filtered seawater | 44.83 L | $ZnSO_4 \cdot 7H_2O$ | 0.99 mg |
| $NaNO_3$ | 3.36 g | $CoCl_2 \cdot 6H_2O$, | 0.45 mg |
| $NaH_2PO_4$ | 0.25 g | $MnCl_2 \cdot 4H_2O$ | 8.07 mg |
| EDTA-$Na_2 \cdot 2H_2O$ | 0.20 g | Vitamin B1 | 4.48 mg |
| $CuSO_4 \cdot 5H_2O$ | 0.44 mg | Vitamin H | 0.02 mg |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.28 mg | Vitamin B12 | 0.02 mg |
| Glass (flasks) | 28.21 g | Compressed air (1.5% $CO_2$) | 4.20 kg |
| *S3. Cultivation* | | | |
| Polycarbonate (20 L carboy) | 68.49 g | Compressed air (1.5% $CO_2$) | 76.01 kg |
| Polypropylene (carboy) | 0.31 g | | |
| *S4. Harvesting* | | | |
| Tap water | 448.36 kg | KCl | 3.01 g |
| Milli-Q water | 14.95 g | $Na_2HPO_4 \cdot 2H_2O$ | 21.55 g |
| NaCl | 119.66 g | $KH_2PO_4$ | 3.58 g |
| Glass (flasks) | 0.25 kg | Polypropylene (flasks) | 2.14 kg |
| *S5. Extraction* | | | |
| Chloroform | 11.43 L | Milli-Q water | 11.43 L |
| Methanol | 5.71 L | $Na_2S_2O_3$ crystals | 0.57 kg |
| Glass (beads) | 1.43 kg | Amber glass (tubes) | 5.31 kg |
| Tap water | 269.01 L | | |
| **Energy** | | **Transport** | |
| Electricity from Belgian grid | | Truck 3.5-7.5 t (chemicals) | 6.67 tkm |
| S2. Preparation of inoculum and culture medium | 37.27 kWh | Truck 3.5-7.5 t (washing agents) | 0.03 tkm |
| S3. Cultivation | 101.32 kWh | Truck 3.5-7.5 t (lab materials) | 4.21 tkm |
| S4. Harvesting | 1.20 kWh | Truck 3.5-7.5 t (wastes) | 1.13 tkm |
| S5. Extraction | 101.35 kWh | | |

**Table 3.1.** Inventory data for the lab-scale production of EPA by *P. tricornutum* (FU=1 g EPA, 36% of total PUFAs) (*Cont.*)

| **OUTPUTS to TECHNOSPHERE** | | | |
|---|---|---|---|
| **Product** | | **By-product** | |
| EPA from *P. tricornutum* (36% of total PUFAs) | 1 g | Remaining *P. tricornutum* dry biomass | 72.55 g |
| **Wastes to landfill** | | | |
| Polypropylene | 15.84 kg | Glass (tubes) | 5.58 kg |
| Polycarbonate | 68.50 g | Glass (beads) | 1.43 kg |
| **OUTPUTS TO ENVIRONMENT** | | | |
| **Water emissions** | | | |
| *S1. Cleaning and sterilization* | | | |
| Wastewater | 97.14 L | Mucasol (soap) | 37.36 mL |
| Norvanol (83% ethanol) | 18.68 mL | Chlorine tablet (NaClO) | 0.26 kg |
| *S2. Preparation of the culture medium* | | | |
| Wastewater | 0.73 L | $ZnSO_4 \cdot 7H_2O$ | 5.73 μg |
| $NaNO_3$ | 19.54 mg | $CoCl_2 \cdot 6H_2O$, | 2.61 μg |
| $NaH_2PO_4$ | 1.46 mg | $MnCl_2 \cdot 4H_2O$ | 46.90 μg |
| EDTA-$Na_2 \cdot 2H_2O$ | 1.14 mg | Vitamin B1 | 26.06 μg |
| $CuSO_4 \cdot 5H_2O$ | 2.55 μg | Vitamin H | 0.13 μg |
| $Na_2MoO_4 \cdot 2H_2O$ | 1.14 μg | Vitamin B12 | 0.13 μg |
| *S4. Harvesting* | | | |
| Wastewater | 507.20 L | $MnCl_2 \cdot 4H_2O$ | 2.63 mg |
| $NaNO_3$ | 1.09 g | Vitamin B1 | 1.46 mg |
| $NaH_2PO_4$ | 0.08 g | Vitamin H | 0.01 mg |
| EDTA-$Na_2 \cdot 2H_2O$ | 0.06 g | Vitamin B12 | 0.01 mg |
| $CuSO_4 \cdot 5H_2O$ | 0.14 mg | NaCl | 119.66 g |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.09 mg | KCl | 3.01 g |
| $ZnSO_4 \cdot 7H_2O$ | 0.32 mg | $Na_2HPO_4 \cdot 2H_2O$ | 21.55 g |
| $CoCl_2 \cdot 6H_2O$, | 0.14 mg | $KH_2PO_4$ | 3.58 g |
| *S5. Extraction* | | | |
| Wastewater | 280.67 L | Methanol | 5.71 L |
| Chloroform | 11.43 L | | |
| **Air emissions** | | | |
| *S2. Preparation of inoculum and culture medium* | | *S3. Cultivation* | |
| Air | 4.14 kg | Air | 74.86 kg |
| $CO_2$ | 0.05 kg | $CO_2$ | 1.04 kg |

**Table 3.2.** Inventory data for the pilot-scale production of EPA by *P. tricornutum* (FU=1 kg EPA, 36% of total PUFAs)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Cleaning and sterilization* | | | |
| Distilled water | 4850 L | NaClO | 12.03 kg |
| Tap water | 4847 L | $Na_2S_2O_3$ | 242.47 g |
| Polyvinyl chloride | 37.58 g | Polyamide | 206.72 g |
| *S2. Preparation of inoculum and culture medium* | | | |
| Filtered seawater | 47700 L | $Na_2MoO_4$ | 19.75 g |
| $NaNO_3$ | 16.36 kg | $CoCl_3$ | 1.58 g |
| $NaH_2PO_4 \cdot 2H_2O$ | 1.50 kg | $CuSO_4$ | 1.52 g |
| EDTA | 0.94 kg | Thiamine | 3.36 g |
| $C_6H_5FeO_7$ | 0.47 kg | Biotin | 0.97 g |
| $ZnCl_2$ | 13.08 g | Vitamin B12 | 0.28 g |
| $MnCl_2$ | 12.08 g | Polyethylene (HDPE) | 83.42 g |
| Inoculum | 30.06 g | Lamps | 30.81 g |
| *S3. Cultivation* | | | |
| Polymethyl methacrylate | 7.14 kg | Lamps | 229.33 g |
| $CO_2$ (aeration) | 114.17 kg | | |
| *S4. Harvesting* | | | |
| Stainless steel | 6.81 kg | | |
| *S5. Extraction* | | | |
| Hexane | 0.31 L | Stainless steel | 0.98 kg |
| **Energy** | | | |
| Electricity from Belgian grid | | | |
| S1. Cleaning and sterilization | 52.59 kWh | S4. Harvesting | 56.35 kWh |
| S3. Cultivation | 886.54 kWh | S5. Extraction | 360.08 kWh |
| **Transport** | | | |
| *S1. Cleaning and sterilization* | | | |
| Truck 3.5-7.5 t (wash. agents) | 1.36 tkm | Truck 3.5-7.5 t (equipment) | 0.01 tkm |
| Truck 3.5-7.5 t (wastes) | 0.01 tkm | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Truck 3.5-7.5 t (nutrients) | 1.91 tkm | Truck 3.5-7.5 t (equipment) | 0.01 tkm |
| Truck 3.5-7.5 t (seawater) | 1908 tkm | Truck 3.5-7.5 t (wastes) | 0.003 tkm |
| Oceanic tanker (seawater) | 1908 tkm | | |

**Table 3.2.** Inventory data for the pilot-scale production of EPA by *P. tricornutum* (FU=1 kg EPA, 36% of total PUFAs) (*Cont.*)

| | | | |
|---|---|---|---|
| **INPUTS from TECHNOSPHERE** | | | |
| **Transport** | | | |
| *S3. Cultivation* | | | |
| Truck 3.5-7.5 t (equipment) | 0.41 tkm | Truck 3.5-7.5 t (wastes) | 0.22 tkm |
| *S4. Harvesting* | | | |
| Truck 3.5-7.5 t (equipment) | 0.37 tkm | Truck 3.5-7.5 t (wastes) | 0.20 tkm |
| *S5. Extraction* | | | |
| Truck 3.5-7.5 t (equipment) | 0.05 tkm | Truck 3.5-7.5 t (wastes) | 0.03 tkm |
| Truck 3.5-7.5 t (solvents) | 0.07 tkm | | |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Product** | | **By-product** | |
| EPA from *P. tricornutum* (36% of total PUFAs) | 1 kg | Remaining algal biomass (wet) | 31.42 g |
| **Wastes to landfill** | | **Wastes to municipal incineration** | |
| Polymethyl methacrylate | 7.14 kg | Polyethylene (HDPE) | 83.41 g |
| Polyvinyl chloride (PVC) | 37.58 g | **Wastes to specific treatment** | |
| Polyamide | 206.72 g | Lamps | 30.81 g |
| Stainless steel | 7.79 kg | | |
| **OUTPUTS TO ENVIRONMENT** | | | |
| **Water emissions** | | | |
| *S1. Cleaning and sterilization* | | | |
| Wastewater | 9697 L | NaClO | 11.03 g |
| $Na_2S_2O_3$ | 242.47 g | | |
| *S4. Harvesting* | | | |
| Wastewater | 47700 L | $Na_2MoO_4$ | 2.67 g |
| $NaNO_3$ | 2.19 kg | $CoCl_3$ | 0.21 g |
| $NaH_2PO_4 \cdot 2H_2O$ | 200.94 g | $CuSO_4$ | 0.21 g |
| EDTA | 126.58 g | Thiamine | 0.45 g |
| $C_6H_5FeO_7$ | 63.11 g | Biotin | 0.13 g |
| $ZnCl_2$ | 1.75 g | Vitamin B12 | 0.04 g |
| $MnCl_2$ | 1.61 g | | |
| *S5. Extraction* | | | |
| Hexane | 0.31 L | | |

**Table 3.3.** Summary of data sources for the background system of the production of EPA by *P. tricornutum*

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Energy | Electricity (Belgian electricity profile) | Ecoinvent database (Dones et al., 2007) |
| Water | Filtered seawater<br>Tap water<br>Distilled water<br>Milli-Q water | Ecoinvent database (Althaus et al., 2007) |
| Chemicals | $NaNO_3$ | Inventoried according to Pokorny et al. (2000) with Ecoinvent processes (Frischknecht et al., 2007) |
| | $NaH_2PO_4 \cdot H_2O$<br>EDTA<br>$CuSO_4 \cdot 5H_2O$<br>$Na_2MoO_4 \cdot 2H_2O$<br>$CoCl_2 \cdot 6H_2O$<br>$MnCl_2 \cdot 5H_2O$<br>Thiamine (vitamin B1)<br>Biotin (vitamin H)<br>Cyanocobalamin (vitamin B12)<br>NaCl<br>$Na_2HPO_4 \cdot 2H_2O$<br>$KH_2PO_4$<br>Chloroform<br>Methanol | Ecoinvent database (Althaus et al., 2007) |
| | $ZnSO_4 \cdot 7H_2O$ | Ecoinvent database (Hischier et al., 2007) |
| | KCl | Ecoinvent database (Nemecek and Kägi, 2007) |
| | Hexane | Ecoinvent database (Jungbluth et al., 2007) |
| | Ethanol (assimilated to Norvanol) | Ecoinvent database (Sutter, 2007) |
| | Soap (assimilated to Mucasol) | Ecoinvent database (Zah and Hischier, 2007) |

**Table 3.3.** Summary of data sources for the background system of the production of EPA by *P. tricornutum* (*Cont.*)

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Lab materials (flasks, reactor) | High density polyethylene (HDPE) | Ecoinvent database (Hischier, 2007) |
| | Polypropylene | |
| | Polycarbonte resin | |
| | Glass (white, amber) | |
| | Polymethyl methacrylate | |
| | Lamps | Ecoinvent (Hischier et al., 2007) |
| Other materials | Glass (beads) | Ecoinvent database (Hischier, 2007) |
| | Sodium sulfate crystals | Ecoinvent database (Althaus et al., 2007) |
| Transport | Truck 3.5-7.5 t | Ecoinvent database (Spielmann et al., 2007) |
| Waste treatment | Sanitary landfill | Ecoinvent database (Doka, 2007) |
| | Inert landfill | |
| | Specific treatment of electronic waste | |
| | Municipal incineration | |

Algae are a photosynthetic source of aquatic biomass. Therefore, they are agents which capture $CO_2$ throughout their growth. The promotion of algae cultivation using residual $CO_2$ enriched gaseous streams derived from industrial processes such as electric power plants is receiving special attention in recent years due to their $CO_2$ fixation capacity (Aresta et al., 2005). Therefore, in this study, the cultivation at pilot scale was assumed to be performed with an enriched $CO_2$ air flow derived from a nearby power plant. Production of this flue stream rich in $CO_2$ has not been included within the system boundaries since it was considered as a waste from other industrial process and all the environmental burdens should be allocated to its process of origin.

*Allocation procedures*

According to the goal and scope, *P. tricornutum* cultivation was only focused on PUFAs production. Thus, all the environmental burdens were allocated to the amount of EPA produced (36% of total PUFAs extracted). However, the residual algal biomass, obtained after lipid extraction, is an accessible source of minerals (e.g. nitrogen, phosphorus and potassium) which can be used as fertilizer for agricultural applications (Mulbry et al., 2005) or as a potential source of biomass for biogas production (Collet et al., 2011). With this regard, a system expansion approach was considered in the discussion section, considering the environmental benefits of using the remaining algal biomass as fertilizer and the production of biogas in the anaerobic digestion process.

### 3.2.3. Environmental impact assessment

An attributional LCA was carried out using the CML 2 baseline 2001 V2.05 method for the life cycle impact assessment (Guinée et al., 2002). This method quantifies the environmental impacts of the system under assessment in different impact categories. In this case, ten impact categories were considered: abiotic depletion potential (ADP), acidification potential (AP), eutrophication potential (EP), global warming potential over a 100 year timeframe (GWP), ozone layer depletion potential (ODP), photochemical oxidants formation potential (POFP) and toxicity related impact categories: human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP) and terrestrial ecotoxicity (TEP). The software SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008).

Among the phases defined by the LCA standard methodology (ISO 14040, 2006) only classification and characterization stages were undertaken, since normalization and weighing are optional (and, to some extent, subjective) steps that provide no additional information according to the goal and scope of the study. The characterization results for the production of 1 kg EPA (representing 36% of the PUFAs produced by *P. tricornutum*) at lab and pilot scale are shown in **Table 3.4**. Although the functional unit is not representative of a lab-scale process, it is used for the two processes to analyze the influence of upscaling.

Regardless of the considered impact category, **Table 3.4** shows a remarkable improvement in the simulated pilot scenario (based on extrapolations) with respect to the baseline lab-scale system, mainly linked to the low yield in relation to the required inputs (lab materials, electricity, etc.) due to the inherent inefficiency and equipment oversizing of laboratory processes. Thus, environmental impacts in most categories are reduced by at least two orders of magnitude when considering the process at pilot scale. The only exceptions are AP, EP and POFP, with still significant contributions 10, 21 and 46 times lower for the pilot system than for the lab-scale process. The relative contributions of the different stages and manufacturing processes involved in the two scenarios are discussed in detail below.

Despite the evident environmental benefits of upscaling, it should be noted that the results here discussed depend on assumptions and extrapolations made for the simulation of the pilot process. A more accurate LCA based on field data after the real implementation of the pilot process could help to confirm the conclusions and give more reliable information. Such analysis will be conducted in Chapter 4.

**Table 3.4.** Environmental impact assessment results (characterization step) associated with the production of 1 kg EPA from *P. tricornutum* at lab scale and in an 80 L pilot scale bubble column reactor

| Impact category | Unit | Lab-scale process | Pilot-scale process |
|---|---|---|---|
| ADP | kg Sb eq | 1717 | 14.08 |
| AP | kg $SO_2$ eq | 645 | 61.44 |
| EP | kg $PO_4^{-3}$ eq | 334 | 15.67 |
| GWP | kg $CO_2$ eq | 215875 | 2064 |
| ODP | kg CFC-11 eq | 20.39 | 0.0002 |
| HTP | kg 1,4-DB eq | 322032 | 1814 |
| FEP | kg 1,4-DB eq | 89884 | 489 |
| MEP | kg 1,4-DB eq | 53070 | 338 |
| TEP | kg 1,4-DB eq | 47.22 | 0.27 |
| POFP | kg $C_2H_4$ eq | 35.20 | 0.77 |

❖ **Lab-scale results**

The cultivation of *P. tricornutum* was assessed at lab scale to identify the potential environmental hot spots associated with the production of EPA. A breakdown of the relative contribution to each impact category of the production stages and related processes of the lab scale production of EPA is presented in **Figure 3.3**.

According to these results, the extraction is clearly the main hot spot among the five stages of the process for nine impact categories. In most of them, the cultivation has a secondary contribution ranging between 15% and 30%. The cleaning stage only has a remarkable influence in the category of TEP (68%), mainly due to the use of Mucasol. Regarding the GWP reduction potential of microalgal growth, $CO_2$ uptake is rather limited in this case compared to the high GHG emissions that are linked with the inefficiency of the lab-scale process.

Among the related processes, the production of the electricity requirements and chemicals used throughout the life cycle are the key environmental factors in almost all the analyzed categories, followed by the production of lab materials and the waste treatment. The high contribution of electricity in categories such as ADP, AP, EP, GWP and POFP is mainly linked to the remarkable dependence of the Belgian grid (which is here considered as the energy supplier) on fossil fuels. The production of chemicals also has a significant influence in ADP, GWP and POFP, and is the main contributor to ODP (99.9%), mainly due to the production of chloroform, which is used as extraction solvent. The treatment of the wastes generated within the lab scale is the main cause of high FEP and MEP values, specifically due to the disposal of polypropylene materials (lab ware) into sanitary landfills. On-site emissions cause a remarkable impact to HTP, related to the chloroform waste flow from extraction, whereas the production of lab ware materials affect some categories such as ADP, AP and POFP.

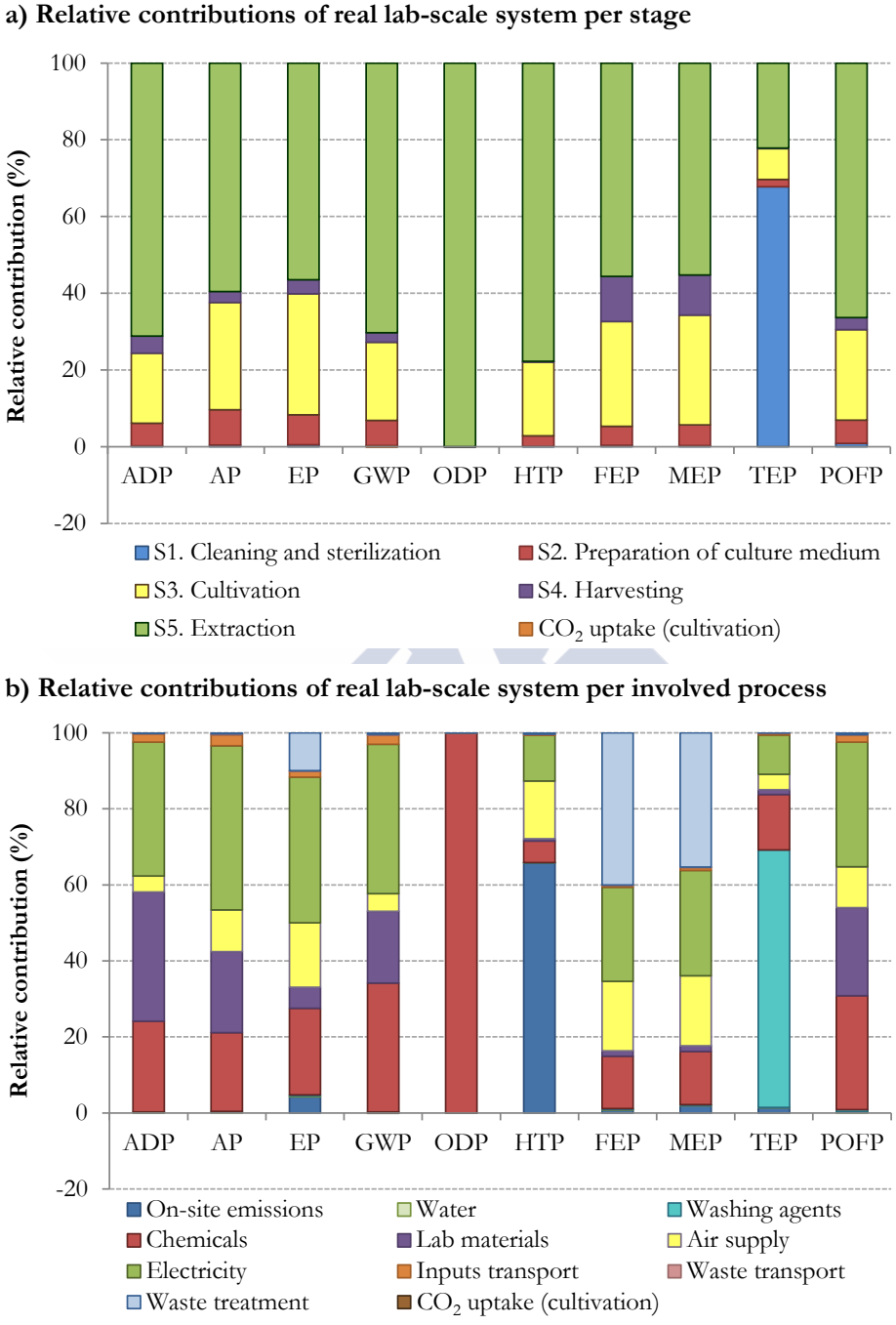



**Figure 3.3.** Relative contributions of the lab-scale production of EPA by *P. tricornutum* to each impact category per a) stage and b) involved process.

❖ **Pilot-scale results**

The production of *P. tricornutum* at pilot scale was simulated in an indoor vertical bubble column PBR with a working volume of 80 L. After 60 days, the algal biomass was harvested and subjected to extraction with hexane. Approximately 45% of the total lipid fraction was PUFAs and 36% of the PUFAs fraction corresponded to EPA. **Figure 3.4** depicts the contributions of the stages and processes involved in the production line in order to identify the most important contributors for each impact category.

According to the results, the preparation of the inoculum and culture medium present the highest contribution to all categories, with impacts ranging between 30% (TEP) up to 96% (AP). Among the secondary stages, cultivation has some effect on ADP (26%), GWP (24%), FEP (23%) and MEP (21%), whereas harvesting has a relatively high impact to HTP (29%) and FEP (19%) and the cleaning and sterilization dominates the contributions to TEP. The extraction step has a limited effect (lower than 10%) for all categories.

The high contribution derived from the preparation of the inoculum and culture medium is mainly due to the production of the nitrogen source required for the microalgal growth (specifically sodium nitrate). The electricity required for reactor lighting is the key factor responsible for the environmental impact of the cultivation whereas the production of materials for the equipment (namely steel) is responsible for most of the contribution of the harvesting step. The production of natural sodium nitrate is responsible for 90% and 87% of total acidifying and eutrophying emissions, and 65% of total electricity consumption at pilot scale takes place at the cultivation step.

The contribution from the transport activities related with the delivery of inputs (e.g. nutrients, sodium nitrate and seawater) to the plant is also important, especially for ADP (45%), GWP (44%) and ODP (65%). Although transport can be a key environmental role depending on the availability of natural seawater, they are often excluded from the system boundaries in the available LCA studies (Collet et al., 2011; Kadam, 2002; Khoo et al., 2011; Lardon et al., 2009). In this case, the contribution of transport activities could be reduced by installing the pilot facility in close proximity to natural seawater sources.

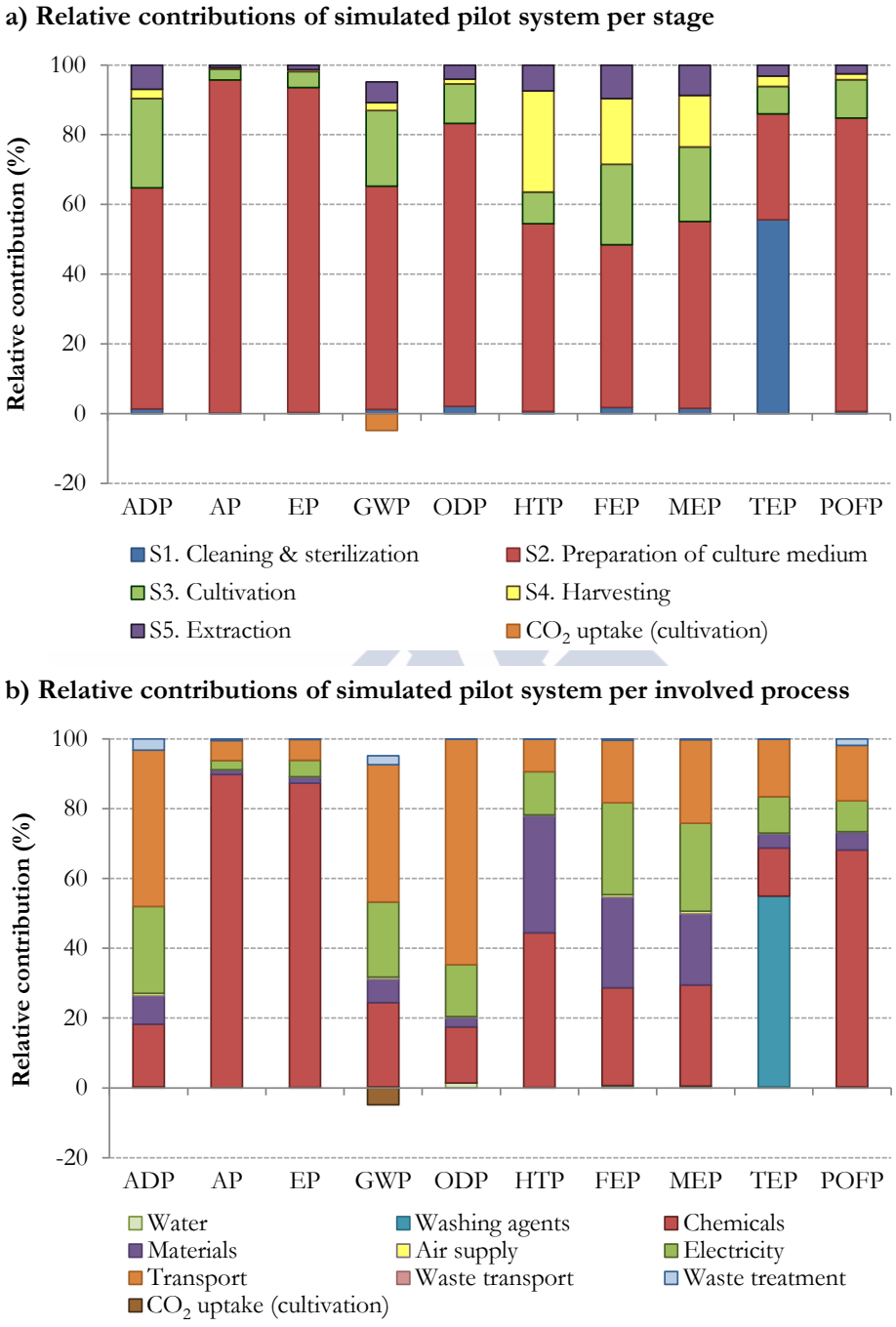



**Figure 3.4.** Relative contributions of the pilot-scale production of EPA by *P. tricornutum* to each impact category per a) stage and b) involved process.

Regarding the derived substances from the different processes involved in the production systems, $NO_x$ emissions are the main cause of the AP and EP, followed by $NH_3$ emissions. Both emissions are mainly derived from the production of the nitrogen source (sodium nitrate). In terms of ODP, emissions of Halon 1301 and Halon 1211 dominate the environmental impact, especially linked to transport activities (diesel production). POFP mostly derives from CO and $SO_2$ emissions, again associated with the production of sodium nitrate. The main contribution to GWP is related to fossil $CO_2$ derived from the mentioned processes, as well as from the production of electricity. Concerning toxicity related categories (HTP, FEP, MEP and TEP), air emissions of polycyclic aromatic hydrocarbons dominated the contributions to HTP, water emission of nickel ion were the main cause of MEP and FEP, and soil emission of cypermethrin had the highest contribution to TEP.

### 3.2.4. Discussion and recommendations

❖ **Lab-scale system**

In the previous section, the processes that dominate the environmental burdens were identified (**Figure 3.3**). The dominating environmental burdens at laboratory scale were attributed to the production of electricity, the production of chemicals (mainly organic solvents for extraction), the treatment of wastewater and the production of lab materials. Since electricity and lab ware were not produced on site and the lab was not responsible for their production, no improvement alternatives were proposed. With regard to the solvents for EPA extraction, those chemicals represent one of the highest contributions to the environmental burdens associated with the production of EPA by *P. tricornutum* at lab scale, mainly in terms of ODP (99.9%), HTP (71%) and GWP (34%), taking into account both their production and the associated waste effluents. Among them, the main cause of the impact is the chloroform used for extraction, contributing to the total environmental profile from 13% in FEP to 99.9% in ODP. For this reason, a sensitivity assessment is performed, and alternative extraction methods with better environmental profile are indicated.

Many methods have been proposed for EPA extraction and purification (Molina Grima et al., 2003), although most of them require complex processing operations which may reduce the recovery and enhance the derived costs (Belarbi et al., 2000; Ibáñez González et al., 1998; Ramírez Fajardo et al., 2007). Besides being nontoxic, easy to handle and safe, the alternative method should avoid heating and oxidation, as PUFAs are highly unstable under these conditions (Ramírez Fajardo et al., 2007).

Among the proposed alternatives, Ganga et al. (1998) analyzed the extraction of PUFAs from sardine oil considering a two-step winterization, saponification and urea fractionation. Wilson et al. (1993) evaluated the elution of PUFAs with hexane and dichloromethane using aminopropyl solid phase extraction columns. Brunner and Reichmann (1998) used aluminium oxide stationary phase and carbon dioxide (supercritical or liquid) as mobile phase for the extraction. Silica gel and silver impregnated silica gel could be also considered in column chromatography for the extraction of PUFAs (Hayashi and Kishimura, 1993).

Belarbi et al. (2000) proposed a simple and scalable process for EPA extraction consisting of three steps where both freeze dried and moist microalgal biomass could be employed. First, a simultaneous extraction and transesterification of the microalgal biomass with hexane was performed. The concentrated extract was then loaded on an argentated silica gel column for chromatography in a second step. Finally, pigments were removed in a second chromatographic step. Different chemicals could be used for the extraction including methanol, acetyl chloride, hexane and acetone. Although the authors reported only small amounts of silver contamination, they proposed the treatment of the silver silica gel column with sodium aluminate to overcome silver leaching.

Similarly, Ibáñez González et al. (1998) proposed the isolation of fatty acids at lab scale in *P. tricornutum* using an optimized three step method with a fatty acid yield of 87%. In a first step, wet biomass was directly saponified with a potassium hydroxide/ethanol mixture. Next, the unsaponificable lipids were extracted by hexane and finally the fatty acids were purified by acidification of the solution with chlorhydric acid followed by extraction with hexane. Highly efficient, this method would allow a reduction up to 20% of the production costs in comparison with other methods.

Furthermore, an extraction and purification method based on selective enzymatic esterification has been proposed using lipases as catalysts at lab scale. Lipases were considered since they are enzymes with low activity on PUFAs (Yang et al., 1990).

Among all methods listed, ethanol and hexane are typical solvents of low toxicity that are frequently used nowadays. In this study, the use of hexane as substitute extraction solvent for the mixture chloroform:methanol is proposed. Hexane can be recovered by evaporation of the eluted fractions and can be recycled so that only a small amount of hexane should leave the system with the biomass extracted. According to the experiments, the amount of hexane required to extract 0.75 g of PUFAs (0.27 g EPA) per batch should be 15.29 g.

The environmental results obtained for the hexane-scenario compared with the chloroform-scenario are reported in **Figure 3.5**. According to the results, the environmental profile should considerably improve, especially in terms of ODP (99.9% reduction of impact) and HTP (71% reduction of impact) since both categories were significantly influenced by the use of chloroform.

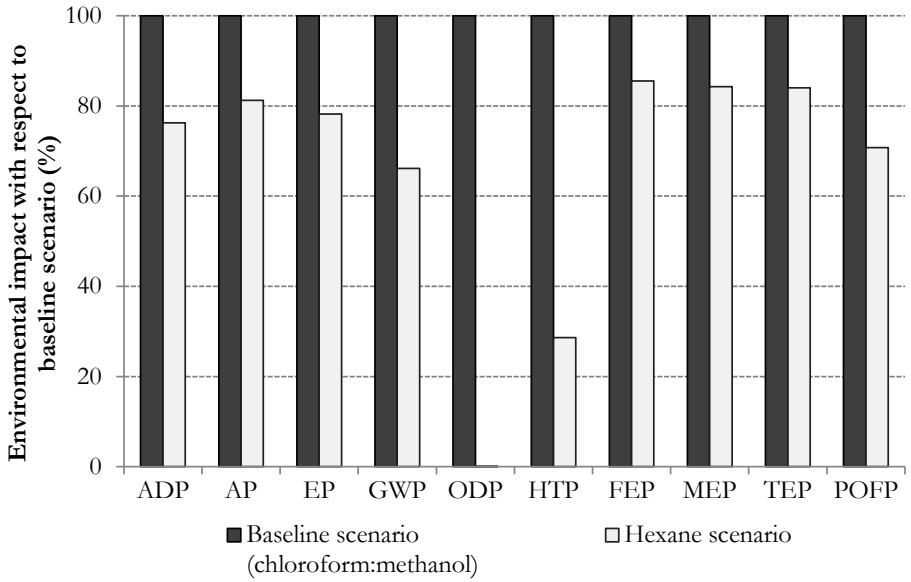



**Figure 3.5.** Comparative environmental profile considering chloroform:methanol and hexane extraction methods at lab scale.

❖ **Pilot scale system**

Up to date, LCA analyzing microalgal products have addressed biodiesel production and biogas production, respectively. In the former case, biodiesel production at industrial scale is documented and the environmental profiles are estimated from LCA specific simulation software tools (Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2010; Khoo et al., 2011; Lardon et al., 2009; Sander and Murthy, 2010) LCA for biogas production from the anaerobic digestion of algae in a hypothetical system has been also reported, based on extrapolations at lab scale (Collet et al., 2011).

In a similar way, this study integrates both lab scale data and pilot scale data which allow the identification of the hotspots (lab scale) and their quantification (pilot scale). In this section, several improved scenarios are evaluated, including the substitution of the main nutrient source and the use of the remaining algal biomass (here treated as a by-product) for potential applications:

i) Use of alternative mineral nitrogen fertilizers to sodium nitrate

As previously reported, sodium nitrate is the main environmental factor for all the impact categories under assessment and is commonly used as nutrient supply for algae growth (Lardon et al., 2009; Ras et al., 2011). Alternative nitrogen based fertilizers were proposed here in order to identify the best option from an environmental point of view. Among the alternative nitrogen based fertilizers, calcium ammonium nitrate (CAN scenario), urea (urea scenario) and synthetic sodium nitrate, produced by the neutralization of nitric acid with sodium hydroxide or sodium carbonate (synthetic $NaNO_3$ scenario) were selected as substitute nitrogen sources for microalgae cultivation and compared with the use of natural sodium nitrate (baseline scenario) (Kadam, 2002; Lardon et al., 2009; Stephenson et al., 2010). Inventory data regarding the production of the alternative nitrogen sources have been taken from the Ecoinvent database (Althaus et al., 2007).

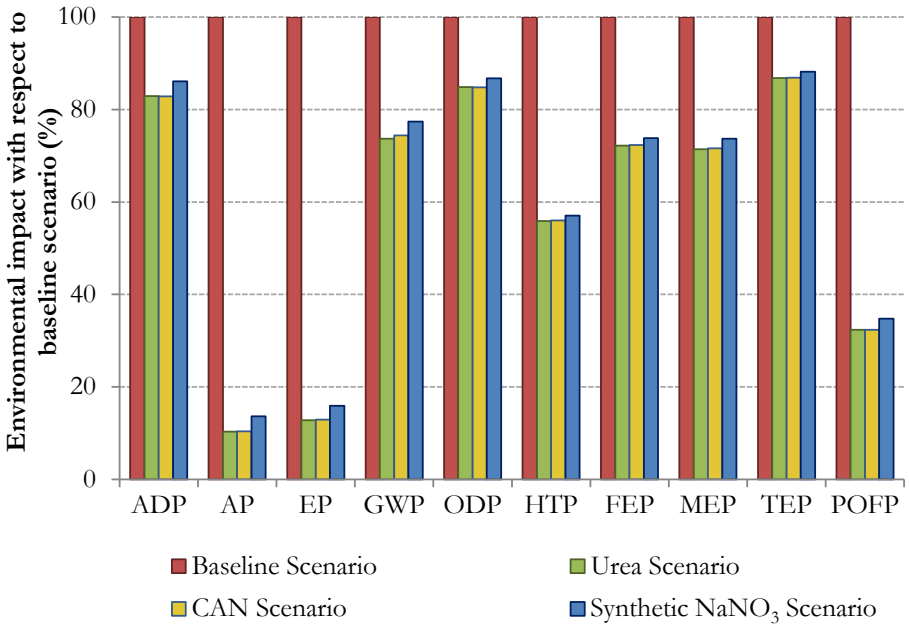



**Figure 3.6.** Comparative environmental profile considering the use of alternative mineral nitrogen based fertilizers.

Comparative environmental profiles for the different scenarios are displayed in **Figure 3.6**. According to these results, changing from sodium nitrate to any of the other nitrogen based fertilizers involved significant reductions (up to 80%) in all the assessed categories particularly those strongly affected by the production of chemicals in the baseline scenario, such as AP (86-90% reduction), EP (84-87% reduction) and POFP (65-68% reduction).

The main reason for these improved profiles is the reduced energy requirements upon production of the alternative fertilizers in comparison with the extraction of natural sodium nitrate from mines (environmental profile shown in **Figure 3.4**).

ii) Use of the residual algal paste in substitution of mineral fertilizer

The remaining algae that has been depleted of its lipid fraction after solvent extraction contains a high content of nitrogen and phosphorous. The recycling of this paste for agricultural purposes as mineral fertilizers could have environmental benefits.

According to Mulbry et al. (2005), the elemental composition of dry algal biomass is 7% N and 1% P. Thus, a production of 2.2 kg N and 0.72 kg $P_2O_5$ derived from mineral fertilizers (e.g. urea and triple superphosphate respectively) could be avoided if 1 kg of EPA was produced. If the residual algal paste was used as soil conditioner, a slight environmental improvement could be achieved (less than 1%) in all the categories.

iii) Biogas production from residual algal paste

Besides being a good source of N and P useful for the agricultural sector, an important application of the residual algal paste consists in using it as a raw material for anaerobic digestion in the production process of biogas (Collet et al., 2011).

The anaerobic digestion of *Chlorella vulgaris*, a freshwater microalgae, has been assessed in detail by Collet et al. (2011) and Ras et al. (2011). In this case, *P. tricornutum* biomass could present high doses of salt which could inhibit the anaerobic digestion process. Therefore, the dry algal residual biomass should be washed with freshwater/PBS in order to remove the salt content (water could be recycled). An alternative to this step could be the combination of the microalgae with a manure stream (co-digestion) in order to dilute the salt and reduce the water requirements.

Due to the lack of inventory data, the conditions described by Collet et al. (2011) for *C. vulgaris* are assumed. Therefore, the new "expanded" system under assessment should include a new step: the production of biogas (and the following conversion into electricity) from the residual algal paste (**Figure 3.7**).

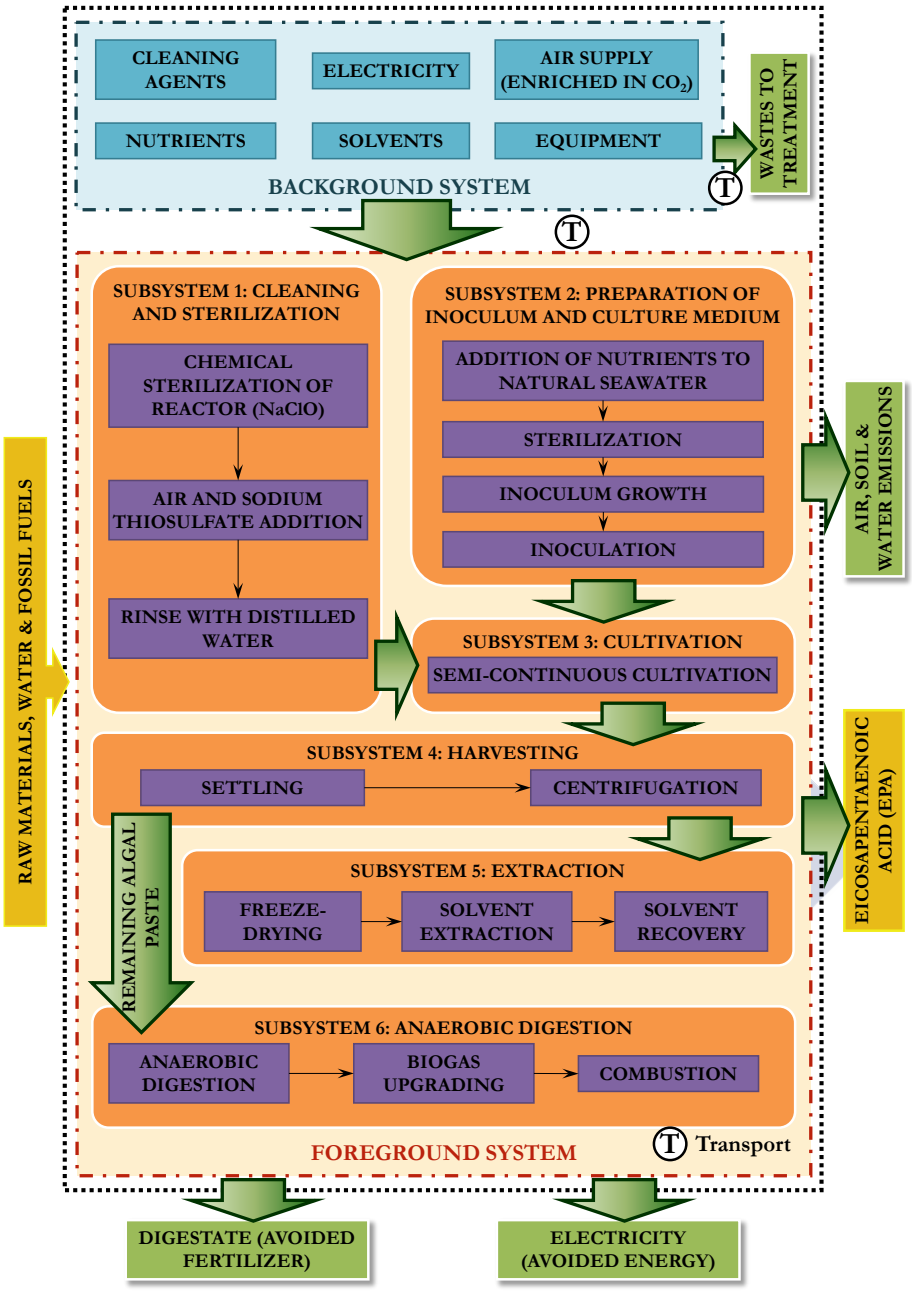



**Figure 3.7.** System boundaries of the production of EPA by *P. tricornutum* at pilot scale coupled to the production of biogas from remaining algal paste.

The algal paste with an initial concentration of 0.66 $g \cdot L^{-1}$ was left to settle, reaching a concentration of 13 $g \cdot L^{-1}$. The electrical consumption associated with this stage was 0.153 $kWh \cdot kg^{-1}$ of algae paste pumped. After the settling, around 80% of biomass is sent to centrifugation (the overflow could be recycled as nutrients source). The paste was then centrifuged in order to obtain a concentration of 50 $g \cdot L^{-1}$, corresponding to an electricity consumption of 0.042 $kWh \cdot kg^{-1}$ of algae centrifuged after which, the algae paste was added to the anaerobic digester. Finally, biogas with a composition of 70% of $CH_4$ and 30% of $CO_2$ was produced in the anaerobic digester. Around 3.93 $m^3$ of biogas was produced per kg PUFAs (36% EPA). Heat required for the anaerobic digestion process came from the combustion of 0.88 $m^3$ of biogas in a boiler. Heat consumption in the anaerobic digestion plant was 6.15 kWh.

The biogas had to be upgraded in order to increase the methane content (~96%). This process could be performed by bubbling the biogas in pressurized water since $CO_2$ is highly soluble in water but not methane. According to Collet et al. (2011), the electricity and water consumption in the purification step would be 0.083 kWh and 0.067 $m^3$ per kg of algae paste digested. Finally, the biogas stream was combusted in order to produce electricity, which should avoid the consumption of the equivalent amount from the national grid.

The anaerobic digestion produces digestate as a co-product. Its use as a fertilizer on arable land has already been evaluated (González-García et al., 2013). In this simulation, two different digestate streams (liquid and solid), both rich in organic and mineralized matter, were produced together with electricity. The solid stream could be applied as soil conditioner while the liquid one could serve as fertilizer for algae cultivation. Information concerning the digestate composition in terms of N, P and K was taken from Collet et al. (2011). Ecoinvent processes for the production of ammonium sulfate, diammonium phosphate and potassium chloride, were used to model the N, P and K fertilizers, respectively (Nemecek and Kägi, 2007).

**Table 3.5** presents the most relevant energy and mass data of the biogas production step. According to the results (**Figure 3.8**), the anaerobic digestion step of the remaining algal paste would lead to a very limited environmental improvement in all the impact categories. The reduction of the impacts would

be lower than 1% in all categories due to the high electricity requirements for the cultivation and extraction with respect to the low electricity production from the algal paste, as well as the large environmental burdens derived from the production of the nitrogen source. Thus, special attention should be paid to the electricity consumption for the algae culturing (68% of total requirements) since improvements on the environmental impacts are considerably affected by this process. The same conclusion was also drawn from Collet et (2011) and Khoo et al.(2011).

**Table 3.5.** Mass and energy data related to the production of biogas from algal biomass (31.42 kg remaining algae per kg EPA)

| **Inputs** | |
|---|---|
| Settling | |
| Electricity (pumping) | 3.83 kWh |
| Centrifugation | |
| Electricity (injecting algae in digester) | 1.06 kWh |
| Anaerobic digestion | |
| Electricity (mixing in digester) | 2.71 kWh |
| Electricity (centrifugation of digestate) | 0.63 kWh |
| Heat (from produced biogas) | 17.08 kWh |
| Upgrading | |
| Electricity | 7.56 kWh |
| Water | 1.68 $m^3$ |
| **Outputs** | |
| Biogas (net production, after subtracting biogas for heat to anaerobic digestion) | 5.03 $m^3$ (50.08 kWh) |
| Solid digestate | 30.17 L |
| Liquid digestate | 472.5 L |
| Water with $CO_2$ | 1.94 $m^3$ |

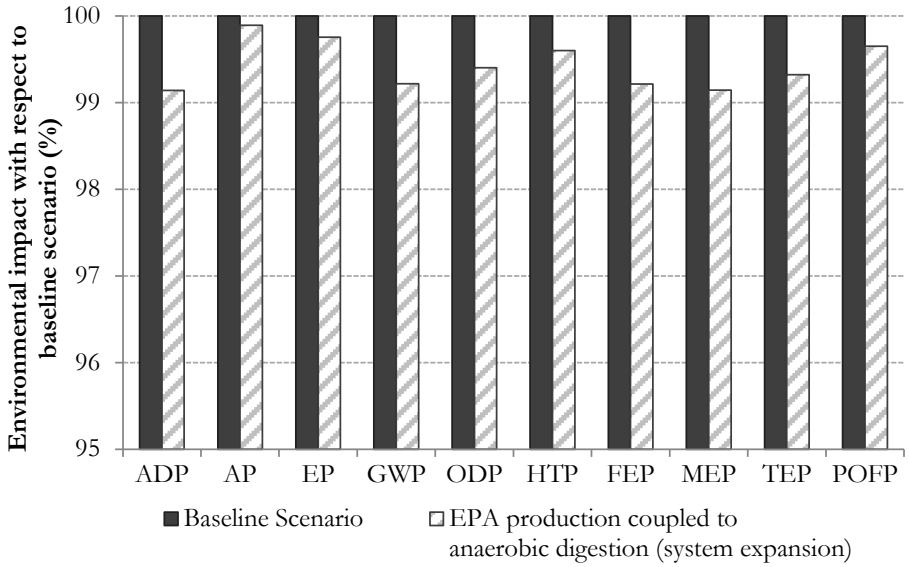



**Figure 3.8.** Comparative environmental profile considering the production of biogas by anaerobic digestion from residual algal paste.

A key aspect of this study that differs from previous literature on LCA of microalgal processes is the fact that, in this case, microalgae were cultured in a closed PBR. As reported, this type of reactor is recommended when contamination problems must be avoided, in this case for the production of high value EPA (Brennan and Owende, 2010; Greenwell et al., 2010). Due to the use of this configuration, artificial lighting was needed, which implied elevated electricity consumption.

Nowadays, research focuses on the exploration of alternative bioreactor combinations by either proposing alternative light sources (e.g. solar collection devices or energy efficient diodes) or combining a closed PBR with an open raceway (Greenwell et al., 2010; Khoo et al., 2011). Depending on the final application, *P. tricornutum* could be produced in different types of bioreactor. However, open pond systems are not recommended for its cultivation for food purposes. Hence, in this specific application, more research should be required on this topic so as to develop more efficient production systems.

## 3.2. Bioactive compounds from *Tetraselmis suecica*

*Tetraselmis suecica* is a unicellular flagellated green algae that lives in marine habitats. It is extensively used in aquaculture as feed for bivalve mollusks, shrimp larvae and rotifers (Chini Zittelli et al., 2006; Jo et al., 2012). *Tetraselmis* genus has been found to present a wide spectrum of antimicrobial activity, as well as potential as probiotic (Chini Zittelli et al., 2006). It also exhibits a high content of vitamin E, which makes it a good source for human and animal consumption, and accumulates organic pigments (such as carotenoids, chlorophyll and tocopherol) that can be extracted from it (Carballo-Cárdenas et al., 2003; Chini Zittelli et al., 2006). Moreover, it is considered to be a robust high-lipid productivity microalgae (Montero et al., 2011; Rodolfi et al., 2009). Thus, besides its potential application for biodiesel production, *T. suecica* constitutes an optimal source of long-chain PUFAs, especially EPA (Guzmán et al., 2010; Rodolfi et al., 2009).

Regarding pigments, the research on the production of compounds such as carotenoids or chlorophyll by microalgae is partially related to the increasing number of regulations on the use of synthetic dyes for culinary applications in the food sector (Del Campo et al., 2000). In the case of PUFAs, as previously mentioned, their commercial uses comprise the production of human food and nutraceuticals, including functional foodstuffs and special preparations such as maternized milk among others, as well as applications in cosmetics and pharmaceutical industries (Gebauer et al., 2006; Guzmán et al., 2010).

The productivity and composition of *T. suecica* are strongly affected by the cultivation conditions (Guzmán et al., 2010). Therefore, several studies on the conditions to maximize the production (especially in terms of PUFAs) have already been published (Chini Zittelli et al., 2006; Fábregas et al., 2001; Go et al., 2012; Guzmán et al., 2010). Although the cultivation of *T. suecica* has been performed under different indoor and outdoor reactor configurations (open ponds, cylindrical photobioreactors, annular photobioreactors…), closed photobioreactors are the recommended option for the production of high value metabolites (e.g. for food and pharmaceutical uses) from specific strains to avoid contamination problems (Brennan and Owende, 2010; Cerón García et al., 2005; Chini Zittelli et al., 2006).

In particular, this study focuses on the environmental evaluation of the cultivation of *T. suecica* as potential feedstock for the production of bioactive compounds, according to a LCA approach. The study presents a detailed life cycle inventory that includes the production and extraction of the pigments α-tocopherol, chlorophyll, β-carotene and polyphenols, together with PUFAs from *T. suecica*. The listed pigments are currently used as food and feed additives. In addition, β-carotene can act as preventive agent for a variety of human diseases (Del Campo et al., 2000). Polyphenols are used as dyes (in food and fabric sectors) and as precursors in green chemistry (Bener et al., 2010). Moreover, they show strong antioxidant activity, which make them promising compounds for the development of functional foods and the prevention of some diseases (El Gharras, 2009).

Concerning the remaining algal paste after the extraction of valuable compounds, it has a significant mineral and carbohydrate composition after lipid extraction (Sander and Murthy, 2010). Thus, the use of the algal paste obtained after extraction is considered for biogas production (baseline scenario) or as an alternative nutrient source. The analysis of the different scenarios will be finally taken as a basis to propose improvement actions in order to reduce the environmental profile of the production system.

### 3.3.1. Goal and scope definition

The function of this system is the production of five different bioactive compounds (PUFAs, α-tocopherol, chlorophyll, β-carotene and polyphenols) from *T. suecica*. Although the production of these compounds is the driven force of the cultivation of this algae, a large amount of residual algal biomass, rich in minerals and carbohydrates, is also co-produced after the extraction steps (Collet et al., 2011). The valorization of this residual stream should also be considered.

Hence, this study aims to identify the environmental profile associated with the complete production process, including all the co-products and the uses of the remaining algal biomass. Since there are no available data on the growth of this microalgae on a commercial scale for the production of these compounds, the study was based on a real pilot scale production according to a cradle-to-gate

perspective. Additional information on the cultivation and extraction stages, as well as the anaerobic treatment of the residual algal paste was also considered to model certain aspects of a scaled-up simulated system (Collet et al., 2011; Khoo et al., 2011; Pérez-López et al., 2014b).

The selected functional unit is 1 kg of *T. suecica* biomass. In this case, an intermediate product was chosen as the functional unit instead of a final product due to the multifunctionality of the process, which allows the combined production of five bioactive compounds with similar economic interest that can be extracted from the microalgae. However, the results of the environmental assessment will also be reported per kg of extracted biocompound by taking into account a mass allocation approach.

Regarding the definition of the system boundaries, the cultivation of *T. suecica* for the production of high value added biomolecules was performed in a real indoor vertical PBR (bubble column) with the same volume and dimensions as those indicated for the pilot-scale production of *P. tricornutum* (80 L, 150 cm × 60 cm × 10 cm). The production process was divided into the following five steps:

i) S1. Cleaning and sterilization: Firstly, the bioreactor was sterilized with a solution of sodium hypochlorite (5 $mL \cdot L^{-1}$) in 100 L tap water for 24 h. After that, residual chlorine was removed with air and 100 L of sodium thiosulfate solution (0.1 $g \cdot L^{-1}$). Finally, 200 L distilled water were used to wash the PBR before cultivation.

ii) S2. Preparation of inoculum and culture medium: The inoculum (8 L, corresponding to 10% of the pilot-scale reactor volume) was grown indoors in high density polyethylene (HDPE) bags with artificial light source. As described by Ulloa et al. (2012), the culture medium consisted of sterilized natural seawater (15 psi for 20 min) with 3.5% salinity containing $NaNO_3$ (4 mM), $NaH_2PO_4 \cdot 2H_2O$ (0.2 mM) as macronutrients and $ZnCl_2$ (2 μM), $MnCl_2$ (2 μM), $Na_2MoO_4$ (2 μM), cobalt (II) chloride ($CoCl_3$) (0.2 μM), $CuSO_4$ (0.2 μM), thiamine (70 $\mu g \cdot L^{-1}$), EDTA (52.8 μM), iron citrate (40 μM), biotin (20 $\mu g \cdot L^{-1}$) and vitamin B12 (6 $\mu g \cdot L^{-1}$).

iii) S3. Cultivation step: Once the culture in HDPE bags achieved the desired concentration (1.55 $g_{DW} \cdot L^{-1}$), the 80 L PBR was inoculated and 72 L filtered seawater enriched with nutrients were added to give a biomass concentration of 0.155 $g_{DW} \cdot L^{-1}$ at the start-up. A semi-continuous cultivation was performed, with enriched $CO_2$ air flow of 4.5 $L \cdot min^{-1}$.

During the semi-continuous operation, the culture was daily supplemented with 32 L of seawater, according to a renewal rate of 40%. The PBR was illuminated with a 12:12 regime (alternative 12 h periods of light, with light intensity of 596 μmol photon$\cdot m^{-2} \cdot s^{-1}$ supplied by cool white and fluorescent lights and 12 h of darkness). Temperature was maintained at 20ºC and pH was adjusted at 7-8 by $CO_2$ supply. The semi-continuous regime was maintained for 60 days, to obtain a production of 1.55 kg biomass throughout the whole period.

iv) S4. Harvesting step: After cultivation, 80% of the total biomass was recovered from the culture medium by settling at 4ºC. The harvested biomass was centrifuged, and then frozen at 20ºC before freeze-drying.

v) S5. Extraction step: In this system, the extraction of bioactive compounds from the freeze dried algal paste was carried out by subsequent conventional solvent extractions. After each extractive process, the algal paste was centrifuged for 5 min with an average efficiency of 90%.

Firstly, the lipid fraction (16% total biomass) was extracted from the freeze dried biomass using hexane as solvent (Sander and Murthy, 2010). The crude extract had a PUFAs content of 45% and was separated from hexane in a rotary evaporator. A hexane loss of 0.01 kg per kg lipid was assumed, according to average values between 0.003-0.015 kg hexane per kg oil (Lardon et al., 2009; Stephenson et al., 2010). Afterwards, the algal paste was centrifuged and washed with ethanol. Chlorophyll and β-carotene were recovered by solvent extraction with an acetone:methanol (2:1 v/v) solution, using 1.7 mL per mg freeze dried biomass. Samples were kept in darkness at 4ºC for 24 h.

For the extraction of α-tocoferol from 1 g freeze dried biomass, 20 mL of methanolic solution (0.5 M KOH) were added and kept at 80ºC for 15 min. After cooling, 5 mL distilled water and 20 mL hexane were added and the mixture was vortexed. The samples were centrifuged, the upper phase was recovered and the solvent was evaporated.

The extraction of polyphenols required the addition of 33 mL ethanol per gram of freeze dried biomass. Samples were kept in an ultrasound bath for 45 min and then centrifuged for 10 min. Finally, the supernatant was filtered with a 0.45 μm membrane and the solvent was evaporated.

Since several of the described stages were performed at lab scale, which has been found to present significantly higher environmental impacts than the corresponding large scale equivalent process, two different scenarios are analyzed in the next sections. The process chain and system boundaries are summarized in **Figure 3.9**.

Firstly, the environmental burdens and hot spots of the pilot process were determined according to the real information provided. With the outcome of these results, an optimized scenario was simulated, to obtain a more realistic evaluation that is expected to reflect the commercial implementation of the described system. Apart from changes in some specific raw materials and energy flows, an anaerobic digestion step was proposed, in order to use the remaining algal paste after the extraction stage.

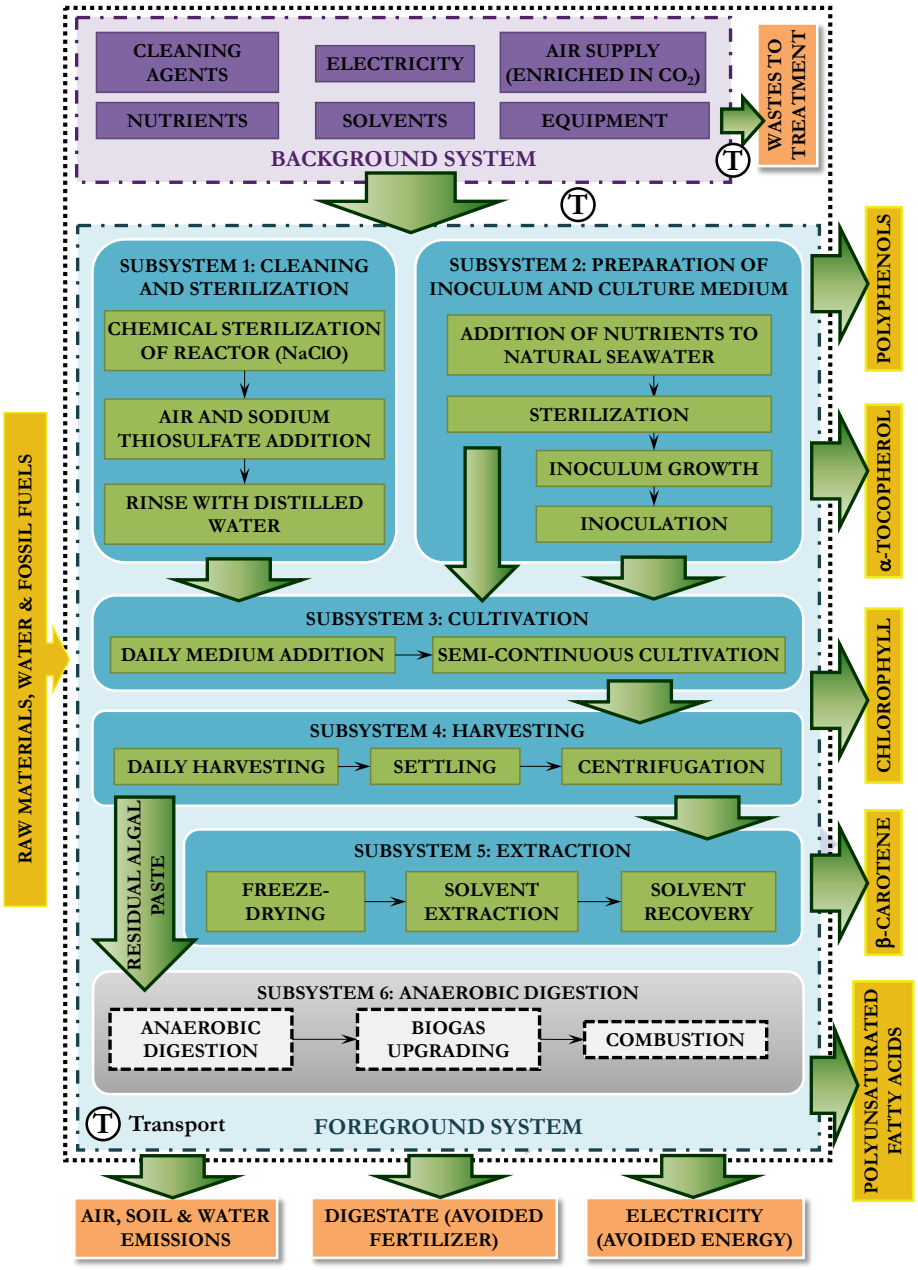



**Figure 3.9.** System boundaries of the production of high value added biocompounds by *T. suecica* in a real pilot and a simulated optimized scenario (blocks in grey with discontinuous lines refer to steps that are only performed in the simulated scenario).

The anaerobic digestion of the algal paste was simulated according to Collet et al. (2011) and Ras et al. (2011), and included in the optimized scenario. The process consisted of a settling step, in which the remaining algal paste from the extraction stage was concentrated, followed by a centrifugation step, after which the biomass was introduced in the digester. The anaerobic digestion led to the production of 0.16 $m^3$ biogas per kg digested algae, with a composition of 70% of $CH_4$ and 30% of $CO_2$. The heat required for the anaerobic digestion was provided by a fraction of the produced biogas. Finally, the biogas was upgraded to increase the methane content, and combusted to produce electricity.

In addition, the anaerobic digestion step involved the production of a solid digestate rich in organic and mineralized matter. The digestate was assumed to be a potential organic fertilizer due to its composition in N (4.4 $kg \cdot m^{-3}$), P (0.6 $kg \cdot m^{-3}$) and K (0.5 $kg \cdot m^{-3}$), avoiding the production of the equivalent amount of mineral fertilizers (Mulbry et al., 2005).

### 3.3.2. Life cycle inventory, data quality and assumptions

The quality of a LCA study considerably depends on the quality and representation of data handled. In this study, different sources and collection methods were taken into account, including field data, interviews with workers, research reports and literature, in order to ensure the reliability of the study.

For the real pilot scenario, the production of bioactive compounds from *T. suecica* was analyzed by field data (i.e. primary data). Therefore, information concerning the foreground system of the preparation of inoculum and culture medium, algal cultivation, harvesting and extraction was collected by on-site measurements for the quantification of mass and energy balances to obtain the life cycle inventory.

This information included the amount of chemicals (nutrients, solvents, washing agents), the lab materials and equipment (e.g. HDPE bags for inoculum, polymethyl metacrylate PBR, lighting system), as well as the transport distances corresponding to the distribution of inputs (nutrients, solvents, equipment, seawater and washing agents) and the energy consumption (electricity from Spanish grid). Information on the materials of the equipment from the pilot real system was taken from other LCA studies (Pérez-López et al., 2014b), whereas

information concerning transport distances and modules was provided by the workers at the real pilot plant. **Table 3.6** describes the transport activities involved in this system. The corresponding combustion emissions were calculated considering the combustion factors reported in the Ecoinvent database (Spielmann et al., 2007).

**Table 3.6.** Transport activities related to the distribution of inputs and outputs of the microalgae pilot facility

| Materials | Transport mode | Capacity (t) | Average distance (km) |
|---|---|---|---|
| Washing agents | Diesel lorry, Euro 4 | 3.5-7.5 t | 100 |
| Nutrients | Diesel lorry, Euro 4 | 3.5-7.5 t | 1140 |
| Seawater | Diesel lorry, Euro 4 | 3.5-7.5 t | 100 |
| Seawater | Transoceanic tanker | NA | 100 |
| Solvents | Diesel lorry, Euro 4 | 3.5-7.5 t | 40 |
| Equipment | Diesel lorry, Euro 4 | 3.5-7.5 t | 40 |
| Wastes to treatment | Diesel lorry, Euro 4 | 3.5-7.5 t | 50 |

Water emissions derived from the cultivation and extraction steps were calculated assuming that the remaining fraction of the nutrients from the algae growth medium was discharged into a sewage wastewater plant. An identical assumption was taken for air emissions. As previously mentioned, microalgae are one of the most efficient converters of $CO_2$ into biomass (Khoo et al., 2011). According to available literature, algal biomass can sequester between 1.50 to 1.83 kg $CO_2$ per kg of dry algal biomass, since half of the dry weight of microalgal biomass is carbon (Chisti, 2007). The species under assessment presents a remarkable C content of 81%, and thus requires about 3 kg $CO_2$ per kg of dry biomass (Ho et al., 2003). **Table 3.7** summarizes the most relevant inputs and outputs corresponding to the real pilot process.

**Table 3.7.** Inventory data for the pilot-scale production of bioactive compounds by *T. suecica* (FU=1 kg harvested algal biomass)[1]

| INPUTS from TECHNOSPHERE | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Cleaning and sterilization* | | | |
| Distilled water | 129 L | NaClO | 0.36 kg |
| Tap water | 129 L | $Na_2S_2O_3$ | 6.45 g |
| Polyvinyl chloride | 1.00 g | Polyamide | 5.52 g |
| *S2. Preparation of inoculum and culture medium* | | | |
| Filtered seawater | 1269 L | $Na_2MoO_4$ | 0.52 g |
| $NaNO_3$ | 0.43 kg | $CoCl_3$ | 0.04 g |
| $NaH_2PO_4 \cdot 2H_2O$ | 39.6 g | $CuSO_4$ | 0.04 g |
| EDTA | 24.9 g | Thiamine | 0.09 g |
| $C_6H_5FeO_7$ | 12.4 g | Biotin | 0.03 g |
| $ZnCl_2$ | 0.35 g | Vitamin B12 | 0.01 g |
| $MnCl_2$ | 0.32 g | HDPE | 3.44 g |
| Inoculum | 0.8 g | Lamps | 0.83 g |
| *S3. Cultivation* | | | |
| Polymethyl methacrylate | 0.19 kg | Lamps | 0.27 kg |
| $CO_2$ (aeration) | 4.48 kg | | |
| *S4. Harvesting* | | | |
| Stainless steel | 0.18 kg | | |
| *S5. Extraction* | | | |
| Hexane | 1.64 g | Ethanol | 156.71 g |
| Methanol | 2.72 kg | KOH | 3.34 g |
| Stainless steel | 25.97 g | Acetone | 5.24 kg |
| **Energy** | | | |
| Electricity from Spanish grid | | | |
| *S1. Cleaning and sterilization* | | | |
| Electricity, autoclaving (water)[1] | 1.56 kWh | | |
| *S3. Cultivation* | | | |
| Electricity, lighting[1] | 426 kWh | Air blowing[1] | 464 kWh |
| Electricity, medium pumping[1] | 28 kWh | | |

[1]Inputs for which the value changes in the simulated optimized scenario

**Table 3.7.** Inventory data for the pilot-scale production of bioactive compounds by *T. suecica* (FU=1 kg harvested algal biomass) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Energy** | | | |
| *S4. Harvesting* | | | |
| Settling at 4ºC[1] | 6.85 kWh | Freezer[1] | 30.95 kWh |
| Centrifugation[1] | 38.39 kWh | | |
| *S5. Extraction* | | | |
| Freeze drying[1] | 17.48 kWh | Extraction[1] | 253 kWh |
| **Transport** | | | |
| *S1. Cleaning and sterilization* | | | |
| Truck 3.5-7.5 t (wash. agents) | 40 kg·km | Truck 3.5-7.5 t (wastes) | 0.16 kg·km |
| Truck 3.5-7.5 t (equipment) | 0.65 kg·km | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Truck 3.5-7.5 t (seawater)[1] | 50.8 tkm | Truck 3.5-7.5 t (nutrients) | 50.5 kg·km |
| Oceanic tanker (seawater) | 50.8 tkm | Truck 3.5-7.5 t (wastes) | 0.11 kg·km |
| Truck 3.5-7.5 t (equipment) | 0.43 kg·km | | |
| *S3. Cultivation* | | | |
| Truck 3.5-7.5 t (equipment)[1] | 46.0 kg·km | Truck 3.5-7.5 t (wastes)[1] | 11.5 kg·km |
| *S4. Harvesting* | | | |
| Truck 3.5-7.5 t (equipment) | 18.1 kg·km | Truck 3.5-7.5 t (wastes) | 4.53 kg·km |
| *S5. Extraction* | | | |
| Truck 3.5-7.5 t (equipment) | 2.60 kg·km | Truck 3.5-7.5 t (wastes) | 0.65 kg·km |
| Truck 3.5-7.5 t (solvents) | 9.25 tkm | | |

[1]Inputs for which the value changes in the simulated optimized scenario

**Table 3.7.** Inventory data for the pilot-scale production of bioactive compounds by *T. suecica* (FU=1 kg harvested algal biomass) (*Cont.*)

| **OUTPUTS to TECHNOSPHERE** | | | |
|---|---|---|---|
| **Products** | | **Wastes to landfill** | |
| α-tocopherol | 0.07 g | Polymethyl methacrylate | 0.19 kg |
| Polyphenols | 3.76 g | Polyvinyl chloride (PVC) | 1.00 g |
| β-carotene | 3.22 g | Polyamide | 5.52 g |
| Chlorophyll | 8.76 g | Stainless steel | 0.21 kg |
| Lipids (45% PUFAs) | 164 g (73.8 g) | **Wastes to municipal incineration** | |
| | | HDPE | 3.44 g |
| **By-product** | | **Wastes to specific treatment** | |
| Remaining algal biomass | 820 g | Lamps | 0.27 kg |
| **OUTPUTS TO ENVIRONMENT** | | | |
| **Water emissions** | | | |
| *S1. Cleaning and sterilization* | | | |
| Wastewater | 258 L | NaClO | 11.03 g |
| $Na_2S_2O_3$ | 242.47 g | | |
| *S4. Harvesting* | | | |
| Wastewater | 1268 L | $Na_2MoO_4$ | 0.07 g |
| $NaNO_3$ | 58.2 g | $CoCl_3$ | 5.66 mg |
| $NaH_2PO_4 \cdot 2H_2O$ | 5.34 g | $CuSO_4$ | 0.07 g |
| EDTA | 45.57 g | Thiamine | 0.01 g |
| $C_6H_5FeO_7$ | 2.7 g | Biotin | 3.43 mg |
| $ZnCl_2$ | 0.05 g | Vitamin B12 | 1.03 mg |
| $MnCl_2$ | 0.04 g | | |
| *S5. Extraction* | | | |
| Wastewater | 0.71 L | Ethanol | 156.71 g |
| Hexane | 1.64 g | KOH | 3.34 g |
| Methanol | 2.72 kg | Acetone | 5.24 kg |

[1]Inputs for which the value changes in the simulated optimized scenario

Regarding the simulated optimized scenario, the electricity consumption in the different stages (reactor sterilization, lighting, air blowing, medium pumping, etc) was estimated from the literature (Brentner et al., 2011; Khoo et al., 2011; Sander and Murthy, 2010). The hypothetical facility was assumed to be placed in a location close to the sea, in order to reduce the transport of seawater by truck (from 40 to 5 km). The remaining algal paste after extraction was assumed to be converted into biogas by anaerobic digestion, which was then upgraded and combusted in an internal combustion engine for electricity production. The conversion process was simulated following the descriptions reported by Collet et al.(2011). A short description of the inventory data concerning the anaerobic digestion step is displayed in **Table 3.8**.

**Table 3.8.** Energy and mass flows associated with the production of biogas from algal paste (0.91 kg remaining algae)

| **Inputs** | |
|---|---|
| Settling | |
| Electricity (pumping) | 0.10 kWh |
| Centrifugation | |
| Electricity (injecting algae in digester) | 0.03 kWh |
| Anaerobic digestion | |
| Electricity (mixing in digester) | 0.07 kWh |
| Heat (from produced biogas) | 0.45 kWh |
| Upgrading | |
| Electricity | 0.21 kWh |
| Water | 1.68 $m^3$ |
| **Outputs** | |
| Biogas (net production, after subtracting biogas for heat to anaerobic digestion) | 0.131 $m^3$ (1.23 kWh) |
| Water with $CO_2$ | 44 L |
| *Avoided products* | |
| Ammonium sulfate | 57.8 g N |
| Diammonium phosphate | 17.9 g $P_2O_5$ |
| Potassium chloride | 7.7 g $K_2O$ |

Concerning air emissions derived from the processes in the biogas generation step, it was assumed that approximately 1% of methane was discharged as diffuse emissions from the anaerobic reactor (Dressler et al., 2012). Inventory data related to emissions derived from the biogas combustion were taken from comparable processes (Hartmann, 2006; Poeschl et al., 2012).

Information concerning the infrastructure requirements at the anaerobic digestion step was taken from the literature (Jungbluth et al., 2007). All the changes in the input and output flows throughout the different stages of the simulated scenario with respect to the original pilot scale scenario are indicated in **Table 3.9**.

For both scenarios, the background system (represented in **Figure 3.9**) includes the processes related to the production of chemicals, equipment, materials and electricity (Spanish profile), as well as the solid waste treatment and the production of transport modules. Inventory data were taken from the Ecoinvent database (Althaus et al., 2007; Doka, 2007; Hischier et al., 2007; Jungbluth, 2007; Spielmann et al., 2007).

Information concerning the production of the nitrogen source ($NaNO_3$) was taken from Pokorny et al. (2000), corresponding to the production of natural sodium nitrate by the Chilean industry and completed with reports from the Ecoinvent database (Althaus et al., 2007).

**Table 3.9.** Changes in simulated optimized scenario with respect to the real pilot-scale system (1 kg harvested algae)

| | **Real pilot system** | **Optimized system** |
|---|---|---|
| **Inputs** | | |
| Materials | | |
| Lamps (S3) | 0.27 kg | 6.08 g |
| Energy | | |
| Electricity from Spanish grid | | |
| S1. Cleaning and sterilization | 1.56 kWh | 0 kWh |
| S3. Cultivation | 919 kWh | 40.05 kWh |
| S4. Harvesting | 76 kWh | 0 kWh |
| S5. Extraction | 271 kWh | 28.65 kWh |
| S6. Anaerobic digestion | 0 kWh | 0.41 kWh |
| Heat supplied by biogas from anaerobic digestion: | | |
| S6. Anaerobic digestion | 0 kWh | 0.45 kWh |
| Transport | | |
| S2. Preparation of culture medium, seawater transport, truck | 50.7 kg·km | 6.4 kg·km |
| S3. Cultivation, equipment | 46.0 kg·km | 19.6 kg·km |
| S3. Cultivation, wastes | 11.5 kg·km | 4.9 kg·km |
| **Outputs** | | |
| Avoided products | | |
| Electricity | 0 kWh | 1.23 kWh |
| Ammonium sulfate | 0 kWh | 57.8 g N |
| Diammonium phosphate | 0 kWh | 17.9 g $P_2O_5$ |
| Potassium chloride | 0 kWh | 7.7 g $K_2O$ |
| Wastes to treatment | | |
| Lamps | 0.27 kg | 0.01 kg |

*Allocation procedures*

In this study, an allocation procedure was required since *T. suecica* cultivation was focused on the production of several bioactive compounds. Thus, all the environmental burdens were allocated among the high value-added co-products since their integrated production was the driven force of the process. In this case, a combined allocation procedure was proposed. Firstly, a mass based allocation was considered to allocate mass and energy flows common to different co-products for all the steps except for the extraction. Mass allocation was proposed due to the lack of information concerning the market prices of all these compounds since their production from microalgae at commercial scale is not available yet. As mentioned, the PUFAs were extracted together with the lipid fraction, which was considered as a waste. In the case of extraction, each compound requires a specific procedure, with different solvents and energy flows. Therefore, mass and energy flows from the extraction step were allocated to each specific product depending on the required technique and solvent.

However, not only high added value compounds but also electricity from the biogas and digestate are produced in the simulated scenario. Accordingly, a system expansion approach was considered, identifying credits for substitute processes. The system expansion was used to describe the environmental benefits of using the digestate as a byproduct and the electricity from the combustion engine. The digestate contains active fertilizer ingredients of diammonium phosphate (expressed as $P_2O_5$), potassium chloride (in terms of $K_2O$) and ammonium nitrate (expressed as N-$NH_4$). Thus, the environmental burdens associated with the production of an identical quantity of mineral fertilizers, which could be substituted with the digestate, were subtracted from the total burdens of the whole production system. The amount of mineral fertilizers substituted by the digestate was defined considering the amount of digestate and the corresponding nutrient content. Concerning the electricity from the biogas combustion in an internal combustion engine, a similar approach was considered. The environmental impacts of generating electricity from natural gas were credited to the system.

### 3.3.3. Environmental impact assessment

As in the previous case study, the environmental results associated with the production of bioactive compounds from *T. suecica* were quantified using the CML 2 baseline 2001 V2.05 method (Guinée et al., 2002). The environmental profile is here presented in terms of ten impact categories that have already been considered in other LCA studies on microalgae (Clarens et al., 2010; Collet et al., 2011; Lardon et al., 2009): ADP, AP, EP, GWP, ODP, POFP and toxicity related impact categories: HTP, FEP, MEP and TEP. The software SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008). The characterization results for the real pilot and optimized scenario are presented in **Table 3.10**.

**Table 3.10.** Environmental impact assessment results (characterization step) associated with the production of 1 kg *T. suecica* for the real pilot and simulated optimized scenario

| Impact category | Unit | Real pilot scenario | Simulated optimized scenario |
|---|---|---|---|
| ADP | kg Sb eq | 4.65 | 0.57 |
| AP | kg $SO_2$ eq | 6.84 | 1.90 |
| EP | kg $PO_4^{-3}$ eq | 1.50 | 0.45 |
| GWP | kg $CO_2$ eq | 625 | 68.36 |
| ODP | kg CFC-11 eq | $3.69 \cdot 10^{-5}$ | $4.17 \cdot 10^{-6}$ |
| HTP | kg 1,4-DB eq | 142.9 | 45.3 |
| FEP | kg 1,4-DB eq | 174.5 | 17.5 |
| MEP | kg 1,4-DB eq | 110.7 | 11.5 |
| TEP | kg 1,4-DB eq | 0.03 | 0.01 |
| POFP | kg $C_2H_4$ eq | 0.21 | 0.03 |

As expected, according to the outcome of the environmental assessment of EPA production in the previous section, the results for all impact categories show a remarkable improvement for the optimized scenario with respect to the real operation of the pilot system. However, the environmental impacts for the simulated optimized process are between 3 and 10 times lower than the

corresponding contributions of the real pilot system, showing the importance of research on the optimal conditions for an efficient development of microalgal processes.

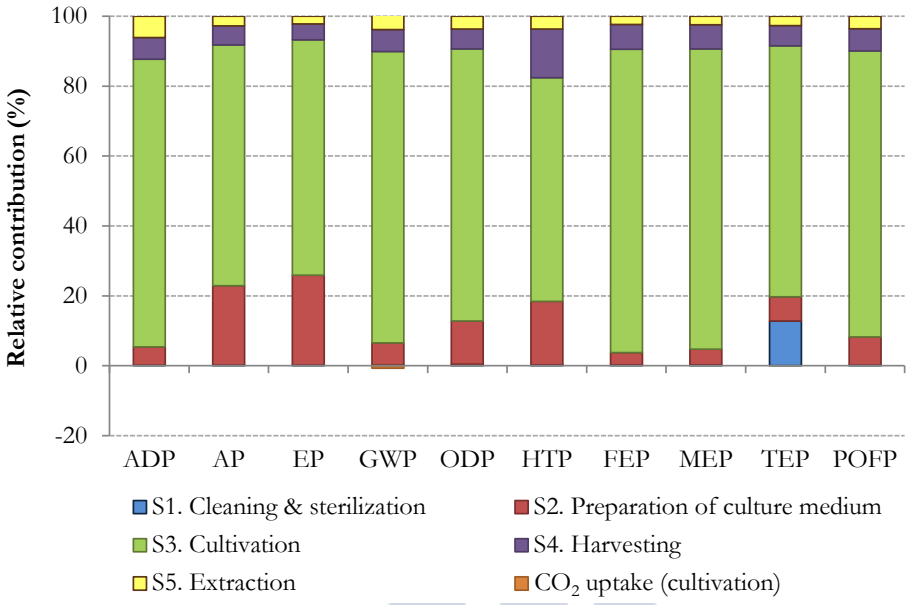
According to these findings, the improvement in the environmental profile associated with the optimized scenario is in this case more limited than that of the previous case study (in which the lab scale scenario showed impacts at least two orders of magnitude higher than those of the simulated pilot process). This is due to the change in scale of production that was considered in the study on the production of EPA by *P. tricornutum*, which compared values of lab and pilot scale, whereas the present assessment focuses on two processes conducted in the same pilot scale but under different conditions (current vs optimized). The detailed discussion on the relative contributions of the different stages and manufacturing processes for the two analyzed scenarios are discussed below.

❖ **Real pilot system**

According to the results depicted in **Table 3.10**, the cultivation stage (S3) is clearly the main hot spot of the real pilot scale process for the production of high value added biocompounds from *T. suecica*. This stage dominates the impacts in all the assessed categories, with relative contributions ranging between 64% and 87%. Among the secondary processes, only the preparation of inoculum and culture medium exceeds 15% of the relative contribution in the categories of AP (23%), EP (26%) and HTP (18%).

The main cause of the high impacts of cultivation is the production of electricity required during the growth of the algae (which represents between 80 and 93% of the total environmental impact of S3). The electricity requirements for air supply represent 51% of the total consumption of the cultivation stage, whereas the PBR lighting is responsible for 46%.

**a) Relative contributions of real pilot-scale system per stage**




**b) Relative contributions of real pilot-scale system per involved process**

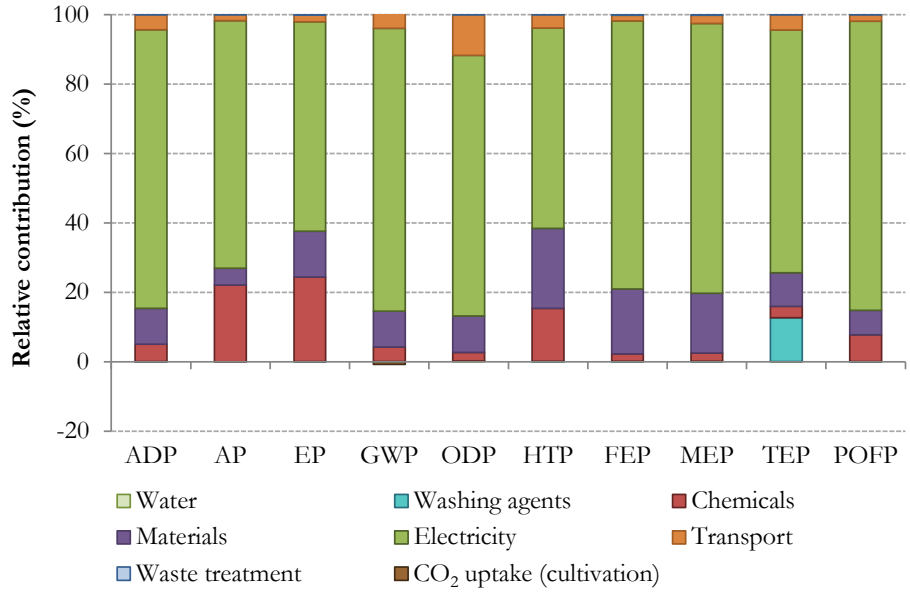



**Figure 3.10.** Relative contributions of the real pilot-scale production of bioactive compounds by *T. suecica* to each impact category per a) stage and b) involved process.

❖ **Simulated optimized scenario**

**Table 3.11** shows the contribution of each product to the environmental impact in each category according to the allocation procedure described in section 3.3.2. Since PUFAs were the compounds produced at the largest quantity, they show the highest contribution, associated with the selected allocation approach. The high contribution to some categories of β-carotene and chlorophyll is mainly linked to the use of an acetone:methanol solution for their extraction, which requires a higher volume of solvent than the extraction of other compounds.

**Table 3.11.** Relative contributions (%) to the environmental impact of each biocompound from *T. suecica* according to the proposed allocation approach

| Impact category | PUFAs | α-tocopherol | β-carotene | Chlorophyll | Polyphenols |
|---|---|---|---|---|---|
| ADP | 54.21 | 0.78 | 11.35 | 30.87 | 2.79 |
| AP | 79.24 | 0.13 | 4.46 | 12.13 | 4.04 |
| EP | 80.43 | 0.16 | 4.11 | 11.19 | 4.10 |
| GWP | 63.04 | 0.51 | 8.93 | 24.28 | 3.24 |
| ODP | 69.18 | 0.61 | 7.15 | 19.45 | 3.61 |
| HTP | 80.12 | 0.19 | 4.19 | 11.41 | 4.09 |
| FEP | 79.77 | 0.22 | 4.28 | 11.66 | 4.08 |
| MEP | 79.16 | 0.23 | 4.45 | 12.10 | 4.05 |
| TEP | 79.15 | 0.19 | 4.47 | 12.15 | 4.05 |
| POFP | 73.43 | 0.85 | 5.91 | 16.07 | 3.75 |

According to **Table 3.7**, around 74 g of PUFAs can be extracted per kg of microalgae harvested, which is significantly higher than the quantities of the other products, ranging from 70 mg of α-tocopherol and 9 g of chlorophyll. The low yield of α-tocopherol is the main reason of the negligible contribution to the environmental profile associated to this compound when considering a mass allocation approach.

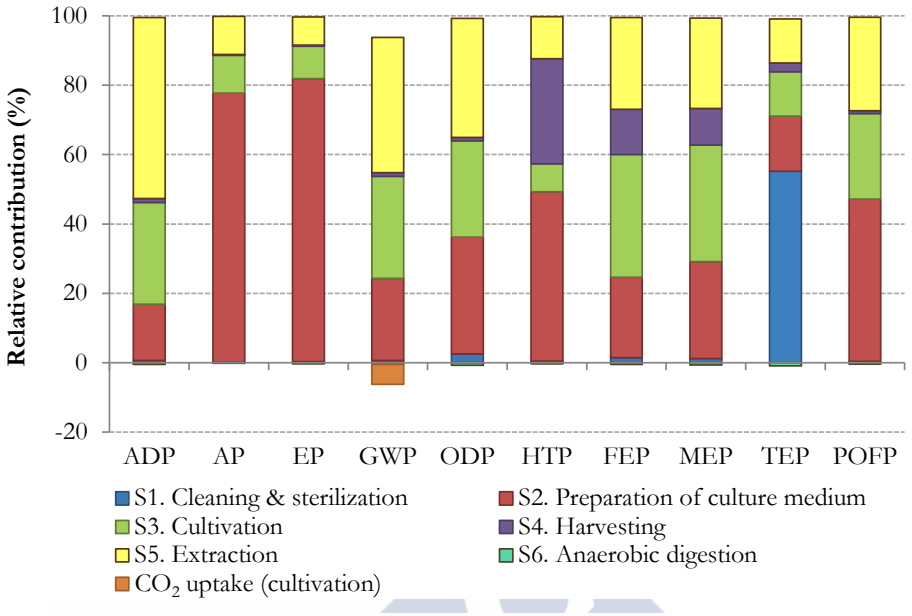
**Table 3.12** displays the environmental profile per functional unit (1 kg of microalgae) for the simulated optimized scenario, together with the individual results for 1 g of each bioactive compound in order to facilitate the environmental results per substance. With this regard, the production of PUFAs has the lowest impact per mass unit for all the assessed impact categories. This is mainly due to the use of hexane, an efficient solvent with a high extraction capacity that is in this case recovered and reused (Demirbas, 2009). Chlorophyll and β-carotene have virtually the same environmental profile, since both are extracted with the same extraction solution (including acetone and methanol). The environmental impacts of α-tocopherol per mass unit are the highest of the analyzed products, associated with the requirement of different solvents for the extraction of a very limited quantity of final bioactive compound.

Regarding the distribution of impacts among the different stages of the process, the contributions of the steps involved over the life cycle of the system are depicted in **Figure 3.11**. In this case, the preparation of the inoculum and culture medium (S2) and the cultivation (S3) are the main contributors to most categories. Thus, the sum of both stages exceeds 50% of the total contribution in eight impact categories. Indeed, S2 dominates the impacts to AP (78%), EP (82%), HTP (49%) and POFP (47%), mainly due to the production of chemicals (especially sodium nitrate). However, there is a significant reduction in the relative contributions of cultivation, which range from 8% to 35%, associated with the remarkable decrease in electricity requirements of the optimized scenario in comparison with the real pilot system. For this reason, an increase in the relative contribution of the extraction step (S5) in categories such as ADP (52%), GWP (39%) and ODP (34%) is here observed, although the absolute impact is nearly the same.

**Table 3.12.** Allocated environmental impacts (characterization step) associated with the production of each bioactive compound by *T. suecica* for the simulated optimized scenario

| Impact category | Unit | 1 kg *T. suecica* biomass | 1 g PUFAs | 1 g α-tocopherol | 1 g β-carotene | 1 g chlorophyll | 1 g polyphenol |
|---|---|---|---|---|---|---|---|
| ADP | kg Sb eq | 0.57 | $4.18 \cdot 10^{-3}$ | $6.35 \cdot 10^{-2}$ | $2.01 \cdot 10^{-2}$ | $2.01 \cdot 10^{-2}$ | $4.23 \cdot 10^{-3}$ |
| AP | kg $SO_2$ eq | 1.90 | $2.04 \cdot 10^{-2}$ | $3.44 \cdot 10^{-2}$ | $2.63 \cdot 10^{-2}$ | $2.63 \cdot 10^{-2}$ | $2.04 \cdot 10^{-2}$ |
| EP | kg $PO_4^{-3}$ eq | 0.45 | $4.85 \cdot 10^{-3}$ | $1.03 \cdot 10^{-2}$ | $5.69 \cdot 10^{-3}$ | $5.69 \cdot 10^{-3}$ | $4.86 \cdot 10^{-3}$ |
| GWP | kg $CO_2$ eq | 68.36 | 0.58 | 4.97 | 1.90 | 1.90 | 0.59 |
| ODP | kg CFC-11 eq | $4.17 \cdot 10^{-6}$ | $3.91 \cdot 10^{-8}$ | $3.65 \cdot 10^{-7}$ | $9.25 \cdot 10^{-8}$ | $9.25 \cdot 10^{-8}$ | $4.00 \cdot 10^{-8}$ |
| HTP | kg 1,4-DB eq | 45.3 | 0.49 | 1.20 | 0.59 | 0.59 | 0.49 |
| FEP | kg 1,4-DB eq | 17.5 | 0.19 | 0.54 | 0.23 | 0.23 | 0.19 |
| MEP | kg 1,4-DB eq | 11.5 | 0.12 | 0.38 | 0.16 | 0.16 | 0.12 |
| TEP | kg 1,4-DB eq | 0.01 | $7.72 \cdot 10^{-5}$ | $1.96 \cdot 10^{-4}$ | $9.99 \cdot 10^{-5}$ | $9.99 \cdot 10^{-5}$ | $7.75 \cdot 10^{-5}$ |
| POFP | kg $C_2H_4$ eq | 0.03 | $3.07 \cdot 10^{-4}$ | $3.75 \cdot 10^{-3}$ | $5.66 \cdot 10^{-4}$ | $5.66 \cdot 10^{-4}$ | $3.08 \cdot 10^{-4}$ |

**a) Relative contributions of simulated system per stage**




**b) Relative contributions of simulated system per involved process**

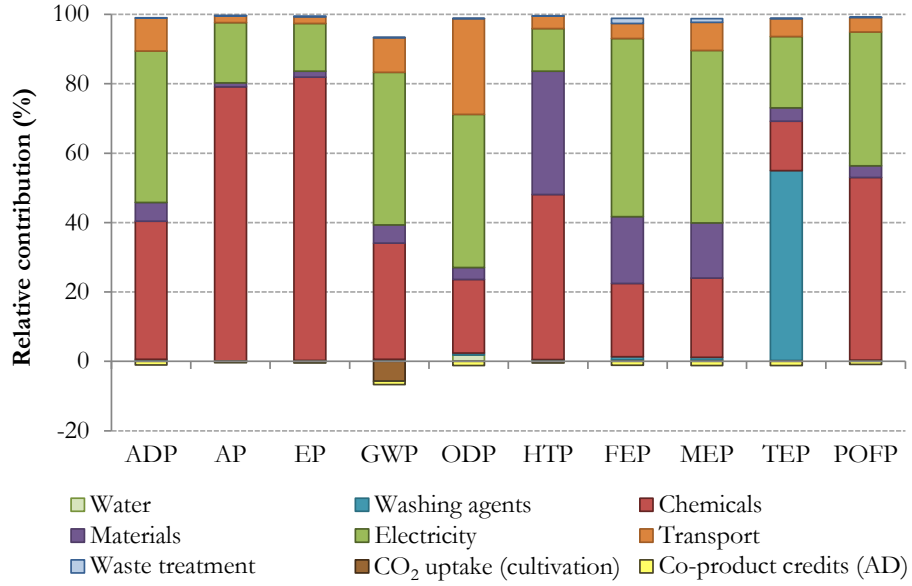



**Figure 3.11.** Relative contributions of the simulated optimized production of bioactive compounds by *T. suecica* to each impact category per a) stage and b) involved process.

The extraction step involves the production and distribution of the solvents required in each extraction process (hexane, methanol, ethanol, potassium hydroxide and acetone) and the production of the corresponding electricity required for the extraction. Among these processes, the main contributors to the environmental impacts of S5 are the production of acetone, used for the extraction of chlorophyll and β-carotene, and the production of electricity, with corresponds to 42% of the total electricity consumed throughout the whole cultivation and extraction process. Thus, the production of acetone is responsible for 54% and 38% of the impact of S5 to ADP and GWP, respectively. The contributions of the production of electricity range between 35% (for ADP) and 80% (for FEP and MEP). Most of this electricity consumption (99%) is linked to the recovery of solvents by evaporation.

Among secondary processes, the production of materials (including lamps, stainless steel, polymethyl metacrylate for the PBR and other materials of the equipment) has a relevant contribution to HTP (36%), FEP (19%) and MEP (16%), whereas the transport of inputs to the facility has contributions of 10% to GWP and 28% to ODP. It should be remarked that the relatively low effect of transport corresponds to an optimized scenario with a significant reduction of impact related to the selection of an appropriate location to minimize the transport of seawater for the culture medium. In case of maintaining the transport distances of the real scenario (40 km by oceanic tanker and 40 km by truck), transport would have contributions of 28% for ADP, 29% for GWP and 58% for ODP, with values ranging between 10 and 20% for five of the remaining categories. Due to the lower absolute values for the environmental impacts of this optimized scenario, $CO_2$ sequestration potential during the cultivation step is here higher than for the original pilot system and exceeds 5% of the total contributions to GWP.

Regarding the main substances related to the environmental burdens, the production of sodium nitrate (identified as one of the hot spots in terms of AP, EP and GWP) is the main cause of $NO_x$ and $NH_3$ emissions. In addition, it is, together with electricity, one of the main sources of $SO_2$ emissions. $NO_x$, $NH_3$ and $SO_2$ are related to the impacts in AP. Both sodium nitrate production and electricity are also responsible of eutrophying substances emissions together

with transport related activities. In this case study, EP is associated with $NO_x$, $NH_3$ and $PO_4^{-3}$ emissions derived from these processes. The 90% of GHG emissions is $CO_2$, which is related to transport activities and the production of chemicals. Halon 1301 and Halon 1211 emissions, which contribute to ODP, mainly derived from the transport activities, sodium nitrate and electricity production. Concerning ecotoxicity categories, air emissions of polycyclic aromatic hydrocarbons are the main cause of HTP, whereas water emission of nickel ion had the highest contribution to MEP and FEP, and soil emission of cypermethrin was the main contributor for TEP. Finally, POFP is affected by the emission of CO and $SO_2$, mainly derived from the production of sodium nitrate and electricity requirements.

### 3.3.4. Discussion and recommendations

The production of bioactive compounds with applications in pharmaceutical, nutraceutical, cosmetic and food industries were assessed in detail in this study, considering *T. suecica* as the producer organism. Microalgae have been assessed with special focus on the production of biogas (Collet et al., 2011; Langlois et al., 2012) and algal biodiesel (Brentner et al., 2011; Khoo et al., 2011; Lardon et al., 2009; Stephenson et al., 2010).

Although there are no similar reports on the production of bioactive compounds from microalgae, common findings were obtained in this study compared to previous work, such as the remarkable contribution to the environmental impact associated to the large use of nutrients (i.e. fertilizers) and electricity (Khoo et al., 2011; Lardon et al., 2009). Due to the relevance of the contributions from sodium nitrate in the global environmental profile, alternative nitrogen sources are proposed for the simulated optimized scenario and discussed below.

i) Alternative nitrogen sources for the simulated scenario

The alternative nitrogen sources are urea (Urea Scenario), calcium ammonium nitrate (CAN scenario), and synthetic sodium nitrate, produced by the neutralization of nitric acid with sodium hydroxide or sodium carbonate (synthetic $NaNO_3$ scenario). These sources were assumed to be added in a percentage to meet the needs (71.1 g N) for the production of 1 kg *T. suecica*.

Additionally, linked to the recommendation of several authors on the use of wastewater, digestate or even the residual algal paste as nutrient source in microalgal systems, another improved scenario is proposed (Aresta et al., 2005; Collet et al., 2011; Langlois et al., 2012; Mulbry et al., 2005). The "Recycled digestate scenario" considers the use of 57.8 g N recovered in the digestate (**Table 3.8**) as a nitrogen source for the algal growth in the PBR. In this scenario, 13.3 g N would be supplied as sodium nitrate (to meet the total requirement of 71.1 g N). The results of the four scenarios are presented in **Figure 3.12**.

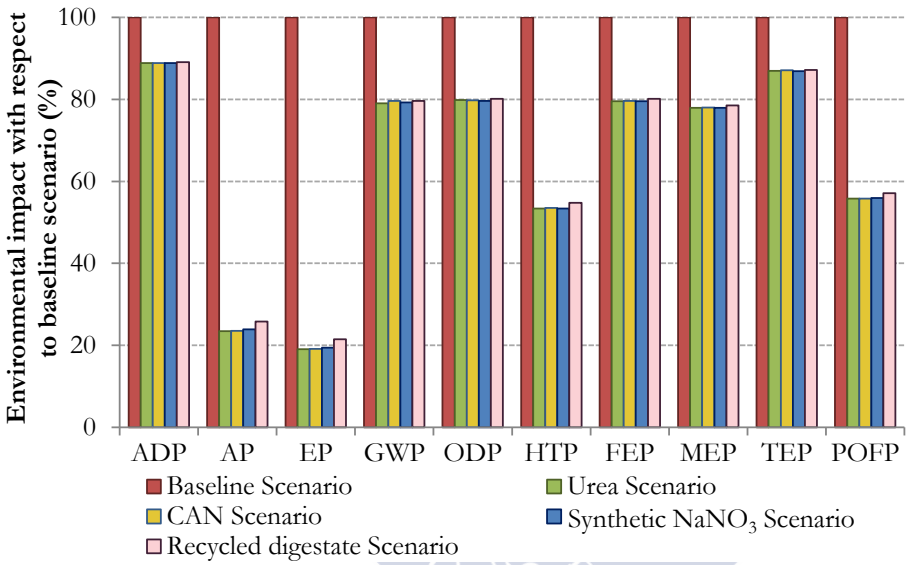



**Figure 3.12.** Comparative environmental profile (for the simulated optimized scenario) considering alternative mineral nitrogen-based fertilizers and the recycling of the digestate from the anaerobic digestion for *T. suecica* cultivation.

According to the results, the use of alternative nitrogen sources may lead to remarkable reductions of impacts in most of the assessed categories. This improvement is particularly significant for the categories of AP (with reductions of approximately 75%) and EP (with reductions around 80%). Other important changes are associated with HTP (45-47% reduction) and POFP (43-44%). The reduction of the impact is attributed to the reduction in energy consumption associated with the production of sodium nitrate.

Among all the proposed alternatives, the use of the digestate as nitrogen source is the slightly worst option from an environmental perspective although remarkable improvements are achieved in comparison with the baseline scenario. The main reason is that not all the nitrogen requirement can be satisfied by means of the digestate; as a result, around 19% has to be supplied as sodium nitrate.

ii) Alternative uses of algal paste

Sander and Murthy (2010) reported the possibility of using the residual algal paste as feedstock to produce ethanol due to its high carbohydrate content, similarly to wheat straw as both of them have similar glucan content and the algal paste does not contain lignin. Thus, the co-production of ethanol from this residual stream could be an interesting alternative to be taken into account in the future.

iii) Changes in reactor configuration

As previously mentioned, one of the environmental key factors in the large-scale production of microalgae is the large energy demand (Khoo et al., 2011; Lardon et al., 2009), specifically in the cultivation and extraction processes. Current research is focused on novel methods of PBR illumination which could maximize the production of biomass and the content in target products, in order to improve the environmental profile. These alternatives include solar collection devices such as light guides and Fresnel lenses or energy-efficient monochromatic light-emitting diodes (Greenwell et al., 2010). In addition, special attention is being paid to combined configurations where a closed PBR is operated in order to produce inoculum to be then used in open raceways (Greenwell et al., 2010; Khoo et al., 2011). Therefore, this alternative is here discussed for *T. suecica* system. For this approach (PBR + Raceway Scenario), information concerning the microalgae growth and energy requirements were taken from Khoo et al. (2011). In this case, the inoculation and cultivation stages should require a total electricity consumption of 1.42 kWh per kg of microalgae, of which around 77% should be consumed in the PBR and the remaining 23% in the raceway pond. Thus, the total electricity consumption (from sterilization to extraction steps) should be 30.45 kWh.

**Figure 3.13** shows the comparative environmental results between the baseline scenario and the PBR + Raceway pond scenario. The reduction of the electricity requirements (up to 56% of total electricity for the baseline scenario) associated with the combined used of a PBR for the preparation of the inoculum followed by the cultivation in a raceway pond is mainly reflected in categories such ADP, GWP, FEP and MEP, with reductions of impact between 25-30%.

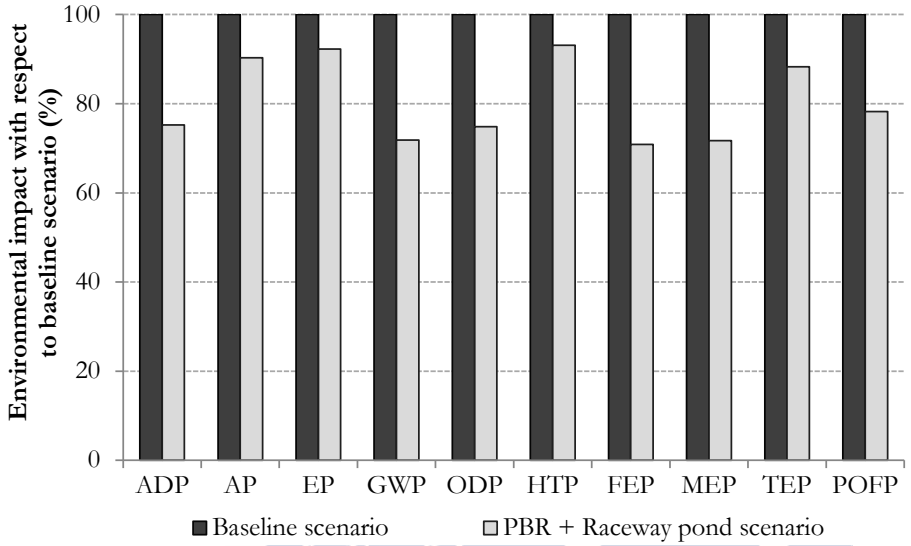
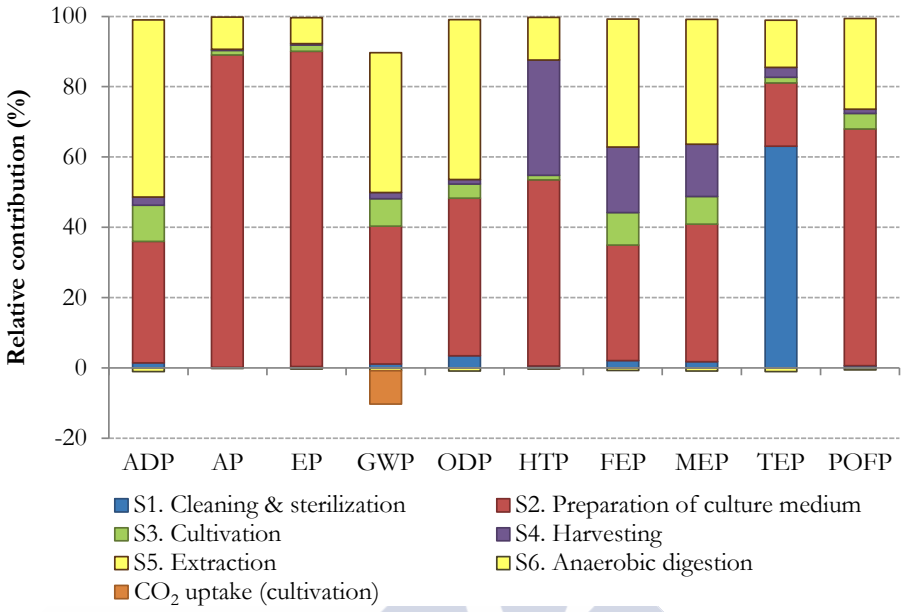



**Figure 3.13.** Comparative environmental profile considering a combination of PBR + Raceway pond instead of the optimized scenario.

The analysis of the alternative scenario (**Figure 3.14**) indicates that the preparation of the inoculum would continue being the main stage responsible for the environmental burdens, despite the significant reduction in electricity consumption. The remaining impacts are mainly due to the contribution from the sodium nitrate production. However, a considerable increase in the relative contribution of other stages (such as extraction) and processes (such as transport related activities of the production of materials for the equipment), can be observed. Thus, alternative nitrogen sources to the sodium nitrate should reduce the contribution from this product as previously discussed. In fact, if this alternative scenario was combined with one of the alternative nitrogen sources, reductions of impact would range between 25% up to 85%.

**a) Relative contributions of simulated PBR+Raceway pond system per stage**




**b) Relative contributions of simulated PBR+Raceway pond system per involved process**

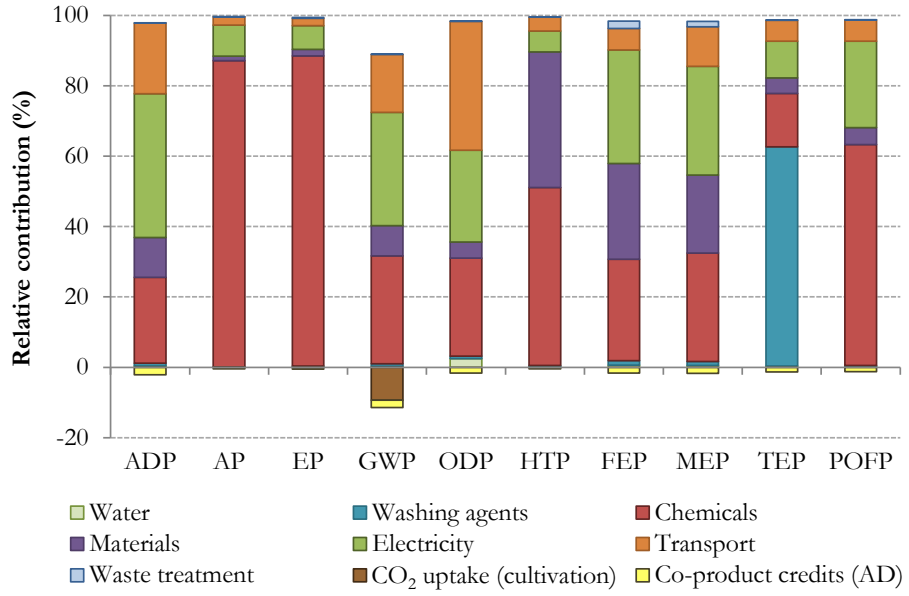



**Figure 3.14.** Relative contributions of the PBR + Raceway pond scenario to each impact category per a) stage and b) involved process.

## 3.4. Conclusions

The aim of these studies was to perform a complete life cycle assessment on the production of different high value added molecules and bioactive compounds from selected promising microalgae. The environmental burdens of these novel processes were assessed and detailed life cycle inventories were obtained. Moreover, the work allowed identifying the main factors (or hot spots) responsible of the production of valuable compounds with pharmaceutical, cosmetic and food applications at different levels of production, including lab scale production and pilot scale production based on real on-field data, as well as estimations on future optimized systems.

Regardless of the process and scale of production, the preparation of inoculum and culture medium and the cultivation in photobioreactors with artificial illumination were identified as the major causes of environmental impacts. Moreover, the production of nutrient sources and electricity were found to be the activities with the highest contributions to the environmental profile.

The outcome of the assessment served as a basis to propose alternative scenarios with significant potential for the improvement of the production systems. Among them, the use of alternative nitrogen sources with lower impacts and the optimization of the cultivation systems to minimize the electricity consumption constituted the most promising examples for the achievement of sustainable production systems in the future.

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# Chapter 4

# Sustainability LCA integrating environment, society and economy: From lab to pilot scale[1]


***Summary***

The freshwater green microalga *Haematococcus pluvialis* is the richest source of natural astaxanthin, a high-value red carotenoid pigment commonly used in the food, feed and cosmetics industries due to its antioxidant and anti-inflammatory properties. This study assesses the sustainability of the production of natural astaxanthin from *H. pluvialis*, including an environmental LCA of real processes (for which the algal cultivation was performed in closed airlift photobioreactors at lab, semi-pilot and pilot scale), as well as a socio-economic assessment according to the Social LCA and cost-benefit approaches. The study allowed the identification of the production of electricity, mainly required in the cultivation step, as the major contributor to the environmental impacts. In addition, a remarkable improvement associated with the scale-up of the process was observed. The conducted socio-economic evaluation completed the sustainability assessment, identifying the main strengths of the process from a holistic perspective.


[1] **Pérez-López P**, González-García S, Jeffryes C, Agathos SN, McHugh E, Walsh D, Murray P, Moane S, Feijoo G, Moreira MT. Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. Journal of Cleaner Production 2014, 64:332-344.

# Index

## 4.1. Production of the red carotenoid astaxanthin by the freshwater microalga *Haematococcus pluvialis*

As previously mentioned in Chapter 3, microalgae are considered as a potential feedstock for the production of a wide diversity of compounds, ranging from high value added products such as food and cosmetics active ingredients or pharmaceuticals to next-generation biodiesel (Cerón García et al., 2006; Olaizola, 2003; Wijffels et al., 2010; Woertz et al., 2014).

The freshwater green microalga *Haematococcus pluvialis* is the richest source of natural astaxanthin, a carotenoid (or pigment) commonly found in marine animals and traditionally used as a pigmentation source for fish aquaculture and poultry (García-Malea et al., 2009; Solovchenko et al., 2013; Yuan et al., 2011). Astaxanthin (3,3'-dihydroxy-β,β-carotene-4,4'-dione; $C_{40}H_{52}O_4$) is a high-value red keto-carotenoid pigment, which starts accumulating in the lipid vesicles of *H. pluvialis* during the transition between green vegetative cells and red aplanospores after exposure to stress conditions (Aflalo et al., 2007; Fábregas et al., 2001; Hong et al., 2015).

Due to its excellent antioxidant properties, astaxanthin has numerous applications; from its use as an additive in food and feed industries, nutraceuticals to cosmetics market (Koller et al., 2012). Recently, its anti-inflammatory and anti-cancer activities confirmed its importance in the medical sector (Aflalo et al., 2007; Guerin et al., 2003).

However, astaxanthin production can be hampered by the low cell growth rate, the sensitivity of the cells to hydrodynamic stress and changes in cell morphology under various environmental conditions (Hata et al., 2001). In order to enhance productivity and thus reduce the production costs of microalgal astaxanthin, several options have been proposed, including *H. pluvialis* cultivation in two-stage systems (Aflalo et al., 2007) or fed-batch culture with exponential nutrient feeding combined with light stress conditions (Kang et al., 2010).

A number of large scale facilities produce natural astaxanthin from *H. pluvialis* (AlgaeHealth, 2015; Algatechnologies Ltd., 2015; Cyanotech Corp., 2015), despite the competition with the cheaper synthetic astaxanthin from petrochemical sources that dominates 95% of the astaxanthin world market, estimated at US$250 million in 2010 (Murray et al., 2013; Spolaore et al., 2006). Consumer growing demand for natural products makes synthetic routes less desirable (Herrero et al., 2006; Lorenz and Cysewski, 2000), which justifies the considerable effort that is being paid on the promotion of biotechnological alternatives with environmental friendly production systems (Olaizola, 2003; Rodríguez-Sáiz et al., 2010). The production costs of the natural process are expected to be more competitive in the short term, after the optimization of the production technology. In this sense, Li et al. (2011) have estimated a production cost of $718·kg$^{-1}$ natural astaxanthin in a conceptually designed plant of 900 kg astaxanthin per year, which is significantly lower to $1000·kg$^{-1}$ synthetic astaxanthin from companies such as DSM, BASF and NHU.

Furthermore, synthetic astaxanthin consists of a racemic mixture with a stereoisomeric ratio of 1:2:1 for the 3R,3'R/meso/3S,3'S isomers, whereas natural astaxanthin mainly corresponds to 3S,30S isomer (Wang et al., 2008). This difference influences several properties related to the biological function of astaxanthin, such as the antioxidant potential or the shelf life, which makes natural astaxanthin more valuable than the synthetic alternative in nutraceuticals and pharmaceutical markets, reaching prices up to $100,000 kg$^{-1}$ (Chen et al., 2007; Chew and Park, 2006; Olaizola, 2003; Spolaore et al., 2006).

Despite the potential, the sustainability of the production of natural astaxanthin by *H. pluvialis* needs to be measured. Although several LCA studies related to biotechnological processes as well as to the production of biologically active molecules have been already published (Jegannathan and Nielsen, 2013; Pietrzykowski et al., 2013), there are no available LCA studies focused on the production of astaxanthin by *H. pluvialis* that provide a complete life cycle inventory and assessment analyzing internationally-accepted categories. Therefore, an integrated assessment based on the Life Cycle Thinking philosophy is here proposed to evaluate the environmental, social and economic sustainability of the process.

## 4.2. Environmental assessment of astaxanthin from *H. pluvialis*

This sections includes a detailed life cycle inventory and quantification of the related environmental impacts of the production of high-value natural astaxanthin from *H. pluvialis* using a photobioreactor (PBR) with artificial illumination. Moreover, the assessment is based on information gathered by on-site measurements in existing facilities at lab, semi-pilot and pilot scale, whereas previous LCA studies (only applied to the evaluation of biofuel production) were mainly performed by extrapolation of lab conditions due to the absence of real data at industrial scale (Brentner et al., 2011; Campbell et al., 2011; Lardon et al., 2009). Therefore, this study will allow a more realistic view of the environmental issues related to microalgal processes than the available literature linked to the use of primary data from real operating systems.

### 4.2.1. Goal and scope

Similarly to previous works (Pietrzykowski et al., 2013), this study aims to perform a comparative assessment of the environmental impacts associated with the production of *H. pluvialis* astaxanthin for nutraceutical or pharmaceutical uses at lab and pilot scale in airlift PBRs with artificial illumination. This dual approach will allow evaluating the differences between both perspectives, considering the influence of scale-up as well as the effectiveness of the changes introduced in the real pilot process after the lab-scale experiments. Additionally, the different stages and processes involved throughout the process will be evaluated, and the most problematic issues or hot spots of the life cycle of the product will be identified.

In a first stage, the environmental impacts associated with the operation of a 15 L tubular airlift PBR will be evaluated. In this case, astaxanthin was obtained as a pure compound after a conventional solvent extraction. The lab experiments for the cultivation of *H. pluvialis* were carried out by the Bioengineering Group of the Earth and Life Institute at the University of Louvain (Belgium) while the extraction processes were developed by the Shannon Applied Biotechnology Centre at the Limerick Institute of Technology (Ireland).

After the lab-scale cultivation, *H. pluvialis* was produced in a semi-pilot system consisting of an annular 80 L PBR. Supercritical $CO_2$ extraction was considered for the separation of the target compound from the algal biomass. The cultivation process was performed by the company Greensea (France), whereas the extraction procedure was carried out in the Shannon Applied Biotechnology Centre at the Limerick Institute of Technology (Ireland).

Finally, a pilot-scale process was developed by the biotechnological company AlgaeHealth (Ireland). This process used the information obtained from the smaller systems as a basis for the scale-up of a pilot process operated in two stages that was performed in consecutive 1000 L airlift PBRs. The target product from the pilot process was a nutraceutical oleoresin with a content of 10% astaxanthin.

The LCA study starts with the selection of the functional unit, to which all inputs and outputs to the system are referred. In this case, the production of 1 kg astaxanthin was chosen as the functional unit for the three scenarios. Although the functional unit is not a realistic reference value for the lab and semi-pilot scale processes (which produce approximately 1 g and 22 g astaxanthin per batch respectively), the results are referred to the same unit as the pilot system to facilitate the comparison between the three scenarios, since the main objective of this LCA is analyzing the influence of scale-up from laboratory to pilot-scale process in the environmental profile. In the three systems, the study extends from the production of the different inputs to the system, the cleaning of the reactor, the preparation of the culture medium, as well as microalgal cultivation, harvesting and final extraction of the carotenoid.

❖ **Lab-scale production scenario**

Lab-scale production in one step was initially considered in order to identify the main stages of the system and the most relevant hot spots that may also affect the pilot process. The system for the production of astaxanthin from *H. pluvialis* was divided into five stages, which are described below: i) cleaning and sterilization, ii) preparation of the inoculum and culture medium, iii) cultivation of the microalgae, iv) harvesting and v) extraction of astaxanthin. **Figure 4.1** shows the different stages and processes that were included in the system boundaries.

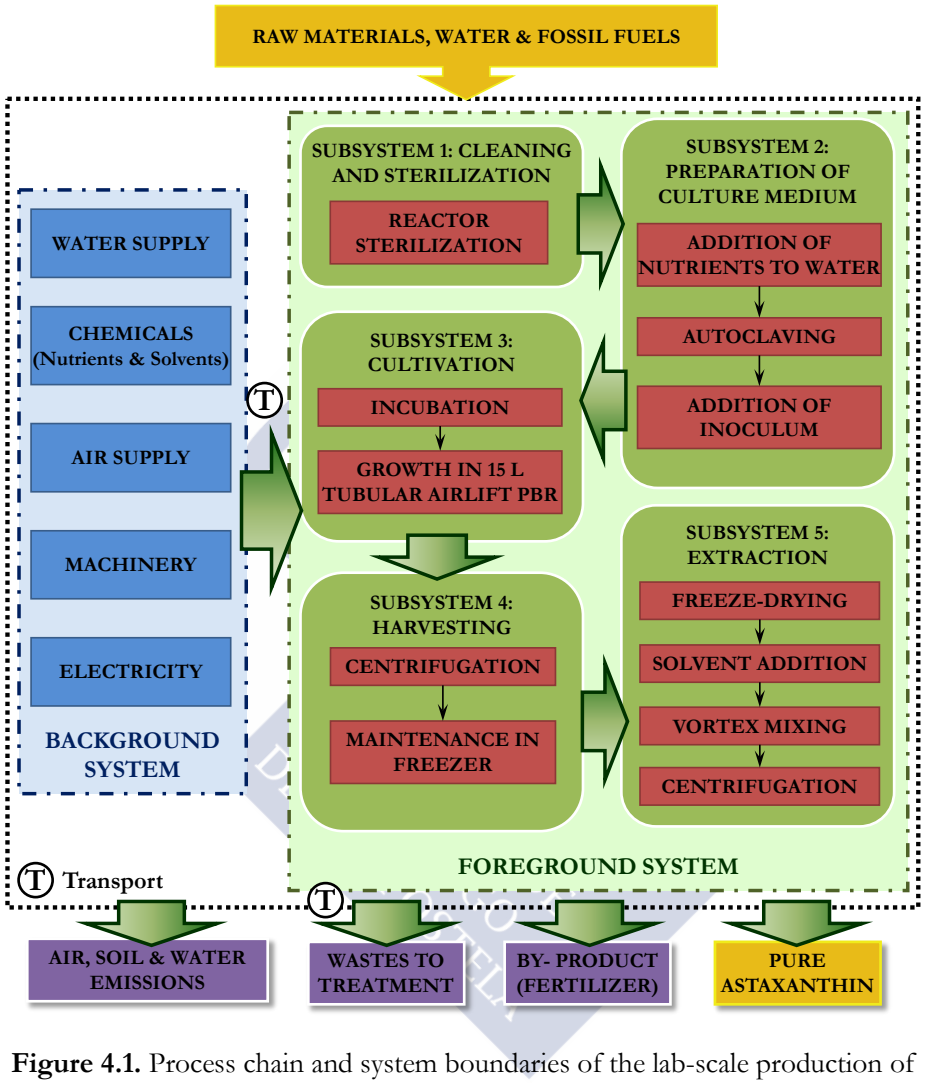



**Figure 4.1.** Process chain and system boundaries of the lab-scale production of astaxanthin by *H. pluvialis* in a 15 L tubular airlift PBR.

i) S1. Cleaning and sterilization: At lab-scale, the use of bleaching agents was considered to clean the reactor. For this purpose, 20 g of sodium hypochlorite, as well as 50 L of tap water and 30 L of sterile autoclaved water were required.

ii) S2. Preparation of culture medium: The culture medium comprised deionised water containing 0.75 $g \cdot L^{-1}$ sodium nitrate ($NaNO_3$) 0.025 $g \cdot L^{-1}$ calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 0.075 mM magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.025 $g \cdot L^{-1}$ sodium chloride (NaCl), 0.075 $g \cdot L^{-1}$ potassium phosphate dibasic trihydrate ($K_2HPO_4 \cdot 3H_2O$), 0.175 $g \cdot L^{-1}$ potassium phosphate monobasic ($KH_2PO_4$), 0.0012 $g \cdot L^{-1}$ vitamin B1, 0.00001 $g \cdot L^{-1}$ vitamin B12, 0.0045 $g \cdot L^{-1}$ ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-$Na_2 \cdot 2H_2O$) and trace metals (less than 0.005 $g \cdot L^{-1}$). This stage required the addition of nutrients in the specified amounts to deionised water, followed by the sterilization of the culture medium in an autoclave, as well as the addition of the initial inoculum in 150 mL culture flasks under a sterile flow hood. Volumes of 1.5 L for the inoculum and 13.5 L of culture medium for the PBR were required.

iii) S3. Cultivation step: Firstly, 150 mL cell cultures were statically incubated in flasks at 20ºC and 20 µmol photons$\cdot m^{-2} \cdot s^{-1}$ of light intensity from four fluorescent lights (15 W). Cells were subcultured and fresh medium was added in order to increase the cell culture density from 0.3 dry weight ($g_{DW}$)$\cdot L^{-1}$ to 2 $g_{DW} \cdot L^{-1}$. Once the required density was reached, the inoculum was added to the culture medium in the PBR.

The lab-scale PBR consisted of a polyvinyl chloride (PVC) tubular airlift reactor with a volume of 15 L. The reactor was illuminated by six fluorescent bulbs of 36 W and aerated by 1.5 $L \cdot min^{-1}$ of compressed air enriched with 0.5% $CO_2$ in the feed gas. In this case, 30 g of dried biomass were produced in one batch after 14 days of operation in a single stage, with an astaxanthin content of 4%.

iv) S4. Harvesting step: The produced biomass was initially harvested by centrifugation with an efficiency of 95%. A volume reduction of 97% was obtained, with final moisture of 94%. The resulting biomass was kept in a freezer before being freeze-dried to 2% moisture.

v) S5. Extraction step: The lab-scale separation process consisted of a conventional solvent extraction with dimethyl sulfoxide (DMSO). After DMSO addition, the mixture was heated to 55ºC and vortexed before separating the pigment phase by centrifugation. Finally, 1 g astaxanthin was obtained with a purity of 95%. As in the case of other marine organisms (Spångberg et al., 2013), the algal residue was considered as a fertilizer due to its content in nitrogen and phosphorous. However, it is important to remark here that this residue contained bioactive components with antioxidant and antimicrobial activities that may have more valuable potential applications.

❖ **Semi-pilot production scenario**

In the second system, *H. pluvialis* was grown in an 80 L annular PBR for 22 days. After each batch, 77 $g_{DW}$ biomass were produced, corresponding to a production of 2.7 g astaxanthin (3.5% astaxanthin within *H. pluvialis* biomass).

In order to obtain applicable conclusions from the lab-scale study, the pilot system was divided into the same five stages as the lab production of *H. pluvialis* astaxanthin, as shown in **Figure 4.2**. The deviations from the original lab process are detailed below.

i) S1. Cleaning and sterilization: Before cultivation, the reactor is chemically sterilized. To do so, 20 L of tap water were consumed. Additionally, and due to the lack of information, it was considered that 15% hypochlorite solution (NaClO) with a density of 1.22 $kg \cdot L^{-1}$ was used, to give a final concentration in the water of 5 $mg \cdot L^{-1}$ of active chlorine.

ii) S2. Preparation of culture medium: The culture medium consisted of deionized water containing 0.200 $g \cdot L^{-1}$ potassium nitrate ($KNO_3$), 0.050 $g \cdot L^{-1}$ sodium bicarbonate ($NaHCO_3$), 0.025 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, 0.0125 $g \cdot L^{-1}$ $K_2HPO_4$, 0.005 $g \cdot L^{-1}$ sodium carbonate ($Na_2CO_3$),

0.001 g·L$^{-1}$ EDTA-$Na_2$, 0.00002 g·L$^{-1}$ cobalt (II) chloride hexaydrate ($CoCl_2 \cdot 6H_2O$), 0.00002 g·L$^{-1}$ zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), 0.00001 g·L$^{-1}$ $CaCl_2 \cdot 6H_2O$, 0.0004 g·L$^{-1}$ manganese (II) chloride tetrahydrate ($MnCl_2 \cdot 4H_2O$) and 0.000001 g·L$^{-1}$ boric acid ($H_3BO_3$). This stage required the addition of nutrients in the specified amounts to deionized water, followed by the sterilization of the culture medium in an autoclave, as well as the addition of the initial inoculum. As the cultivation was performed in four stages (10 mL tube followed by 0.2 L flask, 4 L flask and finally 80 L PBR), the total required medium for one batch was 84 L, with an initial inoculum of 1 mL to the 10 mL tubes.

iii) S3. Cultivation: Firstly, 1 mL cell culture was transferred to a 10 mL tube with the culture medium. This tube was incubated in a culture chamber at 20°C and 500 lux for 168 h (7 days). After that, this culture was inoculated in a 0.2 L flask and culture medium (0.190 L) was added. Again, the cell culture was placed in the culture chamber at the same conditions for 168 h. In the third step of the culture, the content of the 0.2 L flask was added to fresh medium to give a 4 L culture growth for 168 h. In this case, a 0.76 kW compressor was required for aeration at 1.8 L·min$^{-1}$ with 1% $CO_2$ enriched air; illumination and temperature were controlled with 2 fluorescent tubes (58 W each) and a 0.069 kW temperature controller.

The cell culture was transferred to the 80 L PBR and maintained for 528 h (22 days) with aeration of 4 L·min$^{-1}$ (0.5% $CO_2$) provided by a 0.21 kW compressor. In this case, temperature control (0.138 kW) and fluorescent tubes (4x58 W each) were also required. The cultivation system consisted of an annular PBR with internal diameter of 14.8 cm, external diameter of 29.8 cm and height of 2 m. A final amount of 77 g biomass was obtained with an astaxanthin content of 4% (estimated from lab-scale data, real composition unknown).

iv) S4. Harvesting: As in the lab-scale process, the biomass was initially harvested by centrifugation with approximately 95% efficiency to accomplish a water reduction of 90%. After spray-drying the algal paste, the moisture content was reduced to 5%.

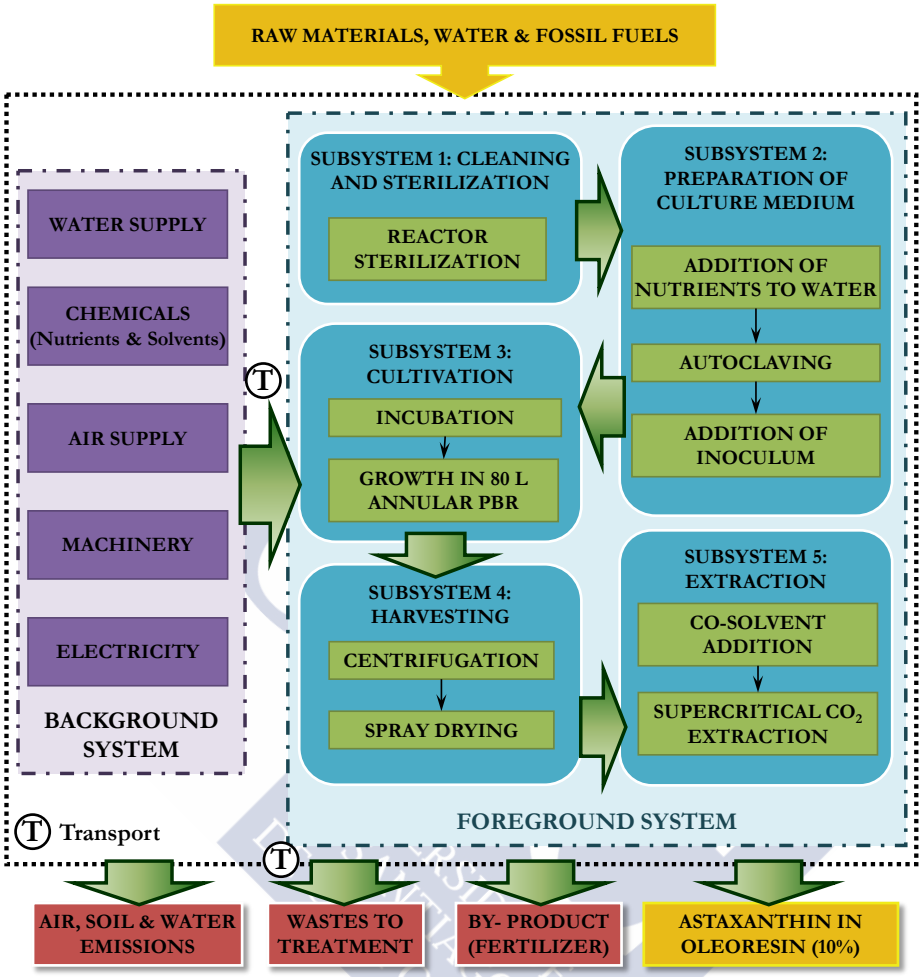



**Figure 4.2.** Process chain and system boundaries of the semi-pilot scale production of astaxanthin by *H. pluvialis* in an 80 L annular PBR.

v) S5. Extraction: The lab-scale separation process consisted of a conventional extraction with DMSO. However, this method is not suitable for the production of astaxanthin used in food or pharmaceutical industries due to the DMSO residue (Ni et al., 2007). Therefore, a supercritical $CO_2$ extraction was chosen to isolate astaxanthin from the algal paste obtained in the pilot-scale process. To do so, the cells were mixed with a dispersing and drying agent prior extraction. Both fish and vegetable oils could be used as a co-solvent.

In this case, fish waste oil was used with a ratio of 25%. The final product was an oleoresin with 10% astaxanthin. However, the final processing was excluded from the system boundaries to make the results comparable to those of lab-scale process. The algal residue was considered as a fertilizer.

❖ **Pilot-scale production scenario**

After the optimization of operational parameters, a two-stage pilot system was designed. The process consisted of a first growth stage with nutrient excess followed by a second stress stage limited in phosphate and nitrate. In both stages, the excess culture medium was recycled, so that at least five cultures were performed with the initial water. A complete cycle of five cultures produced approximately 800 g astaxanthin. The process is depicted in **Figure 4.3**.

i) S1. Cleaning and sterilization: In the case of the pilot process, two options were evaluated for the cleaning and sterilization stage. Firstly, the use of bleaching agents was considered (sodium hypochlorite, NaClO in a 5% w/v aqueous solution and sodium thiosulfate, $Na_2S_2O_3$ in a concentration of 0.1 $g \cdot L^{-1}$). Another possibility was assessed, consisting of the circulation of ozonized water through the reactor for a 4 h period, which seems to be a more appropriate option for a commercial scale production.

ii) S2. Preparation of culture medium: The culture medium for the pilot process was prepared with river and rain water, previously purified by reverse osmosis and UV filter to remove undesired salts and microorganisms. As the cultivation was carried out in two stages, namely growth and stress stage, two cultivation mediums were prepared.

In the medium for the growth stage, the added nutrients were 0.875 $g \cdot L^{-1}$ $NaNO_3$, 0.1975 $g \cdot L^{-1}$ $K_2HPO_4$, 0.0875 $g \cdot L^{-1}$ $KH_2PO_4$, 0.0305 $g \cdot L^{-1}$ $CaCl_2$, 0.141 $g \cdot L^{-1}$ $MgSO_4$, 0.0125 $g \cdot L^{-1}$ NaCl, 0.004 $g \cdot L^{-1}$ citric acid, 0.05 $g \cdot L^{-1}$ $Na_2CO_3$, 0.00275 $g \cdot L^{-1}$ EDTA-$Na_2$, 0.00143 $g \cdot L^{-1}$ $H_3BO_3$ and trace elements (less than 0.001 $g \cdot L^{-1}$).

The culture medium used for the stress stage was equivalent to the previous one, except for the absence of $K_2HPO_4$ and $KH_2PO_4$, as well as for the concentration of $NaNO_3$, which was reduced to 0.0875 $g \cdot L^{-1}$.

iii) S3. Cultivation: In this system, the preparation of inoculum had a negligible effect, since the facility was operated in a semi-continuous mode and the inoculum from each cycle was obtained by taking a small fraction of the biomass produced in the first PBR during the previous cycle.

In the first stage, the microalgae was grown for 8 days in a 1000 L airlift PBR with excess of nutrients. The reactor was internally illuminated with a 16:8 regime (600 W) and continuously aerated (50 W, 24 h). Afterwards, 50% of the cell culture was taken to an analogous reactor and microalgal cells were allowed to settle, enabling the drainage of 96% of the water, which could be recirculated to the original tank. In the second tank, a stress medium with limiting phosphate and nitrate nutrients was added, inducing astaxanthin accumulation. This stressing cycle required 1200 W for illumination, as well as 50 W for stirring motor and 50 W for the aeration pump at a continuous rate during 8 days.

At the end of the period, the microalgae had turned red and accumulated 4-5% astaxanthin. After settling of the culture broth, approximately 80% water was recovered and recirculated. The remaining 20% water was then poured off and sent to harvesting stage.

iv) S4. Harvesting step: As in the previous systems, the biomass was harvested by centrifugation with approximately 95% efficiency. However, in this case a settling step was carried out before centrifugation to preconcentrate the biomass. Therefore, the starting moisture for the centrifugation was lower than that of the lab-scale and semi-pilot processes, and consequently a lower moisture of 80% was obtained in the pilot process. After spray-drying the algal paste, the moisture content was reduced to 5%.

v) S5. Extraction step: Again, supercritical $CO_2$ extraction was considered for the separation of astaxanthin from the algal paste obtained in the pilot-scale process. Fish waste oil was also used as a co-solvent in a 25% ratio. The final processing for the formulation of the oleoresin with 10% astaxanthin was excluded from the system boundaries and the fertilizer potential of the remaining algal paste was taken into account.

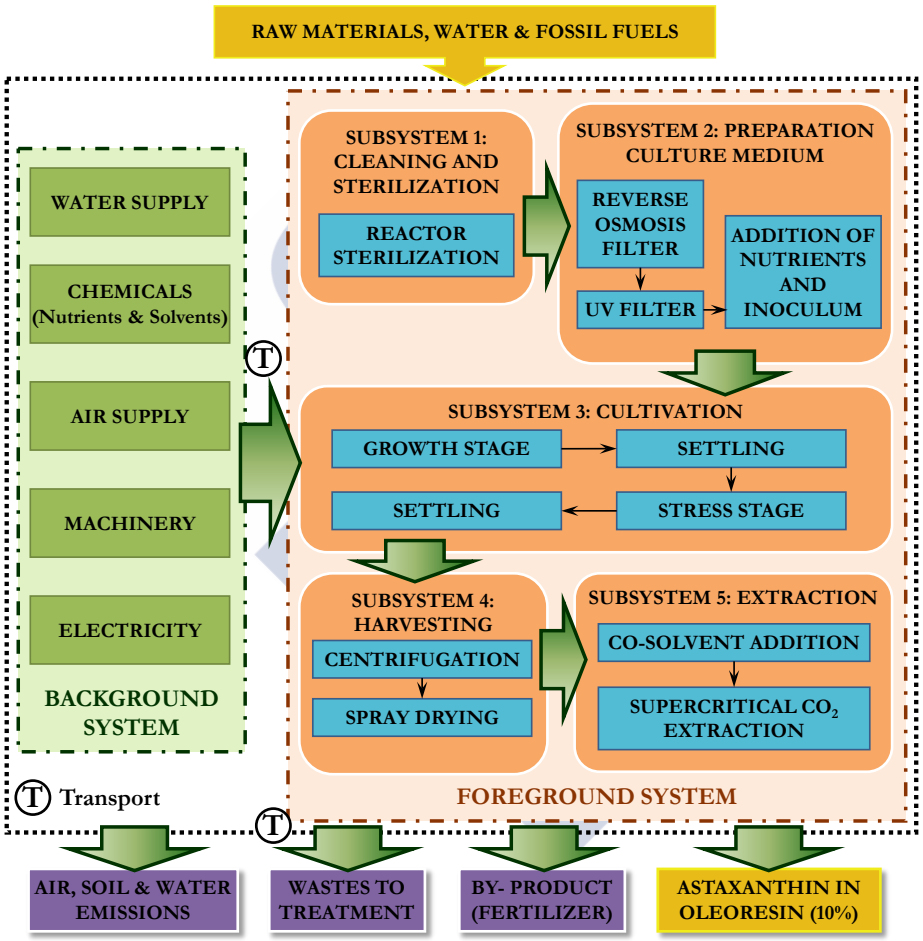



**Figure 4.3.** Process chain and system boundaries of the pilot-scale production of astaxanthin by *H. pluvialis* in a two-stage process with 1000 L internally illuminated airlift PBRs in series.

### 4.2.2. Life cycle inventory, data quality and assumptions

The Life Cycle Inventory (LCI) data for the foreground system (i.e. chemicals and electricity consumptions as well as transport distances) consisted of average data obtained by on-site measurements. Concerning water emissions, they were calculated assuming that the nutrients supplied in the culture medium which are not depleted during the algae growth, are directly discharged to water. An identical assumption was made for air emissions. Concerning the background system, the corresponding inventory data for the production of all the inputs to the system were taken from Ecoinvent database (Frischknecht et al., 2007), except from metal components, that were taken from IDEMAT (2001). These inputs included the production of the chemicals required for the preparation of the culture medium, the electricity used in the different production stages, the distribution of inputs up to the lab gate, lab materials and equipment (flasks, PBR, fluorescent tubes, electronic devices) and waste disposal. A detailed description of the corresponding database reports is shown in **Table 4.1**.

**Table 4.1.** Summary of data sources for the background system of the production of astaxanthin by *H. pluvialis*

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Energy | Electricity (Belgian electricity profile)<br>Electricity (Irish electricity profile) | Ecoinvent database (Dones et al., 2007) |
| Air supply | Compressed air | Ecoinvent database (Steiner and Frischknecht, 2007) |
| | Carbon dioxide | Ecoinvent database (Althaus et al., 2007) |
| Materials | PVC<br>Polystyrene<br>High Density Polyethylene (HDPE)<br>Polyethylene terephthalate (PET) | Ecoinvent database (Hischier et al., 2007) |
| | Gro-lux fluorescent tubes (36 W) | Ecoinvent database (Hischier et al., 2007) |
| | Stainless steel<br>Galvanized steel | Ecoinvent database (Classen et al., 2007) |
| Water | Tap water<br>Distilled water | Ecoinvent database (Althaus et al., 2007) |

**Table 4.1.** Summary of data sources for the background system of the production of astaxanthin by *H. pluvialis* (*Cont.*)

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Chemicals | $NaNO_3$ | Inventoried according to the synthetic route described by UNIDO/IFDC (1998) and Bhat et al. (1994) with Ecoinvent processes (Frischknecht et al., 2007) |
| | $CaCl_2$<br>$MgSO_4$<br>NaCl<br>$K_2HPO_4$<br>$KH_2PO_4$<br>Thiamine (vitamin B1)<br>Cyanocobalamin (vitamin B12)<br>EDTA<br>$FeCl_3$<br>$MnCl_2$<br>$ZnCl_2$<br>$CoCl_2$<br>$Na_2MoO_4$<br>Citric acid<br>Ferric ammonium citrate<br>$H_3BO_3$<br>$CuSO_4$<br>$Co(NO_3)_2$<br>NaClO | Ecoinvent database (Althaus et al., 2007) |
| | $ZnSO_4$ | Ecoinvent database (Hischier et al., 2007) |
| | $Na_2CO_3$ | Ecoinvent database (Sutter, 2007a) |
| | DMSO | Ecoinvent database (Sutter, 2007b) |
| Transport | Truck 3.5-7.5 Euro 4<br>Freight ship | Ecoinvent database (Spielmann et al., 2007) |
| Waste treatment | Inert landfill<br>Sanitary landfill<br>Electronic waste | Ecoinvent database (Doka, 2007) |

For the equipment, different life spans were considered, according to manufacturers' specifications. An average transport distance of 800 and 600 km within continental Europe was estimated for chemicals and materials, respectively, with an average sea distance of 1,400 km from the continental Europe to Ireland in the pilot-scale process and a shorter distance of 50 km for waste transport distance. Disposal in sanitary landfill was considered for all plastic waste, whereas steel components and lamps were sent to either inert landfills or specific waste treatment. Incineration was considered for the filter membrane (polyamide).

With regard to $NaNO_3$ production, this process is not defined in the Ecoinvent database. Therefore, the considered inventory data correspond to the synthetic process described in UNIDO/IFDC (1998). The method, developed by GIAP, consists of an oxidation of ammonia in the presence of platinum catalyst followed by the absorption of nitrogen oxide produced in an aqueous solution of sodium carbonate and the separation of sodium nitrate and sodium nitrite. Finally, nitric acid is added to convert sodium nitrite to sodium nitrate. Sodium nitrate is separated from the solution and dried in a rotary dryer. Inventory data for the raw materials were taken from Ecoinvent database, whereas energy requirements from Bhat et al. (1994) were taken.

Fish oil, required for the supercritical extraction stage in the pilot-scale process, is not available in the Ecoinvent database. Fish oil is a by-product of fisheries, obtained from the discarded fraction of marine fish such as mackerel, salmon, tuna and cod (Lin and Li, 2009). In this case, the inventory data from Iribarren et al. (2012) were considered.

*Allocation procedures*

In this study, all the environmental burdens were allocated to the amount of astaxanthin produced since algal cultivation was only focused on this biocompound. A system expansion approach was considered to include the potential use of residual algal biomass as fertilizer. The biomass content of nitrogen and phosphorous were calculated according to Mulbry et al. (2005). Thus, a nitrogen content of 7% in algal biomass was considered, with 30% of total nitrogen as plant available nitrogen. Regarding phosphorus, it was assumed a content of 1% present in biomass, with 60% as plant available phosphorus.

Once the fertilizer potential was estimated, the equivalent amount of a typical fertilizer (ammonium sulfate as N source and diammonium phosphate as P source) was considered in the model as avoided product, which resulted in negative impacts (environmental credits) that were subtracted from the environmental burdens.

The inventory data for lab, semi-pilot and pilot-scale processes are shown in **Tables 4.2**, **4.3** and **4.4**.

**Table 4.2.** Inventory data for the lab-scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Cleaning and sterilization* | | | |
| Tap water | 47.11 $m^3$ | NaClO | 18.84 kg |
| Deionized water | 28.27 $m^3$ | Stainless steel | 4.70 kg |
| *S2. Preparation of inoculum and culture medium* | | | |
| Deionized water | 14.13 $m^3$ | Vitamin B12 | 0.14 g |
| $NaNO_3$ | 10.57 kg | EDTA-$Na_2$·$2H_2O$ | 63.42 g |
| $CaCl_2$·$2H_2O$ | 0.35 kg | $FeCl_3$ | 4.91 g |
| $MgSO_4$·$7H_2O$ | 1.06 kg | $MnCl_2$ | 3.47 g |
| NaCl | 0.35 kg | $ZnCl_2$ | 0.42 g |
| $K_2HPO_4$·$3H_2O$ | 1.06 kg | $CoCl_2$·$6H_2O$ | 0.17 g |
| $KH_2PO_4$ | 2.50 kg | $Na_2MoO_4$·$2H_2O$ | 0.34 g |
| Thiamine | 16.91 g | Stainless steel | 106.08 kg |
| Polystyrene | 3.37 t | HDPE | 124.37 kg |
| PET | 0.96 kg | Polyurethane foam | 34.26 kg |
| Fluorescent lamps | 3.04 kg | | |
| *S3. Cultivation* | | | |
| Fluorescent lamps | 16.15 kg | PVC | 40.47 kg |
| Compressed air | 67.39 t | $CO_2$ (0.5% in air) | 514.46 kg |
| *S4. Harvesting* | | | |
| Distilled water | 4.71 $m^3$ | Polycarbonate | 422.11 kg |
| PP | 819.72 kg | Polyurethane foam | 3.00 kg |
| Galvanized steel | 32.96 kg | Stainless steel | 20.70 kg |
| Anodized aluminum | 0.85 kg | | |

**Table 4.2.** Inventory data for the lab-scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S5. Extraction* | | | |
| DMSO | 2.63 $m^3$ | Galvanized steel | 8.16 kg |
| Stainless steel | 0.31 kg | Anodized aluminum | 0.22 kg |
| Cast metal | 0.94 kg | Polycarbonate | 211.05 kg |
| **Energy** | | | |
| Electricity from Belgian grid | | | |
| *S1. Cleaning and sterilization* | | | |
| Autoclaving | 1.11 MWh | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Autoclaving | 0.78 MWh | Addition of inoculum in laminar flow hood | 0.19 MWh |
| Incubation (excluding lights) | 40.70 MWh | Lighting in incubation stage | 9.50 MWh |
| *S3. Cultivation* | | | |
| Lighting in PBR | 68.38 MWh | Aeration | 3.73 MWh |
| *S4. Harvesting* | | | |
| Centrifugation | 10.99 MWh | Freezer | 5.65 MWh |
| Freeze-drying | 2.26 MWh | | |
| *S5. Extraction (from Irish grid)** | | | |
| Heating of solvent | 0.39 MWh | Vortex mixing | 0.05 MWh |
| Centrifugation | 1.28 MWh | | |
| **Transport** | | | |
| *S1. Cleaning and sterilization* | | | |
| Truck 3.5-7.5 t (chemicals) | 15.08 tkm | Truck 3.5-7.5 t (materials) | 2.82 tkm |
| Truck 3.5-7.5 t (wastes) | 0.23 tkm | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Truck 3.5-7.5 t (chemicals) | 12.76 tkm | Truck 3.5-7.5 t (materials) | 2115 tkm |
| Truck 3.5-7.5 t (wastes) | 176.24 tkm | | |
| *S3. Cultivation* | | | |
| Truck 3.5-7.5 t (materials) | 104.32 tkm | Truck 3.5-7.5 t (wastes) | 8.69 tkm |
| *S4. Harvesting* | | | |
| Truck 3.5-7.5 t (materials) | 779.60 tkm | Truck 3.5-7.5 t (wastes) | 64.97 tkm |
| *S5. Extraction* | | | |
| Truck 3.5-7.5 t (chemicals) | 2317 tkm | Truck 3.5-7.5 t (materials) | 132.41 tkm |
| Truck 3.5-7.5 t (wastes) | 11.03 tkm | | |

**Table 4.2.** Inventory data for the lab-scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin) (*Cont.*)

| **OUTPUTS to TECHNOSPHERE** | | | |
|---|---|---|---|
| **Product** | | **Avoided product** | |
| Astaxanthin from *H. pluvialis* | 1 kg | N-fertilizer[1] | 0.57 kg |
| | | P-fertilizer[1] | 0.38 kg |
| **Wastes to sanitary landfill** | | | |
| Polystyrene | 3.73 t | PVC | 40.47 kg |
| HDPE | 124.37 kg | PP | 819.72 kg |
| PET | 0.95 kg | Aluminum | 1.07 kg |
| Polyurethane foam | 37.26 kg | Polycarbonate | 633.16 kg |
| **Wastes to inert landfill** | | **Wastes to specific treatment** | |
| Steel | 173.85 kg | Fluorescent lamps | 19.18 kg |
| **OUTPUTS TO ENVIRONMENT** | | | |
| **Air emissions** | | | |
| Air (excluding $CO_2$) | 67.39 t | $CO_2$ | 480.99 kg |
| **Water emissions** | | | |
| *S1. Cleaning and sterilization* | | | |
| Wastewater | 75.38 $m^3$ | NaClO | 18.84 kg |
| *Total non-consumed nutrients (from S4. Harvesting + S5. Extraction)* | | | |
| Wastewater | 18.37 $m^3$ | Vitamin B12 | 6.36 mg |
| $NaNO_3$ | 0.48 kg | EDTA-$Na_2{\cdot}2H_2O$ | 2.86 g |
| $CaCl_2{\cdot}2H_2O$ | 15.90 g | $FeCl_3$ | 0.22 g |
| $MgSO_4.7H_2O$ | 47.70 g | $MnCl_2$ | 0.16 g |
| NaCl | 15.90 g | $ZnCl_2$ | 19.08 mg |
| $K_2HPO_4.3H_2O$ | 47.70 g | $CoCl_2{\cdot}6H_2O$ | 7.63 mg |
| $KH_2PO_4$ | 111.29 g | $Na_2MoO_4.2H_2O$ | 15.27 mg |
| Thiamine | 0.76 g | | |
| *S5. Extraction* | | | |
| Wastewater | 471.10 L | DMSO | 2.63 $m^3$ |

[1] N and P fertilizer dosage is equivalent to 27.266 kg of residual biomass.

**Table 4.3.** Inventory data for the semi-pilot scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Cleaning and sterilization* | | | |
| Tap water | 7.51 $m^3$ | NaClO | 37.54 g |
| *S2. Preparation of inoculum and culture medium* | | | |
| Deionized water | 1.43 $m^3$ | EDTA-$Na_2$ | 31.47 g |
| $KNO_3$ | 6.29 kg | $CoCl_2 \cdot 6H_2O$ | 0.63 g |
| $Na_2CO_3$ | 0.16 kg | $ZnSO_4 \cdot 7H_2O$ | 0.63 g |
| $NaHCO_3$ | 1.57 kg | $CaCl_2 \cdot 6H_2O$ | 0.31 g |
| $K_2HPO_4$ | 0.39 kg | $MnCl_2 \cdot 4H_2O$ | 12.58 g |
| $MgSO_4 \cdot 7H_2O$ | 0.79 kg | $H_3BO_3$ | 31.47 mg |
| Compressed air | 8.05 t | $CO_2$ (1% in air) | 122.97 kg |
| *S3. Cultivation* | | | |
| Fluorescent lamps | 6.74 kg | PVC | 19.81 kg |
| Compressed air | 56.38 t | $CO_2$ (1% in air) | 429.42 kg |
| *S5. Extraction* | | | |
| Drying agent (pelletized diatomaceous earth) | 53.71 kg | Co-solvent (fish/vegetable oil) | 44.17 kg |
| **Energy** | | | |
| Electricity from French grid | | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Sterilization (autoclaving) | 1.25 MWh | Incubation | 20.53 MWh |
| Lighting | 7.32 MWh | Temperature control | 4.35 MWh |
| Air supply | 47.94 MWh | | |
| *S3. Cultivation* | | | |
| Lighting | 45.99 MWh | Temperature control | 27.36 MWh |
| Aeration | 41.63 MWh | | |
| *S4. Harvesting* | | | |
| Centrifugation | 0.04 MWh | Spray drying | 3.42 MWh |
| *S5. Extraction (from Irish grid)** | | | |
| Supercritical $CO_2$ extraction | 4.73 MWh | | |

**Table 4.3.** Inventory data for the semi-pilot scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin) (*Cont.*)

| | | | |
|---|---|---|---|
| **INPUT to TECHNOSPHERE** | | | |
| **Transport** | | | |
| *S1. Cleaning and sterilization* | | | |
| Truck 3.5-7.5 t (chemicals) | 0.20 tkm | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Truck 3.5-7.5 t (chemicals) | 7.40 tkm | | |
| *S3. Cultivation* | | | |
| Truck 3.5-7.5 t (materials) | 21.24 tkm | Truck 3.5-7.5 t (wastes) | 1.33 tkm |
| *S5. Extraction* | | | |
| Truck 3.5-7.5 t (chemicals) | 78.30 tkm | Truck 3.5-7.5 t (wastes) | 2.69 tkm |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Product** | | **Avoided product** | |
| Astaxanthin from *H. pluvialis* | 1 kg | N-fertilizer[1] | 0.57 kg |
| | | P-fertilizer[1] | 0.38 kg |
| **Wastes to sanitary landfill** | | **Wastes to specific treatment** | |
| PVC (S3. Cultivation) | 19.81 kg | Fluorescent lamps | 6.74 kg |
| **Wastes to inert landfill** | | | |
| Inert material (S5. Extraction) | 53.71 kg | | |
| **OUTPUTS TO ENVIRONMENT** | | | |
| **Air emissions** | | | |
| Air (excluding $CO_2$) | 64.43 t | $CO_2$ | 519.25 kg |
| **Water emissions** | | | |
| *S1. Cleaning and sterilization* | | | |
| Wastewater | 75.38 $m^3$ | NaClO | 18.84 kg |
| *Total non-consumed nutrients (from S4. Harvesting + S5. Extraction)* | | | |
| Wastewater | 29.97 $m^3$ | EDTA-$Na_2$ | 3.00 g |
| $KNO_3$ | 0.60 kg | $CuCl_2 \cdot 6H_2O$ | 0.06 g |
| $Na_2CO_3$ | 14.98 g | $ZnSO_4 \cdot 7H_2O$ | 0.06 g |
| $NaHCO_3$ | 0.15 kg | $CaCl_2 \cdot 6H_2O$ | 0.03 g |
| $K_2HPO_4$ | 37.46 g | $MnCl_2 \cdot 4H_2O$ | 1.20 g |
| $MgSO_4 \cdot 7H_2O$ | 74.92 g | $H_3BO_3$ | 3.00 mg |

[1] N and P fertilizer dosage is equivalent to 27.266 kg of residual biomass.

**Table 4.4.** Inventory data for the pilot-scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Cleaning and sterilization* | | | |
| OPTION 1 | | | |
| Tap water | 5.01 $m^3$ | NaClO | 5.01 kg |
| OPTION 2 | | | |
| Stainless steel | 0.25 kg | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| $NaNO_3$ | 5.58 kg | $H_3BO_3$ | 17.08 g |
| $K_2HPO_4$ | 1.14 kg | $ZnSO_4$ | 1.31 g |
| $KH_2PO_4$ | 0.51 kg | $CuSO_4$ | 0.48 g |
| $CaCl_2$ | 0.36 kg | $Co(NO_3)_2$ | 0.30 g |
| $MgSO_4$ | 1.68 kg | $FeCl_3$ | 3.48 g |
| NaCl | 0.15 kg | $ZnCl_2$ | 0.18 g |
| Citric acid | 35.83 g | $CoCl_2$ | 0.07 g |
| Ferric ammonium citrate | 35.83 g | $MnCl_2$ | 12.28 g |
| $Na_2CO_3$ | 0.60 kg | $Na_2MoO_4$ | 1.46 g |
| EDTA-$Na_2$ | 32.85 g | Stainless steel | 0.43 kg |
| PVC | 26.59 g | UV lamps | 21.92 g |
| Polyamide | 146.16 g | | |
| *S3. Cultivation* | | | |
| Fluorescent lamps | 0.16 kg | Stainless steel | 10.44 kg |
| *S4. Harvesting* | | | |
| Stainless steel | 4.80 kg | | |
| *S5. Extraction* | | | |
| Drying agent | 44.32 kg | Co-solvent (fish/vegetable oil) | 5.83 kg |
| Stainless steel | 0.69 kg | | |
| **Energy** | | | |
| Electricity from Irish grid | | | |
| *S1. Cleaning and sterilization (OPTION 2)* | | | |
| Sterilization with ozonized water | 0.15 kWh | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Reverse osmosis filtration | 9.64 kWh | UV filtration | 0.44 kWh |

**Table 4.4.** Inventory data for the pilot-scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin) (*Cont.*)

| | | | |
|---|---|---|---|
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Energy** | | | |
| Electricity from Irish grid | | | |
| *S3. Cultivation* | | | |
| Lighting in PBR | 1924 kWh | Aeration | 120.3 kWh |
| Agitation | 120.3 kWh | | |
| *S4. Harvesting* | | | |
| Centrifugation | 1.88 kWh | Spray drying | 103.4 kWh |
| *S5. Extraction* | | | |
| Supercritical $CO_2$ extraction | 197.8 kWh | | |
| **Transport** | | | |
| Electricity from Irish grid | | | |
| *S1. Cleaning and sterilization* | | | |
| Truck 3.5-7.5 t (chemicals) | 4.01 tkm | Truck 3.5-7.5 t (materials) | 0.15 tkm |
| Freight ship (chemicals) | 7.02 tkm | Freight ship (materials) | 0.35 tkm |
| Truck 3.5-7.5 t (wastes) | 0.01 tkm | | |
| *S2. Preparation of inoculum and culture medium* | | | |
| Truck 3.5-7.5 t (chemicals) | 8.13 tkm | Truck 3.5-7.5 t (materials) | 0.38 tkm |
| Freight ship (chemicals) | 14.23 tkm | Freight ship (materials) | 0.88 tkm |
| Truck 3.5-7.5 t (wastes) | 0.03 tkm | | |
| *S3. Cultivation* | | | |
| Truck 3.5-7.5 t (materials) | 6.36 tkm | Truck 3.5-7.5 t (wastes) | 0.53 tkm |
| Freight ship (materials) | 14.85 tkm | | |
| *S4. Harvesting* | | | |
| Truck 3.5-7.5 t (materials) | 2.88 tkm | Truck 3.5-7.5 t (wastes) | 0.24 tkm |
| Freight ship (materials) | 6.72 tkm | | |
| *S5. Extraction* | | | |
| Truck 3.5-7.5 t (chemicals) | 40.12 tkm | Truck 3.5-7.5 t (materials) | 0.41 tkm |
| Freight ship (chemicals) | 70.21 tkm | Freight ship (materials) | 0.96 tkm |
| Truck 3.5-7.5 t (wastes) | 2.25 tkm | | |
| **INPUTS from ENVIRONMENT** | | | |
| **Materials** | | | |
| Biomass | 20.88 g | Air (excluding $CO_2$) | 543.40 t |
| River/rain water | 3.483 $m^3$ | $CO_2$ | 0.33 t |

**Table 4.4.** Inventory data for the pilot-scale production of astaxanthin by *H. pluvialis* (FU=1 kg astaxanthin) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Product** | | **Avoided product** | |
| Astaxanthin from *H. pluvialis* | 1 kg | N-fertilizer[1] | 0.54 kg |
| | | P-fertilizer[1] | 0.35 kg |
| **Wastes to inert landfill** | | | |
| Steel (OPTION 1) | 16.37 kg | Steel (OPTION 2) | 16.62 kg |
| Diatomaceous earth | 44.32 kg | | |
| **Wastes to sanitary landfill** | | **Wastes to municipal incineration** | |
| PVC | 0.02 kg | Textiles (polyamide) | 0.15 kg |
| **Wastes to specific treatment** | | | |
| Fluorescent lamps | 0.16 kg | | |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Air emissions** | | | |
| Air (excluding $CO_2$) | 543.40 t | $CO_2$ | 297.46 kg |
| **Water emissions** | | | |
| *S1. Cleaning and sterilization* | | | |
| Wastewater | 5.01 $m^3$ | NaClO | 5.01 kg |
| *Total non-consumed nutrients (from S3. Cultivation + S4. Harvesting + S5. Extraction)* | | | |
| Wastewater | 3.483 $m^3$ | EDTA-$Na_2$ | 0.46 g |
| $NaNO_3$ | 111.37 g | $H_3BO_3$ | 0.24 g |
| $K_2HPO_4$ | 24.27 g | $ZnSO_4$ | 18.47 mg |
| $KH_2PO_4$ | 10.75 g | $CuSO_4$ | 6.71 mg |
| $CaCl_2$ | 5.12 g | $Co(NO_3)_2$ | 4.20 mg |
| $MgSO_4$ | 23.67 g | $FeCl_3$ | 48.85 mg |
| NaCl | 2.10 g | $ZnCl_2$ | 2.52 mg |
| Citric acid | 0.50 g | $CoCl_2$ | 1.01 mg |
| Ferric ammonium citrate | 0.50 g | $MnCl_2$ | 17.26 mg |
| $Na_2CO_3$ | 8.39 g | $Na_2MoO_4$ | 20.48 mg |

[1] N and P fertilizer dosage is equivalent to 25.632 kg of residual biomass.

### 4.2.3. Environmental impact assessment

As in the previous chapter, an attributional LCA was carried out according to the ISO standards (ISO 14040, 2006). Among the steps defined to accomplish the life cycle impact assessment, classification and characterization were undertaken here. Normalization and weighing were considered to provide no additional objective information for the aim of the study.

The assessment was conducted using the CML 2 baseline 2001 V2.05 method (Guinée et al., 2002). The ten impact categories included were: abiotic depletion potential (ADP), acidification potential (AP), eutrophication potential (EP), global warming potential over a 100 year timeframe (GWP), ozone layer depletion potential (ODP), photochemical oxidants formation potential (POFP) and toxicity related impact categories: human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP) and terrestrial ecotoxicity (TEP). The software SimaPro 7.3 was used for the implementation of the collected inventories (Goedkoop et al., 2008).

The characterization results of the production of astaxanthin by *H. pluvialis* in the three systems (lab, semi-pilot and pilot) according to a cradle-to-gate perspective are shown in **Table 4.5**, including the two options evaluated for the cleaning and sterilization stage in the pilot-scale scenario. It should be remarked, as mentioned before, that the functional unit (1 kg astaxanthin) is only representative for the pilot-scale scenario and is here used for the lab and semi-pilot processes exclusively for comparative purposes.

The obtained values demonstrate the strong dependence of environmental impacts on the production scale, in accordance with the previous outcome from the comparison of lab-scale and simulated pilot systems in Chapter 3. Thus, the total contributions were found to be from 10% up to 4 times higher for lab-scale process than for semi-pilot system. With regard to the pilot two-stage process, the semi-pilot system has impacts between 10 and 100 times higher, whereas the lab process shows contributions between 25 and 122 times above those of the pilot production process. The most remarkable reductions are associated with the toxicity potentials.

**Table 4.5.** Impact assessment results (characterization step) associated with the production of astaxanthin by *H. pluvialis* at lab, semi-pilot and pilot scale (FU= 1 kg astaxanthin)

| Impact category | Unit | Lab-scale process | Semi-pilot scale process | Pilot-scale process | |
|---|---|---|---|---|---|
| | | | | Chemical disinfection | Ozone sterilization |
| ADP | kg Sb eq | 716.11 | 166.27 | 16.99 | 16.94 |
| AP | kg $SO_2$ eq | 444.70 | 156.07 | 15.16 | 15.16 |
| EP | kg $PO_4^{-3}$ eq | 157.22 | 70.84 | 2.37 | 2.35 |
| GWP | kg $CO_2$ eq | 87189 | 24756.4 | 2329.74 | 2322.91 |
| ODP | kg CFC-11 eq | $4.79 \cdot 10^{-3}$ | $1.22 \cdot 10^{-3}$ | $1.62 \cdot 10^{-4}$ | $1.62 \cdot 10^{-4}$ |
| HTP | kg 1,4-DB eq | 48608.4 | 40332.5 | 401.67 | 398.50 |
| FEP | kg 1,4-DB eq | 33786.8 | 16244.1 | 326.99 | 323.76 |
| MEP | kg 1,4-DB eq | 20208.1 | 10224.7 | 237.16 | 235.19 |
| TEP | kg 1,4-DB eq | 4.72 | 4.30 | 0.11 | 0.11 |
| POFP | kg $C_2H_4$ eq | 15.40 | 6.19 | 0.61 | 0.61 |

Despite the significant differences, these values show a slightly more limited improvement linked to the scale-up than the one observed for the hypothetical pilot system from extrapolations (i.e. production of eicosapentaenoic acid by *P. tricornutum*), which reduced the contributions to most categories in at least two orders of magnitude for the pilot scale with respect to the lab scale (Section 3.2.3).

This behavior may be related to the fact that the extrapolations and assumptions considered in the previous chapter are based on available studies that mainly analyze the production of biofuels rather than high value compounds. These bioactive compounds require very specific operating conditions such as light stress, which is likely to lead to higher energy requirements, and nutrient limitation, which may result in low productivity for similar quantities of inputs to the system.

In addition, most of the existing reports, from which the information for the scale up was taken, refer to large scale systems rather than pilot scale facilities, and mainly consider production in outdoor reactors (commonly raceway ponds) that present lower energy requirements due to the geometry but also to the use of natural sunlight instead of artificial illumination. In order to identify the specific stages and activities that have the greatest influence in these results, the relative contributions for the three systems are individually discussed in this section.

❖ **Lab-scale results**

According to **Figure 4.4**, the contribution from the cultivation stage (S3) is the main factor responsible for the environmental burdens derived from the production of astaxanthin with remarkable contributions of more than 44% to all impact categories under assessment. Among the secondary stages, the preparation of the medium plays a significant role in categories such as ADP, EP, GWP or POFP, whereas the extraction is only relevant in terms of AP and ODP, and harvesting mainly contributes to ecotoxicity categories.

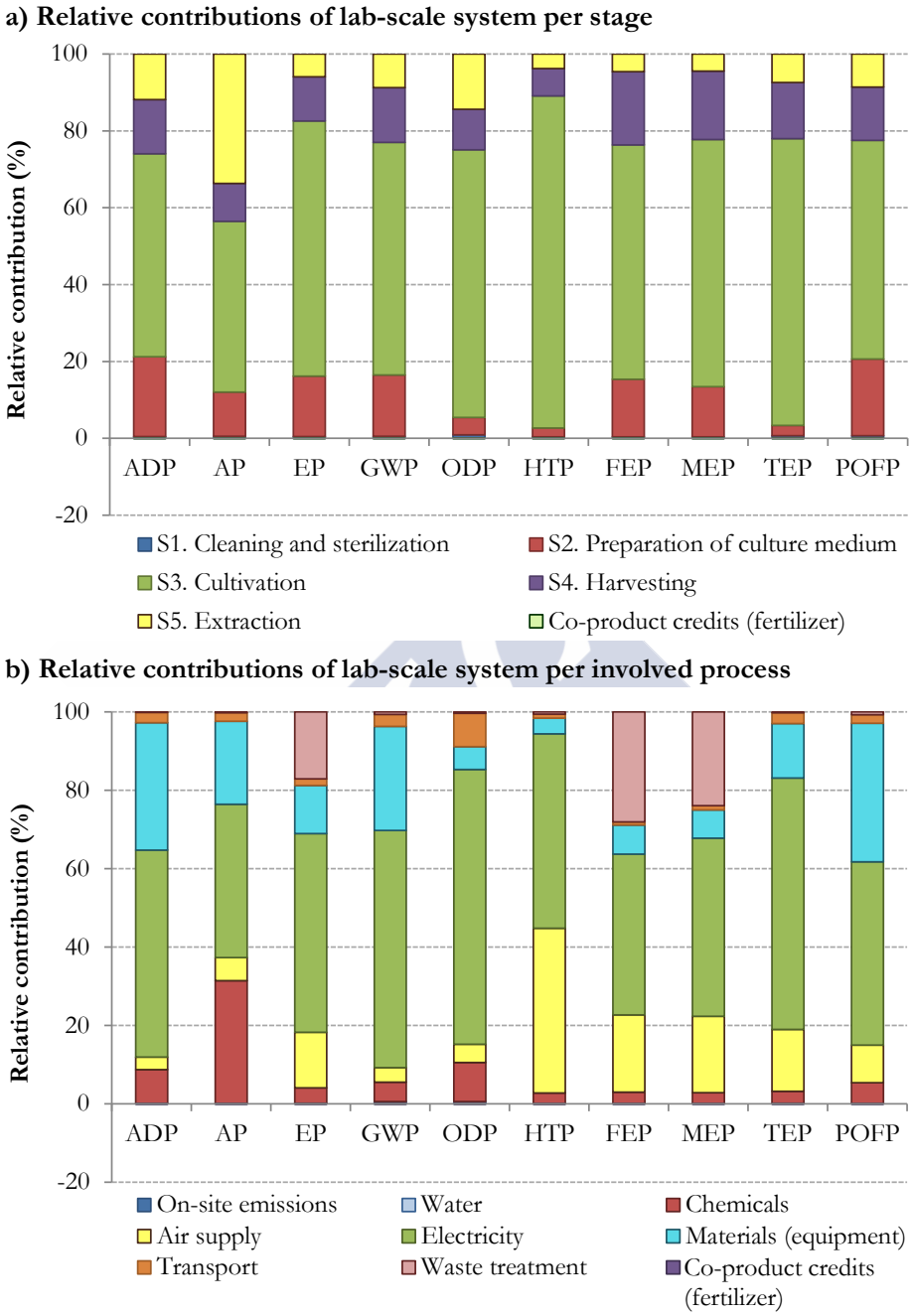



**Figure 4.4.** Relative contributions of the lab-scale production of astaxanthin by *H. pluvialis* (15 L tubular PBR) to each impact category per a) stage and b) involved process.

Concerning the involved processes, the production of electricity required throughout the whole process is the main cause of the environmental impacts, with contributions ranging from 39% (for AP) to 70% (ODP). The production of materials has some significant relative contributions between 20% and 35% in categories such as ADP, AP, GWP and POFP (mainly linked to the production of polystyrene and steel), whereas air supply is responsible for 42% HTP and between 15-20% for FEP, TEP and MEP. The production of chemicals only exceeds 10% for AP (31%), while waste treatment processes have a noticeable influence in EP (17%), FEP (28%) and MEP (24%), especially associated with the management of polystyrene and polypropylene in sanitary landfills. With regard to the co-product credits, considered to include the potential of remaining algal biomass as fertilizers according to a system expansion approach, the results show a very low benefit (less than 0.1% for all categories) due to the limited quantity of algae produced (from which only 2% can be used as N-fertilizer and 1% can be used as P-fertilizer) in comparison with the high input requirements.

In terms of emitted substances, $SO_2$ emissions from coal power plants have the highest contribution to AP, followed by $H_2S$ from DMSO production and $NO_x$ emissions. EP is mainly affected by emissions of phosphate (66%) and organic matter (17%) to water, as well as $NO_x$ (13%) to air. The main substance responsible for GWP is fossil $CO_2$ (90%), especially related to the production of electricity, whereas Halon 1211 (43%) emitted to air during the transport of natural gas in the production of electricity and DMSO was the main cause of ODP. The impact in HTP is mainly associated with emissions to air of arsenic (33%), chromium VI (27%) and polycyclic aromatic hydrocarbons (16%). Emissions of metals (e.g. vanadium, nickel and beryllium) to water cause significant impacts to FEP (41%) and MEP (45%), and most of TEP is due to the emission of mercury to the air derived from the use of coal for electricity generation (41%) and chromium VI to the soil from the distribution network (32%). The main contributing substances to POFP are $SO_2$ (59%) and CO (15%).

❖ **Semi-pilot scale results**

According to the results depicted in **Figure 4.5**, the environmental impacts for the semi-pilot process are in the same order of magnitude as those of the lab-scale system, but significantly lower in all the assessed categories, with reductions of impact from 9% (TEP) up to 75% (ADP and ODP). This may be partially due to the scale-up effect, but also to other changes in the process, mainly concerning the extraction stage.

Again, the contribution from the cultivation stage (S3) is the major cause of the environmental burdens derived from the production of astaxanthin, with much higher relative contributions than those of lab-scale process (more than 90% to all impact categories under assessment). Although the cultivation in the 80 L annular PBR has lower absolute impacts than the lab-scale process in all categories except for TEP (with reductions of impact from 8% up to 66%), the higher reduction observed in other stages is the reason for the still large relative contributions of S3. Thus, stages such as extraction show improvements between 56% and 96% with respect to the lab-scale extraction process. This demonstrates the efficiency of novel extraction technologies (i.e. supercritical $CO_2$ extraction) compared to conventional extraction using DMSO as solvent. Concerning the fertilizer potential of the residual algal biomass, the credits obtained according to the system expansion approach are still well below 0.1%, regardless of the considered impact category. As in the lab-scale process, the main reason for that is the low amount of algae produced compared to the high input requirements.

As in the previous system, electricity was clearly the hot spot, with relative contributions considerably higher than those of the lab-scale process. Thus, between 83% and 94% of environmental impacts were due to the electricity requirements. Among secondary processes, only air supply exceeds 10% in the category of HTP (12%), whereas its contribution to other categories ranges between 4% and 10%. Most categories also have a minor contribution between 4 and 8% associated with the production of materials.

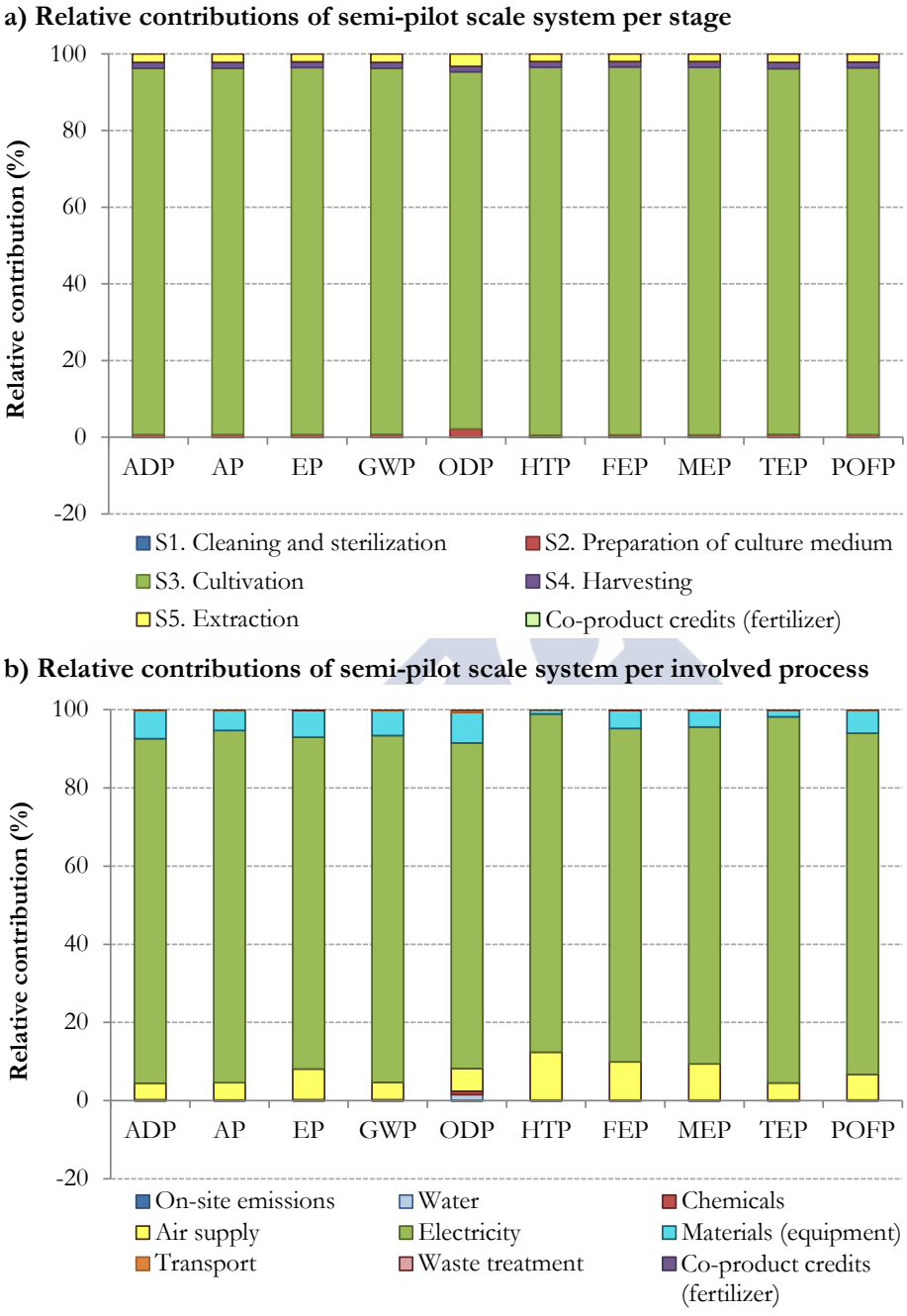



**Figure 4.5.** Relative contributions of the semi-pilot production of astaxanthin by *H. pluvialis* (80 L annular PBR) to each impact category per a) stage and b) involved process.

Regarding the involved substances, contributions to ADP and AP are linked to the use of fossil fuels, including coal and natural gas, as the main energy sources according to the French electricity profile. Indeed, $SO_2$ and $NO_x$ emissions from coal power plants are responsible for 77% and 21% of impact to AP. Most of the impacts to EP are related to phosphate emissions to water and $NO_x$ to air, whereas $CO_2$ (93%) is the main contributor to GWP. Halon 1211 (50%) emitted to air during the transport of natural gas and Halon 1301 (39%) released during the production of crude oil were the main causes of ODP. Contributions to toxicity categories are especially related to the emissions of metals. In the case of HTP, the impact is mainly due to the emissions to air of arsenic (41%) and chromium VI (31%), while the emissions to water of nickel, beryllium and vanadium strongly affect FEP and MEP. TEP is associated with chromium VI emissions to soil and mercury and vanadium emissions to water. $SO_2$ is again the main contributor to POFP (77%).

**Figure 4.6** shows that cultivation in 80 L PBR requires 56% of total electricity, comprising lighting (23%), aeration (20%) and temperature control (13%). The growth step in 4 L flask also has a significant contribution, with 29% of the total electricity consumed, where 80% of this contribution is due to aeration. Other stages, such as harvesting, extraction or sterilization during the preparation of the medium have secondary contributions, lower than 5%.

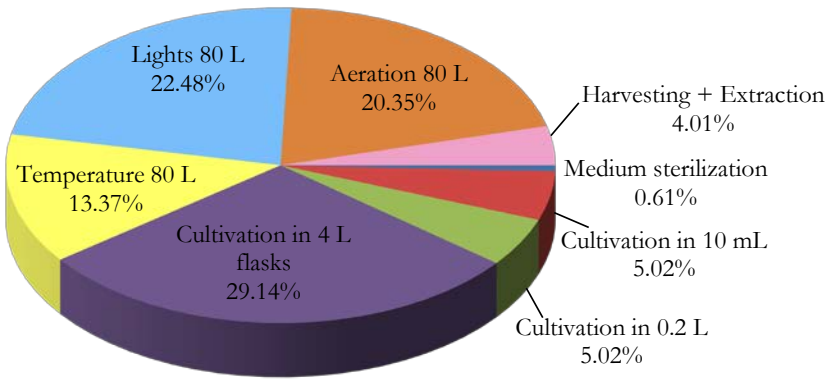



**Figure 4.6.** Relative contributions of the different steps to the total electricity requirements of the semi-pilot scale astaxanthin production by *H. pluvialis*.

❖ **Pilot-scale results**

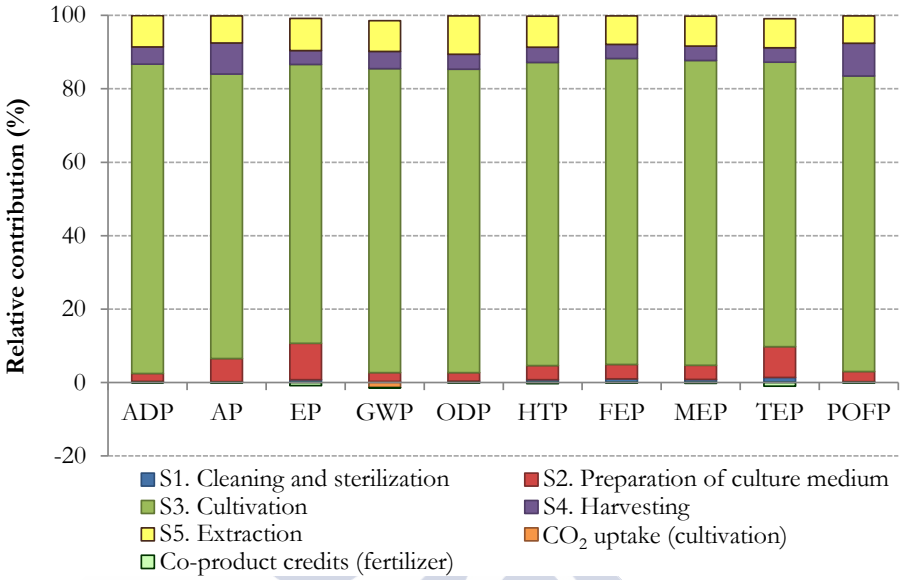
The results from **Table 4.5** show that there is no significant difference between the environmental impacts of both pilot-scale alternatives in all the assessed categories, although a slight improvement is observed for the case of ozone sterilization. The relative contributions of stages and involved processes are depicted in **Figures 4.7** and **4.8**. As the results differ in less than 1.5%, the numeric relative contributions discussed below are only provided for the second option, ozone sterilization, as this seems the most realistic scenario for a commercial scale plant.

In the pilot system, the cultivation (S3) is again the main stage responsible for the environmental burdens derived from the production of astaxanthin. As for the semi-pilot system, S3 presents even higher relative contributions with respect to the other stages of the pilot process (more than 70% for all the impact categories) than those of the lab-scale cultivation.

However, when comparing absolute values of lab and semi-pilot processes with those of the pilot system, impact reductions up to 95% and 90% respectively were observed in all the evaluated categories, related to the substitution of the cultivation in a single reactor by the two-stage cultivation process (thus, increasing the biomass productivity and astaxanthin accumulation), but also to the avoidance of oversizing problems (and resulting inefficiency) associated with laboratory equipment.

Other stages such as the preparation of the culture medium (S2) or the extraction (S5) show a moderate reduction of their relative contributions, probably due to the optimization of the process and the different technologies applied. Hence, the total environmental impacts for S2 and S5 associated with the pilot astaxanthin production decrease up to 90% with respect to the lab scenario and between 48% and 95% with respect to the semi-pilot process (except S2 for AP, with a 9% reduction).

**a) Relative contributions of pilot system per stage (chemical sterilization)**




**b) Relative contributions of pilot system per involved process (chemical sterilization)**

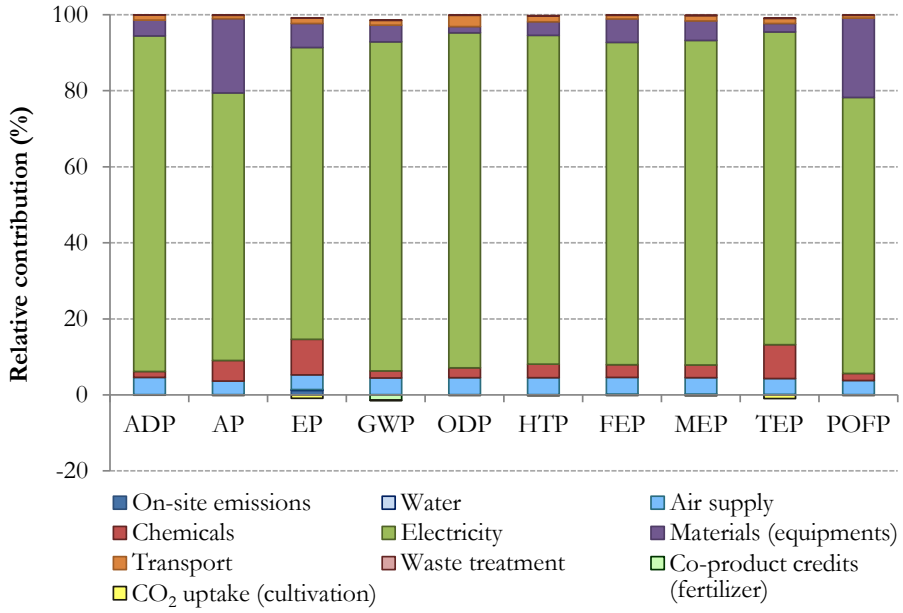
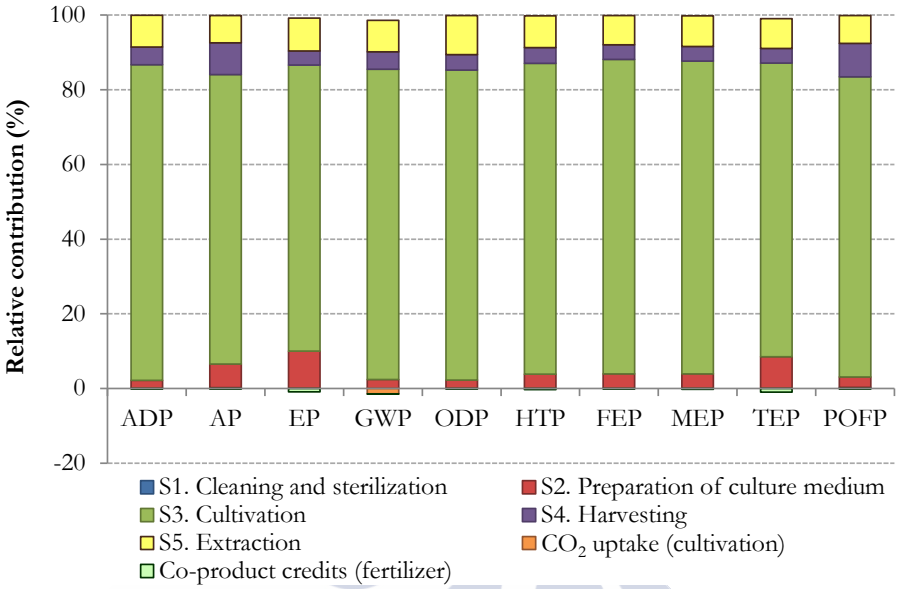



**Figure 4.7.** Relative contributions of the pilot-scale production of astaxanthin by *H. pluvialis* with chemical sterilization to each impact category per a) stage and b) involved process.

**a) Relative contributions of pilot system per stage (ozone sterilization)**




**b) Relative contributions of pilot system per involved process (ozone sterilization)**

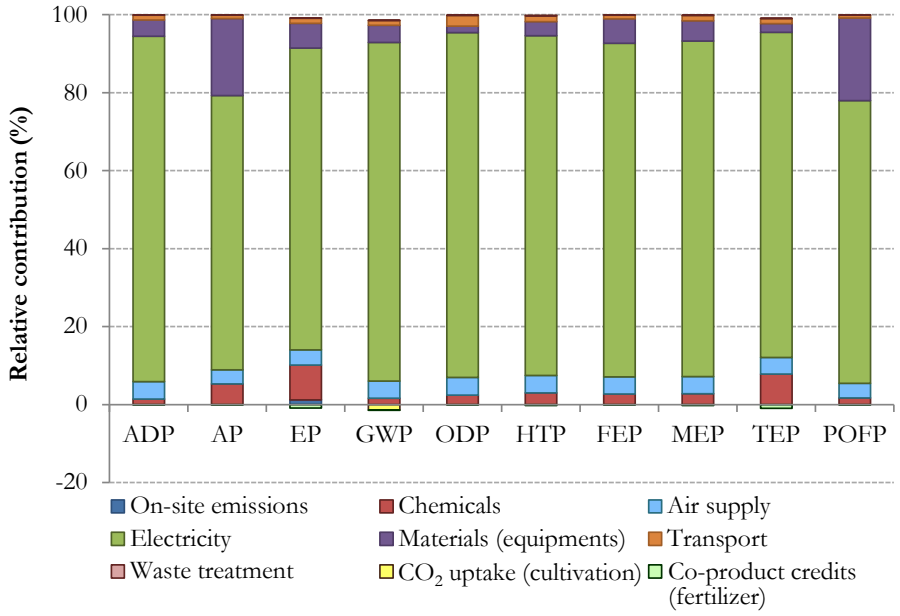



**Figure 4.8.** Relative contributions of the pilot-scale production of astaxanthin by *H. pluvialis* with ozone sterilization to each impact category per a) stage and b) involved process.

Particularly, supercritical technology has already been highlighted as a less energy-consuming separation alternative than organic solvent extraction (Aresta et al., 2005). Regarding this issue, Brentner et al. (2011) found an energy demand 4.5 times higher for hexane extraction than the corresponding value for supercritical $CO_2$, whereas supercritical methanol extraction allowed an additional reduction in energy consumption, 5% lower than that of supercritical $CO_2$. In this case, the environmental impacts of the extraction stage in the pilot process are about 50 times lower than in the lab process for most categories. The highest reduction (99% less impact of S5 in the pilot system) corresponds to the category of AP. The main reason may be the substitution of DMSO, associated with acidifying emissions of $H_2S$ during the production process.

Similarly to the previous systems, the production of electricity is the main hot spot in all categories (70%-89% contributions). Although these relative contributions are well above those of the lab scale, they are slightly lower than for the semi-pilot system. This may indicate a moderate improvement of the efficiency due to the optimization of the two-stage process. Other processes with considerable contributions in the lab-scale scenario show remarkable reductions. For example, the production of materials had a relative contribution to ADP of 33% in the lab-scale system that decreases to 4% in the pilot system. This may be linked, to some extent, to the longer life span of materials for the equipment in a large scale facility compared to that of lab-scale devices. The production of chemicals also exhibits a remarkable reduction of its relative contributions in the category of AP, which was associated to 34% of the impact in the lab process and is responsible for only 5% in the pilot process). The effect of waste treatment on FEP and MEP is reduced from contributions of 28% and 24% in the lab scenario to less than 0.1% of impacts in the pilot scenario.

The main substances affecting the categories are similar to the contributors of the previous scenarios. Thus, $SO_2$ emissions are the main cause of AP and POFP, whereas phosphates to water and $NO_x$ to air are responsible for EP. Most GWP is linked to $CO_2$ from electricity production. Although the $CO_2$ sequestration potential was considered, the results show a limited capability (only 1%) to reduce the impact. Halon 1211 is the main contributor to ODP. Emissions of metals are related to the contributions in all the toxicity categories.

### 4.2.4. Discussion and recommendations

According to the results shown in the previous section, the production of electricity required within the whole life cycle of the astaxanthin production at lab and pilot scale dominated the environmental burdens in all the impact categories. Several processes involved in the lab-scale process had significant contributions in some specific categories (e.g. materials affected considerably to ADP and POFP, waste treatment had a relevant impact in FEP and MEP). Nevertheless, the only secondary process that had relevant contributions to some categories in the case of pilot-scale system was the production of materials for the equipment, which affected to AP and POFP.

The findings of this study are consistent with the outcome of other works related with high value added products from microalgae (Pérez-López et al., 2014a; Pérez-López et al., 2014b), presented in Chapter 3. In the mentioned studies, cultivation stage was already identified as a key factor in terms of environmental impacts, and electricity was highlighted as one of the hot spots. However, the influence of electricity for these simulated pilot scale processes is relatively more limited than the contributions found in the production of astaxanthin. There are several reasons behind this difference. Firstly, the simulated scenarios used extrapolated data from studies that are based on the production of biofuels in outdoor systems with simple configurations that commonly have a much lower electricity consumption. Moreover, the production of astaxanthin is a particularly energy intensive process, since it requires strong light stress conditions (Aflalo et al., 2007; Katsuda et al., 2006).

No straight-forward comparison between the results from this study and available literature from other authors can be made due to the lack of reports regarding the production of high value molecules. Up to date, the related papers on microalgal LCA aim at the identification of the environmental performance of biodiesel production from microalgae (Brentner et al., 2011; Lardon et al., 2009; Stephenson et al., 2010), which is performed under different conditions and has a much lower value than the compounds analyzed in this thesis.

❖ **Sensitivity assessment of electricity requirements**

According to Stephenson et al. (2010) and Jorquera et al. (2010), the choice of cultivation system (e.g. air-lift tubular bioreactor, raceway pond…) considerably influences the environmental results associated to microalgal production (specifically in terms of energy requirements and GWP). This is mainly due to the energy-intensive nature of the cultivation, which is the stage over the life cycle of biodiesel production with the highest requirements (cradle-to-grave perspective).

**Figure 4.9** shows a breakdown of the contribution of the electricity for all the stages of the production process that have consumption higher than 1% of the total. According to the results, the two step approach for cultivation is responsible for the highest ratios of the environmental burdens derived from the electricity production, due to the high light intensity requirements. Both stages need 82% of the total electricity consumption (20% associated to the growth stage and 62% for the stress stage), while any of the other stages contributes less than 10% to the impact.

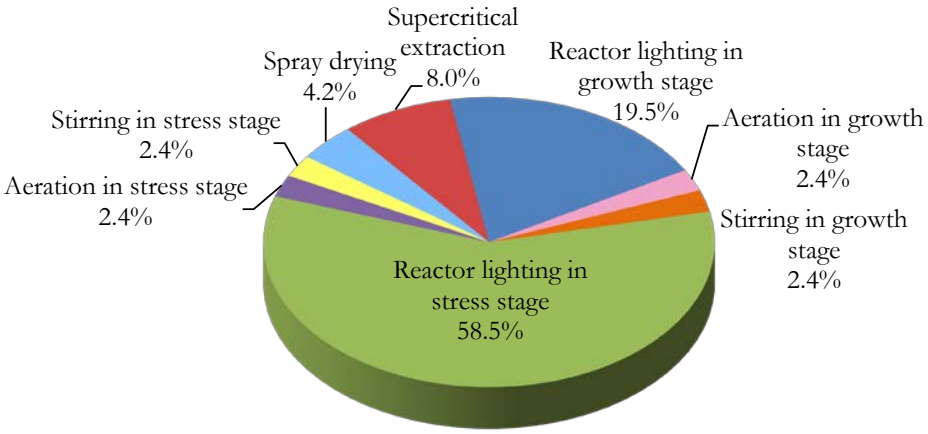



**Figure 4.9.** Relative contributions of the different steps to the total electricity requirements of the pilot-scale production of astaxanthin by *H. pluvialis*.

It could be possible to propose improvement alternatives in order to reduce the electricity requirement in the bioreactor. However, not only the electricity requirement must be taken into account in a decision process but also other important variables such as water consumption, contamination risks, light utilization rate as well as the production yield and capacity. This is the case of the ORPs which commonly require less electricity but present lower culture productivity.

Obviously, for practical, economic and environmental reasons, sunlight is preferred in extensive systems (Pruvost et al., 2011). In this pilot-scale process, two airlift PBRs were used for the experiment using artificial illumination. The use of solar illumination could be a cheap alternative although it presents limitations due to the diurnal fluctuations of light intensity that may result in a decrease in the total biomass concentration as high as 35% (Chen et al., 2011; Ogbonna et al., 1999).

Specifically in Ireland, algae can only be produced outdoors for less than five months a year and the installation should be moved indoors and use artificial lighting to work all the year. Moreover, it is important to point out that the number of days required to obtain the same amount of microalgae cell paste under solar light conditions are considerably higher than under artificial illumination since the growth of microalgae and the composition of biomass are strongly dependent on the light supply (light source and light intensity) (Ogbonna and Tanaka, 2000; Yeh et al., 2010). For these reasons, closed controlled indoor photobioreactors illuminated with artificial light are being currently applied for high value products including astaxanthin (Lorenz and Cysewski, 2000; Patil et al., 2008).

Several related studies have analyzed and compared differences on energy requirement and operational parameters between different types of PBRs under solar radiation (Brentner et al., 2011; Jorquera et al., 2010; Sierra et al., 2008). Based on these results, a sensitivity assessment was carried out considering other alternatives for the PBRs used here, which have been considered as internally-illuminated annular photobioreactors according to productivity and electricity requirements. In this study, artificial illumination is supplied to the PBR by means of fluorescent bulbs for 8 days and 16 $h \cdot day^{-1}$ for the growth stage and

for other 8 days and 24 $h \cdot day^{-1}$ for the stress stage. According to Brentner et al. (2011), reductions up to 96% in the energy consumption can be achieved if flat-panel PBRs are used instead of annular PBRs when solar radiation is used as light source and considering the same amount of biomass production. Moreover, a reduction in the biomass and astaxanthin yields is also considered, taking into account the highest residence times required in a PBR under sunlight conditions in comparison with artificial light in order to obtain identical levels of biomass (Pruvost et al., 2011). Since the exposure to solar light is only diurnal (~10 hours per day), it was assumed a reduction on the biomass production for the same period of time as in annular PBRs under artificial light (8 days for each cultivation stage) of 50% for each of the annular PBRs under sunlight. Based on Brentner et al. (2011), algal cultivation in flat-panel PBRs require the same residence time than in annular PBRs under identical conditions. Consequently, it was assumed the same production of biomass in both annular and flat-panel PBRs.

Therefore, in the sensitivity assessment, annular and flat-panel PBRs with sunlight are proposed as potential alternatives to the artificially illuminated PBRs, assuming no differences in other LCI data (Brentner et al., 2011). The four evaluated options are:

i) Sc 1: baseline annular PBRs with artificial light in growth and stress stages and 800 g of astaxanthin production
ii) Sc 2: annular PBRs with sunlight in growth and stress stage and 400 g of astaxanthin production
iii) Sc 3: flat-panel PBRs with artificial light in growth and stress stages and 800 g of astaxanthin production
iv) Sc 4: flat-panel PBRs with sunlight in growth and stress stages and 400 g of astaxanthin production

Although longer lifetimes for the reactor materials are expected for the flat-panel PBRs (50 years in comparison with 40 years considered for the annular PBRs) as well as larger required areas for similar biomass yield, these values have not been taken into account due to their minimal contribution to most impact categories.

**Figure 4.10** shows the comparative environmental result per impact category and PBR models assumed. According to the results, all the proposed scenarios would allow significant environmental benefits with respect to the case study. AP is the category with the lowest impact reductions, whereas GWP is associated with the most significant improvement.

In the first alternative scenario, in which the use of annular PBRs was considered and artificial illumination was substituted by sunlight illumination, significant improvements were obtained, ranging between 15% (for AP) and 46% (GWP). In the case of selecting a flat-panel configuration with sunlight illumination, reductions of impact could be from 29% to 64. The ideal situation would be the use of two flat-panel PBRs with artificial illumination, which would permit a decrease between 62% and 79% of the impacts depending on the evaluated category.

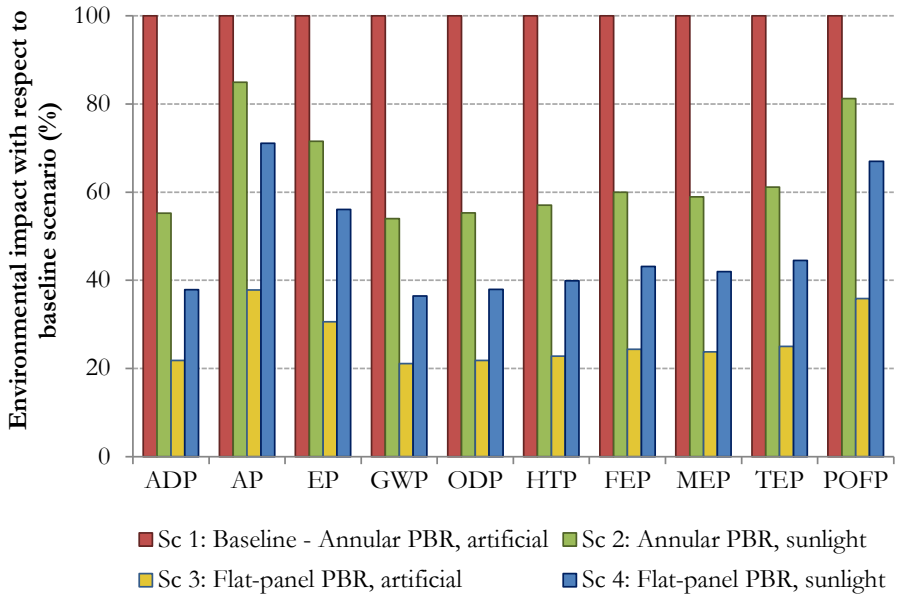



**Figure 4.10.** Sensitivity analysis of the environmental performance considering four different configurations for the two PBRs: annular and flat-panel PBRs with artificial or solar illumination.

## 4.3. Socio-economic assessment

According to the internationally accepted global nature of sustainability, the evaluation of economic and social dimensions is essential to attain a holistic assessment of systems. This approach may contribute to the decision-making process with a balanced combination of criteria that include the three pillars (Jiménez-González and Woodley, 2010; Paracchini et al., 2011). For this reason, a set of social and economic indicators are following proposed to reflect the global sustainability of the astaxanthin production process previously analyzed in environmental terms.

### 4.3.1. Social assessment

The social dimension is frequently considered as the weakest pillar of sustainable development, as reflected by the limited analytical and theoretical tools for its evaluation (Lehtonen, 2004). Until recently, accounting for social issues has been a challenge for practitioners, due to the qualitative nature of most social aspects, the absence of consensus on relevant criteria (Von Geibler et al., 2006), as well as the lack of data, models and tools (Benoît Norris, 2014).

In order to develop a standardized methodology, UNEP-SETAC published the Guidelines for Social Life Cycle Assessment of Products, together with The methodological sheets for subcategories in Social Life Cycle Assessment (SLCA) (UNEP-SETAC, 2009,2013). The methodological sheets contain all the necessary information to collect data for 31 defined impact subcategories (analogous to relevant characterized social issues) used to classify the social impacts within five categories of stakeholders, which are listed in **Table 4.6**. The given information includes a definition of each subcategory and an explanation of issues associated with it, as well as examples of inventory indicators, units of measurement and data sources (UNEP-SETAC, 2013).

**Table 4.6.** List of impact subcategories for social LCA associated with each stakeholder category

| Stakeholder category | Social impact subcategory |
|---|---|
| 1. Workers | Freedom of association and collective bargaining |
| | Child Labor |
| | Fair Salary |
| | Working Hours |
| | Forced Labor |
| | Equal opportunities/Discrimination |
| | Health and Safety |
| | Social Benefits/Social Security |
| 2. Consumers | Health & Safety |
| | Feedback Mechanism |
| | Consumer Privacy |
| | Transparency |
| | End of life responsibility |
| 3. Local community | Access to material resources |
| | Access to immaterial resources |
| | Delocalization and Migration |
| | Cultural Heritage |
| | Safe & healthy living conditions |
| | Respect of indigenous rights |
| | Community engagement |
| | Local employment |
| | Secure living conditions |
| 4. Society | Public commitments to sustainability issues |
| | Contribution to economic development |
| | Prevention and mitigation of armed conflicts |
| | Technology development |
| | Corruption |
| 5. Value chain actors (excluding consumers) | Fair competition |
| | Promoting social responsibility |
| | Supplier relationships |
| | Respect of intellectual property rights |

Since social and socio-economic mechanisms can take different forms, there are three possible types of indicators to evaluate socio-economic issues that depend on the goal of the study: quantitative, semi-quantitative and qualitative indicators. Quantitative indicators describe the analyzed issue based on numbers, whereas qualitative indicators describe an issue using words. Finally, semi-quantitative indicators categorize qualitative indicators into a Yes/No form or a scoring system (UNEP-SETAC, 2009).

Once the indicators are selected and the inventory data are gathered, the impact assessment is carried out in a similar way as the methodology of the environmental LCA. Thus, the inventory results are assigned to a specific stakeholder and impact category (classification step) and the indicator results are converted to common units and aggregated within particular impact categories (characterization step).

❖ **Inventory analysis**

In this case, the methodological sheets were taken as a basis to perform the social assessment of the *H. pluvialis* astaxanthin production. Thus, a specific questionnaire was developed, dealing with key issues and possible indicators related to the subcategories that were relevant for the scope of the assessment. The questionnaire was fulfilled by two small and medium enterprises (SMEs): AlgaeHealth (Ireland) and Greensea (France)[2].

After the collection of data, a set of the most characteristic indicators for the considered scope and framework was selected and analyzed in detail. According to the specific context of microalgal processes developed by SMEs, three stakeholder groups were considered as the most representative: workers, consumers and society. For each of them, the selected indicators were grouped into subcategories and measured in quantitative or semi-quantitative terms. All the subcategories and indicators considered in the assessment are listed in **Table 4.7**.

[2] For confidentiality reasons, the companies are referred to as SME 1 and SME 2 in the results section.

**Table 4.7.** List of impact subcategories for social LCA associated with each stakeholder category

| Stakeholder category | Subcategory | Indicator | Type of indicator |
|---|---|---|---|
| 1. Workers | Equal opportunities | Women-to-man ratio of labor force participation | Quantitative |
| | | Women-to-man-ratio of salary (for similar work) | Quantitative |
| | Fair salary | Annual salary | Quantitative |
| | | Women-to-man-ratio of salary (for similar work) | Quantitative |
| | Working hours | Total working hours per week | Quantitative |
| 2. Consumers | Health & Safety | Tests performed to check safety | Semi-quantitative |
| | | Fulfilled existing regulations | Semi-quantitative |
| | | Safety data sheet provided | Semi-quantitative |
| | Transparency | Information on the formulation, use and effects | Semi-quantitative |
| | | Customer service | Semi-quantitative |
| | | Number of complaints related to lack of transparency | Semi-quantitative |
| | Benefits of the product | Value added of the product (according to its applications) | Semi-quantitative |
| | | Product from natural source | Quantitative |

**Table 4.7.** List of impact subcategories for social LCA associated with each stakeholder category (*Cont.*)

| Stakeholder category | Subcategory | Indicator | Type of indicator |
|---|---|---|---|
| 3. Society | Contribution to economic development | Importance of pharmaceutical sector in the country | Semi-quantitative |
| | | Importance of marine biotechnology in the country | Semi-quantitative |
| | | Potential market share of the company | Semi-quantitative |
| | Public commitments to sustainability issues | Available certification about sustainability issues | Semi-quantitative |
| | | Certification regarding safety of the company | Semi-quantitative |
| | | Signed principles or codes of conduct related to sustainability | Semi-quantitative |
| | | Performed environmental assessments (LCA, risk assessment...) | Semi-quantitative |

As in previous social LCA works (Benoît Norris et al., 2012; Dreyer et al., 2010), each indicator was expressed according to a numeric index based on the risk level in order to better understand the social impact information. In this case, the index for each indicator ranged from 1 to 4, being 1 the corresponding value for the worst scenario (highest risk) and 4 the index for the ideal scenario (no risk at all).

In the case of stakeholder "workers", all the selected indicators were quantitative. The index for each indicator was calculated with respect to minimum and maximum risk levels in the world according to the values reported by OECD (2015), Statista (2015) and the World Economic Forum (2013). For each subcategory, the index was then obtained as the average index of the set of indicators assigned to this subcategory. For the stakeholders "consumers" and "society", most indicators had a Yes/No format; therefore, all the impacts related to these subcategories were converted into semi-quantitative terms through a scoring system. For Yes/No indicators, a value of 1 was

assigned to negative response and a value of 4 was considered for affirmative response. In the case of some specific indicators, intermediate values were assigned according to expertise knowledge. Finally, the results per subcategory were depicted in a spider chart to obtain a visual representation and identify the hot spots or most problematic social aspects of the process.

❖ **Results of the social impact assessment**

The indexes for the selected social indicators of each stakeholder category are listed in **Tables 4.8**, **4.9** and **4.10** for the two SMEs. As shown in **Figure 4.11**, the results of the social impact assessment show the profiles for both SMEs, with most indexes near from the maximum possible value. However, the outcome significantly differs depending on the strategic management of the company, although this deviation is highly dependent on the considered stakeholder category. Thus, while the performance related to workers and consumers show quite different profiles for the two companies, the subcategories related to society present a similar behavior. This is due to the specific characteristics of the corresponding indicators and subcategories that are explained below.

**Table 4.8.** Social indexes for the selected indicators in the stakeholder category "workers"

| Subcategory | Indicator | Social index | |
|---|---|---|---|
| | | SME 1 | SME 2 |
| Equal opportunities | Women-to-man ratio of labor force participation | 2.78 | 1.56 |
| | Women-to-man-ratio of salary (for similar work) | 4.00 | 4.00 |
| Fair Salary | Annual salary | 3.58 | 3.39 |
| | Women-to-man-ratio of salary (for similar work) | 4.00 | 4.00 |
| Working Hours | Total working hours per week | 4.00 | 3.86 |

**Table 4.9.** Social indexes for the selected indicators in the stakeholder category "consumers"

| Subcategory | Indicator | Social index | |
|---|---|---|---|
| | | SME 1 | SME 2 |
| Health and safety | Tests performed to check safety | 4.00 | 4.00 |
| | Fulfilled existing regulations | 4.00 | 1.00 |
| | Safety data sheet provided | 4.00 | 4.00 |
| Transparency | Information on the formulation, use and effects | 4.00 | 4.00 |
| | Customer/Quality service | 1.00 | 2.50 |
| | Number of complaints related to lack of transparency | 4.00 | 4.00 |
| Benefits of the product | Value added of the product (according to its applications) | 3.50 (*) | 3.50 (*) |
| | Product from natural source | 4.00 | 4.00 |

** Use as nutraceutical considered for the obtained oleoresin, index of 4.00 would correspond to the use as pharmaceutical*

**Table 4.10.** Social indexes for the selected indicators in the stakeholder category "society"

| Subcategory | Indicator | Social index | |
|---|---|---|---|
| | | SME 1 | SME 2 |
| Contribution to economic development | Importance of pharmaceutical sector in the country | 3.50 | 4.00 |
| | Importance of marine biotechnology in the country | 4.00 | 3.50 |
| | Potential market share of the company | 1.50 | 1.50 |
| Public commitments to sustainability issues | Available certification about sustainability issues | 1.00 | 2.00 (in progress) |
| | Certification regarding safety of the company | 1.00 | 1.00 |
| | Signed principles or codes of conduct related to sustainability | 1.00 | 1.00 |
| | Performed environmental assessments (LCA, risk assessment) | 4.00 | 4.00 |

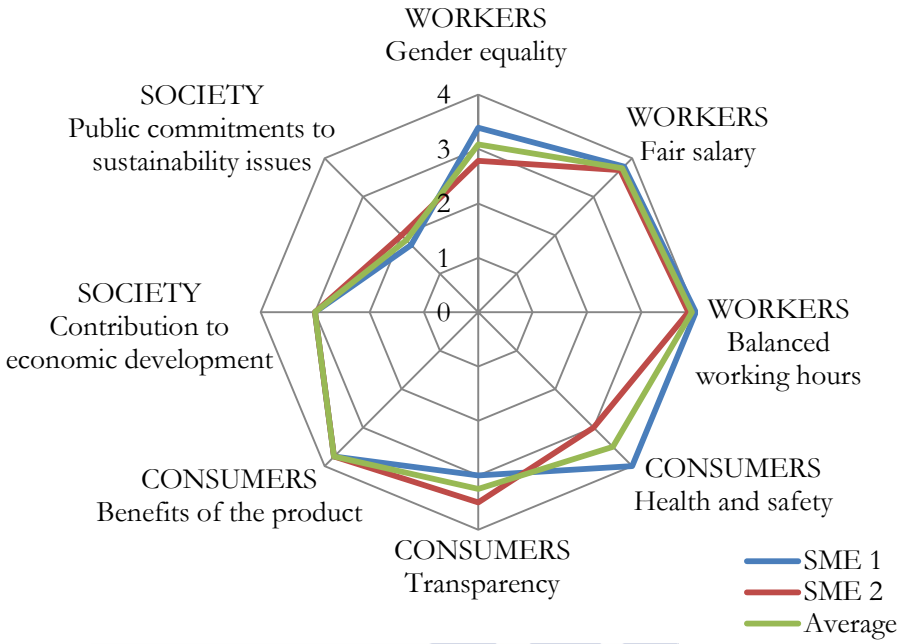



**Figure 4.11.** Radar chart representing prominent social issues of the corporate strategy of two small and medium enterprises (SMEs) involved in the production of *H. pluvialis* astaxanthin.

i) Workers

In the case of the subcategories related to workers, SME 1 shows some slight strengths, especially in terms of gender equality. The reason for the difference between the two companies is mainly linked to the indicator "women-to-men ratio". Although both of them are below the world average ratio of female labor force (0.73), SME 1 has 40% of female employees, whereas only 25% of workers at SME 2 are women. This is probably related to the specific characteristics of the industrial sector, which traditionally employs a larger number of male workers. In addition, it shall be highlighted the small size of the companies: as only 5 and 8 employees work for SME 1 and SME 2 respectively. With so limited staff, it is more difficult to maintain balanced gender equality than in large companies. On the other hand, both companies have equal salary, which corresponds to a social index of 4 (no risk of discrimination), regardless of the gender of the worker.

Furthermore, the indicator of women-to-men salary ratio affects not only the subcategory of gender equality, but is also used to measure the level of fair salary. This subcategory has a very good score in both companies, although the performance of SME 1 shows a slightly higher value. Indeed, the average salaries in both companies expressed in terms of purchasing power parity[3] (around $40,000) are well above the world average ($18,500).

With respect to the working hours, the two companies are in the optimum level. In both cases, the total working hours are below the average value in the same country. Thus, the schedule in SME 1 is up to 3 h lower than the national average (40.5 h), whereas the working hours in SME 2 (39 h) are nearly 2 h lower than the average in the country (41 h). Indeed, the total weekly working hours in SME 1 is even lower (37.5 h) to the minimum value per country, which corresponds to Denmark (38.3 usual weekly working hours for full-time employment), according to the Organization for Economic Co-operation and Development (OECD, 2015).

Besides the mentioned quantitative indicators, other important issues should be considered in qualitative terms when analyzing the social impacts related to workers. Among these aspects, social benefits to workers such as sick leaves or medical insurance as well as education and training programs at the companies and the implementation of policies to minimize occupational accidents can result in remarkable reductions in the social impacts of the company. Although these policies are probably more common in large corporations, their consideration in early stages of development of the company may facilitate to incorporate those criteria to the philosophy of the firm and help to improve the quality of the management strategy once it is fully consolidated.

[3] *Purchasing power parity* (PPP): specific economic term to refer the salary of a country in equivalent PPP $ to take into account the different values of basic goods and thus give representative data of the life quality that a salary allows in a specific country. The value is obtained by dividing the real salary in the specific currency of the country by the purchasing power parity factor, that is 0.94 for Ireland and 0.89 for France (Source: The World Bank, 2015).

ii) Consumers

The results obtained for the stakeholder "consumers" are the most dependent on the specific company. Thus, whereas SME 1 clearly makes the highest effort in health and safety issues, SME 2 is slightly above in terms of transparency. The reason for the difference in the first subcategory is that SME 1 indicates the fulfillment of specific European regulations affecting the product that SME 2 has not reported.

On the contrary, regarding transparency SME 2 mentions the presence of a quality service to solve consumer's incidents, although this service has not been valuated with the maximum score due to the lack of knowledge about the offered services. Other indicators related to transparency (information on the formulation through safety data sheets, no complaints related to lack of transparency) show a good performance for both companies.

As expected, one of the most outstanding social aspects of the product is linked to its benefits for the consumers. With this regard, both companies present exactly the same index, since they produce the same compound to be used in equivalent applications. In this case, the major potential use considered is as nutraceutical, according to the properties of the obtained product. For this reason, the assigned index to the indicator associated with the value of the product was slightly lower than the maximum value, which would correspond to its use as pharmaceutical. Nevertheless, the benefits linked to its natural origin were recognized with the highest possible score.

iii) Society

The benefits of the processes in the society probably constitute the weakest aspect of the analyzed companies. The reasons for the lower indexes in these subcategories are not linked to an insufficient effort from the assessed companies but mainly to the limited size of both SMEs and, in the case of SME 1, to its recent creation (it was established in 2009).

In terms of contribution to economic development, the highest scores correspond to the importance of pharmaceutical and marine biotechnology sectors in the countries. Regarding the pharmaceutical industry, France and Ireland are among the largest producers in Europe (Finesco, 2012). Moreover,

France also has one of the highest consumption of medicines in the world. With respect to marine biotechnology, both countries are well-positioned thanks to their strategic location.

Indeed, they have already recognized the importance of marine sector as a strategic field on a national scale. Thus, a recent report from the French Ministry of Higher Education and Research (MESR) points out the use of marine sources in the development of applications for healthcare and cosmetic sectors as well as for energy-related processes (MESR, 2013). In the case of Ireland, the national strategy related to marine biotechnology is even more developed and was launched earlier, as reflects the report "Sea change – A marine knowledge, research & innovation strategy for Ireland 2007-2013" from the Marine Institute (2006).

The most limited benefit of the two companies related to the contribution to economic development is their low potential market share, due to their small size. However, if the business volume increases in the next years, both SMEs will achieve a very advantageous position that could result in a significantly positive effect for the local community in particular and for the society as a whole.

The weakest corner of the radar chart corresponds to the subcategory of "public commitments to sustainability issues". There is still an important lack of certifications and codes of conduct of the two SMEs related to sustainability issues and safety of the company, although some valuable steps towards the accomplishment of this aspect have already taken place. In this sense, SME 2 is currently working on a certification about sustainability issues, whereas both companies have collaborated to carry out the sustainability assessment of their processes by applying LCA methodology. These assessments have allowed determining the main hot spots of the processes in order to focus the optimization of the technologies on the most problematic aspects.

### 4.3.2. Economic evaluation

As economic aspects cannot be neglected in life cycle based sustainability assessment, a Cost-Benefit approach is here proposed to evaluate this dimension. Cost-Benefit Analysis (CBA) is a basic decision-making tool included by Huppes et al. (2004) among the methods to address the economic dimension of sustainability. It allows the comparison between different proposals according to the net profit of each option. As the integration of both CBA and LCA is now being widespread for the combined assessment of economic and environmental aspects (Birol et al., 2010; Molinos-Senante et al., 2011; Morrissey and Horne, 2011; Weidema, 2006), this methodology has been selected for the evaluation of astaxanthin production process.

CBA aims to compare the economic feasibility of a project or process by taking into account the costs and benefits over its life time (Huppes et al., 2004; Molinos-Senante et al., 2011). The starting point of the tool is the premise that a project should only be developed if all the benefits exceed the aggregated costs. This premise is checked out by considering the net profit of a process as the difference between benefits and costs:

$$NP = \sum B_i + \sum C_i \quad (1)$$

Where $NP$ is the net profit, $B_i$ is the value of the benefit item i and $C_i$ is the value of the cost item i. Thus, if the result of the calculation is NP>0, then the project is economically viable, whereas if NP<0 the project is not viable in economic terms.

The implementation of CBA requires that all benefits and costs are expressed in the same units. In projects related to environmental issues (e.g. operation of wastewater treatment plants), this restriction may require a complex homogenization method for the quantification in monetary terms. However, in the case of the addressed process, the only benefit corresponded to the production of a high value molecule with biological properties, so the benefits could be measured in the same units as for costs (monetary units) and no method of homogenization was needed.

Firstly, the assessment followed the CBA approach proposed by Molinos-Senante et al. (2011), and considered only the variable operating costs of the process in terms of energy consumption, chemicals, staff and waste management in comparison with the benefit (expressed as total revenue from the product).

In the second stage of the analysis, a more global perspective was taken into account to conduct a study of economic feasibility throughout the whole life time of the process. In this step, four different types of costs were included:

i) Investment costs, including machines and expensive tools necessary for the operation of the plant.

ii) Overhead costs, related to renting activities, insurances, travel costs, taxes and interests...

iii) Variable operating costs (already considered in the previous stage) associated with the consumption of water, chemicals and other raw materials (material costs), as well as energy, operating labor costs, and also disposal costs.

iv) Research and development costs (calculated in relation to revenue).

With this information, the economic feasibility of the process was evaluated by considering two traditional parameters: the net present value (NPV) and the pay-back period.

$$\text{Total } NPV \text{ of the project} = \sum_{n=1}^{n=t} \frac{\text{Estimated cash flow in year t}}{(1+r)^n} \qquad (2)$$

Where "*n*" is the number of years of analysis and "*r*" the discount rate. The determination of cash flows was carried out according to the algorithm shown in **Table 4.11**.

**Table 4.11.** Algorithm for the calculation of cash flows in the determination of the net present value of a project

| |
|---|
| (+) Revenue |
| (-) Variable operating costs |
| (-) Overhead costs |
| (-) Amortization |
| **BENEFITS BEFORE TAXES** |
| (-) Taxes |
| **BENEFITS BEFORE INTERESTS** |
| (-) Interests |
| **NET BENEFIT** |
| (+) Amortization |
| (-) Investment |
| **NET CASH FLOW** |

Regarding the pay-back period of a project, this indicator is defined as the period of time during which a facility must operate to recover the initial investment, according to the total capital costs and the estimated annual profits (Bakos and Tsagas, 2003; Ibáñez-Forés et al., 2013; Sánchez et al., 2012). It is determined by accumulating the annual profits until an equal value to the capital sum invested is obtained (Bakos and Tsagas, 2003).

### ❖ Inventory analysis

The economic assessment is based on information from the same astaxanthin producers, which was gathered in a specific questionnaire. The collected data included the four groups of costs previously listed (investment costs, overhead costs, variable production costs and R&D costs), together with the final revenue. The values are summarized in **Table 4.12**.

**Table 4.12.** Summary of production costs and outcome for the operation of a producer of *H. pluvialis* astaxanthin (expressed on an annual basis)

| **Type of cost** | **Value** |
|---|---|
| Investment costs | 500,000 € |
| Overhead costs | |
| - Rent | 34,000 € |
| - Others (insurance, taxes, travel costs…) | 84,000 € |
| Variable production costs | |
| - Materials | 5,000 € |
| - Energy | 53,500 € ([1]) |
| - Operating labor | 200,000 € ([2]) |
| - Disposal costs | 2,000 € |
| Research and development costs (13% total revenue) | 156,000 € |
| **Revenue** | 1,200,000 € |

[1] Estimated from a consumption of 2478 kWh·kg$^{-1}$ according to the life cycle inventory, considering the production of 120 kg·year$^{-1}$ and a price of 0.18 €·kWh$^{-1}$.

[2] Staff of 5 workers, with a salary of 40,000 € per person.

### ❖ Results of the cost-benefit analysis

According to the first CBA approach, and considering the variable operating costs, the annual net profit corresponds to:

$$NPV = 1{,}200{,}000\ € - (5{,}000\ € + 53{,}500\ € + 200{,}000\ € + 2{,}000\ €) = 939{,}500\ € \quad (3)$$

Since the obtained NP>>0, the process would be economically feasible. Among the different groups of costs, the variable production costs are logically the main contribution to the process in economic terms and are responsible for up to 75% of the total cost. As shown in **Figure 4**, the highest fraction of these variable operating costs corresponds to the staff, which represents more than three fourths of the production costs. Among the other items, energy consumption would be the most relevant cost, with 20% of the total.

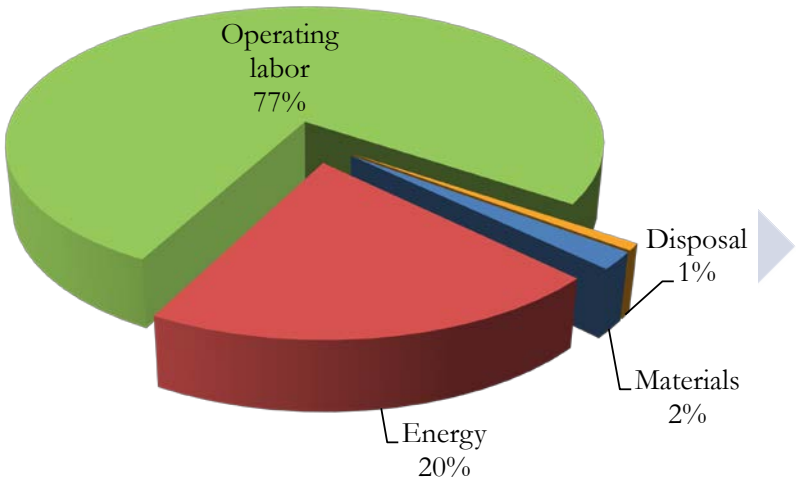



**Figure 4.12.** Relative distribution of the variable operating costs.

Regarding the global feasibility assessment, all the cash flows are detailed in **Table 4.13**, considering a 12.5% discount rate, with a 1.7% inflation rate (average inflation rate in the country for the year 2012). According to the determined cash flows, under the assumed conditions the obtained NPV is 2,068,203 €, which means that the assessed process would be economically feasible.

**Table 4.13.** Annual cash flows for *H. pluvialis* astaxanthin production process

| YEAR | Initial nvestment | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 | Year 6 | Year 7 | Year 8 | Year 9 | Year 10 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (+) Revenue | | 1,200,000 | 1,220,400 | 1,241,147 | 1,262,246 | 1,283,704 | 1,305,527 | 1,327,721 | 1,350,293 | 1,373,248 | 1,396,593 |
| (-) Variable operating costs | | 260,500 | 264,929 | 269,432 | 274,013 | 278,671 | 283,408 | 288,226 | 293,126 | 298,109 | 303,177 |
| (-) Overhead costs | | 274,000 | 278,658 | 283395 | 288,213 | 293,113 | 298,095 | 303,163 | 308,317 | 313,558 | 318,889 |
| (-) Amortization | | 42,500 | 43,223 | 43,957 | 44,705 | 45,465 | 46,237 | 47,023 | 47,823 | 48,636 | 49,463 |
| **BENEFITS BEFORE TAXES** | | 623,000 | 633,591 | 644,362 | 655,316 | 666,457 | 677,786 | 689,309 | 701,027 | 712,944 | 725,064 |
| (-) Taxes | | 203,175 | 206,882 | 210,652 | 214,486 | 218,385 | 222,350 | 226,383 | 230,484 | 234,656 | 238,898 |
| **BENEFITS BEFORE INTERESTS** | | 419,825 | 426,709 | 433,710 | 440,831 | 448,072 | 455,436 | 462,926 | 470,543 | 478,289 | 486,167 |
| (-) Interests | | 25,000 | 25,425 | 25,857 | 26,297 | 26,744 | 27,198 | 27,661 | 28,131 | 28,609 | 29,096 |
| **NET BENEFIT** | | 394,825 | 401,284 | 407,853 | 414,534 | 421,328 | 428,238 | 435,265 | 442,411 | 449,680 | 457,071 |
| (+) Amortization | | 42,500 | 43,223 | 43,957 | 44,705 | 45,465 | 46,237 | 47,023 | 47,823 | 48,636 | 49,463 |
| (-) Inversion | 500,000 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **NET CASH FLOW** | | 437,325 | 444,507 | 451,810 | 459,238 | 466,792 | 474,475 | 482,288 | 490,234 | 498,315 | 506,534 |

Additionally, when considering the economic indicator of the pay-back period, **Figure 4.13** shows that one year and four months of operation of the facility, would be a sufficient period of time to recover the total initial investment according to the estimated costs and revenues from **Table 4.12**.

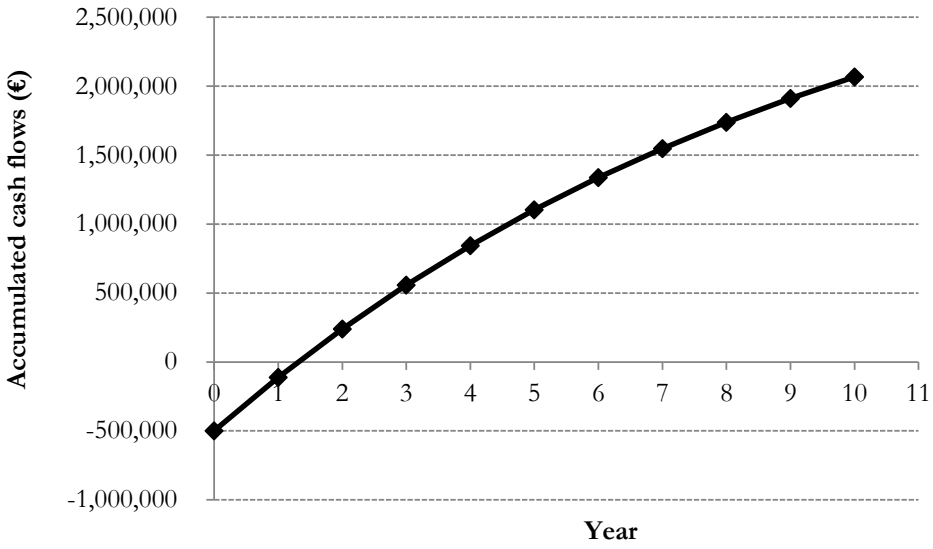



**Figure 4.13.** Relative distribution of the variable operating costs.

The results obtained for the two selected indicators (net present value and pay-back period) suggest that the production of astaxanthin from microalgae could allow significant economic benefits. Although the performed economic assessment is subjected to a considerable level of uncertainty, related to the estimation of the different costs and final revenue, it should be pointed out that all the evaluated indicators show a remarkable margin of error. Therefore, no significant change in the results is expected unless a dramatic variation in the conditions takes place. Moreover, additional co-products may be obtained from the residual algal paste in the future, increasing the potential revenues of the process. Hence, the cost-benefit analysis confirms that the production of astaxanthin by *H. pluvialis* could also be feasible from an economic perspective of sustainability.

## 4.4. Conclusions

Nowadays special interest is being paid to microalgal production for several reasons: sustainable energy, foodstuffs, industrial chemicals, pharmaceuticals and nutraceuticals production. The life cycle impacts of microalgal cultivation considerably depend on the production scale, according to the results presented in this study. Moreover, several remarkable improvements observed in the pilot process can be related to changes implemented in the system as a result of lab-scale environmental assessment, such as the substitution of compressed air supply or the use of supercritical $CO_2$ extraction as a more suitable separation technique. In the lab-scale process, several inputs affected the environmental profile, whereas in the case of pilot system, electricity dominated the contributions to all categories.

From an environmental perspective, the choice of the PBR for the algal cultivation stage is one of the most important environmental issues to be taken into account due to the large differences of electricity requirements, which can also affect some economic aspects of the process. Moreover, if the microalgae are cultivated in order to obtain a specific compound such as carotenoids, the extraction method considered, whether it is based on organic solvents or supercritical fluids, has significant influence on the environmental results.

Due to the high contribution of the electricity, a sensitivity assessment was proposed in order to identify the best reactor configuration for the *H. pluvialis* astaxanthin production system from an environmental point of view. According to the results, the scenarios based on the use of sunlight instead of artificial illumination allowed significant reductions of impact. However, the improvements observed in these cases were limited by the decrease in biomass productivity associated with sunlight culture systems. Therefore, the optimal production system would consist of two flat-panel PBRs with artificial illumination, which would allow reductions between 62% and 79% of the impact depending on the considered category. As this study included the evaluation of a pilot-scale process, the results allow the identification of specific environmental hot spots which are likely to affect the industrial scale processes and, thus, that must be solved before the implementation of the commercial process.

Moreover, the conducted socio-economic assessment allows the holistic evaluation of the future commercial process. The use of a wide range of indicators ensures the robustness of the assessment and demonstrates the feasibility of the processes according to social and economic criteria. In the social assessment, several strengths of the involved companies were identified, mainly related to workers and consumers. Moreover, the evaluation found some key issues that can potentially improve by adopting the appropriate managment strategies. With respect to the economic assessment, the considered approaches reflect a very profitable process, that is likely to compensate the initial investment in a relatively short period of time.

The combined outcome of the environmental, social and economic assessments suggests that the novel systems for the production of *H. pluvialis* astaxanthin constitute a valuable basis for the successful incorporation of sustainability criteria in the development of marine biotechnological processes. Technological advantages are rapidly occurring in the microalgae-related industries. The results of this study should be considered in order to produce in a more sustainable manner not only *Haematococcus* astaxanthin but also other microalgae-derived compounds.

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# Chapter 5

# Biocompounds from macroalgae[1,2]


***Summary***

The utilization of macroalgae currently comprises a large market and shows an increasing potential associated with their ability to produce bioactive molecules with similar applications to those of microalgae in pharmaceutical, food or cosmetics sectors.

The use of macroalgae as a renewable source for valuable compounds includes two approaches: wild harvesting and cultivation. While certain algae species such as the invasive brown seaweed *Sargassum muticum* (Yendo) can be collected from the natural environment and subjected to extraction processes for the desired molecules, others can be cultivated in photobioreactors under controlled conditions for maximum target compound production and biomass growth. The production of essential terpene oils with potential applications in food and pharmaceutical industries by the macroalga *Ochtodes secundiramea* is an example of the latter.

Both cases demonstrate the value of LCA as a decision-making tool, especially in the development of novel processes. The outcomes may serve to improve current techniques towards the optimal valorization of natural resources.


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[1] **Pérez-López P**, Balboa EM, González-García S, Domínguez H, Feijoo G, Moreira MT. Comparative environmental assessment of valorization strategies of the invasive macroalgae *Sargassum muticum*. Bioresource Technology 2014, 161:137-148.

[2] **Pérez-López P**, Jeffryes C, Agathos SN, Feijoo G, Rorrer G, Moreira MT. Environmental life cycle optimization of essential terpene oils produced by the macroalga *Ochtodes secundiramea* (Submitted for publication).

# Index

## 5.1. Applications of seaweed natural products

Macrophytic marine algae (known as macroalgae or seaweeds) have been extensively cultured and collected from natural aquatic habitats, especially in Asian countries, as a source of food and chemicals (Aresta et al., 2005; Rorrer and Cheney, 2004). Their utilization worldwide involves a multi-billion dollar industry, mainly related to the production of agar, carrageenan and alginate. Although most of the commercial exploitation is linked to hydrocolloids used in various industries for their gelling, water-retentive and emulsifive properties, new applications are receiving increasing attention (Smit, 2004).

One of these uses corresponds to the production of supplements for functional foods, associated with their richness in polysaccharides, minerals and certain vitamins, as well as other bioactive substances such as proteins, lipids and polyphenols with properties as antibacterial, antifungal and antiviral agents, among others (Andrade et al., 2013; Holdt and Kraan, 2011). Macroalgae are also well-established on the cosmetics market, including products such as anti-aging and regenerative creams, anti-irritants, sun protection and hair care products (Balboa et al., 2015).

Moreover, the biological activities are currently being investigated by pharmaceutical firms for the development of new drugs from natural products (Andrade et al., 2013; Smit, 2004). Besides the aforementioned antibacterial, antifungal and antiviral properties, macroalgae produce metabolites with anti-inflammatory, antimutagenic, antidiabetic, antihyperthensive, antilipidemic, antithrombotic, antitumor and antioxidant activities (Andrade et al., 2013; Jung et al., 2013). Among the most promising compounds are sulphated polysaccharides with antiviral properties, halogenated furanones from *Delisea pulchra* as antifouling compounds and kahalalide F from the green algae *Bryopsis* sp. as possible treatment of lung cancer, tumors and AIDS (Chennubhotla et al., 2013; Smit, 2004).

Currently, macroalgae are also considered an attractive renewable source for biofuel production with several advantages over terrestrial biomass (Wei et al., 2013). As in the case of microalgae, seaweed have superior photon conversion efficiency and can synthesize biomass from sunlight, $CO_2$ and inorganic

nutrients faster than terrestrial plants. They show high production yields per unit area and high rates of $CO_2$ fixation. In addition, their depolymerization is easier since they lack hemicellulose and lignin, which is present in terrestrial plants for structural support (Aresta et al., 2005; Wei et al., 2013).

As explained in Chapter 1, there are two approaches for macroalgal cultivation. One option corresponds to the vegetative cultivation, in which small pieces of algae are grown in aquatic systems under appropriate conditions (e.g. temperature, light, salinity, nutrients) and later harvested (Fasahati et al., 2015; McHugh, 2003; Wei et al., 2013). In other cases (e.g. *Laminaria* sp.), they must be cultivated by a separate reproductive cycle that requires the initial generation of embryonic sporophytes in land-based facilities under controlled conditions before the stage of vegetative cultivation (McHugh, 2003; Wei et al., 2013).

The main options for farming sites are offshore farms, near-shore coastal farms and land-based ponds. Although offshore farming has been successfully tested, its cost is still high. Near-shore farms are common in some countries (e.g. China and Japan), but pose some environmental concerns for which government regulations in other areas have prevented its use. Land-based pond systems present several advantages including the easy control of nutrients and conditions, as well as the possibility to integrate their production with other aquaculture species, while avoiding adverse weather, disease and predation. However, the use of these systems requires the optimization of technology and the reduction of construction costs in scaled-up systems (Wei et al., 2013).

After cultivation, algae can be harvested by conventional manual methods or recently developed harvesting systems for large quantities (Bruton et al., 2009; Wei et al., 2013). Finally, suitable extraction techniques need to be selected for the efficient separation and purification of target compounds, including conventional techniques based on heat or solvent use and novel options such as supercritical fluid extraction or pressurized liquid extraction (Kadam et al., 2013).

In order to evaluate the feasibility of macroalgal processes, the application of LCA methodology for the optimization of two novel systems is developed in the next sections, focused on the macroalgae *Sargassum muticum* and *Ochtodes secundiramea*.

## 5.2. Valorization of invasive seaweed *Sargassum muticum*

Invasive seaweed are currently considered a major threat to native species and ocean's resources worldwide (Schaffelke et al., 2006). The introduction of non-indigenous species may affect the existing habitats due to shifts in communities and trophic chains, which results in the decline of biodiversity and the alteration of the ecological stability of invaded ecosystems (Walker and Kendrick, 1998). Although biological invasion takes place naturally, anthropological activities such as heavy naval traffic, import of shellfish products or aquaculture have sharply accelerated this process and made it more frequent in both terrestrial and marine ecosystems over the last decades (Anderson, 2007; Schaffelke et al., 2006; Walker and Kendrick, 1998). Therefore, different strategies have been studied in order to control and prevent the proliferation of invasive species with different outcomes, essentially based on several mechanical removal procedures, but even considering the use of heat, chemicals (copper, chlorine, salt) or biological control by herbivorous mollusks (Anderson, 2007).

*Sargassum muticum* (Yendo) Fensholt is an invasive brown seaweed native to Japan which was introduced in North America by 1940s and in Europe during 1970s (Kraan, 2008; Walker and Kendrick, 1998). Nowadays, due to its extensive reproductive capacity, *S. muticum* is almost worldwide distributed, including different areas of the Pacific coast from Alaska to Mexico, the North Sea (Belgium, Denmark, the Netherlands...), major areas in Portugal, Spain, France and Ireland, as well as the English Channel coast or the Mediterranean Sea (Davis et al., 2004; Kraan, 2008). Moreover, several studies have already highlighted the effect that *S. muticum* has on native communities (Britton-Simmons, 2004; Kraan, 2008). Although the influence on other species is limited in the foreshore, native populations are strongly affected by the organism according to studies in the subtidal zone (deepest area of the shore), probably related to shading effects (Britton-Simmons, 2004).

Seasonal harvesting appears as an alternative to control algae proliferation (Kraan, 2008). Nevertheless, this measure entails the accumulation of large quantities of biomass that needs to be treated or utilized for valuable applications. The potential valorization of the resulting biomass lies in the

capability of *Sargassum* sp to produce numerous high-value compounds with potential pharmaceutical applications. Particularly *S. muticum* exhibits a significant amount of phenolic compounds with biological activities, such as antifouling or antioxidant properties (González-López et al., 2012; Plouguerné et al., 2010). In addition, the seaweed contains polysaccharides, namely alginate and fucoidans, and fucoxanthin, with antioxidant, anticoagulant, antithrombic, antitumor and antiviral activities (Balboa et al., 2013; Conde et al., 2012).

The use of these functional compounds from macroalgae requires the selection of a suitable extraction method, according to several criteria such as selectivity, cost-effectiveness and environmental performance. Specifically for alginate, the standard extraction method consists of a neutral extraction with hydrochloric acid and sodium hydroxide and final precipitation with sodium chloride and ethanol. Other methods have also been applied with comparable results in terms of alginate yield, including alkaline extraction at room and high temperatures (Davis et al., 2004). Solvent extraction is the most widely used technique for phenols, polysaccharides and fucoxanthin, though it requires long extraction times as well as the use of aqueous organic solvents such as methanol, ethanol and acetone (Garcia-Salas et al., 2010; Kadam et al., 2013). Moreover, organic solvents can also damage the functional properties of the extract, so alternative methods have been proposed such as supercritical fluid extraction, pressurized liquid extraction, ultrasound-assisted extraction and membrane separation (Garcia-Salas et al., 2010; Ye et al., 2008), which typically render extracts with fewer polar impurities and, therefore, an easier subsequent purification procedure (Conde et al., 2012).

In this study, the processing scheme described by González-López et al. (2012) was evaluated from an environmental perspective together with three alternative configurations with the aim of identifying the most suitable valorization route. The process consisted of consecutive extraction stages of the valuable biologically active compounds (fucoxanthin-containing extract by supercritical fluid extraction, alginate by alkaline extraction and antioxidant extract by non-isothermal autohydrolysis) to achieve an integral utilization of *S. muticum*. Life Cycle Assessment (LCA) standardized methodology was used to evaluate the environmental aspects and potential impacts of the process (ISO 14040, 2006).

This methodology has already been applied in a small number of studies related to the potential of macroalgal biomass as a feedstock in the production of biogas and bioethanol (Alvarado-Morales et al., 2013; Aresta et al., 2005). Although the production of high value bioactive molecules from other marine sources has also been evaluated through a LCA perspective (Pérez-López et al., 2014a; 2014b), there are no available studies focused on the production of these biocompounds from macroalgae harvested from nature.

### 5.2.1. Goal and scope

This study aims at identifying the environmental profile associated with the valorization of the invasive seaweed *S. muticum* in four different scenarios. Depending on the selected alternative, three main products were obtained: sodium alginate, antioxidant extract and fucoxanthin-containing extract. Additionally, the remaining algal residue resulting from each process was considered as by-product due to its potential use as fertilizer. The selected functional unit (FU) was 1 kg of final valorized biomass.

Four alternative routes of extraction were assessed to valorize the seaweed biomass. The processes were evaluated according to a cradle-to-gate perspective, including the production of the different inputs to the system, as well as the harvesting of seaweed biomass, cleaning and preparation of the harvested biomass and further extraction and purification. The involved stages and obtained products are summarized in **Figure 5.1** and following described.

i) Scenario 1 (Sc 1): Baseline scenario described by González-López et al. (2012), consisting of the valorization of dry algal biomass by alkaline extraction and precipitation of alginate followed by a non-isothermal autohydrolysis to separate an antioxidant extract rich in phenolics and polysaccharides, with potential applications in cosmetics industry.

ii) Scenario 2 (Sc 2): Valorization of wet algal biomass by alkaline extraction and precipitation of alginate followed by a non-isothermal autohydrolysis to separate the antioxidant extract.

iii) Scenario 3 (Sc 3): Valorization of wet algal biomass by non-isothermal autohydrolysis to obtain the antioxidant extract.

iv) Scenario 4 (Sc 4): Integral valorization of freeze-dried algal biomass based on the supercritical fluid extraction of fucoxanthin-containing extract followed by alkaline extraction and precipitation of alginate as well as non-isothermal autohydrolysis to obtain the antioxidant extract.

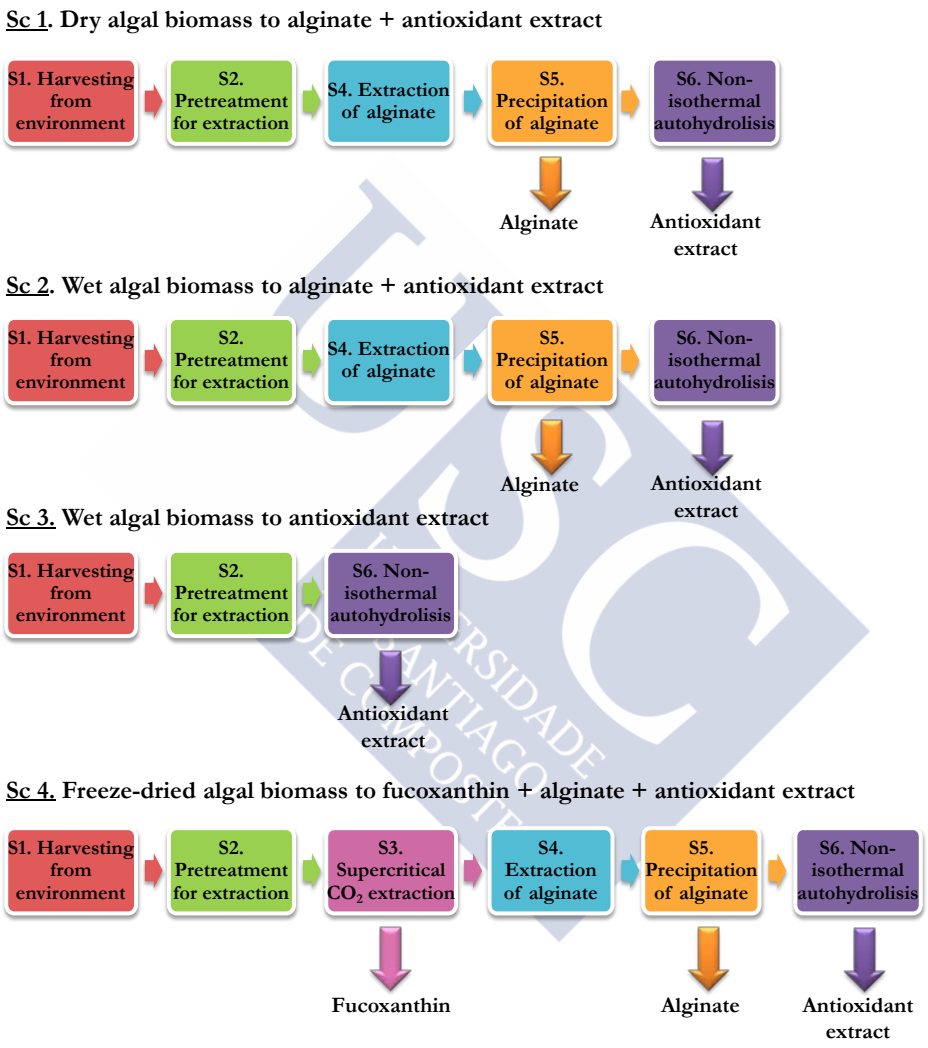


**Figure 5.1.** Schematic view of the stages performed and products obtained in each of the compared scenarios.

The system under study consisted of six main stages: i) harvesting of the macroalgae from the natural environment, ii) pretreatment for extraction, iii) supercritical extraction of fucoxanthin-containing extract, iv) extraction of alginate from the algal biomass, v) precipitation of alginate and vi) non-isothermal autohydrolysis to obtain the antioxidant extract. The stages and unit processes included within the system boundaries are depicted in **Figure 5.2** and described below.

i) S1. Harvesting of macroalgae from the natural environment by two methods: (i) direct manual harvesting of macroalgae that arrived at the beach due to tides or (ii) collection from the sea by boat. The amount of biomass collected by each procedure was estimated as 20% by boat and 80% at the beach (Manuel Loureiro, Conservas y Ahumados Lou SL, March 2013, personal communication). The system boundaries include materials and fuel, as well as emissions to environment associated with vessel operations. In addition, water to clean and rinse the collected biomass to remove impurities (sand, epiphytes…) was considered, as well as the use of polyethylene and nylon for nets.

ii) S2. Pretreatment for extraction: The clean biomass was kept in the freezer for a week (as average) before additional rinsing with water. The next stage depended on the considered scenario: in the baseline scenario (Sc 1), the algal biomass was dried in oven for 2 h before being grinded for 1 h, which reflects the protocol followed by some algal canning factories to process algae for food uses.

   Experimental work showed the possibility to perform the extractions with wet algae, so drying stage was not considered in Sc 2 and Sc 3, and grinding was substituted by mincing. Finally, when carrying out the supercritical extraction before alginate and antioxidant extractions (Sc 4), biomass had to be previously freeze-dried and grinded.

iii) S3. Supercritical extraction of fucoxanthin-containing extract: This stage is only included in Sc 4. In this scenario, a fraction of the fucoxanthin present in the biomass (12 mg from a total of 55.1 mg fucoxanthin per 100 g dry weight seaweed biomass) was separated through supercritical $CO_2$ extraction (P=20-40 MPa and T=40-55ºC; 140 kg $CO_2 \cdot kg^{-1}$

valorized biomass with no recycling system), using ethanol as co-solvent (21 $L \cdot kg^{-1}$ valorized algae with no recovery system) and operating the system for 1 h. In this case, 90% recovery and reuse of both $CO_2$ and ethanol were assumed. The obtained extract contained 5-10% fucoxanthin.

iv) S4. Extraction of alginate from algal biomass: In all scenarios except for Sc 3, the remaining algal biomass was then extracted at room temperature with consecutive additions of formaldehyde 1% (15 h), sulfuric acid 0.2 N (4 h) and sodium carbonate 1% (15 h) in a sequential process with intermediate filtrations and washings of solids using distilled water. Stirring in all the extractions was also included within the system boundaries.

v) S5. Precipitation of alginate: Once the liquid fraction containing sodium alginate (11.4% algal biomass in dry weight) was separated, a precipitation process was performed. The process consisted of the addition of ethanol 95% (15 min stirring, 1 h resting) and a washing step with ethanol and acetone, followed by a drying step in oven. A solvent recovery of 90% was assumed for ethanol (total consumption of 106 $L \cdot kg^{-1}$ valorized algae with no recovery system), whereas no acetone recovery was considered, due to the low need for this solvent (8 $L \cdot kg^{-1}$ valorized algae).

vi) S6. Non-isothermal autohydrolysis to obtain antioxidant extract: The solid fraction obtained after the last filtration in S4 (or after mincing in S2 for Sc 3) was rich in antioxidant extract. In the four scenarios, this solid was dried for two days in an oven at 50ºC and treated with water in a batch reactor under non-isothermal conditions (final temperature of 170ºC, which renders to the maximum content in fucoidans within the extract that corresponds to 20-30% of the product) at a liquid/solid ratio of 60:1 $g \cdot g^{-1}$. Once the selected temperature was reached, the biomass was kept in the reactor for 30 min and then cooled to 50-60ºC. The resulting antioxidant extract (21% algal biomass in dry weight) was recovered by filtration and freeze-dried, whereas the algal paste with potential use as fertilizer was dried in oven for two days.

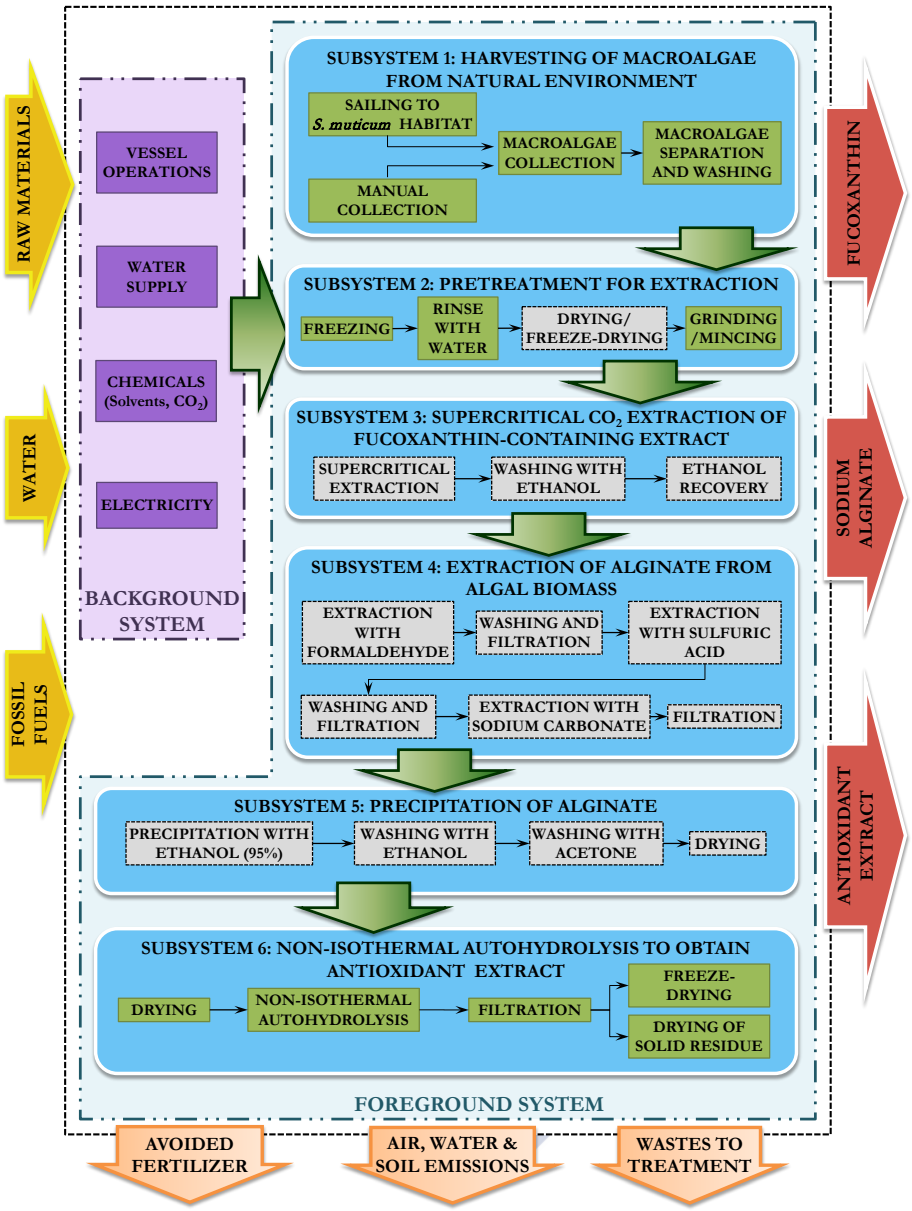


**Figure 5.2.** Process chain and system boundaries of the integral valorization of the seaweed *S. muticum* by sequential extraction of fucoxanthin-containing extract, alginate and antioxidant extract (blocks in grey with discontinuous lines refer to steps that are not common to the four assessed scenarios).

According to the described protocols, only stages S1, S2 and S6 were common to the four analyzed scenarios; whereas S3 was only performed when fucoxanthin-containing extract was one of the target products and S4 and S5 were not required in case alginate was not extracted.

The protocols for the different alternatives of separation and purification were developed by the Group EQ2 of Chemical Engineering (www.grupoeq2.es) in the Faculty of Sciences in Ourense, at the University of Vigo (Spain).

### 5.2.2. Life cycle inventory, data quality and assumptions

Foreground data for the six stages were collected from different sources and procedures, as indicated in **Table 5.1**.

**Table 5.1.** Summary of data sources for the foreground system of the integral valorization of *S. muticum* biomass

| Stage | Raw material/Energy | Data source |
|---|---|---|
| S1. Harvesting from natural environment | Polyester<br>Steel | Manufacturers' specifications, personal communication, Hospido and Tyedmers (2005). |
| | Antifouling<br>Paint<br>Lubricant oil | Manufacturers' specifications, Vázquez-Rowe et al. (2010). |
| | Tap water | Experimental data, consumption at canning facility. |
| | Low density polyethylene (LDPE)<br>Nylon | Experimental data, on-site measurement. |
| | Emissions | Estimated according to Vázquez-Rowe et al. (2010), Hospido and Tyedmers (2005). |
| | Wastes to treatment | Calculated from mass balances. |
| S2. Pretreatment for extraction | Tap water | Experimental data, on-site measurement. |
| | Electricity consumption | Estimated from power of equipment and duration of stage. |
| | Emissions | Calculated from mass balances. |

**Table 5.1.** Summary of data sources for the foreground system of the integral valorization of *S. muticum* biomass (*Cont.*)

| Stage | Raw material/Energy | Data source |
|---|---|---|
| S3. Supercritical extraction of fucoxanthin-containing extract | Ethanol<br>Carbon dioxide | Experimental data, on-site measurement. Recovery or recycling. |
| | Electricity consumption | Estimated from power of equipment and duration of stage. |
| | Emissions | Calculated from mass balances. |
| S4. Separation of alginate from antioxidant fraction | Tap water<br>Distilled water<br>Formaldehyde<br>Sulfuric acid<br>Disodium carbonate | Experimental data, on-site measurements. Gonzalez-López et al. (2012). |
| | Electricity consumption | Estimated from power of equipment and duration of stage. |
| | Emissions | Calculated from mass balances. |
| S5. Precipitation of alginate | Ethanol<br>Acetone<br>Distilled water | Experimental data, on-site measurements. Gonzalez-López et al. (2012). |
| | Electricity consumption | Estimated from power of equipment and duration of stage. |
| | Emissions | Calculated from mass balances. |
| S6. Non-isothermal autohydrolysis to obtain antioxidant extract | Distilled water | Experimental data, on-site measurements. |
| | Electricity consumption | Estimated from power of equipment and duration of stage. |
| | Emissions | Calculated from mass balances. |

The inputs for the collection of biomass from natural environment (S1), including fuel consumption, as well as materials of the vessel and chemicals associated with maintenance, were obtained from manufacturers' specifications and personal communications with expert advisors. Materials for the vessel were estimated according to average dimensions and weights. A shared used of the boat was considered (1600 h per year) and three months of operation corresponded to the seasonal harvest of *S. muticum* (480 h). Materials for hull

and engine were increased by 25% and 50% respectively, and life spans of 30 and 15 years were considered (Hospido and Tyedmers, 2005). Chemicals related to vessel operations (i.e. paint, anti-fouling paint, marine lubricant oil), as well as water and air emissions from fuel combustion discharged to the environment were inventoried according to Vázquez-Rowe et al. (2010), considering manufacturers' specifications. For paint and anti-fouling emitted to marine environment, a loss of two thirds of the total amount used was considered (Hospido and Tyedmers, 2005).

For the next stages (S2 to S6), chemicals and water consumptions were estimated from experimental data obtained by on-site measurements and completed with information from González-López et al. (2012). Electricity consumptions were extrapolated on the basis of the power of the equipment, processing capacity and duration of each stage. As the inventory is associated with a hypothetical facility placed in shore, transport of equipment and chemicals was considered negligible. Water and air emissions were calculated on the hypothesis that the non-depleted chemicals were directly discharged.

Concerning the background system, the corresponding inventory data for the production of all the inputs to the system were taken from Ecoinvent database (Frischknecht et al., 2007). These inputs included the production of chemicals required for the extraction stages, the production of electricity used within the stages of the process, as well as the materials for the vessel needed for the algae collection and waste disposal. A detailed description of the corresponding database reports considered is shown in **Table 5.2**.

Finally, all the scenarios allow obtaining a biomass residue with potential applications as fertilizer. To do so, the content in carbon and nitrogen, as well as the ratio C:N were determined. The measured content of carbon was 45.6±0.2% (dry weight) and the content of nitrogen was 1.5±0.2% (dry weight). Since the obtained C:N ratio is higher than 25, all the nitrogen present in the residual biomass can be uptaken by the plants. Once the fertilizer potential was estimated, the equivalent amount of a typical fertilizer (containing ammonium sulfate as N source) was considered in the model as avoided product, which resulted in negative impacts that were subtracted from the environmental burdens. The inventory data of the four scenarios are shown in **Table 5.3**.

*Allocation procedures*

According to the selected FU (1 kg valorized algae), no allocation procedure was required to distribute the impacts among co-products. A system expansion was considered to include the fertilizer potential of the residual biomass.

**Table 5.2.** Summary of data sources for the background system of the integral valorization of *S. muticum* biomass

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Energy | Diesel | Ecoinvent database (Jungbluth, 2007) |
| | Electricity (Spanish electricity profile) | Ecoinvent database (Dones et al., 2007) |
| Chemicals related to vessel operation | Anti-fouling<br>Boat paint<br>Marine lubricant oil | Vázquez-Rowe et al. (2010) |
| Materials for harvesting from natural environment | Glass fibre reinforced plastic, polyester resin | Ecoinvent database (Kellenberger et al., 2007) |
| | Steel | Ecoinvent database (Classen et al., 2007) |
| | Polyethylene<br>Nylon | Ecoinvent database (Hischier, 2007) |
| Chemicals | Formaldehyde<br>Sulfuric acid<br>Acetone<br>Carbon dioxide | Ecoinvent database (Althaus et al., 2007) |
| | Sodium carbonate | Ecoinvent database (Sutter, 2007a) |
| | Ethanol | Ecoinvent database (Sutter, 2007b) |
| Water supply | Tap water<br>Distilled water | Ecoinvent database (Althaus et al., 2007) |
| Waste treatment | Inert landfill<br>Sanitary landfill<br>Municipal incineration | Ecoinvent database (Doka, 2007) |
| Avoided product: fertilizer | Ammonium sulfate | Ecoinvent database (Nemecek and Kägi, 2007) |

**Table 5.3.** Inventory data for the valorizaton of the invasive seaweed *S. muticum* (FU=1 kg valorized biomass)

| **INPUTS from TECHNOSPHERE** | | | | |
|---|---|---|---|---|
| | Sc 1. Alginate + antioxidant extract from dry alga | Sc 2. Alginate + antioxidant extract from wet alga | Sc 3. Antioxidant extract from wet alga | Sc 4. Fucoxanthin+ antioxidant+alginate from freeze-dried alga |
| **Materials** | | | | |
| *S1. Harvesting from natural environment* | | | | |
| Polyester | 65.37 g | | 101.13 g | 65.34 g |
| Steel | 19.61 g | | 30.34 g | 19.60 g |
| Antifouling | 41.61 g | | 64.37 g | 41.59 g |
| Paint | 10.47 g | | 16.19 g | 10.46 g |
| Lubricant oil | 51.92 g | | 80.33 g | 51.91 g |
| Tap water | 260.82 kg | | 403.50 kg | 260.72 kg |
| LDPE | 32.23 g | | 49.86 g | 32.22 g |
| Nylon | 17.75 g | | 27.45 g | 17.74 g |
| *S2. Pretreatment for extraction* | | | | |
| Tap water | 167.67 kg | | 259.40 kg | 167.61 kg |
| *S3. Supercritical extraction of fucoxanthin-containing extract* | | | | |
| Carbon dioxide | 0 | | 0 | 45.15 kg |
| Ethanol | 0 | | 0 | 63.08 kg |
| *S4. Extraction of alginate from antioxidant fraction* | | | | |
| Tap water | 1051.36 kg | | 0 | 1050.97 kg |
| Distilled water | 522.97 kg | | 0 | 522.78 kg |
| Formaldehyde | 1.43 kg | | 0 | 1.43 kg |
| Sulfuric acid | 1.74 kg | | 0 | 1.74 kg |
| Disodium carbonate | 1.75 kg | | 0 | 1.75 kg |
| *S5. Precipitation of alginate* | | | | |
| Ethanol | 39.64 kg | | 0 | 39.62 kg |
| Acetone | 20.79 kg | | 0 | 20.78 kg |
| Distilled water | 1.63 kg | | 0 | 1.63 kg |
| *S6. Non-isothermal autohydrolysis to obtain antioxidant extract* | | | | |
| Distilled water | 119.98 kg | | 288.22 kg | 121.82 kg |

**Table 5.3.** Inventory data for the valorizaton of the invasive seaweed *S. muticum* (FU=1 kg valorized biomass) (*Cont.*)

| | Sc 1. Alginate + antioxidant extract from dry alga | Sc 2. Alginate + antioxidant extract from wet alga | Sc 3. Antioxidant extract from wet alga | Sc 4. Fucoxanthin+ antioxidant+alginate from freeze-dried alga |
|---|---|---|---|---|
| **INPUTS from TECHNOSPHERE** | | | | |
| **Energy** | | | | |
| *S1. Harvesting from natural environment* | | | | |
| Diesel | 23.25 kg | 23.25 kg | 35.97 kg | 23.24 kg |
| *S2. Pretreatment for extraction (electricity from Spanish grid)* | | | | |
| Cooler | 1.22 kWh | 1.22 kWh | 1.88 kWh | 1.22 kWh |
| Freeze-drying | 0 | 0 | 0 | 294.55 kWh |
| Drying | 256.97 kWh | 0 | 0 | 0 |
| Grinding | 2.63 kWh | 0 | 0 | 2.35 kWh |
| Mincing | 0 | 1.38 kWh | 2.14 kWh | 0 |
| *S3. Supercritical extraction (electricity from Spanish grid)* | | | | |
| Supercritical extraction equipment | 0 | 0 | 0 | 2087.61 kWh |
| *S4. Extraction of alginate (electricity from Spanish grid)* | | | | |
| Stirring | 97.95 kWh | 97.95 kWh | 0 | 97.92 kWh |
| *S5. Precipitation of alginate (electricity from Spanish grid)* | | | | |
| Drying | 1.30 kWh | 1.30 kWh | 0 | 1.30 kWh |
| Stirring | 0.63 kWh | 0.63 kWh | 0 | 0.63 kWh |
| *S6. Non-isothermal autohydrolysis (electricity from Spanish grid)* | | | | |
| Drying | 96.91 kWh | 211.53 kWh | 212.02 kWh | 94.52 kWh |
| Autohydrolysis in Parr reactor | 81.12 kWh | 81.12 kWh | 194.87 kWh | 82.36 kWh |
| Freeze-drying | 532.15 kWh | 532.15 kWh | 1167.99 kWh | 540.38 kWh |
| Drying | 4.19 kWh | 4.19 kWh | 7.10 kWh | 4.20 kWh |
| **INPUTS from ENVIRONMENT** | | | | |
| **Materials** | | | | |
| Macroalgal biomass | 3.11 $kg_{DW}$ | | 4.81 $kg_{DW}$ | 3.11 $kg_{DW}$ |
| Sand and residues | 155.25 g | | 240.18 g | 155.19 g |
| Seawater | 29.42 kg | | 45.52 kg | 29.41 kg |

**Table 5.3.** Inventory data for the valorizaton of the invasive seaweed *S. muticum* (FU=1 kg valorized biomass) (*Cont.*)

| | Sc 1. Alginate + antioxidant extract from dry alga | Sc 2. Alginate + antioxidant extract from wet alga | Sc 3. Antioxidant extract from wet alga | Sc 4. Fucoxanthin+ antioxidant+alginate from freeze-dried alga |
|---|---|---|---|---|
| **OUTPUTS to TECHNOSPHERE** | | | | |
| **Products** | | | | |
| Fucoxanthin | 0 | | 0 | 0.37 g |
| Alginate | 0.35 kg | | 0 | 0.35 kg |
| Antioxidant extract | 0.65 kg | | 1.00 kg | 0.65 kg |
| **Avoided product**[1] | | | | |
| Nitrogen-rich fertilizer (as kg N) | 8.10 g | | 12.53 g | 8.10 g |
| **Wastes to landfill** | | | | |
| *S1. Harvesting from natural environment* | | | | |
| Polyester | 65.37 g | | 101.13 g | 65.34 g |
| Steel | 19.61 g | | 30.34 g | 19.60 g |
| LDPE | 32.23 g | | 49.86 g | 32.22 g |
| **Wastes to municipal incineration** | | | | |
| *S1. Harvesting from natural environment* | | | | |
| Nylon (textile) | 17.75 g | | 27.45 g | 17.74 g |
| **OUTPUTS to ENVIRONMENT** | | | | |
| **Air emissions** | | | | |
| *S1. Harvesting from natural environment* | | | | |
| Carbon dioxide | 73.65 kg | | 113.94 kg | 73.62 kg |
| Sulfur dioxide | 0.05 kg | | 0.07 kg | 0.05 kg |
| Non-methane volatile organic compounds | 0.69 kg | | 1.06 kg | 0.69 kg |
| Methane | 4.19 kg | | 6.47 kg | 4.18 kg |
| Nitrogen oxides (NOx) | 0.55 kg | | 0.84 kg | 0.55 kg |
| Carbon monoxide | 0.17 kg | | 0.27 kg | 0.17 kg |
| Particulate matter | 0.04 kg | | 0.07 kg | 0.04 kg |

[1]N dosage is equivalent to 1.5% (dry weight) nitrogen content within biomass. Since C/N ratio is higher than 25, all present nitrogen can be uptaken by plants.

**Table 5.3.** Inventory data for the valorizaton of the invasive seaweed *S. muticum* (FU=1 kg valorized biomass) (*Cont.*)

| **OUTPUTS to ENVIRONMENT** | | | | |
|---|---|---|---|---|
| | Sc 1. Alginate + antioxidant extract from dry alga | Sc 2. Alginate + antioxidant extract from wet alga | Sc 3. Antioxidant extract from wet alga | Sc 4. Fucoxanthin+ antioxidant+alginate from freeze-dried alga |
| **Water emissions** | | | | |
| *S1. Harvesting from natural environment* | | | | |
| Xylene | 3.71 g | | 5.748 g | 3.71 g |
| Cobalt | 1.59 mg | | 2.46 mg | 1.59 mg |
| Copper | 8.62 g | | 13.34 g | 8.62 g |
| Zinc | 3.90 g | | 6.03 g | 3.90 g |
| Ethylbenzene | 0.97 g | | 1.50 g | 0.97 g |
| Sea nine 211 | 0.42 g | | 0.64 g | 0.42 g |
| 4-methylpentan-2-one | 0.42 g | | 0.64 g | 0.42 g |
| Wastewater | 267.35 kg | | 413.61 kg | 267.25 kg |
| *S2. Pretreatment for extraction* | | | | |
| Wastewater | 185.58 kg | 167.67 kg | 259.40 kg | 185.88 kg |
| *S3. Supercritical extraction of fucoxanthin* | | | | |
| Ethanol | 0 | | 0 | 67.08 kg |
| *S4. Extraction of alginate* | | | | |
| Formaldehyde | 1.43 kg | | 0 | 1.43 kg |
| Sulfuric acid | 1.74 kg | | 0 | 1.74 kg |
| Wastewater | 1373.87 kg | | 0 | 1373.36 kg |
| *S5. Precipitation of alginate* | | | | |
| Ethanol | 39.91 kg | | 0 | 39.90 kg |
| Acetone | 20.79 kg | | 0 | 20.78 kg |
| Wastewater | 165.87 kg | | 0 | 165.80 kg |
| Disodium carbonate | 1.63 kg | | 0 | 1.63 kg |
| *S6. Non-isothermal autohydrolysis to obtain antioxidant extract* | | | | |
| Wastewater | 132.99 kg | 150.90 kg | 316.53 kg | 134.45 kg |
| Disodium carbonate | 0.13 kg | | 0 | 0.13 kg |

### 5.2.3. Environmental impact assessment

Among the phases defined by LCA standard methodology for the life cycle impact assessment stage (ISO 14040, 2006) only classification and characterization stages were undertaken, since normalization and weighting were not considered to provide any additional information according to the goal and scope of the study. The impact assessment methodology reported by the Centre of Environmental Science of Leiden University (CML 2001 method) and previously used for assessing microalgal process, was applied (Guinée et al., 2002). Analogously, the potential impact categories analyzed were: abiotic depletion (ADP), acidification (AP), eutrophication (EP), global warming (GWP), ozone layer depletion (ODP), human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP), terrestrial ecotoxicity (TEP) and photochemical oxidants formation (POFP). The software SimaPro 8 was used to implement the inventory and obtain the impact assessment (Goedkoop et al., 2013).

❖ **Comparative environmental performance of the valorization strategies of *S. muticum* biomass**

LCA characterization results of the evaluated scenarios are summarized in **Table 5.4** with reference to 1 kg of valorized biomass as FU. According to the results depicted in **Figure 5.3**, valorization by extracting sodium alginate and antioxidant fraction from wet algae would be the most appropriate route with respect to all impact categories except for FEP. Thus, Sc 2 presents contributions between 4% (for FEP) and 12% (for EP, MEP or TEP) lower than the impacts of the baseline scenario Sc 1.

Moreover, the environmental profiles of both strategies Sc 1 and Sc 2 are better than the performance of Sc 3. Indeed, the contributions when extracting only the antioxidant fraction are from 14% to 44% higher than those of Sc 1 and exceed the values of Sc 2 in a range of 26% up to 64%, except for FEP that presents a contribution 52% lower than Sc 1 and 49% lower than Sc 2. Sc 4 was found as the alternative with the highest contributions in all the evaluated categories, being these contributions more than 1.5 times higher for FEP and nearly 3 times higher for HTP, MEP or TEP in comparison with the other three scenarios.

**Table 5.4.** Environmental impact assessment results (characterization step) associated with 1 kg of valorized *S. muticum* in the four evaluated scenarios

| Impact category | Unit | FU: 1 kg valorized *Sargassum muticum* | | | |
|---|---|---|---|---|---|
| | | **Sc 1** | **Sc 2** | **Sc 3** | **Sc 4** |
| ADP | kg Sb eq | 6.73 | 6.11 | 7.70 | 17.54 |
| AP | kg $SO_2$ eq | 8.13 | 7.31 | 9.75 | 20.58 |
| EP | kg $PO_4^{-3}$ eq | 1.43 | 1.27 | 1.97 | 4.05 |
| GWP | kg $CO_2$ eq | 821.61 | 736.70 | 1072.52 | 2245.94 |
| ODP | kg CFC-11 eq | $47.40 \cdot 10^{-6}$ | $42.82 \cdot 10^{-6}$ | $67.52 \cdot 10^{-6}$ | $120.03 \cdot 10^{-6}$ |
| HTP | kg 1,4-DB eq | 197.12 | 173.14 | 274.74 | 602.29 |
| FEP | kg 1,4-DB eq | 605.06 | 578.95 | 292.88 | 1007.06 |
| MEP | kg 1,4-DB eq | 131.14 | 114.61 | 188.37 | 385.95 |
| TEP | kg 1,4-DB eq | $45.23 \cdot 10^{-3}$ | $39.84 \cdot 10^{-3}$ | $61.61 \cdot 10^{-3}$ | $128.10 \cdot 10^{-3}$ |
| POFP | kg $C_2H_4$ eq | 0.31 | 0.28 | 0.35 | 0.86 |

Sc 1. Alginate + antioxidant extract from dry algae

Sc 2. Alginate + antioxidant extract from wet algae

Sc 3. Antioxidant extract from wet algae

Sc 4. Fucoxanthin-containing extract + alginate + antioxidant extract from freeze-dry algae

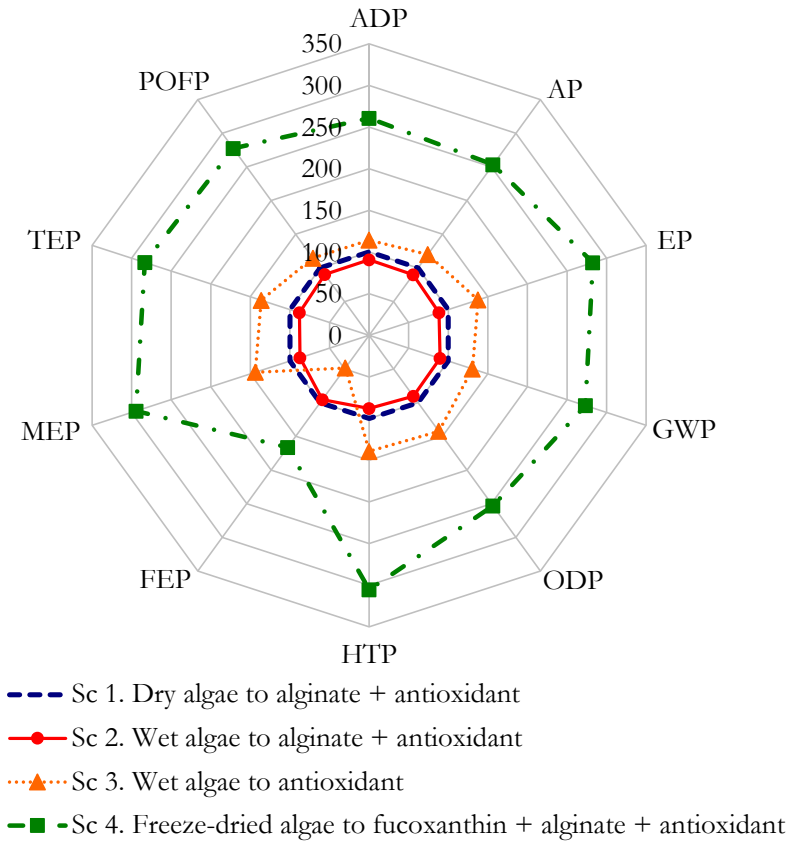


**Figure 5.3.** Relative environmental profile of the compared valorization scenarios with Sc 1 as the baseline (index = 100) for 1 kg valorized algae as FU.

❖ **Identification of hot spots for the valorization strategies of *S. muticum* biomass**

**Figure 5.4** depicts the most problematic stages contributing to the environmental impacts of the four valorization alternatives. In the case of Sc 1, the non-isothermal hydrolysis (S6) is the major contributor to most impact categories that accounts from 45% up to 60%, except for FEP, which is dominated by the extraction of alginate from the algal biomass (S4) with 70% of the contribution. S4 also has a significant effect in terms of AP (21%). Among the secondary stages, the pretreatment of algal biomass for extraction (S2) has remarkable contributions, especially in EP (21%), HTP (22%), MEP (23%) and TEP (22%). The collection of algae from natural environment (S1) is

responsible for 11% of impact to GWP and nearly 23% ODP, whereas the precipitation of alginate (S5) has rather limited effects for most categories, despite being the second cause of ADP and POFP (22% each).

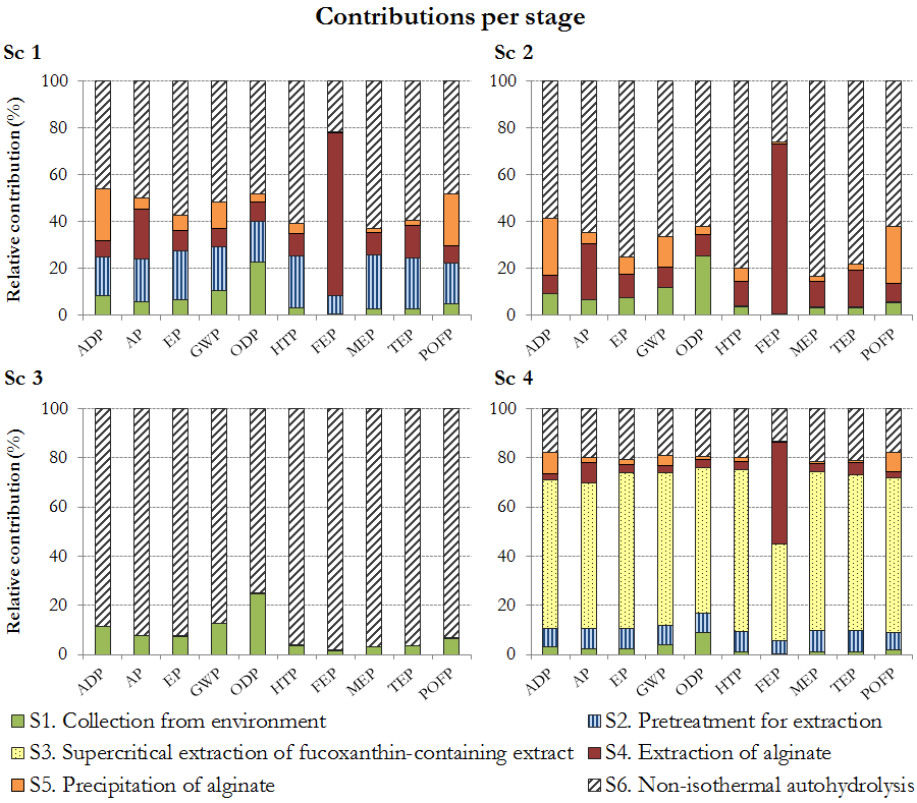


**Figure 5.4.** Relative contributions per stage to the environmental profile of the compared scenarios for 1 kg valorized algae as FU.

Regarding the activities associated with these impacts, shown in **Figure 5.5**, the production of electricity is clearly the hot spot in Sc 1. This activity exhibits a global contribution ranging from 68% to 95% of the total impacts in all the categories except for FEP, which presents 66% of the impact related to waste streams (specifically linked to organic solvent emissions to water in S4). The highest electricity consumption (66% of total electricity) corresponds to S6 that has three energy-intensive steps with significant electricity requirements (drying, autohydrolysis in reactor itself and, to a larger extent, freeze-drying of the

obtained extract), followed by S2 (24% of total electricity) mainly due to drying step for the preparation of the algae for extraction. Among the secondary processes, the production of chemicals constitutes a major contributor in terms of ADP, POFP (23% each) and GWP (12%) related to the production of ethanol and, to a lesser extent, acetone, both required for the precipitation of alginate in S5. Vessel operations cause 11% of the total impact in GWP and 23% of ODP, especially due to the consumption of diesel and the derived greenhouse gas emissions. Although the use of residual biomass as a fertilizer represents a reduction of impact (avoided synthetic fertilizer), the limited amount of material results in a negligible improvement, much lower than 1% of the total impacts in all the categories.

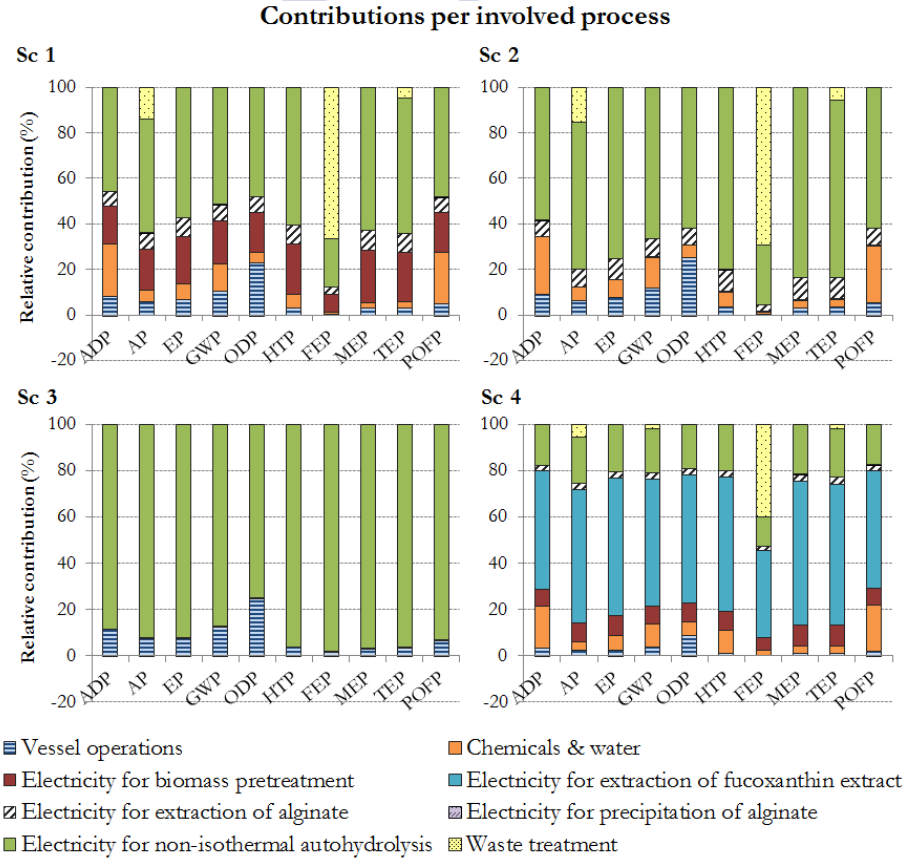


**Figure 5.5.** Relative contributions per involved process to the environmental profile of the compared scenarios for 1 kg valorized algae as FU.

In the case of Sc 2, the effect of S2 is remarkably lower than for Sc 1, decreasing from contributions between 16% and 23% in most categories to less than 0.5%. This change is due to the omission of the drying stage (resulting in a remarkable reduction in electricity consumption) that was proven to be feasible without affecting the extraction stages and yields in scenarios where only alginate and antioxidant extract were obtained. Therefore, S6 dominates the environmental burdens (between 58% and 83% depending on the category) except for contributions to FEP, which are again associated with S4 (73% of total FEP). As in the previous case, S5 is only relevant for ADP (24%) and POFP (24%), whereas S1 affects GWP and ODP in 12% and 25% respectively. Concerning the production processes that are associated with the stages, the production of electricity to meet the energy requirements is again the hot spot with contributions that range between 65% and 94% to all the categories excluding FEP (69% from waste treatment processes), despite the slight decrease of the influence of electricity with respect to Sc 1. Nearly 90% of this electricity consumption is related to S6, due to the combined requirements associated with the steps of drying, autohydrolysis and mostly freeze-drying. Following the same trend as Sc 1, the production of chemicals is responsible for the second highest contribution to ADP, POFP (25%) and GWP (13%), whereas vessel operations affect GWP (12%) and ODP (25%).

Regarding Sc 3, due to the elimination of S4 and S5, the contributions of S1 and S6 exhibit a noticeable increase in relative terms. S6 clearly constitutes the hot spot in all the evaluated categories, ranging from 75% to 98% of the impact depending on the category. S1 mainly affects ODP (25%), and has relatively significant contributions to ADP (11%) and GWP (13%). Regarding the involved activities, the contributions related to electricity are responsible for more than 75% of the impact to all categories, with 99% of these requirements coming from S6. Another significant change in this scenario is the sharp decrease of the impacts from waste treatment (from around 70% in Sc 1 and Sc 2 to 0.3% in Sc 3). This is due to the fact that no organic solvents are emitted to water when the extraction of alginate is not performed.

The environmental profile of Sc 4 is remarkably different compared with the other scenarios as a result of the implementation of a supercritical extraction stage to obtain fucoxanthin-containing extract. In this case, S3 is certainly the major hot spot in all the impact categories with contributions between 39% and 66%. Among the other stages, S6 has significant effects for all categories (between 13% and 22%) and S4 only affects noticeably FEP (42%), while all the other contributions are below 10%. The main reason for this behavior is the need for electricity to satisfy the high energy requirements of the supercritical extraction that affect the impact categories between 38% and 62%. This consumption corresponds to 65% of the total electricity required, whereas more than 22% of the remaining demand is related to S6 and 9% is due to the freeze-drying of biomass in S2 that is necessary to perform the supercritical extraction. Among the processes that are not related to electricity, only three contributions exceed 10% of the impacts: the production of chemicals for ADP (18%) and POFP (20%), jointly with waste flows for FEP (40%).

### 5.2.4. Discussion and recommendations

The comparative analysis conducted in this study shows up the great influence of the extraction pathway in the LCA results. Two main processes are related to the impact: i) the production of electricity to meet the requirements and ii) the production of solvents used for the extraction. Moreover, the results are based on the specific product distribution reported by González-López et al. (2012) for a final temperature of 170ºC during the non-isothermal autohydrolysis. The selected FU and allocation procedures may also affect the outcome of the study. In order to evaluate the possible changes in the environmental profile related to these key factors, four sensitivity assessments are presented:

❖ **Effect of energy optimization (10% electricity reduction)**

Although no similar works related to valuable compounds obtained from macroalgae were found in the literature, the identification of hot spots is consistent with previous findings related to the remarkable effect of electricity consumption on harvesting and extraction processes for other products from marine organisms, such as lipid extraction from microalgae or biofuels from macroalgae (Aresta et al., 2005; Beach et al., 2012). Indeed, a 10% reduction in

electricity requirements could lead to improvements between 3% and 10% of the total impacts depending on the considered category (**Figure 5.6**).

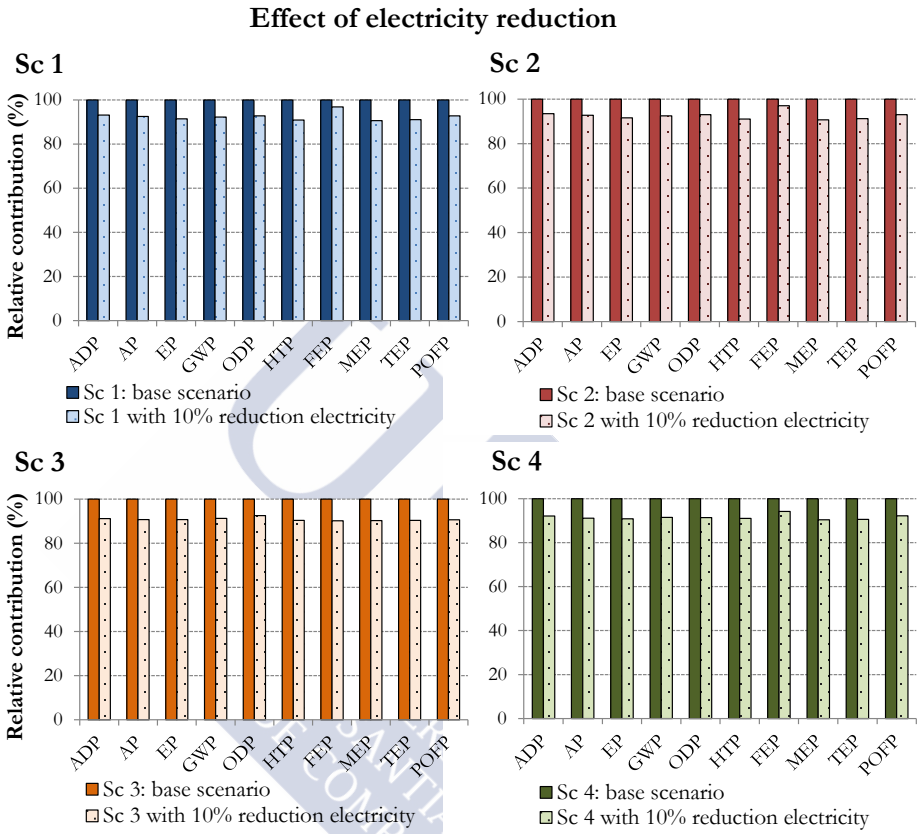


**Figure 5.6.** Relative environmental profile of the compared valorization scenarios with current electricity requirements and with 10% reduction of electricity requirements, being the current scenario the baseline (index=100) for 1 kg valorized alga as FU.

❖ **Effect of solvent recovery**

In addition to the impact associated with the production of electricity, organic solvents can also result in a significant contribution that in this case was observed when considering the precipitation of alginate. With this regard, Raymond et al. (2010) found that the possibility of solvent recovery or reduction could entail up to 90% of reduction in overall emissions.

In this case, ethanol associated with alginate precipitation was the main contribution among the chemicals. As a recovery system was already taken into account for the baseline inventory, a comparison of the assessed scenarios with and without ethanol recovery was conducted (**Figure 5.7**). According to the results, solvent recovery is a key issue in the three scenarios that include the precipitation of alginate (Sc 1, Sc 2 and Sc 4). The omission of this system would cause a remarkable increase in the environmental impacts related to most categories, especially ADP and POPF, which exceed the original value in 25% for Sc 4 and nearly double their contributions when considering Sc 1 and Sc 2.

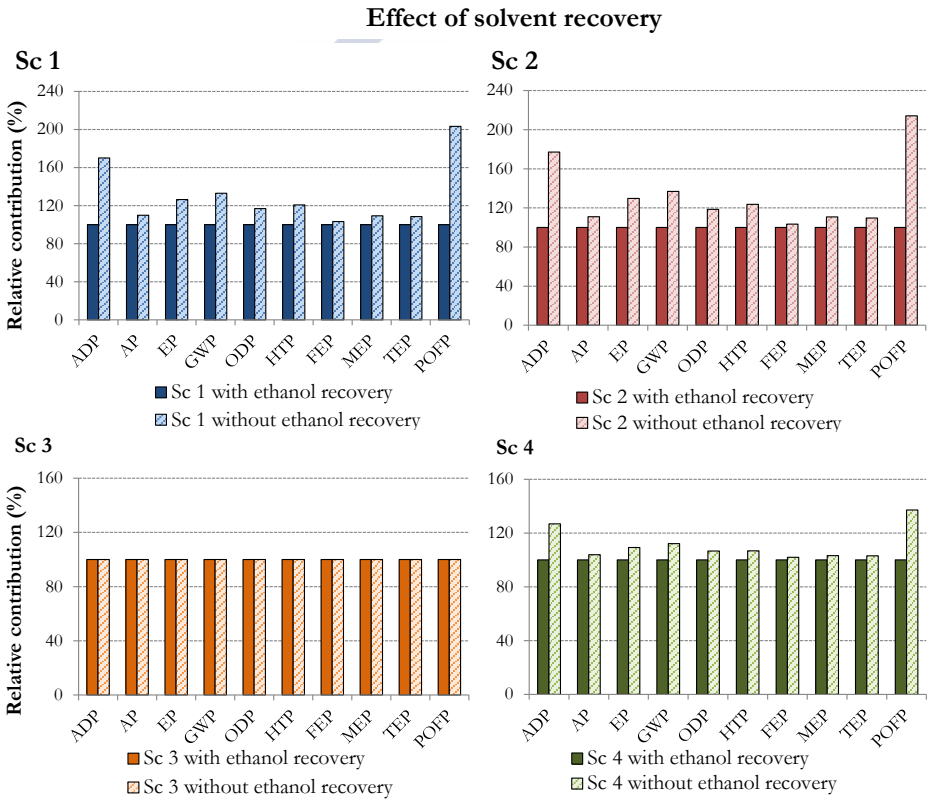


**Figure 5.7.** Relative environmental profile of the compared valorization scenarios with and without ethanol recovery system, being the scenario with ethanol recovery the baseline (index=100) for 1 kg valorized alga as FU.

❖ **Effect of changes in biomass composition**

The environmental results analyzed in the previous sections were calculated for the case in which the antioxidant extract contained the highest concentration of fucoidans (final temperature of 170ºC during non-isothermal autohydrolysis). However, González-López et al. (2012) found a remarkable influence of the final heating temperature on the solubilization of solids and therefore, on the final amount of the antioxidant extract obtained.

As the antioxidant fraction constitutes the main product as much in mass as in economic terms, a change in the obtained amount may significantly affect the global environmental profile of the process. Moreover, the considered quantity of extracted fucoxanthin (12 mg fucoxanthin in 100 g dry algae) corresponds to the maximum experimental yield obtained, although *S. muticum* contains up to 55.1 mg in 100 g dry algae (Conde et al., 2012). Additionally, seasonal variations may also result in important changes in the composition of the biomass, and therefore in the product distribution (Balboa et al., 2013).

For this reason, a sensitivity assessment was conducted. The potential impacts for all the scenarios were calculated in two opposite situations: the maximization of the amount of antioxidant extract (autohydrolysis temperature of 200ºC to obtain 41% dry algae as antioxidant extract) and the operation with minimum amount of antioxidant extract (temperature of 150ºC to obtain 13% dry algae as antioxidant extract). For Sc 4, an additional situation was evaluated, considering the highest content of fucoxanthin in the biomass: 55.1 mg in 100 g algae (**Table 5.5**).

The results reveal the clear dependence of the environmental performance on the operational conditions of S6. Thus, a change of 17% in the final temperature for the non-isothermal autohydrolysis (from 170 to 200ºC) turns into a reduction of impact around 33% for Sc 1 and Sc 2, 44% for Sc 3 and 37% for Sc 4, whereas lowering temperature by 12% (from 170 to 150ºC) involves increases from 25% (for Sc 1, Sc 2 and Sc 4) up to 50% for Sc 3. Concerning Sc 4, the increment in the recovered amount of fucoxanthin has virtually no effect on the environmental profile.

**Table 5.5.** Influence of changes in biomass composition on the environmental profile with respect to baseline scenario for each alternative route

| Impact category | Unit | Characterization results for baseline scenario[1] | | | | % change with minimum antioxidant content[2] | | | | % change with minimum antioxidant content[3] | | | | % change Sc 4, maximum fucoxanthin content[4] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Sc 1 | Sc 2 | Sc 3 | Sc 4 | Sc 1 | Sc 2 | Sc 3 | Sc 4 | Sc 1 | Sc 2 | Sc 3 | Sc 4 | Sc 4 |
| ADP | kg Sb eq | 6.73 | 6.11 | 7.70 | 17.54 | 27.2 | 26.8 | 51.7 | 29.7 | -34.1 | -33.6 | -44.2 | -37.1 | -0.134 |
| AP | kg $SO_2$ eq | 8.13 | 7.31 | 9.75 | 20.58 | 26.9 | 26.4 | 51.5 | 29.5 | -33.6 | -33.0 | -44.0 | -36.9 | -0.134 |
| EP | kg $PO_4^{-3}$ eq | 1.43 | 1.27 | 1.97 | 4.05 | 26.2 | 25.6 | 51.5 | 29.4 | -32.8 | -32.0 | -44.0 | -36.8 | -0.134 |
| GWP | kg $CO_2$ eq | 822 | 737 | 1073 | 2246 | 26.7 | 26.2 | 51.8 | 29.6 | -33.5 | -33.8 | -44.3 | -37.0 | -0.131 |
| ODP | g CFC-11 eq | 0.05 | 0.04 | 0.07 | 0.12 | 27.0 | 26.6 | 52.7 | 29.5 | -33.8 | -33.3 | -45.1 | -37.0 | -0.134 |
| HTP | kg 1,4-DB eq | 197 | 173 | 275 | 602 | 25.9 | 25.2 | 51.2 | 29.5 | -32.5 | -31.5 | -43.8 | -36.9 | -0.134 |
| FEP | kg 1,4-DB eq | 605 | 579 | 292 | 1007 | 29.3 | 29.3 | 51.0 | 30.1 | -36.7 | -36.6 | -43.6 | -37.7 | -0.134 |
| MEP | kg 1,4-DB eq | 131 | 115 | 188 | 386 | 25.7 | 25.0 | 51.1 | 29.3 | -32.2 | -31.2 | -43.7 | -36.8 | -0.134 |
| TEP | g 1,4-DB eq | 0.05 | 0.04 | 0.06 | 0.13 | 26.0 | 25.4 | 51.2 | 29.4 | -32.6 | -31.7 | -43.8 | -36.8 | -0.134 |
| POFP | g $C_2H_4$ eq | 0.31 | 0.28 | 0.35 | 0.86 | 27.0 | 26.6 | 51.4 | 29.7 | -33.8 | -33.2 | -44.0 | -37.2 | -0.134 |

[1]Baseline scenario: T=170ºC, antioxidant extract constitutes 21% algal biomass (dry weight)
[2]Minimum antioxidant content: T=150ºC, antioxidant extract constitutes 13% algal biomass (dry weight)
[3]Maximum antioxidant content: T=200ºC, antioxidant extract constitutes 41% algal biomass (dry weight)
[4]Maximum fucoxanthin content: 55.1 mg in 100 g algal biomass (dry weight)

❖ **Effect of FU choice and allocation procedures on the environmental profiles**

The results from Section 5.3.2 show that Sc 4 presents much higher environmental burdens than the other three alternatives. However, it should be pointed out that in this scenario an additional valuable compound is obtained. Fucoxanthin is a biologically active molecule with a high value, not only in economic terms, but also with potential uses in the pharmaceutical sector. Moreover, Sc 3 has higher environmental impacts than Sc 1 and Sc 2 in most categories per kg valorized biomass, but the obtained product (1 kg antioxidant extract) is significantly more valuable than the product of Sc 1 and Sc 2 (0.65 kg antioxidant extract and 0.35 kg alginate).

The obtained results are based on a FU that focuses on the amount of valorized biomass rather than on the obtained products. Indeed, the choice of the FU is a critical point in a LCA study and several authors consider it as a limitation since it is a subjective matter (Fleischer and Schmidt, 1996; Schau and Fet, 2008). The selected FU considers the maximization of valorized biomass as the main function of the system but does not include the benefits of the process associated with the production of valuable molecules.

Hence, a second approach is presented below, consisting of a FU focused on the products obtained instead of the amount of biomass processed. High-purity fucoxanthin has a market value of up to 9000 €·g$^{-1}$, although the fucoxanthin-containing extract in this case has a significantly lower price ranging between 40 and 240 €·g$^{-1}$. The value of the antioxidant extract is estimated around 170 €·g$^{-1}$ (according to the price of similar extracts from other macroalgae) and that of sodium alginate is lower than 0.10 €·g$^{-1}$ (www.sigmaaldrich.com).

Considering that the antioxidant extract and the alginate were the major components in quantitative terms and the antioxidant extract (obtained in all configurations) had a much higher value, the FU was selected as 1 kg of antioxidant extract. Since two additional co-products (fucoxanthin-containing extract and alginate) were obtained, economic allocation was applied according to the Handbook on Life Cycle Assessment (Guinée et al., 2002) and economic values of the three products were used for allocation. However, not all the stages were associated with the three products (e.g. supercritical extraction -S3-

was only related to fucoxanthin), so allocation factors varied within the stages of each evaluated scenario. A summary of the considered allocation factors is given in **Table 5.6**. The effect of this alternative FU on the environmental profiles is shown in **Table 5.7** and **Figure 5.8**.

According to the considered economic allocation, the results change significantly with respect to the previous analysis. Thus, Sc 4 is not the alternative with the highest impacts and Sc 3 constitutes the least appealing option. However, the differences between scenarios 1, 3 and 4 are lower than 5% in all categories. The integral valorization of biomass by extracting the three high value compounds becomes competitive when considering the product-based FU with economic allocation. Again, Sc 2 is the preferred scenario according to the environmental performance and has contributions between 10% and 15% lower than Sc 1. The most relevant reductions of impact are linked to toxicity categories, because no solvents are needed to obtain the antioxidant extract.

These results are based on an economic allocation (**a**) that assigns a factor of 0 for the impact of the antioxidant extract related to S4 and S5, since these stages are not strictly necessary to obtain the product. Nevertheless, the performance of stage S4 facilitates the non-isothermal autohydrolysis and allows reducing mass and energy consumption in S6 due to the lower quantity of biomass treated.

Therefore, a second allocation approach (**b**) was also assessed, allocating a fraction of the environmental burdens of S4 to alginate and another fraction to the antioxidant extract according to the same criteria applied to stages S1 to S3. The main change when considering the second approach is the reduction of the impact of Sc 3, which has a more favorable environmental profile than Sc 1 and Sc 4 despite the limited differences between them (less than 5% between Sc 1 and Sc 3, and less than 3% between Sc 1 and Sc 4). The decrease of the relative contributions of Sc 3 is due to the impact of the production of chemicals and the effluents from the extraction for Sc 1, 2 and 4, that was not taken into account with the first allocation.

**Table 5.6.** Partitioning fraction for economic allocation in the evaluated scenarios for 1 kg antioxidant extract

| Scenario | Co-product | Produced quantity (g product per kg valorized alga) | Stage 1 | Stage 2 | Stage 3 | Stage 4 | Stage 5 | Stage 6 |
|---|---|---|---|---|---|---|---|---|
| **Sc 1 and Sc 2** | Fucoxanthin-containing extract | 0 | 0% | 0% | 0% | 0% | 0% | 0% |
| | Alginate | 354 | 0.03% | 0.03% | 0.03% | 100% | 100% | 0% |
| | Antioxidant extract | 646 | 99.97% | 99.97% | 99.97% | 0% | 0% | 100% |
| **Sc 3** | Fucoxanthin-containing extract | 0 | 0% | 0% | 0% | 0% | 0% | 0% |
| | Alginate | 0 | 0% | 0% | 0% | 0% | 0% | 0% |
| | Antioxidant extract | 1000 | 100% | 100% | 100% | 100% | 100% | 100% |
| **Sc 4** | Fucoxanthin-containing extract | 0.37 | 0.05% | 100% | 100% | 0% | 0% | 0% |
| | Alginate | 353 | 0.03% | 0% | 0% | 100% | 100% | 0% |
| | Antioxidant extract | 646 | 99.93% | 0% | 0% | 0% | 0% | 100% |

*Estimated market price of 140 €·g$^{-1}$ for fucoxanthin-containing extract, 0.08 €·g$^{-1}$ for alginate and 167.30 €·g$^{-1}$ for antioxidant extract

**Table 5.7.** Impact assessment results (characterization step) associated with 1 kg antioxidant extract and two economic allocation approaches in the four evaluated scenarios

| Impact category | Unit | FU= 1 kg antioxidant extract, approach a) | | | | FU= 1 kg antioxidant extract, approach b) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Sc 1 | Sc 2 | Sc 3 | Sc 4 | Sc 1 | Sc 2 | Sc 3 | Sc 4 |
| ADP | kg Sb eq | 7.36 | 6.41 | 7.70 | 7.66 | 8.09 | 7.14 | 7.70 | 8.39 |
| AP | kg $SO_2$ eq | 9.31 | 8.05 | 9.75 | 9.70 | 11.99 | 10.73 | 9.75 | 12.34 |
| EP | kg $PO_4^{-3}$ eq | 1.88 | 1.63 | 1.97 | 1.96 | 2.07 | 1.82 | 1.97 | 2.15 |
| GWP | kg $CO_2$ eq | 1174.86 | 1043.56 | 1220.82 | 1215.54 | 1271.19 | 1139.89 | 1220.82 | 1311.54 |
| ODP | mg CFC-11 eq | 64.98 | 57.89 | 67.52 | 67.18 | 71.00 | 63.91 | 67.52 | 73.17 |
| HTP | kg 1,4-DB eq | 261.77 | 224.70 | 274.74 | 273.29 | 290.58 | 253.50 | 274.74 | 301.69 |
| FEP | kg 1,4-DB eq | 278.75 | 238.38 | 292.88 | 291.19 | 9292.29 | 888.92 | 292.88 | 941.56 |
| MEP | kg 1,4-DB eq | 179.43 | 153.88 | 188.37 | 187.32 | 198.64 | 173.09 | 188.37 | 206.42 |
| TEP | g 1,4-DB eq | 58.69 | 50.36 | 61.61 | 61.28 | 68.40 | 60.07 | 61.61 | 70.96 |
| POFP | g $C_2H_4$ eq | 376.13 | 329.94 | 392.28 | 390.46 | 410.72 | 364.53 | 392.28 | 423.35 |

Sc 1. Baseline scenario. Alginate + antioxidant extract from dry alga
Sc 2. Alginate + antioxidant extract from wet alga
Sc 3. Antioxidant extract from wet alga
Sc 4. Fucoxanthin-containing extract + alginate + antioxidant extract from freeze-dry alga

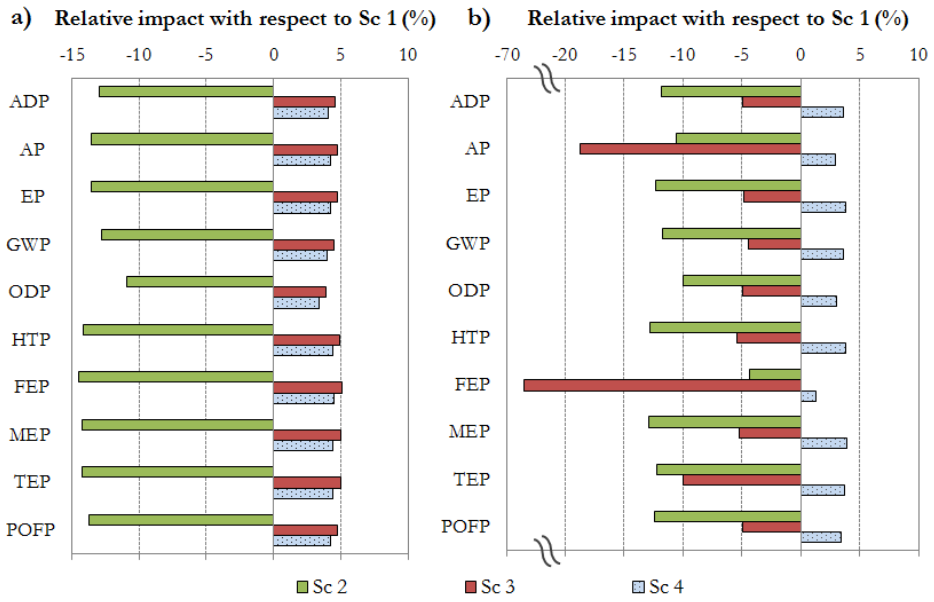


**Figure 5.8.** Relative environmental profile of Sc 2, Sc 3 and Sc 4 with respect to Sc 1 for 1 kg antioxidant extract as FU, with a) economic allocation considering null impact of antioxidant extract related to S4 and S5, and b) economic allocation considering benefits of S4 for antioxidant extraction due to biomass reduction.

The distributions of impacts within stages (**Figures 5.9** and **5.10**) and involved processes (**Figures 5.11** and **5.12**), which are roughly the same for both allocation approaches, are consistent with the findings of Section 5.2.3. Thus, S6 is also the main responsible for most impacts due, to a large extent, to the consumption of electricity in this stage. S2 has significant contributions in some specific categories, especially those related to toxicity, whereas the effect of S1 is limited to the categories of GWP, ODP and POFP, associated with vessel operations. S5 constitutes the main change with respect to the FU based on the valorized biomass, as it has no contribution to the impacts related to the antioxidant extract. S4 follows the same trend as S5 when considering approach **a**, although the behavior of this stage in economic allocation **b** is similar to the results in Section 5.2.3. Thus, in approach **b** S4 is the main responsible for impacts to FEP due to waste treatment associated with solvent residues.

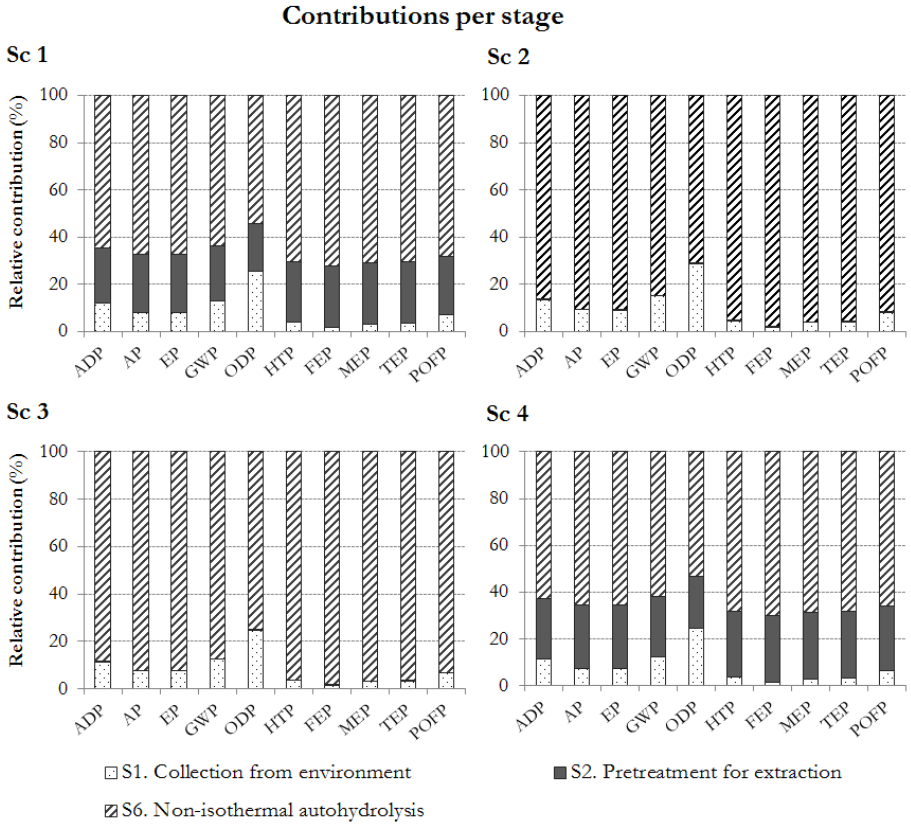


**Figure 5.9.** Relative contributions per stage to the environmental profile of the compared scenarios for 1 kg antioxidant extract as functional unit and economic approach a).

According to the results and sensitivity assessments, the environmental LCA has allowed identifying the combined extraction of alginate and antioxidant from wet algae as the most efficient route for the valorization of *S. muticum*, regardless of the methodological approach. Furthermore, the extremely low content of fucoxanthin within the biomass in relation to the high electricity requirements of the supercritical $CO_2$ extraction system makes its recovery unfeasible from an environmental perspective.

Concerning the relative contribution of the different stages and processes, the production of electricity associated with the non-isothermal autohydrolysis for the antioxidant extraction and the supercritical fucoxanthin extraction (in the case of Sc 4) are clearly the main hot spot for all the analyzed approaches.

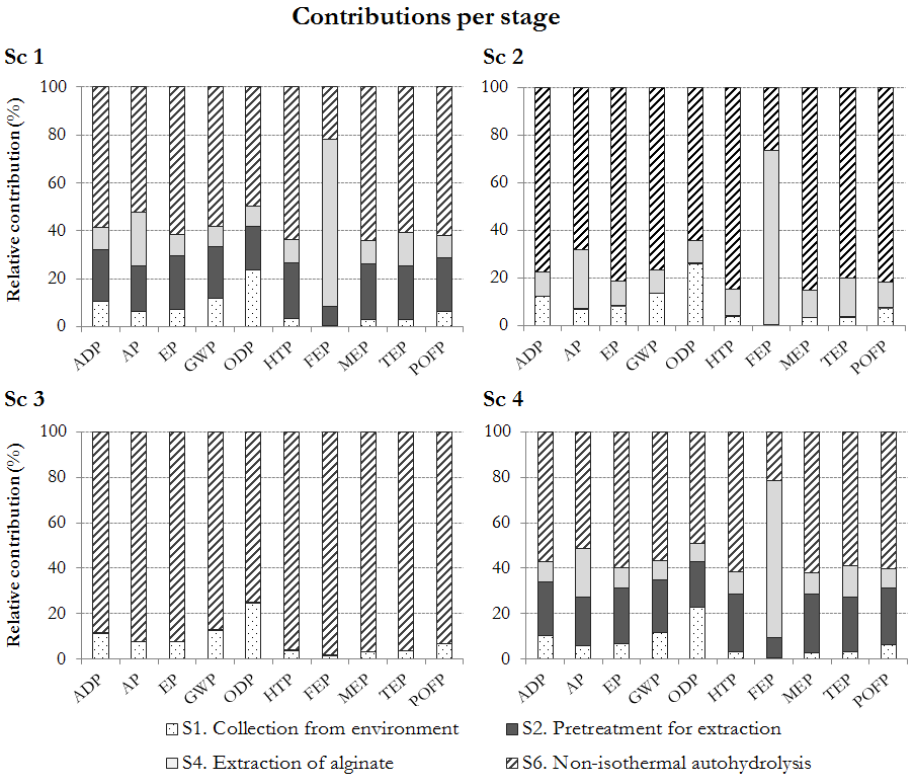


**Figure 5.10.** Relative contributions per stage to the environmental profile of the compared scenarios for 1 kg antioxidant extract as functional unit and economic approach b).

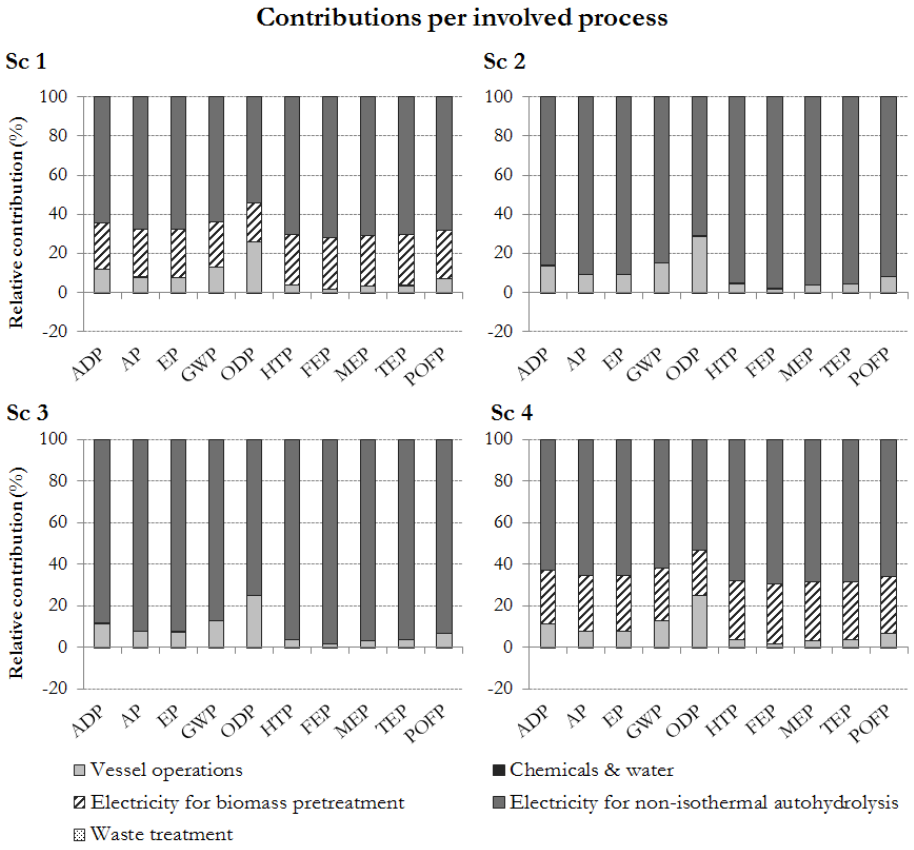


**Figure 5.11.** Relative contributions per involved process to the environmental profile of the compared scenarios for 1 kg antioxidant extract as functional unit and economic approach a).

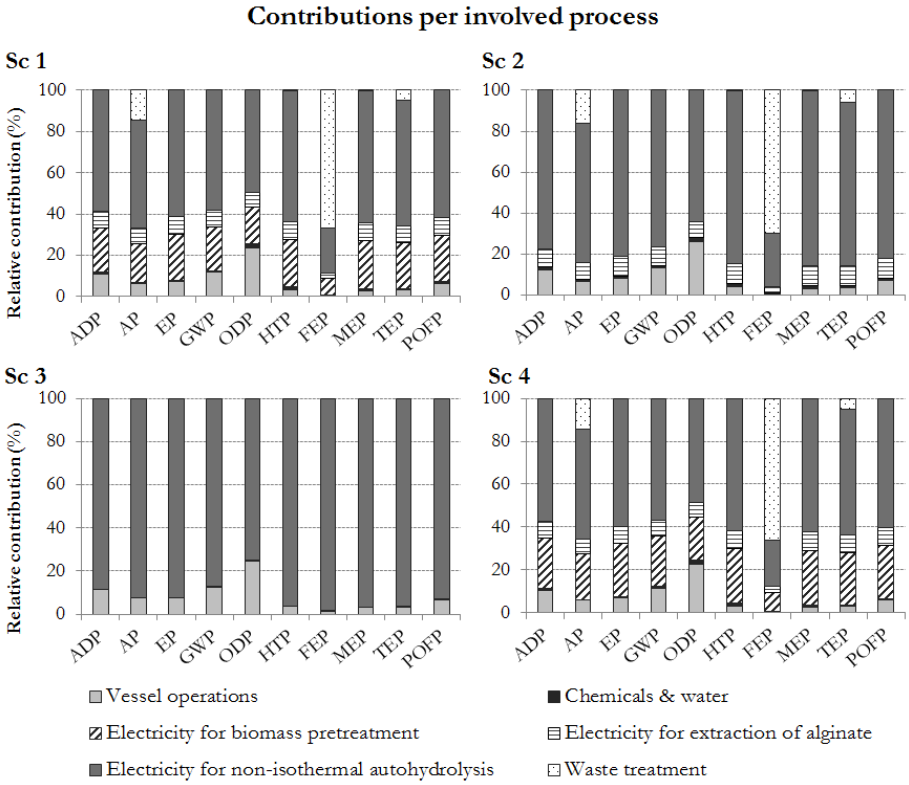


**Figure 5.12.** Relative contributions per involved process to the environmental profile of the compared scenarios for 1 kg antioxidant extract as functional unit and economic approach b).

## 5.3. Essential terpene oils from macroalga *Ochtodes secundiramea*

Despite the great potential of macroalgae as source of numerous bioactive metabolites, produced as a result of their defense and survival mechanisms, the lack of appropriate *in vitro* culture systems for a continuous supply is a major barrier for bioprocess development (Rorrer and Cheney, 2004). With this regard, several artificial systems, such as ponds and enclosed photobioreactors (PBRs), are currently proposed as a suitable alternative to *in situ* conventional farming sites for the commercial implementation of seaweed cultivation (Ahmed and Taha, 2011; Rorrer and Cheney, 2004). As in the case of microalgae, the use of these artificial systems allows the growth of target species to high cell densities on low-value land (Ahmed and Taha, 2011), while avoiding possible impacts of large-scale seaweed farming on biodiversity (Radulovich et al., 2015; Wei et al., 2013).

Cell and tissue cultivation in PBRs enable a continuous and steady production of macroalgal products with high yields and no seasonality barriers (Ahmed and Taha, 2011). Moreover, they provide controlled conditions and a sterile environment, which are required for the production of high value metabolites (Rorrer and Cheney, 2004). The use of these techniques requires the development of three components: i) cell and tissue culture, ii) PBR design and iii) identification of strategies for promoting the production of target metabolites (Ahmed and Taha, 2011; Rorrer and Cheney, 2004).

Despite being considerably underdeveloped, the techniques for cell and tissue cultures are mainly based on those for land plants. Their characteristics depend on the species and its morphology. Thus, the development of cell culture systems for filamentous algae is based on diverse isolation techniques, whereas tissue culture systems usually involve a first step of callus induction from explants of specimens collected on site and a second step of partial regeneration of shoot tissues to form "microplantlets" (Rorrer and Cheney, 2004).

Once developed, the cell or tissue culture is suspended in the PBR. The cultivation requires illumination, nutrient delivery and appropriate gas exchange ($CO_2$ addition and $O_2$ removal), as well as mixing and temperature control. The

main bioreactor configurations reported for macroalgal cultures are airlift PBRs, bubble columns, stirred tanks and tubular recycled PBRs (Rorrer and Cheney, 2004; Yong et al., 2014). Each of them presents advantages and limitations regarding mixing patterns, gas transfer, light efficiency and shear damage potential, so the selection depends on the species and required conditions (Rorrer and Cheney, 2004).

Future research related to the production of metabolites from macroalgae should focus on the development of the third component: the design of appropriate strategies to enhance the production of target compounds. With this regard, some authors have already shown examples of the application of metabolic principles for the regulation of secondary metabolism of some algal species (Maliakal et al., 2001; Polzin et al., 2003; Wargacki et al., 2012).

In this study, a novel system for the controlled cultivation of *Ochtodes secundiramea* (Montagne) MA Howe is proposed. *O. secundiramea* is a red macroalgae that exhibits a significant content of diverse bioactive compounds belonging to the group of essential terpenes (Machado et al., 2014). Terpenes constitute a vast group of natural products, based on the different possible arrangements of bonded isoprene units ($C_5H_8$) (Zwenger and Basu, 2008). The existing arrangements extend from single isoprene units, known as hemiterpenes, to combinations of eight (tetraterpenes) or even more units (polyterpenes), monoterpenes (containing two isoprene units) being the most abundant type, followed by sesquiterpenes (consisting of three isoprene units) (Silvestre and Gandini, 2008; Zwenger and Basu, 2008).

Essential terpene oils are secondary metabolites commonly obtained from herbs and other plants (e.g. rosemary, juniper, pine, eucalyptus), although they are also found in some insects, marine organisms and fungi (Mühlbauer et al., 2003; Silvestre and Gandini, 2008). Among marine sources, macroalgae are increasingly proposed as an alternative source (Barahona and Rorrer, 2003; Mühlbauer et al., 2003). In nature, many terpenes play important ecological roles, such as their defense function as insect repellents or their involvement in symbiotic mechanisms. Moreover, they can be used for a wide variety of applications such as the production of insecticides and polymers, as well as for cosmetics and pharmaceutical products (Silvestre and Gandini, 2008).

In particular, *O. secundiramea* contains considerable amounts of halogenated monoterpenes. A variety of assays performed on *O. secundiramea* extracts have allowed the identification of high antifungal activity linked to these terpenes (Machado et al., 2014). Furthermore, several of these compounds have been found to possess anticancer and anti-microbial bioactivities (Polzin, 2005).

Due to the potential interest of *O. secundiramea* cultivation, a semi-continuous production process is here proposed, together with the subsequent extraction of essential terpene oils, specifically myrcene, 10Z-bromomyrcene, 10E-bromo-3-chloromyrcene, apakaochtodene B and acyclic $C_{10}H_{14}Br_2$. LCA methodology was again selected as a suitable environmental management tool for the evaluation of the impacts of the process throughout its whole life cycle.

### 5.3.1. Goal and scope definition

The aim of this LCA study is to identify the environmental impacts associated with the complete cultivation and extraction process according to a cradle-to-gate perspective. In a first step, the impacts are quantified with respect to the total essential terpene oils. To do so, the selected FU is 700 mg of essential terpene oils (1 batch). Subsequently, the specific impacts of the different fractions are estimated. Since the five bioactive compounds have similar applications and economic value, a mass allocation will be considered to assign the corresponding impacts to each product. Additionally, the different stages and activities involved in the process are analyzed to identify the highest contributions to the environmental profile, and propose alternatives.

The production scheme considered in this assessment is based on the lab process developed by the Bioengineering Group of the Earth and Life Institute at the University of Louvain (Belgium). The cultivation was carried out in a 13 L transparent polyvinyl chloride (PVC) airlift PBR. The process was divided into six main stages, which are described below: i) cleaning and sterilization, ii) preparation of the culture medium, iii) preparation of the inoculum, iv) cultivation in PBR, v) cell separation and vi) cell disruption and extraction. **Figure 5.13** shows the different stages and processes included within the system boundaries.

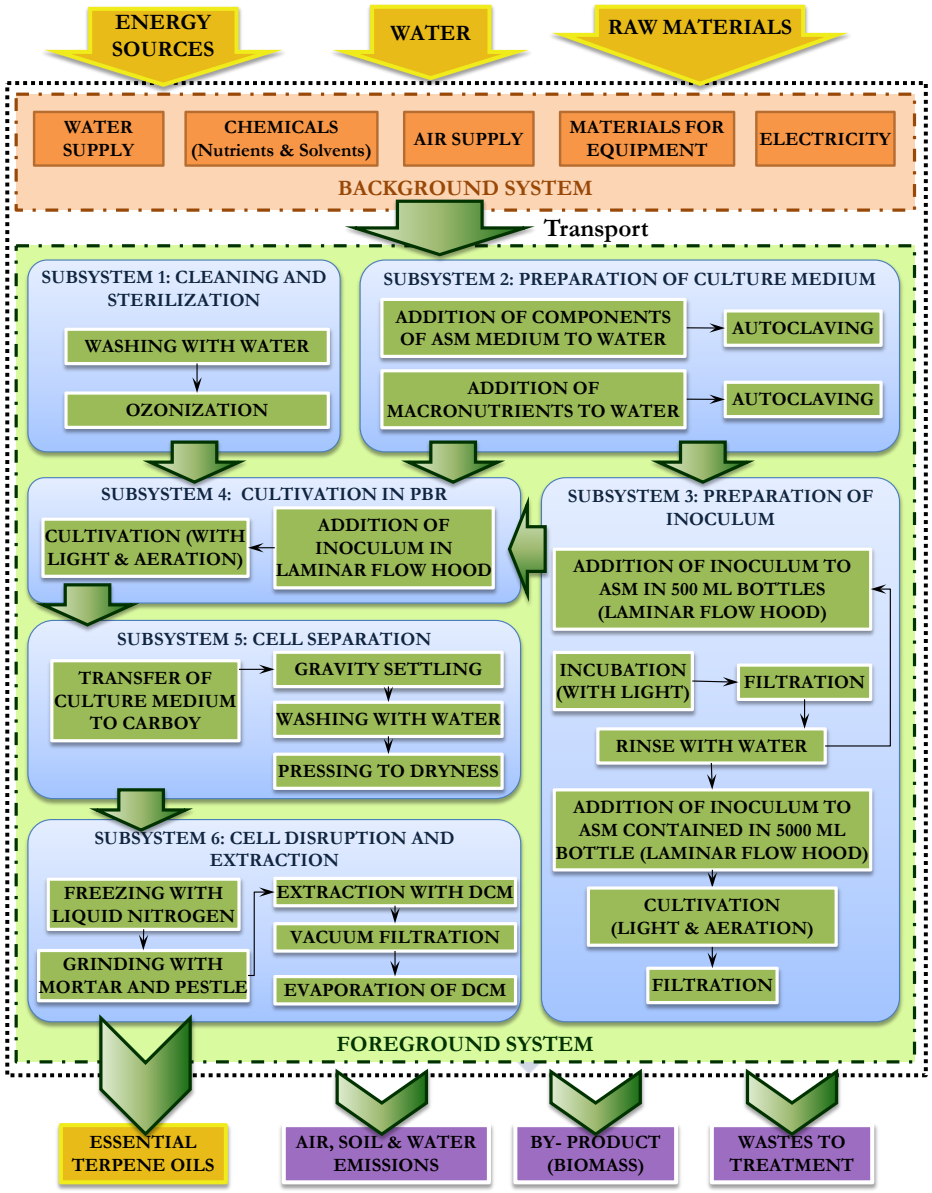


**Figure 5.13.** Process chain and system boundaries of the cultivation of *O. secundiramea* in a 13 L airlift PBR and later extraction of essential terpene oils.

i) S1. Cleaning and sterilization: Before each batch culture in the 13 L PBR, a cleaning stage was carried out. Firstly, 20 L of tap water with about 10 mL soap, followed by 5 L of sterile demineralized water were used to clean the reactor. Subsequently, the PBR was loaded with sterilized culture medium and sparged with 2.0 L $O_2$ $min^{-1}$ (source pressurized oxygen tank, reduced to 1 bar before injection) passed through an $O_3$ generator to further disinfect the inside of the PBR. The reactor was sparged for one additional day with 2.0 L air $min^{-1}$ (compressed house air at 6 bar, reduced to 1 bar before injection) to remove all traces of $O_3$ before inoculation.

ii) S2. Preparation of the culture medium: A total volume of 20 L of sterile seawater was prepared to be used in all steps of the process. The artificial seawater medium (ASM) was composed of 7.64 mM potassium chloride (KCl), 1.97 mM sodium bicarbonate ($NaHCO_3$), 690 μM potassium bromide (KBr), 354 μM boric acid ($H_3BO_3$), 63.3 μM sodium fluoride (NaF), 345 mM sodium chloride (NaCl), 23.8 mM sodium sulfate ($Na_2SO_4$), 44.9 mM magnesium chloride hexahydrate ($MgCl_2 \cdot 6H_2O$), 8.7 mM calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$) and 77.7 μM strontium chloride hexahydrate ($SrCl_2 \cdot 6H_2O$).

The composition of micronutrients in the medium was 30 μM KI, 19.7 μM iron chloride ($FeCl_3$), 20.7 μM ethylenediaminetetraacetic acid disodium salt dihydrate (EDTA-$Na_2 \cdot 2H_2O$), 2.28 μM manganese (II) sulfate tetrahydrate ($MnSO_4 \cdot 4H_2O$), 165 nM zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), 80 nM ammonium metavanadate ($NH_4VO_3$), 35.9 nM sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$), 45.4 nM copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), 0.954 nM sodium selenate ($Na_2SeO_3$), 24.7 nM cobalt (II) sulfate heptahydrate ($CoSO_4 \cdot 7H_2O$) and 6 nM nickel (II) chloride hexahydrate ($NiCl_2 \cdot 6H_2O$).

The composition of macronutrients supplement was 10.6 mM sodium nitrate ($NaNO_3$) and 483 μM sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_2O$). The ASM also contained 1.53 nM thiamine hydrochloride (thiamine HCl), 0.737 pM biotin and 5.71 pM vitamin B12. The salts for the solutions were reagent grade chemicals.

Demineralized water was used to prepare all medium components. The medium was autoclaved for 30 min at 121°C and 1.5 bar.

iii) S3. Preparation of inoculum: Firstly, 400 mL cell cultures were kept in 500 mL culture bottles within an incubator set at 25°C and 60 μmol photons·$m^{-2}$·$s^{-1}$ light intensity from two 18 W fluorescent lights in a 14 h on, 10 h off diurnal cycle. Each bottle was aerated with 300 mL·$min^{-1}$ of sterile air (0.75 vvm). The medium was changed once per week in each culture bottle when the plantlets were separated from the spent medium by coarse filtration, rinsed with 200 mL of water demineralized by ion exchange, and returned to the culture bottle with 400 mL of medium which had been sterilized in autoclave. The cultures were inoculated at 10 g·$L^{-1}$ fresh weight (2 g·$L^{-1}$ dry weight) and grown to 20 g·$L^{-1}$ fresh weight (4 g·$L^{-1}$ dry weight) after two weeks.

After one cell subculture cycle, 32 g of fresh plantlet biomass from the 500 mL culture bottles was used to inoculate a 5 L Scott bottle containing 4 L of fresh medium, aerated with 3 L·$min^{-1}$ of sterile filtered house air and illuminated by two 18 W fluorescent bulbs. At days 7 and 14, 80 mL of macronutrient solution was added to the culture medium. After 21 days the 5 L culture bottle contained 65 g of fresh plantlets, which were used to inoculate the 13 L PBR.

iv) S4. Cultivation in 13 L PBR: The airlift PBR was loaded with 13 L of sterilized culture medium and inoculated with 65 g fresh plantlets (13 g dry mass) which yielded an initial cell culture density of 1 $g_{DW}$·$L^{-1}$. The reactor was illuminated by six fluorescent bulbs of 36 W each and aerated by 2.6 L·$min^{-1}$ of compressed air. The pH was maintained at 8.0 with pure $CO_2$ at 1.5 bar.

Artificial seawater medium and macronutrient solution were fed to the PBR at a rate of 464 and 42 mL·$d^{-1}$, respectively, by the use of an intermittent timer coupled to a peristaltic pump, operated for 15 min every 4 hours. The culture was kept for 28 days, yielding a final biomass of approximately 780 g fresh macroalgae (equivalent to approximately 156 $g_{DW}$).

v) S5. Cell separation: In this step, 13 L of cell culture (780 g of fresh plantlets) was transferred by gravity into a carboy. After 1 min, the plantlets settled to the bottom and approximately 10 L of supernatant were removed. Successive aliquots of the remaining tissues were passed onto a large strainer to remove the liquid.

   From the collected algae, 65 g of fresh tissues were kept to inoculate the next photobioreactor cultivation, while the remaining 715 g of fresh plantlets (containing approximately 700 mg essential terpene oils) were washed first with 13 L of tap water followed by 13 L of deionized water. The plantlets were placed in a filter (cotton cloth) and pressed to absorb the remaining moisture. The cloth was dried at room temperature.

vi) S6. Cell disruption and extraction: 715 g of fresh microplantlets were grinded with a mortar and pestle using 4 L of liquid nitrogen to keep the tissues in a frozen state during the grinding process, until the liquid nitrogen was evaporated (approx. 3 min).

   The biomass was then placed in a screw-cap flask with 1000 mL of dichloromethane (DCM) for 24 h at 22°C under continuous mixing on an orbital shaker at 100 rpm. After collecting the extraction solvent, the solid residue was vacuum filtered and biomass was re-extracted using the same conditions. The combined extract was evaporated under nitrogen gas flow at 22°C to give a total amount of approximately 700 mg of essential oils.

In a semi-continuous process, the required amount of fresh tissue to inoculate the reactor can be supplied by taking a small portion of the produced biomass in S6. Therefore, the subculture in 500 mL flasks (S3) and the cultivation in 5 L bottles would be unnecessary when implementing a semi-continuous process on subsequent batches. For this reason, three possible scenarios are proposed and compared:

i) Discontinuous operation, corresponding to one batch that includes the seven described stages.

ii) Semi-continuous operation for approximately 1 year (12 batches) with stages S3 and S4 only performed for the first batch, whereas the subsequent cultivations in the 13 L PBR are started by re-inoculating a portion of the harvested biomass.

iii) Semi-continuous operation maintained for 10 years (120 batches) with each inoculum coming from a portion of the harvested biomass.

### 5.3.2. Life cycle inventory, data quality and assumptions

The LCI data for the foreground system (i.e. water, chemicals and electricity consumptions as well as transport distances) consisted of average data obtained by on-site measurements. Regarding water emissions derived from the different production stages, they were assumed to be directly discharged to the environment.

The inventory data of the process are shown in **Table 5.8** for the three analyzed scenarios: 1 batch, 1 year of semi-continuous operation (35 days for inoculum preparation followed by 10 batches) and 10 years of semi-continuous operation (35 days for inoculum preparation followed by 120 batches).

Concerning the background system, these inputs include the processes for the production of the different chemicals required for the separation, the electricity used in the different stages (taken from the Irish grid), the distribution of inputs up to the lab gate, laboratory supplies and equipment (flasks, electronic devices) and waste disposal. For the equipment, average weights and life spans were estimated according to manufacturers' specifications. With respect to transport, an average distance of 800 and 600 km within continental Europe was considered for chemicals and materials, respectively. Waste transport distance was estimated at around 50 km. Glass, steel and plastic wastes were assumed to be disposed in sanitary or inert landfills whereas the filter was sent to incineration. Inventory data for all those background processes were taken from Ecoinvent database, as summarized in **Table 5.9**.

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**Table 5.8.** Inventory data for 1 batch production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| | 1 batch | 1 year semi-continuous mode | 10 years semi-continuous mode |
| **Materials** | | | |
| *S1. Cleaning and sterilization* | | | |
| Tap water | 20.00 L | 20.00 L | 20.00 L |
| Sterile water | 5.00 L | 5.00 L | 5.00 L |
| Soap | 10.00 mL | 10.00 mL | 10.00 mL |
| Stainless steel | 32.43 g | 32.43 g | 32.43 g |
| Compressed air | | | |
| *S2. Preparation of culture medium* | | | |
| Demineralized water | 34.53 L | 27.78 L | 27.23 L |
| KCl | 18.91 g | 15.14 g | 14.84 g |
| $NaHCO_3$ | 5.43 g | 4.40 g | 4.31 g |
| KBr | 2.73 g | 2.18 g | 2.14 g |
| $H_3BO_3$ | 0.73 g | 0.58 g | 0.57 g |
| NaF | 0.09 g | 0.07 g | 0.07 g |
| NaCl | 669.24 g | 536.16 | 525.28 g |
| $Na_2SO_4$ | 112.21 g | 89.90 g | 8.07 g |
| $MgCl_2 \cdot 6H_2O$ | 302.99 g | 242.74 g | 237.81 g |
| $CaCl_2 \cdot 2H_2O$ | 42.45 g | 34.01 g | 33.32 g |
| $SrCl_2 \cdot 6H_2O$ | 0.69 g | 0.55 g | 0.54 g |
| KI | 0.17 g | 0.13 g | 0.13 g |
| $FeCl_3$ | 106.06 mg | 84.97 mg | 83.25 mg |
| EDTA-$Na_2 \cdot 2H_2O$ | 255.75 mg | 204.90 mg | 200.74 mg |
| $MnSO_4 \cdot 4H_2O$ | 16.88 mg | 13.52 mg | 13.25 mg |
| $ZnSO_4 \cdot 7H_2O$ | 1.57 mg | 1.26 mg | 1.24 mg |
| $NH_4VO_3$ | 0.31 mg | 0.25 mg | 0.24 mg |
| $Na_2MoO_4 \cdot 2H_2O$ | 0.29 mg | 0.23 mg | 0.23 mg |
| $CuSO_4 \cdot 5H_2O$ | 0.38 mg | 0.30 mg | 0.30 mg |
| $CoSO_4.5H_2O$ | 0.20 mg | 0.16 mg | 0.16 mg |
| $NiCl_2 \cdot 6H_2O$ | 47.34 µg | 37.92 µg | 37.15 µg |
| $Na_2SeO_3$ | 5.48 µg | 4.39 µg | 4.30 µg |

**Table 5.8.** Inventory data for 1 batch production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| | 1 batch | 1 year semi-continuous mode | 10 years semi-continuous mode |
| **Materials** | | | |
| *S2. Preparation of culture medium* | | | |
| Thiamine HCl | 17.13 μg | 13.72 μg | 13.44 μg |
| Biotin | 5.97 ng | 4.79 ng | 4.69 ng |
| Vitamin B12 | 0.26 μg | 0.21 μg | 0.20 μg |
| $NaNO_3$ | 1.20 g | 1.07 g | 1.06 g |
| $NaH_2PO_4 \cdot H_2O$ | 89.04 mg | 79.27 mg | 78.47 mg |
| Stainless steel | 5.49 g | 4.42 g | 4.33 g |
| *S3. Preparation of inoculum* | | | |
| Stainless steel | 2.83 g | 0.24 g | 0.02 g |
| Glass | 36.22 g | 3.02 g | 0.30 g |
| High density polyethylene (HDPE) | 0.51 g | 0.04 g | 0.04 g |
| Lamps | 3.32 g | 0.28 | 0.03 g |
| Polyurethane foam | 43.78 g | 3.65 g | 0.36 g |
| Zinc coated steel | 102.17 g | 8.51 g | 0.85 g |
| Silicon rubber | 1.67 g | 0.14 g | 0.01 g |
| Demineralized water | 20.00 mL | 16.67 mL | 1.67 mL |
| Compressed air (8 bar) | 12.85 $m^3$ | 1.07 $m^3$ | 0.11 $m^3$ |
| *S4. Cultivation in 13 L PBR* | | | |
| Stainless steel | 30.74 g | 30.37 g | 27.98 g |
| PVC | 32.59 g | 32.17 g | 29.43 g |
| Lamps | 34.27 g | 34.27 g | 34.27 g |
| Compressed air (8 bar) | 13.10 $m^3$ | 13.10 $m^3$ | 13.10 $m^3$ |
| *S5. Cell separation* | | | |
| Polypropylene (PP) | 16.52 g | 16.30 g | 14.92 g |
| Filter (cloth) | 7.59 g | 7.49 g | 6.86 g |
| Tap water | 13.00 L | 13.00 L | 13.00 L |
| Deionized water | 13.00 L | 13.00 L | 13.00 L |

**Table 5.8.** Inventory data for 1 batch production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes) (*Cont.*)

| INPUTS from TECHNOSPHERE | | | |
|---|---|---|---|
| | 1 batch | 1 year semi-continuous mode | 10 years semi-continuous mode |
| **Materials** | | | |
| *S6. Cell disruption and extraction* | | | |
| Liquid nitrogen | 4.00 L | 4.00 L | 4.00 L |
| Gaseous nitrogen | 1000 L | 1000 L | 1000 L |
| Dichloromethane | 2.00 L | 2.00 L | 2.00 L |
| Steel | 127.35 g | 127.35 g | 127.35 g |
| Glass | 18.18 g | 18.18 g | 18.18 g |
| **Energy** | | | |
| *S1. Cleaning and sterilization* | | | |
| Autoclaving | 0.19 kWh | 0.19 kWh | 0.19 kWh |
| Ozone generator | 1.92 kWh | 1.92 kWh | 1.92 kWh |
| Air supply | 0.05 kWh | 0.05 kWh | 0.05 kWh |
| *S2. Preparation of culture medium* | | | |
| Autoclaving | 1.29 kWh | 1.05 kWh | 1.03 kWh |
| *S3. Preparation of inoculum* | | | |
| Laminar flow hood | 0.93 kWh | 0.08 kWh | 0.01 kWh |
| Incubation (excluding lights) | 4.48 kWh | 0.37 kWh | 0.04 kWh |
| Aeration | 1.68 kWh | 0.14 kWh | 0.01 kWh |
| Lighting | 12.47 kWh | 1.04 kWh | 0.09 kWh |
| *S4. Cultivation* | | | |
| Laminar flow hood | 0.75 kWh | 0.75 kWh | 0.75 kWh |
| Aeration | 1.72 kWh | 1.72 kWh | 1.72 kWh |
| Lighting | 84.67 kWh | 84.67 kWh | 84.67 kWh |
| Medium pumping | 2.10 kWh | 2.10 kWh | 2.10 kWh |
| *S6. Cell disruption and extraction* | | | |
| Extraction with DCM on orbital shaker | 9.60 kWh | 9.60 kWh | 9.60 kWh |
| Vacuum filtration | 0.13 kWh | 0.13 kWh | 0.13 kWh |
| Solvent evaporation | 0.56 kWh | 0.56 kWh | 0.56 kWh |

**Table 5.8.** Inventory data for 1 batch production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| | 1 batch | 1 year semi-continuous mode | 10 years semi-continuous mode |
| **Transport** | | | |
| Truck 3.5-7.5, euro 4: | | | |
| *S1. Cleaning and sterilization* | | | |
| Equipment | 19.46 kg·km | 19.21 kg·km | 17.62 kg·km |
| Wastes | 1.62 kg·km | 1.60 kg·km | 1.47 kg·km |
| *S2. Preparation of culture medium* | | | |
| Chemicals (nutrients) | 925.89 kg·km | 741.87 kg·km | 726.82 kg·km |
| Materials (equipment) | 3.30 kg·km | 2.65 kg·km | 2.60 kg·km |
| Wastes | 0.27 kg·km | 0.22 kg·km | 0.22 kg·km |
| *S3. Preparation of inoculum* | | | |
| Materials (equipment) | 114.29 kg·km | 9.52 kg·km | 0.95 kg·km |
| Wastes | 9.52 kg·km | 0.79 kg·km | 0.08 kg·km |
| *S4. Cultivation* | | | |
| Materials (equipment) | 58.56 kg·km | 58.08 kg·km | 55.01 kg·km |
| Wastes | 4.88 kg·km | 4.84 kg·km | 4.58 kg·km |
| *S5. Cell separation* | | | |
| Materials (equipment) | 14.47 kg·km | 14.28 kg·km | 13.07 kg·km |
| Wastes | 1.21 kg·km | 1.19 kg·km | 1.09 kg·km |
| *S5. Cell separation* | | | |
| Chemicals (solvents) | 2.13 tkm | 2.13 tkm | 2.13 |
| Materials (equipment) | 87.32 kg·km | 87.32 kg·km | 87.32 kg·km |
| Wastes | 7.28 kg·km | 7.28 kg·km | 7.28 kg·km |
| **INPUTS from ENVIRONMENT** | | | |
| *S3. Preparation of inoculum* | | | |
| Algal biomass for inoculum | 16.00 g | 1.33 g | 0.13 g |
| Carbon dioxide, $CO_2$([1]) | 13.86 g | 1.16 g | 0.11 g |
| *S4. Cultivation* | | | |
| $CO_2$([1]) | 202.31 g | 202.31 g | 202.31 g |

[1]Corresponding to an approximate biomass composition of $CH_{2.11}O_{1.01}N_{0.055}P_{0.002}$.

**Table 5.8.** Inventory data for 1 batch production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes) (*Cont.*)

| OUTPUTS to TECHNOSPHERE | | | |
|---|---|---|---|
| | 1 batch | 1 year semi-continuous mode | 10 years semi-continuous mode |
| **Product** | | | |
| Essential terpene oils | 700 mg | 700 mg | 700 mg |
| **By-product** | | | |
| Cell paste, recycled to PBR | 0 | 59.58 g | 64.46 g |
| Residual cell paste | 779.30 g | 719.72 g | 714.84 g |
| **Wastes to landfill** | | | |
| *S1. Cleaning and sterilization* | | | |
| Steel | 32.43 g | 32.02 g | 29.37 g |
| *S2. Preparation of culture medium* | | | |
| Steel | 5.49 g | 4.42 g | 4.33 g |
| *S3. Preparation of inoculum* | | | |
| Steel | 104.99 g | 8.75 g | 0.87 g |
| Glass | 36.22 g | 3.02 g | 0.30 g |
| HDPE | 0.51 g | 0.04 g | 0.04 g |
| Polyurethane foam | 43.78 g | 3.65 g | 0.36 g |
| *S4. Cultivation in 13 L PBR* | | | |
| Steel | 30.74 g | 30.36 g | 27.98 g |
| PVC | 32.59 g | 32.17 g | 29.43 g |
| *S5. Cell separation* | | | |
| PP | 16.51 g | 16.30 g | 14.92 g |
| *S6. Cell disruption and extraction* | | | |
| Steel | 127.35 g | 127.35 g | 127.35 g |
| Glass | 18.18 g | 18.18 g | 18.18 g |
| **Wastes to municipal incineration** | | | |
| *S3. Preparation of inoculum* | | | |
| Silicon rubber | 1.67 g | 0.14 g | 0.01 g |
| *S5. Cell separation* | | | |
| Filter (cloth) | 7.59 g | 7.50 g | 6.86 g |
| **Wastes to specific treatment** | | | |
| *S3. Preparation of inoculum* | | | |
| Lamps | 3.32 g | 0.28 | 0.03 g |
| *S4. Cultivation in 13 L PBR* | | | |
| Lamps | 34.27 g | 34.27 g | 34.27 g |

**Table 5.8.** Inventory data for 1 batch production of essential terpene oils by *O. secundiramea* cultivated in airlift PBR (FU=700 mg terpenes) (*Cont.*)

| **OUTPUTS to ENVIRONMENT** | | | |
|---|---|---|---|
| | 1 batch | 1 year semi-continuous mode | 10 years semi-continuous mode |
| **Water emissions** | | | |
| Wastewater (from all stages) | 84.95 L | 78.79 | 78.24 L |
| DCM (from S6) | 2 L | 2 L | 2 L |
| *Total non-consumed nutrients (from S3+S4+S5)* | | | |
| KCl | 0.85 g | 0.74 g | 0.74 g |
| $NaHCO_3$ | 0.25 g | 0.22 g | 0.22 g |
| KBr | 0.12 g | 0.11 g | 0.11 g |
| $H_3BO_3$ | 0.03 g | 0.03 g | 0.03 g |
| NaF | 39.86 mg | 34.98 mg | 34.59 mg |
| NaCl | 30.24 g | 26.54 g | 26.24 g |
| $Na_2SO_4$ | 5.07 g | 4.45 g | 4.40 g |
| $MgCl_2{\cdot}6H_2O$ | 13.69 g | 12.02 g | 11.88 g |
| $CaCl_2{\cdot}2H_2O$ | 1.92 g | 1.68 g | 1.66 g |
| $SrCl_2{\cdot}6H_2O$ | 31.07 mg | 27.27 mg | 26.96 mg |
| KI | 7.48 mg | 6.47 mg | 6.47 mg |
| $FeCl_3$ | 4.79 mg | 4.21 mg | 4.16 mg |
| EDTA-$Na_2{\cdot}2H_2O$ | 11.55 mg | 10.14 mg | 10.03 mg |
| $MnSO_4{\cdot}4H_2O$ | 0.76 mg | 0.67 mg | 0.66 mg |
| $ZnSO_4{\cdot}7H_2O$ | 71.16 µg | 62.46 µg | 61.75 µg |
| $NH_4VO_3$ | 14.03 µg | 12.16 µg | 12.16 µg |
| $Na_2MoO_4{\cdot}2H_2O$ | 13.03 µg | 11.43 µg | 11.30 µg |
| $CuSO_4{\cdot}5H_2O$ | 17.00 µg | 14.92 µg | 14.75 µg |
| $CoSO_4.5H_2O$ | 9.08 µg | 7.97 µg | 7.88 µg |
| $NiCl_2{\cdot}6H_2O$ | 2.14 µg | 1.88 µg | 1.86 µg |
| $Na_2SeO_3$ | 0.25 µg | 0.22 µg | 0.21 µg |
| Thiamine HCl | 0.77 µg | 0.68 µg | 0.67 µg |
| Biotin | 0.27 ng | 0.24 ng | 0.23 ng |
| Vitamin B12 | 0.01 µg | 0.01 µg | 0.01 µg |
| $NaNO_3$ | 60.18 mg | 53.58 mg | 53.04 mg |
| $NaH_2PO_4{\cdot}H_2O$ | 4.45 mg | 3.96 mg | 3.92 mg |

**Table 5.9.** Summary of data sources for the background system of the production of essential terpene oils by *O. secundiramea*

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Chemicals | Soap | Ecoinvent database (Zah and Hischier, 2007) |
| | $H_3BO_3$ | Ecoinvent database (Althaus et al., 2007) |
| | NaF | |
| | NaCl | |
| | $Na_2SO_4$ | |
| | $MgCl_2 \cdot 6H_2O$ | |
| | $CaCl_2 \cdot 2H_2O$ | |
| | $SrCl_2 \cdot 6H_2O$ | |
| | $FeCl_3$ | |
| | EDTA-$Na_2 \cdot 2H_2O$ | |
| | $MnSO_4 \cdot 4H_2O$ | |
| | $NH_4VO_3$ | |
| | $Na_2MoO_4 \cdot 2H_2O$ | |
| | $CuSO_4 \cdot 5H_2O$ | |
| | $CoSO_4.5H_2O$ | |
| | $NiCl_2 \cdot 6H_2O$ | |
| | $Na_2SeO_3$ | |
| | Thiamine HCl | |
| | Biotin | |
| | Vitamin B12 | |
| | $NaH_2PO_4 \cdot H_2O$ | |
| | $ZnSO_4 \cdot 7H_2O$ | Ecoinvent database (Hischier et al., 2007) |
| | KCl | Ecoinvent database (Nemecek and Kägi, 2007) |
| | KBr | |
| | KI | |
| | $NaHCO_3$ | Ecoinvent database (Sutter, 2007a) |
| | $NaNO_3$ | Inventoried according to the synthetic route described by UNIDO/IFDC (1998) and Bhat et al. (1994) with Ecoinvent processes (Frischknecht et al., 2007) |

**Table 5.9.** Summary of data sources for the background system of the production of essential terpene oils by *O. secundiramea* (*Cont.*)

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Energy | Electricity (Belgian electricity profile) | Ecoinvent database (Dones et al., 2007) |
| Water | Tap water<br>Distilled water | Ecoinvent database (Althaus et al., 2007) |
| Materials | HDPE<br>PP<br>PVC<br>Glass<br>Polyurethane foam<br>Silicon rubber | Ecoinvent database (Hischier, 2007) |
| | Lamps | Ecoinvent database (Hischier et al., 2007) |
| | Stainless steel | Ecoinvent database (Classen et al., 2007) |
| Transport | Truck 3.5-7.5 t | Ecoinvent database (Spielmann et al., 2007) |
| Waste treatment | Inert landfill<br>Sanitary landfill<br>Electronic waste<br>Municipal incineration | Ecoinvent database (Doka, 2007) |

*Allocation procedures*

Since five specific compounds (i.e. myrcene, 10Z-bromomyrcene, 10E-bromo-3-chloromyrcene, apakaochtodene B and acyclic $C_{10}H_{14}Br_2$) were identified among the essential terpene oils extracted from *O. secundiramea* biomass, an allocation approach was needed to quantify the impacts associated with each of the main compounds. Due to the similar value and potential applications of the different terpenes, a mass allocation approach is here proposed. The product distribution considered to allocate the environmental impacts was estimated from the composition measured by Polzin (2005) for the cultivation of *O. secundiramea* during 28 days with N-source excess.

**Table 5.10.** Partitioning fraction for mass allocation of the five main terpenes extracted from *O. secundiramea*

| Bioactive compound | Fraction (%) |
|---|---|
| Myrcene | 1.6 |
| 10Z-bromomyrcene | 27.9 |
| 10E-bromo-3-chloromyrcene | 25.1 |
| Apakaochtodene B | 8.5 |
| Acyclic $C_{10}H_{14}Br_2$ | 36.9 |

### 5.3.3. Environmental impact assessment

The environmental results associated with the production of essential terpene oils from *O. secundiramea* were again quantified using the CML 2 baseline 2001 V2.05 method for the Life Cycle Impact Assessment (LCIA) (Guinée et al., 2002) with the impact categories aforementioned for *S. muticum*: ADP, AP, EP, GWP, ODP, HTP, FEP, MEP, TEP and POFP. The inventory data were implemented in the software SimaPro 8 to obtain the characterization results for the impact assessment (Goedkoop et al., 2013). The results for the three evaluated scenarios are summarized in **Table 5.11**.

**Table 5.11.** Environmental impact assessment results (characterization step) associated with the production of 700 mg essential terpene oils from *O. secundiramea* cultivated in an airlift PBR operated in 1 batch, 1 year and 10 years

| Impact category | Unit | 1 batch | 1 year | 10 years |
|---|---|---|---|---|
| ADP | kg Sb eq | 0.49 | 0.42 | 0.41 |
| AP | kg $SO_2$ eq | 0.30 | 0.26 | 0.26 |
| EP | kg $PO_4^{-3}$ eq | 0.13 | 0.11 | 0.11 |
| GWP | kg $CO_2$ eq | 70.58 | 61.24 | 60.45 |
| ODP | kg CFC-11 eq | $1.51 \cdot 10^{-4}$ | $1.50 \cdot 10^{-4}$ | $1.50 \cdot 10^{-4}$ |
| HTP | kg 1,4-DB eq | 48.31 | 36.48 | 35..50 |
| FEP | kg 1,4-DB eq | 24.63 | 19.46 | 19.01 |
| MEP | kg 1,4-DB eq | 15.32 | 12.15 | 11.88 |
| TEP | kg 1,4-DB eq | $1.71 \cdot 10^{-2}$ | $1.61 \cdot 10^{-4}$ | $1.55 \cdot 10^{-2}$ |
| POFP | kg $C_2H_4$ eq | $1.25 \cdot 10^{-2}$ | $1.06 \cdot 10^{-2}$ | $1.05 \cdot 10^{-2}$ |

According to these results, the preparation of the inoculum (S3) has a significant effect on the environmental profile for most of the analyzed impact categories. Thus, the implementation of a semi-continuous process in which the inoculum for each cultivation is obtained from a small fraction of the final biomass from the previous culture represents a considerable improvement. The impact reduction is related to the sharing of the total contribution of the inoculum prepared at the start-up among all the batches performed during the semi-continuous process. Hence, the relative impact of S3 decreases when increasing the number of consecutive batches carried out with one single inoculum.

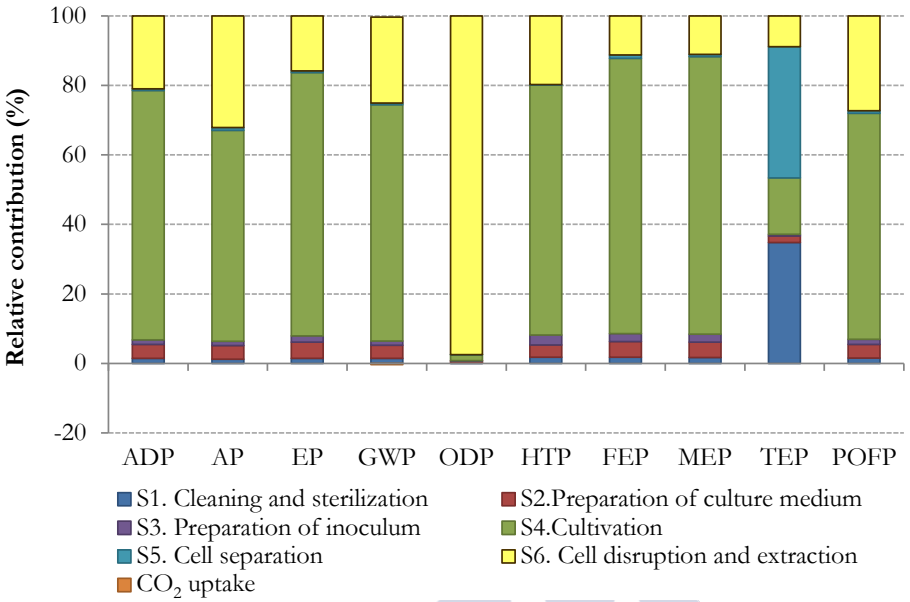
For 1-year operation maintained in these conditions, the characterization results are between 13% and 25% lower than those of a single batch, except for ODP and TEP, with limited reductions below 1% and 6% respectively. This is related to the low relative contribution of S3 for the aforementioned categories. When comparing the environmental profiles of 1 year and 10 years of semi-continuous operation, the differences are very limited in most categories (less than 4% for all impact categories). This finding demonstrates that the relative contribution of S3 is already very low compared to other stages in the 1-year scenario, so any further improvement is nearly negligible.

The relative contributions of all the stages and involved processes are further discussed below. Since the 1-batch scenario is not representative of a large scale process and the difference between 1-year and 10-year scenarios are very limited, the results provided in this section correspond to the semi-continuous operation for 1 year. Despite slight variations in the numerical values, the main findings and identified hot spots are applicable to the three analyzed scenarios.

❖ **Identification of hot spots**

According to the results shown in **Figure 5.14**, cultivation (S4) is the main contributor to the environmental burdens derived from the production of essential terpene oils. The contributions range between 60% and 80% for all categories except for ODP and TEP. The contribution to ODP is dominated by the cell disruption stage (97%), whereas TEP is mainly due to cleaning (35%) and cell separation (38%), followed by cultivation (16%).

**a) Relative contributions of 1-year scenario per stage**




**b) Relative contributions of 1-year scenario per involved process**

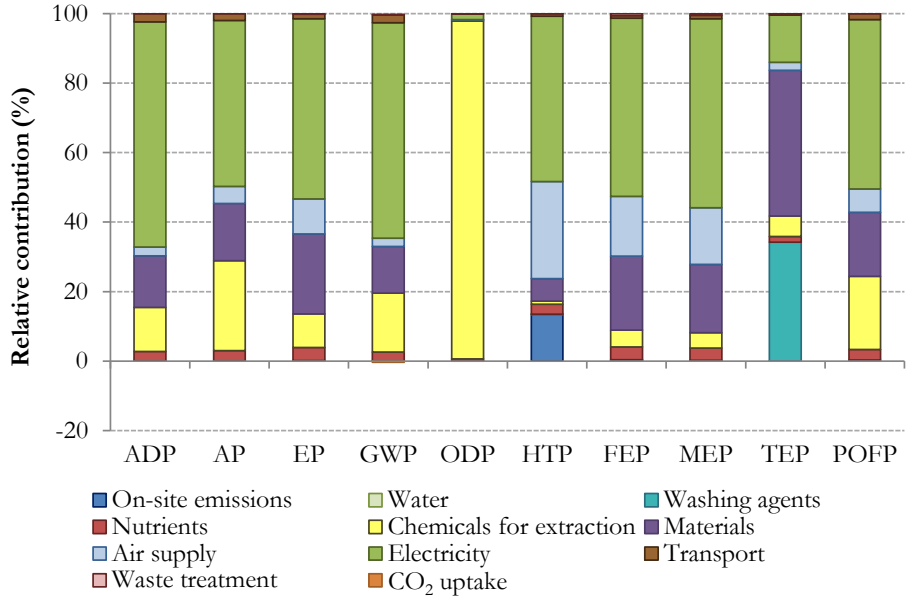



**Figure 5.14.** Relative contributions of the semi-continuous production of essential terpene oils by *O. secundiramea* to each impact category per a) stage and b) involved process.

Electricity production is the main process responsible for the high contributions of the cultivation stage. It involves between 55% and 78% of the total impact of S4, depending on the category. Moreover, electricity required for the cultivation involves 86% of the total consumption throughout the process. In particular, the electricity requirement for the illumination of the PBR corresponds to 81% of the total energy consumed, as depicted in **Figure 5.15**.

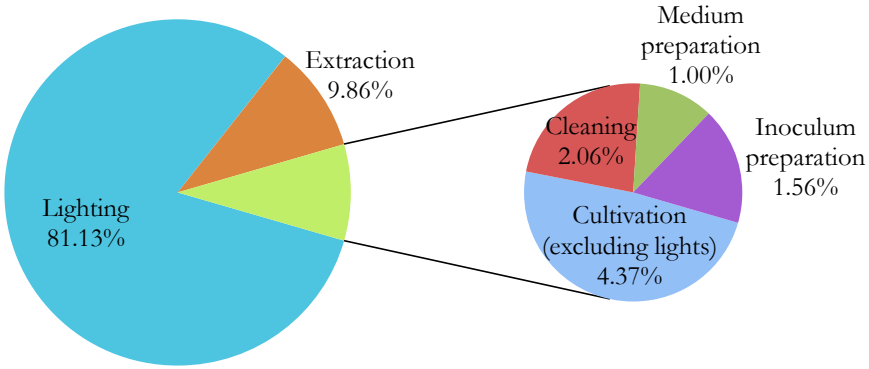



**Figure 5.15.** Relative contributions of the semi-continuous production of essential terpene oils by *O. secundiramea* to each impact category per a) stage and b) involved process.

Regarding other processes, the production of chemicals for the extraction is the most problematic issue in terms of ODP (97%), mainly due to the use of dichloromethane as solvent. The production of soap for cleaning (34%) and the materials (42%, mainly associated with the fabric filter) are important contributors to TEP. The production of materials also has other relevant contributions above 15% in categories such as AP, EP, FEP or MEP related to the production of lamps for reactor lighting. Among other secondary processes, only air supply has a noticeable impact in HTP (28%), FEP (17%) and MEP (16%). Although the carbon sequestration potential of algae during cultivation was considered, the benefit is very limited (<1%) in comparison with the total emissions of the process, mainly due to the high electricity consumption.

**Table 5.12.** Environmental impact assessment results (characterization step) for each terpene from *O. secundiramea* in the 1-year scenario according to the proposed allocation approach

| Impact category | Unit | 700 mg essential terpene oils | Myrcene | 10Z-bromomyrcene | 10E-bromo-3-chloromyrcene | Apakaochtodene B | Acyclic $C_{10}H_{14}Br_2$ |
|---|---|---|---|---|---|---|---|
| ADP | kg Sb eq | 0.42 | 0.01 | 0.12 | 0.11 | 0.04 | 0.16 |
| AP | kg $SO_2$ eq | 0.26 | $4.20 \cdot 10^{-3}$ | 0.07 | 0.07 | 0.02 | 0.10 |
| EP | kg $PO_4^{-3}$ eq | 0.11 | $1.77 \cdot 10^{-3}$ | 0.03 | 0.03 | 0.01 | 0.04 |
| GWP | kg $CO_2$ eq | 61.24 | 0.98 | 17.09 | 15.37 | 5.21 | 22.60 |
| ODP | kg CFC-11 eq | $1.50 \cdot 10^{-4}$ | $2.41 \cdot 10^{-6}$ | $4.20 \cdot 10^{-5}$ | $3.78 \cdot 10^{-5}$ | $1.28 \cdot 10^{-5}$ | $5.55 \cdot 10^{-5}$ |
| HTP | kg 1,4-DB eq | 36.48 | 0.58 | 10.18 | 9.16 | 3.10 | 13.46 |
| FEP | kg 1,4-DB eq | 19.46 | 0.31 | 5.43 | 4.88 | 1.65 | 7.18 |
| MEP | kg 1,4-DB eq | 12.15 | 0.19 | 3.39 | 3.05 | 1.03 | 4.48 |
| TEP | kg 1,4-DB eq | $16.10 \cdot 10^{-3}$ | $2.58 \cdot 10^{-4}$ | $4.49 \cdot 10^{-3}$ | $4.04 \cdot 10^{-3}$ | $1.37 \cdot 10^{-3}$ | $5.94 \cdot 10^{-3}$ |
| POFP | kg $C_2H_4$ eq | $10.65 \cdot 10^{-3}$ | $1.70 \cdot 10^{-4}$ | $2.97 \cdot 10^{-3}$ | $2.67 \cdot 10^{-3}$ | $0.91 \cdot 10^{-3}$ | $3.93 \cdot 10^{-3}$ |

❖ **Contribution of main terpenes**

**Table 5.12** shows the contribution of each product to the total environmental impact in each category according to the allocation procedure described in section 5.3.2. According to this procedure, around 258 mg of acyclic $C_{10}H_{14}Br_2$ could be extracted after one algal culture, together with 195 mg of 10Z-bromomyrcene and 176 mg of 10E-bromo-3-chloromyrcene. Only 60 mg of apakaochtodene and 11 mg myrcene could be obtained. Since the acyclic $C_{10}H14Br_2$ terpene was the compound produced in the largest quantity, it has the highest contribution to the environmental profile, according to the selected mass allocation approach. Thus, it is responsible for nearly 40% of contributions. The compounds 10Z-bromomyrcene and 10E-bromo-3-chloromyrcene have similar contributions between each other, and their sum constitutes up to 80% of the remaining impacts. With regard to myrcene and apakaochtodene, their low content within the total extracted terpenes leads to very limited contributions to the total environmental impact.

### 5.3.4. Discussion and recommendations

While microalgal cultivation processes have already been analyzed in detail as renewable energy sources (Brentner et al., 2011; Collet et al., 2011; Khoo et al., 2011; Langlois et al., 2012; Lardon et al., 2009; Stephenson et al., 2010) and also for the production of high value biomolecules with applications in pharmaceutical, nutraceutical, cosmetic and food industries (Pérez-López et al., 2014a; 2014b; 2014c), few studies deal with the environmental effects of macroalgae (Alvarado-Morales et al., 2013; Aresta et al., 2005; Langlois et al., 2012; Pilicka et al., 2011). Moreover, the environmental assessments on macroalgal processes focus on their use as energy sources by applying diverse conversion technologies that range from direct combustion to gasification or anaerobic digestion (Aresta et al., 2005), biogas production being the most common route (Alvarado-Morales et al., 2013; Langlois et al., 2012; Pilicka et al., 2011).

Despite the lack of similar reports on macroalgae cultivation for the production of bioactive compounds, the obtained results show some findings consistent with previous work on micro and macroalgae. Thus, the cultivation stage was

identified as the main contributor to the environmental impacts (Alvarado-Morales et al., 2013; Pérez-López et al., 2014b), linked to the high electricity requirements of the process (Aresta et al., 2005; Khoo et al., 2011; Pérez-López et al., 2014b). Other secondary processes, such as the production of chemicals for the extraction stage, were found to have a remarkable impact in specific categories, in accordance with LCA studies for microalgae (Pérez-López et al., 2014c). Due to the relevant contributions of the cultivation and extraction stages, two improved scenarios are proposed below. As in the previous section, the improvement is here presented for the 1-year scenario, since the behavior of the other cases is similar and no relevant additional information is expected from their evaluation.

❖ **Energy optimization**

Since the high electricity consumption in the cultivation stages was identified as the main hot spot of the process, similarly to other LCA studies related to bioactive compounds from micro and macroalgae (Pérez-López et al., 2014b; 2014d), an optimization of the total energy required for the production of essential terpene oils is expected to have a great potential in the reduction of the environmental impacts. For this reason, three alternative scenarios involving a change in light regime are proposed. On the first case (Sc 2), a reduction of lighting from 14 h to 10 h is evaluated, whereas the second option (Sc 3) considers the effect of substituting the artificially-illuminated indoor PBR by an outdoor PBR with sunlight as the only source. The third scenario (Sc 4) takes into account a 50% reduction in electricity consumption of lights by substituting current lamps by light-emitting diodes (LEDs) (Chen et al., 2011). As highlighted by Pérez-López et al. (2014b), a reduction of illumination may lead to lower biomass productivities and final yields. Therefore, 10% reduction of final terpene oils per batch was estimated for the 10 h scenario, whereas 30% reduction was considered for the indoor system. In addition, the incubation chamber for the preparation of inoculum in 500 mL flasks was eliminated, since the cultures proved their capability to be maintained in a room at 23-25ºC with no power input thanks to the waste heat from the lighting. This change involved not only a reduction in the energy consumption, but also in the production of materials, transport and waste treatment for the corresponding equipment.

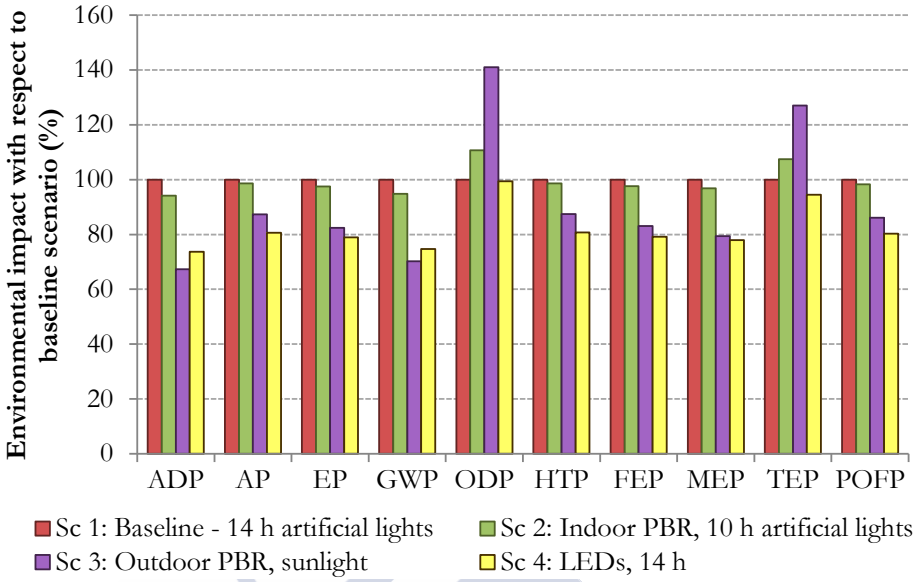



**Figure 5.16.** Effect of energy optimization on the environmental performance of the production of essential terpene oils by *O. secundiramea* considering a 4 h reduction of artificial lighting, solar illumination or lamps substitution by LEDs.

According to the results depicted in **Figure 5.16**, reductions of electricity consumption have a remarkable influence on the environmental profile. Although the benefits of Sc 2 (associated with the changing of light regime from 14 h to 10 h of artificial illumination) are limited, 6% improvement can be achieved in categories such as ADP or GWP, whereas other reductions between 2% and 4% are obtained for other categories such as EP, FEP, MEP or POFP. However, a worse performance than that of the baseline case is observed for ODP and TEP, with impacts 11% and 7% higher. This is due to the estimated lower productivity associated with the reduction in lighting hours, which leads to increased total contributions of other stages. Thus, the total environmental impact of the production of chemicals for extraction in the case of ODP and that of the filter associated with cell separation (S5) increase in 18%. If the productivity is maintained closer to that of the baseline scenario, the impact reductions would range between 11% and 16% for most categories (except for ODP and TEP).

Regarding Sc 3, the significant reduction in electricity consumption results in considerably lower environmental impacts for most categories, despite the associated reduction in productivity. Excluding ODP and FEP, all categories show improvements ranging from 12% (AP or HTP) up to 32% (ADP). Again, ODP and FEP show higher environmental impacts in Sc 3 than in Sc 1. As explained for Sc 2, the reason is the lower productivity caused by the lack of continuous artificial lighting.

Sc 4 is the only option in which all categories show reductions of impact (between 0.7% and 26%), although they are slightly more limited for the specific categories of ADP and GWP than in Sc 3. However, since Sc 3 leads to significantly higher impacts to ODP and TEP, Sc 4 can be considered the most efficient scenario in global terms. This is due to the possibility to maintain (or eventually improve) the biomass productivity and terpenoids yield when using LEDs instead of conventional lamps.

❖ **Changes in downstream processes**

Although the cell separation and extraction stages have limited secondary contributions to most impact categories, some relevant effects were found for specific cases, such as ODP and TEP. Therefore, an alternative scenario (Sc 5) is evaluated, including the following changes:

- Substitution of conventional cotton filter by organic cotton filter (i.e. cotton cultivated with no use of chemicals as pesticides).
- Substitution of current grinding procedure by a typical mechanical grinding stage, with an estimated energy consumption according to Pérez-López et al. (2014d).
- Optimization of solvent dose for the extraction with 20% reduction of initial requirement.
- Reduction of energy consumption for mixing after solvent addition by operating at the maximum capacity of the equipment.
- Alternative solvent evaporation method in chemical hood with no nitrogen gas addition.

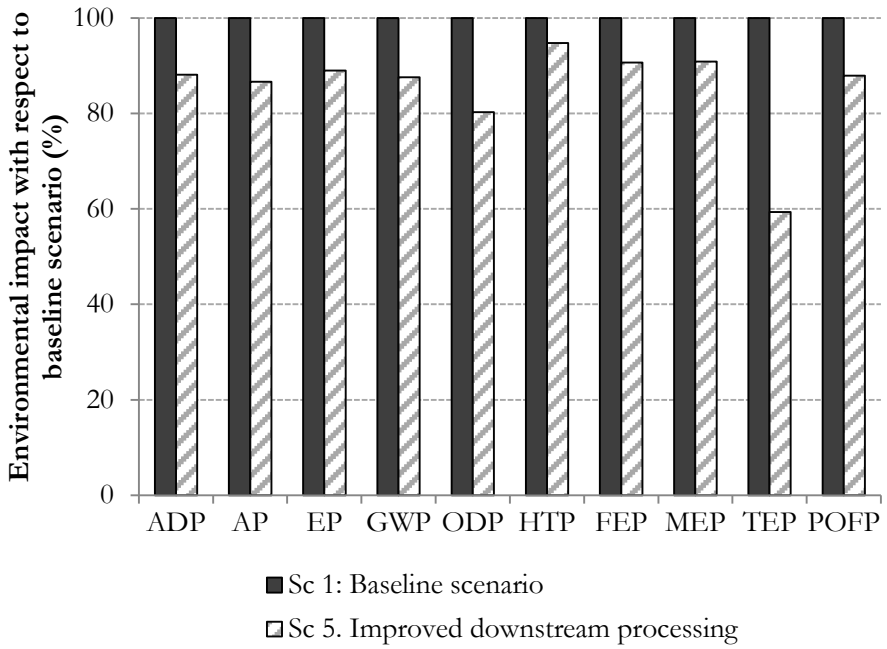



**Figure 5.17.** Effect of changes in downstream processing on the environmental performance of the production of essential terpene oils by *O. secundiramea*.

According to **Figure 5.17**, the proposed changes may help to improve the environmental profile of the analyzed process in all categories. While the reduction associated with the proposed changes on downstream processing is between 5% and 15% for most impacts, contributions to ODP and TEP are remarkably affected. As expected, the change in the material of the filter allows a reduction of up to 40% of the total impact to TEP, whereas the reduction of chemicals for the extraction (especially the 20% decrease for dichloromethane) is the main reason for the improvement in ODP.

❖ **Combined optimized scenarios**

Since the proposed changes can be applied together in an optimized scenario, the combination of improvement actions is globally evaluated below. The two analyzed scenarios are:

- Sc 6. Combined change in the lighting regime consisting of a 4 hour reduction of artificial lighting, together with the removal of the incubating cabinet for the preparation of the inoculum in 500 mL flasks and all the described changes in the downstream processing.
- Sc 7. Substitution of the indoor PBR with artificial lights by an outdoor PBR with sunlight and removal of the incubating cabinet for the preparation of the inoculum combined with the aforementioned optimized downstream processing.
- Sc 8. Combined substitution of conventional lamps by LEDs, removal of the incubating cabinet and optimized downstream processing.

As shown in **Figure 5.18**, the combined implementation of the suggested recommendations would allow important improvements in most impact categories.

Sc 6, which considers the reduction of total lighting by 4 h, may lead to improvements ranging from 8% (for HTP) up to 40% (for TEP), being the contributions to most categories about 15-20% lower than in the baseline scenario.

Sc 7, which includes the substitution of the current indoor PBR by an outdoor system illuminated with sunlight, involves remarkably higher reductions of impact in most cases, with improvements between 20% (HTP) and 50% (ADP). However, the reduction of final product yield associated with the use of sunlight instead of controlled artificial lighting results in a higher impact of the cell disruption and extraction stage, particularly related to the increase in the required amount of dichloromethane per FU.

Finally, the combination of LEDs in the cultivation stage with the removal of the incubation chamber and the optimized downstream processes may lead to significant improvements in all the categories that range between 20% (ODP) and 46% (TEP).

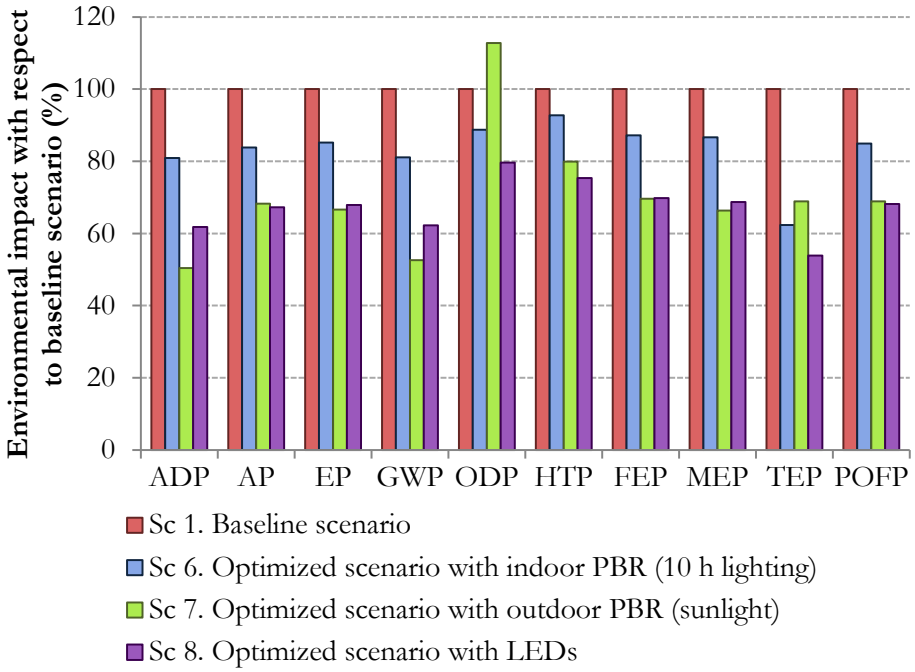



**Figure 5.18.** Environmental profile of the optimized scenarios (including energy reduction and improved downstream processing) for the production of essential terpene oils by *O. secundiramea*.

In addition to the proposed improvements, it should be noticed that the process involves the production of a large quantity of biomass that might be further used to obtain other co-products. Despite the successful application of anaerobic digestion for the use of the remaining biomass as source of energy and fertilizers (Collet et al., 2011), this option was not evaluated in the current process due to the limited effect observed in the case of bioactive compounds (Pérez-López et al., 2014a; Pérez-López et al., 2014c). However, the future identification of additional bioactive compounds that could be extracted from the remaining biomass would substantially help to improve the production process.

## 5.4. Conclusions

The LCA studies presented in this chapter provide the first life cycle inventory and impact assessment results for macroalgae cultivation and extraction applied to the production of high value bioactive molecules with applications in strategic sectors such as pharmaceutical or nutraceutical industries.

In the first case, different routes for the valorization of an otherwise residual biomass (from an invasive seaweed with possible harmful ecological effects) were compared. The high electricity consumption linked to specific stages of the process (i.e. supercritical extraction and non-isothermal autohydrolysis) were identified as the most problematic issues (hot spots) throughout the whole life cycle of the product. Other secondary processes such as vessel operations for the collection of algae and waste treatment of organic solvents only have relevant effects of specific categories. The obtained results considerably depend on the chosen FU and the biomass composition (which can change due to seasonal variations and operating conditions), although the selection of the most suitable scenario is maintained in this case, regardless of the approach. Thus, the integral valorization of biomass, which was initially considered the most attractive scenario, was less efficient than the scenario with the recovery of only two fractions (antioxidant extract and alginate) from wet algae.

The second LCA study evaluates the production of valuable compounds by macroalgae in a complete cultivation and extraction scheme. As for microalgal processes, the cultivation in reactor constitutes a major environmental concern, again linked to the high electricity consumption, together with some relevant contributions of materials and chemicals in specific categories. Moreover, LCA is applied to evaluate potential improvements with respect to the baseline case.

The design of novel processes to valorize natural resources requires objective supporting tools to evaluate the efficiency of available technologies and identify the most suitable options from environmental, economic and social perspectives. The results highlight the usefulness of LCA methodology as a decision-making tool, especially in processes under development related to emergent sectors such as marine biotechnology.

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# Chapter 6

# Pharmaceutical ingredients from marine sponges[1]


*Summary*

Marine sponges are one of the most diverse invertebrates and show a great ability to produce valuable natural products with high biological activities. The main bottleneck for the commercial exploitation is the need of continuous production in sufficient quantities. The main current approaches for the production of sponge biomass and further extraction of biomolecules are here analyzed from an environmental perspective. Firstly, the *in situ* cultivation of the sponge *Sarcotragus spinosulus* in sea-based farming structures was evaluated. The results demonstrated that the cultivation had a relatively efficient performance, whereas the subsequent downstream processes were the main cause of the environmental impacts. A novel process for the cultivation of the sponge *Crambe crambe* in controlled aquaria is then presented. Although the baseline process had remarkable impacts from the cultivation stage due to the high electricity requirements for lighting, the further optimization of the key processes involved allowed significant impact reductions and led to the proposal of two improved processes with combined improvement strategies. Finally, the *in situ* and *ex situ* approaches were compared. Although the *in situ* growth had lower environmental impacts than the baseline process, other optimized *ex situ* scenarios had the best profile.


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[1] **Pérez-López P**, Ternon E, González-García S, Genta-Jouve G, Feijoo G, Thomas OP, Moreira MT. Environmental solutions for the sustainable production of bioactive natural products from the marine sponge *Crambe crambe*. Science of the Total Environment 2014, 475:71-82.

# Index

## 6.1. Biologically active compounds from sponges

Among aquatic organisms, sponges are one of the most diverse invertebrates not only due to the number of species but also to the variety of morphological characters (Blunt et al., 2015; Hooper and Van Soest, 2002). Indeed, between 7000 and 8000 different species have already been described, and at least twice that number is thought to exist (Hooper and Lévi, 1994; Thakur and Müller, 2004). This diversity associated to the fact that these sessile invertebrates produce a large array of secondary metabolites make sponges a good target for the search of high value added molecules (Leal et al., 2012). According to Blunt et al. (2015), up to 7000 natural products have been isolated from marine sponges worldwide, an amount that increases annually. Many of these molecules (e.g. halichondrin B, avarol, crambescidins) have shown high biological activities that make them valuable products for medical drugs development due to their anti-inflamatory, antitumor, immunosuppressive or neurosuppressive, antiviral or antibiotic properties, among others (Bergman et al., 2011a; Bondu et al., 2012; Newman and Cragg, 2004; Sipkema et al., 2005a). Eribulin mesylate is the first drug derived from a sponge natural product that entered the market in 2011 as an anticancer agent (Huyck et al., 2011).

Despite the great potential of bioactive compounds from marine origin and particularly from sponges, steady production is a key limiting factor that may hinder the development of commercial processes (Murray et al., 2013). As bioactive compounds of marine origin are present in small quantities in the producer organisms, fresh material is required in large amounts. Wild harvest only satisfies the demand partially and arises as an unsuitable production route (Bergman et al., 2011a; Ogbonna et al., 1999; Pomponi, 2001). Therefore, unless feasible alternatives to harvesting from the natural environment are developed, many of these target molecules will remain unexploited (Murray et al., 2013). For this reason, the current challenge is to develop efficient culture techniques for small to medium-scale production schemes (Schippers et al., 2012).

The selection of the most appropriate culture technique depends on the nature of the target compound and its concentration within the sponge. Thus, if the organism presents a high concentration of the desired metabolite, the cultivation of adult specimens would be the best choice, while *in vitro* cell cultures may

constitute a more suitable method for products found in low concentrations (Schippers et al., 2012; Sipkema et al., 2005b). However, *in vitro* cultivation systems have been found difficult to maintain in a long-term operation (Müller et al., 2004; Rinkevich, 1999).

Alternatively, aquaculture has been widely proposed as a technique to supply sponge materials, not only for the production of natural bath sponges, but more recently also for biotechnological purposes (Duckworth, 2009; Munro et al., 1999; Osinga et al., 1999; Pronzato and Manconi, 2008). Cultivation of sponges can be performed either *in situ* or *ex situ* (Bergman et al., 2011a; Louden et al., 2007; Sipkema et al., 2005b). Sea-based culture systems (*in situ* systems) involve the construction of a sponge field where small cuttings (explants) from a parent are strung on a support for cultivation in the sea, so as to keep the organisms in their natural environment (Schippers et al., 2012). The main drawbacks of this alternative are the numerous risks sponges are exposed to, including biological factors such as predation and fouling, but also diseases or adverse weather conditions (Schippers et al., 2012; Webster et al., 2002). These risks are turned into very fluctuant survival rates that strongly depend not only on the considered species but also on the location of the sponge field, the season and the aquaculture method (Bergman et al., 2011a; 2011b; De Caralt et al., 2007; 2010; De Voogd, 2007; Louden et al., 2007; Osinga et al., 2010).

In order to circumvent these difficulties, the *ex situ* cultivation of sponges in closed or semi-enclosed systems such as aquarium has been proposed as an alternative strategy (Mohamed et al., 2008; Osinga et al., 2003). Even if this approach avoid seasonality effects and allow controlled conditions, the observed growth rates in aquarium are significantly lower than those of mariculture. The limited progress made in the cultivation of sponges under controlled conditions is due to the scarce knowledge on the optimal environmental conditions and ecological needs required by sponges to develop properly in a non-natural system (Carballo et al., 2010). In this regard, Schippers et al. (2012) suggest that *ex situ* cultivation should be performed in a semi-continuous mode instead of a batch operation, by regularly harvesting a small fraction of the culture.

## 6.2. *In situ* culture systems for the production of prenylhydroquinone from *Sarcotragus spinosulus*

Sea-based farming (also known as *in situ* culture or "mariculture") of sponges has been proposed and successfully applied for several species (Osinga et al., 2010; Page et al., 2011; Sipkema et al., 2005b). Despite the difficult control of culture conditions and the exposure to unfavorable climate phenomena, survival rates between 11% and 100%, depending on the species, location and culture depth, have been reported (De Caralt et al., 2010; Duckworth, 2009; Osinga et al., 2010; Schippers et al., 2012). Growth rates found in literature show a remarkable variability and range from negative values (i.e. size decrease) up to 2000% of the original size per year (Page et al., 2011; Schippers et al., 2012).

The start-up of a sponge *in situ* culture consists of the fragmentation of specimens collected from the wild habitat by cutting them into sponge explants. The explants are then placed on supporting structures for growth (Schippers et al., 2012). Duckworth (2009) provides a detailed description of available systems for aquaculture of both bath sponges and producers of bioactive metabolites. For bath sponges that need to grow with a specific shape, two main methods are used: farmed on ropes or inside mesh, according to the designs presented in **Figure 6.1**. Survival rates are usually higher for sponges farmed in mesh due to the protection provided by the structure, which avoids explant damage. However, this structure and the associated biofouling problems also reduce water flow and therefore nutrient availability, resulting in low growth rates compared to culture on ropes.

Since final explant shape is secondary in the case of sponge aquaculture focused on the production of bioactive metabolites, a wider range of farming methods is possible. Among the proposed techniques, mesh arrays (consisting of mesh tubes divided into alternating pockets), nets stretched over metal frames and small plastic containers on horizontal lines are some of the most promising (Duckworth, 2009; Page et al., 2005; Pronzato, 2004). Moreover, the specific goal of the cultivation allows the application of partially harvesting techniques. This option involves the collection of only a fraction of the organism containing the target metabolite while leaving the rest of the explant to continue its growth on the farming system (Duckworth, 2009).

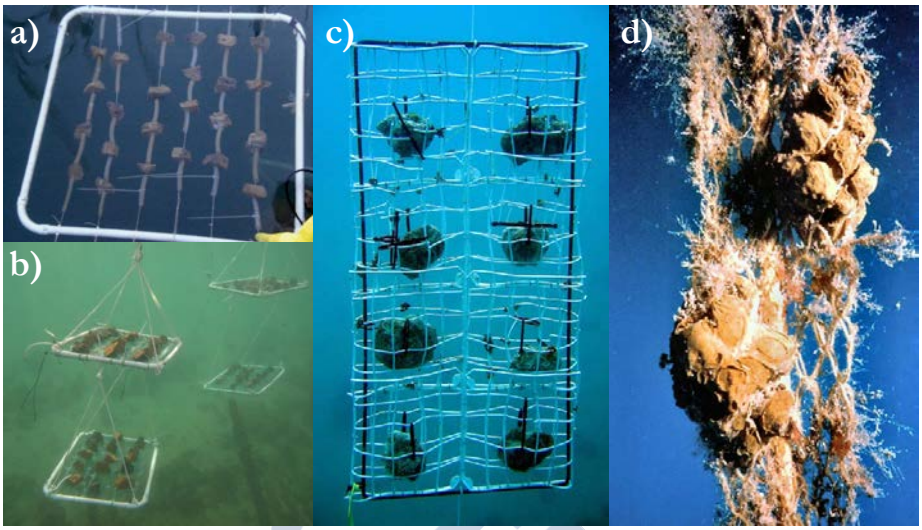



**Figure 6.1.** *In situ* sponge farming systems based on a) ropes, b) nets, c) mesh and d) mesh arrays.
Source: Duckworth (2009) and Ledda et al. (2014).

Besides the high value of the produced metabolites, sponges grown in mariculture systems have filtering ability that may allow bacterial and organic waste removal (Ledda et al., 2014; Page et al., 2011). In this regard, Ledda et al. (2014) reported removal efficiencies up to 80% for the bacterial load of seawater in a polluted harbor. Furthermore, survival and growth rates in these sites were generally similar to those observed in unpolluted sites.

Due to all the aforementioned advantages, the cultivation of the sponge *Sarcotragus spinosulus* is evaluated in this study from a life cycle perspective. *S. spinosulus* is a large and often horizontally flattened sponge belonging to the Irciniidae family (Abed et al., 2011; Mercurio et al., 2013; Murray et al., 2013). It has a regular conulose surface with external color ranging from black to grey and white to light brown interior (Abed et al., 2011; Mercurio et al., 2013). This subspherical demosponge is commonly found in shallow waters and below the tide line, mainly in the Western Mediterranean coasts (Abed et al., 2011; Mercurio et al., 2013; Murray et al., 2013).

*S. spinosulus* is a natural producer of linear polyprenylhydroquinones. This group of aromatic organic compounds exhibit moderate antibacterial, antiviral, anti-inflammatory and cytotoxic activities (Abed et al., 2011). Therefore, the interest on the production lies in their potential therapeutic uses. However, current supply is mainly based on complex synthetic routes in multiple steps (Ling et al., 2002; Molinari et al., 2000; Ran et al., 2001).

In this study, a novel alternative process is evaluated, consisting of the yearly harvest of sponge explants grown in mariculture systems followed by the solvent extraction of a bioactive prenylhydroquinone fraction. The LCA approach according to ISO 14040 standardized methodology (ISO 14040, 2006) was again selected as the tool for the environmental assessment.

### 6.2.1. Goal and scope definition

The purpose of this work is to evaluate the environmental performance of *S. spinosulus* mariculture and following extraction of prenylhydroquinone. According to the experimental conditions, 100 mg of produced bioactive compound was selected as the functional unit (FU), corresponding to one year of cultivation of the specimens collected from the wild habitat in one farming structure. The performed LCA is based on a cradle-to-gate approach, including: i) installation of sea-based farming structures, ii) collection and transport of sponges from natural environment, iii) explants seeding, iv) monitoring of cultured sponges, v) harvesting of sponge explants, vi) preparation of the sponge biomass for extraction and vii) solvent extraction of prenylhydroquinone fraction.

Firstly, the environmental burdens of the initial scenario are quantified and the main hot spots are identified. In addition, the most relevant stages and parameters are further analyzed by conducting sensitivity assessments that include alternative scenarios.

The assessment is based on the farming structures for the mariculture of *S. spinosulus* in the Mediterranean coasts developed by the Dipartimento di Scienze della Terra, dell'Ambiente e della Vita (DISTAV) at the Università degli Studi di Genova (Italy). The target species was cultured on sea-based farming systems consisting of structures built with low cost materials (i.e. nylon,

polyvinylchloride, steel and polyethylene). The system exploited the natural ability of sponges to regenerate from small fragments. The farming systems were installed in coastal areas close to the natural habitat of donor sponges. Since sponges are active and mainly unselective filter feeders, their source of food was the surrounding water column (containing particulate and dissolved organic matter, as well as microalgae and bacteria). **Figure 6.2** depicts the seven stages of the process that are included within the system boundaries in the environmental study:

i) S1. Preparation and installation of sea-based farming structures: At the beginning of the cultivation, the farming structures were built and installed in the selected site. The structures consisted of nylon ropes with a diameter of 2-5 mm (15 m nylon per structure) fastened to 1.5 m wide polyvinylchloride (PVC) square frames (composed of 6 m pipe with 4 annular connections), with polyethylene (LDPE) spacers of 1 cm of diameter to separate the specimens (15 m per structure).

   Each square frame supported 10 nylon lines with 10 sponge fragments each, so 100 sponge explants were cultured in each frame. Three farming structures were used, so a total of 300 sponge explants were transplanted. The settlement of the structures involved the use of an inflatable boat and scuba equipment for 2 h. As an average, the structures could be maintained in a farming site for at least 15 years.

ii) S2. Collection of sponge seeds from natural habitat: Specimens for explant seeding in the mariculture structures were harvested in the natural habitat. In order to avoid ecological damage, only a fraction of each specimen was collected by cutting approximately 50% of total volume of the donor sponge. Due to the regeneration capacity, the remaining donor sponge was able to restore the damaged zone and persist in its habitat.

   The harvest of sponge seed was only needed at the beginning of a sponge culture. In subsequent phases of expansion, the growth of the sponge was sufficient to supply new explants for next cycles, as well as the biomass required to extract the bioactive compound. For the collection and transport of sponges close to the farming site, the same

boat and scuba equipment as for S1 were used. The collection of sponges took place once each three years. For the cultivation in three farming structures, 10 kg of sponge biomass was collected at the beginning of the cultivation cycle.

iii) S3. Preparation of explants and sponge seeding: The collected specimens were fragmented with sterile scalpels to obtain several explants with an approximate volume of 30-50 $cm^3$ each. Before settling, the wet weight of each explant was measured with a portable balance, and the volume was determined either by volume displacement or by image analysis of photographs. The explants were then attached to the farming structures and maintained in the site for growth.

iv) S4. Monitoring and maintenance of farming system: The mariculture systems were periodically monitored to assess the survival and growth rates of the sponge explants. Each explant was monitored with direct measures and photographs. The step was conducted once each three months. Since the structures were located in a site close to the shore, the use of boat was considered negligible in this case.

v) S5. Sponge harvesting and transport to facility for extraction: Once per year, sponges were harvested and transported by passenger car to the laboratory where a solvent extraction was conducted. In this period, an approximate growth of 100% was observed for the surviving specimens. A survival rate of 80% was considered for the baseline scenario, according to the results reported by Ledda et al. (2014).

vi) S6. Preparation of crude extract: The harvested biomass was rinsed with water and cut into small slices. The fragments were grinded and freeze-dried for extraction. During the process, 2 L of tap water and 2 L of distilled water were required per gram of freeze-dried sample. For the preparation of crude extract, 1 L of methanol, together with 0.5 L of n-hexane, chloroform and carbon tetrachloride were used per gram of sample. The extraction was conducted at room temperature and the extract was filtered and concentrated under reduced pressure.

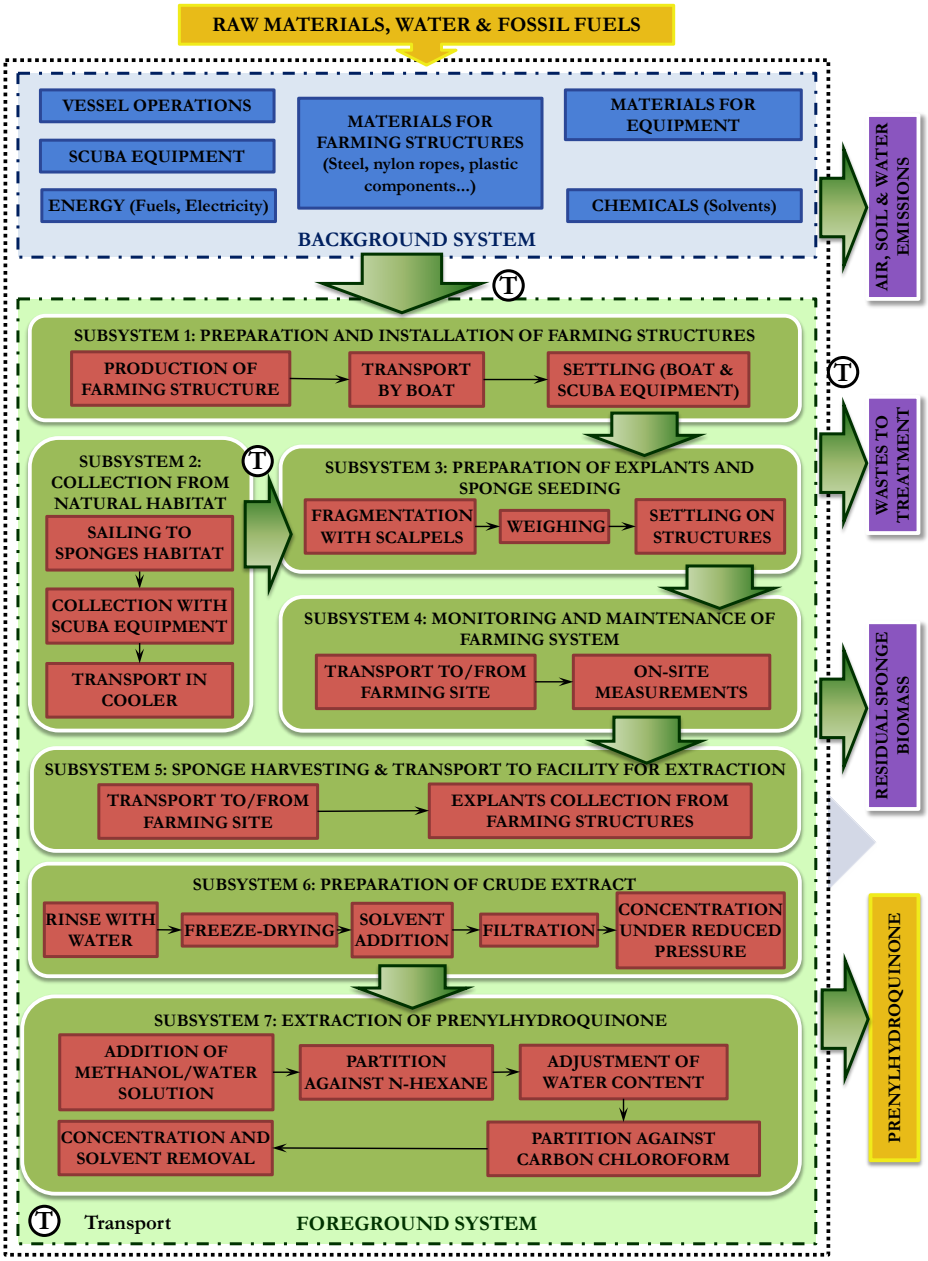



**Figure 6.2.** Process chain and system boundaries of sea-based farming of *S. spinosulus* for the production of prenylhydroquinones.

vii) S7. Extraction of prenylhydroquinone: The crude extract from the previous stage was dissolved in a mixture of methanol and water (9:1 v/v), and partitioned against 1 L n-hexane. The water content of the methanolic fraction was adjusted to 20% and partitioned against chloroform. The extract was finally concentrated to remove the solvent and obtain the prenylhydroquinone fraction.

### 6.2.2. Life cycle inventory, data quality and assumptions

The life cycle inventory (LCI) data for the foreground system (i.e. raw materials for farming structures, chemicals, transport distances, fuel and electricity consumption) consisted of average data obtained by on-site measurements. The air emissions, mainly derived from the combustion of the boat engine, and the water emissions from the different stages, were assumed to be directly discharged to the environment. **Table 6.1** shows the inventory data for the baseline scenario.

The inputs from the background system include the production of the different materials for the farming structures (i.e. nylon ropes, PVC, LDPE and steel snap hooks), the vessel and scuba equipment used for the preparation of farming structures and the sponge collection from the wild habitat, as well as the different chemicals required for the extraction and the materials for the equipment (e.g. freeze-dryer, solvent evaporator). The farming structures have an average life span of 15 years. For the equipment, average weight and life span were estimated according to manufacturers' specifications.

In the case of vessel operations, the emissions from fuel combustion were determined as shown in the EMEP/EEA air pollutant emission inventory guidebook (EMEP/EEA, 2009). Marine lubricant oil needed for the maintenance of the boat engine was inventoried according to Vázquez-Rowe et al. (2010). No other chemicals were consumed, since the vessel was an inflatable boat that required no periodic addition of paint or anti-fouling. Water consumption for the boat washing was included in the LCI. The calculated amount of synthetic rubber (assumed for the hull) and steel required for the engine of the boat were increased by 25% and 50% respectively, to take into account repair and maintenance of the boat. A life span of 10 years was

estimated, according to manufacturer's specifications. For the baseline scenario, boat was considered to be exclusively used for tasks related to the evaluated process.

The background system also provides the energy used in the different production and extraction stages (petrol and electricity from the Italian grid), as well as waste treatment. Solid wastes were assumed to be disposed of in sanitary or inert landfills, except for synthetic rubber, which was sent to incineration. With respect to transport, an average distance of 180 km was considered for chemicals and equipment. Waste transport distance was estimated around 50 km. Inventory data for all those background processes were taken from Ecoinvent database, as summarized in **Table 6.2**.

*Allocation procedures*

In this case, the process aims to the production of only one bioactive fraction: prenylhydroquinone. Thus, all the environmental burdens were associated with the produced prenylhydroquinone (100 mg per year of cultivation in a single sea-based farming structure). However, according to Ledda et al. (2014), the mariculture system showed the ability to act as a natural filter for the treatment of large volumes of water with high organic and bacterial load. This environmental benefit was taken into account in the LCA study by applying a system expansion approach. Thus, the electricity consumption of an ultraviolet (UV) sterilizer with an equivalent function was considered as "avoided product" and the corresponding environmental burdens were subtracted from the total impact of the process. For the quantification, an average water filtration capacity of 20 $mL \cdot h^{-1} \cdot cm^{-3}$ sponge was considered. For a coupled set of three sea-based farming structures, containing a total of 300 specimens of 50 $cm^3$ each, the final volume of filtered water would be approximately 2400 $m^3 \cdot year^{-1}$, which would involve an electricity consumption of 27 $kWh \cdot year^{-1}$ by the UV sterilizer.

Although no additional biomolecules were isolated from *S. spinosulus*, the residual sponge biomass after prenylhydroquinone extraction might contain other valuable products. Further research could allow identifying other biomolecules that would lead to the reduction of the relative impact for each obtained fraction and hence improve the environmental profile of the process.

**Table 6.1.** Inventory data for mariculture and prenylhydroquinone extraction from sponge *S. spinosulus* (FU=100 mg bioactive fraction)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Preparation and installation of farming structures* | | | |
| Synthetic rubber | 1.96 kg | Nylon (farming structures) | 37.50 g |
| Steel (engine and scuba equip.) | 1.21 kg | PVC (farming structures) | 37.50 g |
| Lubricant oil | 2.32 g | Steel (farming structures) | 18.75 g |
| Compressed air (200 bar) | 0.10 kg | LDPE (farming structures) | 18.75 g |
| Water (maintenance) | 3.08 t | | |
| *S2. Collection of sponge seeds from natural habitat* | | | |
| Synthetic rubber | 9.80 kg | Compressed air (200 bar) | 0.73 kg |
| Steel | 6.05 kg | Water (maintenance) | 15.42 t |
| Lubricant oil | 11.49 g | Polypropylene (PP) | 0.55 kg |
| *S3. Preparation of explants and sponge seeding* | | | |
| Steel | 7.44 g | Electric battery | 0.36 g |
| *S4. Monitoring and maintenance of farming system* | | | |
| Steel | 27.50 g | Electric battery | 1.44 g |
| *S5. Preparation of crude extract* | | | |
| Tap water | 500 kg | N-hexane | 81.85 kg |
| Distilled water | 500 kg | Chloroform | 186.25 kg |
| Methanol | 198 kg | Carbon tetrachloride | 198.75 kg |
| Steel | 0.12 kg | Glass | 0.05 kg |
| **Energy** | | | |
| Electricity from Italian grid | | | |
| *S1. Preparation and installation of farming structures* | | *S5. Preparation of crude extract* | |
| Gasoline | 0.77 kg | Electricity for freeze-drying | 7.20 kWh |
| *S2. Collection of sponge seeds from natural habitat* | | Electricity for solvent evaporation | 39.06 kWh |
| Gasoline | 3.86 kg | | |
| **Transport** | | | |
| Truck, 3.5-7.5 t (materials) | 3.58 tkm | Truck, 3.5-7.5 t (chemicals) | 122.2 tkm |
| Passenger car (S1, S2, S4, S5) | 197.31 pkm | Truck, 3.5-7.5 t (wastes) | 0.99 tkm |

**Table 6.1.** Inventory data for mariculture and prenylhydroquinone extraction from sponge *S. spinosulus* (FU=100 mg bioactive fraction) (*Cont.*)

| | | | |
|---|---|---|---|
| **INPUTS from ENVIRONMENT** | | | |
| **Materials** | | | |
| Sponge biomass (wet weight) | 1.04 kg | Seawater | 5.21 L |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Product** | | | |
| Prenylhydroquinone | 100 mg | | |
| **Avoided product** | | | |
| Electricity from Italian grid (UV filter) | 8.44 kWh | | |
| **Wastes to treatment** | | | |
| *S1. Preparation and installation of farming structures* | | | |
| Steel | 1.21 kg | | |
| *S2. Collection of sponge seeds from natural habitat* | | | |
| Steel | 6.05 kg | PP | 0.55 kg |
| *S3. Preparation of explants and sponge seeding* | | | |
| Steel | 7.44 g | Electric battery | 0.36 g |
| *S4. Monitoring and maintenance of farming system* | | | |
| Steel | 27.50 g | Electric battery | 1.44 g |
| *S5. Annual harvesting* | | | |
| Nylon | 37.50 g | LDPE | 18.75 g |
| PVC | 37.50 g | Steel | 18.75 g |
| *S6. Preparation of crude extract* | | | |
| Steel | 0.12 kg | Glass | 0.05 kg |
| **Wastes to municipal incineration** | | | |
| *S1. Preparation and installation of farming structures* | | | |
| Synthetic rubber | 1.96 kg | | |
| *S2. Collection of sponge seeds from natural habitat* | | | |
| Synthetic rubber | 9.80 kg | | |

**Table 6.1.** Inventory data for mariculture and prenylhydroquinone extraction from sponge *S. spinosulus* (FU=100 mg bioactive fraction) (*Cont.*)

| **OUTPUTS to ENVIRONMENT** | | | |
|---|---|---|---|
| **Air emissions** | | | |
| *S1. Preparation and installation of farming structures* | | | |
| Carbon dioxide ($CO_2$) | 2.454 kg | Methane ($CH_4$) | 0.139 g |
| Sulfur dioxide ($SO_2$) | 0.002 kg | Nitrogen oxides ($NO_x$) | 0.031 kg |
| Non-methane volatile organic compounds (NMVOC) | 0.039 kg | Carbon monoxide (CO) | 0.006 kg |
| | | Particulate matter (PM) | 0.002 kg |
| *S2. Collection of sponge seeds from natural habitat* | | | |
| $CO_2$ | 12.270 kg | $NO_x$ | 0.153 kg |
| $SO_2$ | 0.008 kg | CO | 0.029 kg |
| NMVOC | 0.193 kg | PM | 0.012 kg |
| $CH_4$ | 0.695 g | | |
| **Water emissions** | | | |
| *S1. Preparation and installation of farming structures* | | | |
| Wastewater | 3.08 $m^3$ | | |
| *S2. Collection of sponge seeds from natural habitat* | | | |
| Wastewater | 15.42 $m^3$ | | |
| *S6. Preparation of crude extracts* | | | |
| Methanol | 250 L | Carbon tetrachloride | 125 L |
| N-hexane | 125 L | Wastewater | 1000 L |
| Chloroform | 125 L | | |
| *S7. Extraction of prenylhydroquinone* | | | |
| Methanol | 15 L | Chloroform | 1 L |
| N-hexane | 1 L | Wastewater | 3 L |

**Table 6.2.** Summary of data sources for the background system of the mariculture and prenylhydroquinone extraction from sponge *S. spinosulus*

| Type of involved process | Raw material | Data source |
|---|---|---|
| Energy | Diesel | Ecoinvent database (Jungbluth, 2007) |
| | Electricity (from the Italian grid) | Ecoinvent database (Dones et al., 2007) |
| Materials | Steel | Ecoinvent database (Classen et al., 2007) |
| | Synthetic rubber<br>Nylon 6<br>PVC<br>LDP<br>PP | Ecoinvent database (Hischier, 2007) |
| | Electric battery | Ecoinvent database (Hischier et al., 2007) |
| Chemicals | Marine lubricant oil | Vázquez-Rowe et al. (2010) |
| | Methanol<br>Chloroform<br>Carbon tetrachloride | Ecoinvent database (Althaus et al., 2007) |
| | N-hexane | Ecoinvent database (Jungbluth, 2007) |
| Air for scuba equipment | Compressed air | Ecoinvent database (Steiner and Frischknecht, 2007) |
| Water supply | Tap water<br>Distilled water | Ecoinvent database (Althaus et al., 2007) |
| Waste treatment | Inert landfill<br>Sanitary landfill<br>Municipal incineration | Ecoinvent database (Doka, 2007) |

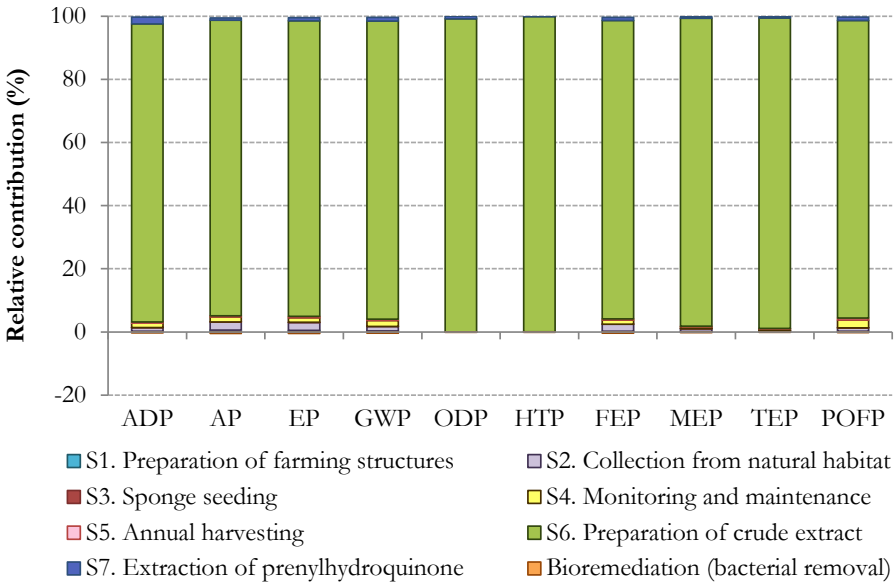
### 6.2.3. Environmental impact assessment

The environmental profile for the production of prenylhydroquinone from *S. spinosulus* cultured *in situ* was assessed by performing classification and characterization stages of the LCA methodology (ISO 14040, 2006). The characterization factors reported by the Centre of Environmental Science of Leiden University (CML 2001 method) were used (Guinée et al., 2002). The impact potentials evaluated according to the CML method were: abiotic depletion (ADP), acidification (AP), eutrophication (EP), global warming (GWP), ozone layer depletion (ODP), human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP), terrestrial ecotoxicity (TEP) and photochemical oxidants formation (POFP). The software SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008). The characterization results for the production of prenylhydroquinone by the sponge *S. spinosulus* in the baseline scenario are shown in **Table 6.3** and split into involved stages and processes in **Figure 6.3**.

**Table 6.3.** Environmental impact assessment results (characterization step) associated with the production of 100 mg prenylhydroquinone by *S. spinosulus*

| Impact category | Unit | Value |
|---|---|---|
| ADP | kg Sb eq | 13.17 |
| AP | kg $SO_2$ eq | 5.26 |
| EP | kg $PO_4^{-3}$ eq | 1.44 |
| GWP | kg $CO_2$ eq | 1522.11 |
| ODP | kg CFC-11 eq | 0.23 |
| HTP | kg 1,4-DB eq | $46.38 \cdot 10^3$ |
| FEP | kg 1,4-DB eq | 257.47 |
| MEP | kg 1,4-DB eq | 371.51 |
| TEP | kg 1,4-DB eq | 0.27 |
| POFP | kg $C_2H_4$ eq | 0.45 |

**a) Relative contributions of prenylhydroquinone from *S. spinosulus* per stage**




**b) Relative contributions of prenylhydroquinone from *S. spinosulus* per involved process**

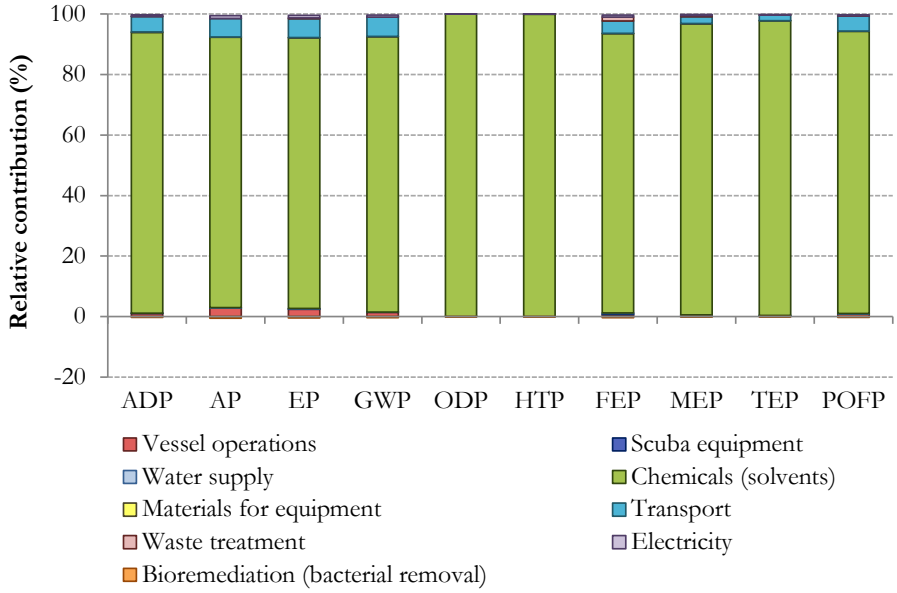



**Figure 6.3.** Relative contributions of the production of prenylhydroquinones by *S. spinosulus* in sea-based farming structures to each impact category per a) stage and b) involved process.

According to the results, the preparation of the crude extract (S6) is the main contributor to the environmental burdens derived from the production of prenylhydroquinones from *S. spinosulus* grown *in situ* in sea-based farming structures. The contributions range between 88% and 99% for all categories. Among the secondary stages, only the relative impact of the collection of specimens from the natural habitat (S2) exceeds 4% in five of the evaluated categories: ADP, AP, EP, GWP and FEP. Other stages such as the preparation and installation of the farming structures (S1) or the monitoring and maintenance of the mariculture system (S4) have marginal contributions of approximately 1%.

Regarding the involved processes, the production of chemicals used as solvents is associated with the highest impacts throughout the whole process. It involves between 84% and 99% of the total impact and is mainly associated with S6. Indeed, the solvents consumed in S6 are responsible for up to 98% of the total environmental burdens for all the categories (especially chloroform, followed by carbon tetrachloride and methanol), whereas the solvents for the extraction of prenylhydroquinone fraction from the crude extract (S7) constitute less than 2% of the total impact of chemicals.

The materials and emissions associated with the "vessel operation", together with the transport are the only secondary activities with contributions above 4% in some categories, including ADP, AP, EP, GWP and FEP. Other processes such as the production of electricity for the downstream processing (mainly the preparation of the crude extract), the tap and distilled water required throughout the process or the production of materials for the scuba and lab equipment have contributions below 1% in nearly all categories.

Although the filtering potential of sponges was taken into account in the study, the benefit linked to the bacterial removal capacity represents less than 1% of the total environmental impact of the process, mainly due to the large amount of solvents required for the extraction stages.

### 6.2.4. Discussion and recommendations

While several LCA studies dealing with the production of valuable metabolites from algae are available (Pérez-López et al., 2014a; Pérez-López et al., 2014b; Pérez-López et al., 2014d), the growth of sponges in mariculture systems has only been evaluated from an economic point of view (Sipkema et al., 2005b). Although no environmental analyses are available, the results of the current work are in line with the findings of the economic evaluation, which already pointed out the relative importance of downstream processing. Thus, according to Sipkema et al. (2005b), the isolation and purification stages to obtain biocompounds from maricultured sponges are associated with 70-90% of the total variable costs of the process.

Due to the large contribution of the extraction stages and particularly the high environmental impact of the production of chemicals used to obtain the crude extract from the harvested sponge biomass, a sensitivity assessment is proposed in this section to evaluate the future steps to be conducted towards the reduction of solvent consumption. Moreover, the life cycle inventory presented in previous sections of the LCA study relies on assumptions and extrapolations from small scale systems that may suffer modifications for the implementation on a continuous mode. The effect of the most influencing assumptions is also considered to define the alternative scenarios analyzed in this section.

❖ **Optimization of solvent consumption**

The production of solvents causes more than 80% of the environmental burdens associated with the production of prenlyhydroquinones from *S. spinosulus*. With this regard, several authors have demonstrated the feasibility of solvent recovery and reuse for the extraction of other metabolites from sponges (Blaicher et al., 1981; Harkrader and Jones, 1998).

Therefore, the recovery of solvents used for the preparation of the crude extract is here evaluated. Thus, the individual recovery of 50% methanol (Sc 2), hexane (Sc 3), chloroform (Sc 4) and carbon tetrachloride (Sc 5) are analyzed. The recovery percentage is based on the reuse scenario proposed by Pérez-Lopez et al. (2014c) for a bioactive molecule from another sponge. In addition, a combined scenario (Sc 6) based on the reuse of the four solvents is proposed.

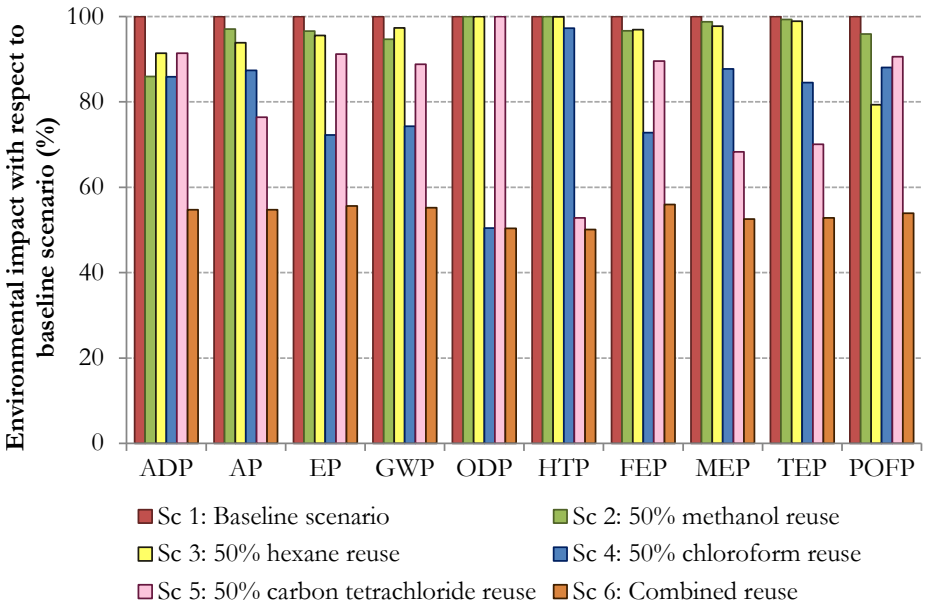



**Figure 6.4.** Effect of solvent reuse during the preparation of the crude extract on the environmental profile of the production of prenylhydroquinones by *S. spinsulus*.

According to the results (**Figure 6.4**), the recovery of the solvents used in stage S6 would involve remarkable reductions of impact. The most limited effect is observed for the reuse of methanol, with an improvement between 1% and 5% in most categories. The environmental performance when reducing the consumption of chloroform presents significant reductions ranging from 3% (for HTP) to 50% (for ODP). The reuse of carbon tetrachloride also involves important reductions between 9% and 47%, except for the category of ODP, which is mainly linked with the production of chloroform. The combined reuse of the three solvents would allow a global improvement from 25% (for POFP) to 50% (for ODP and HTP). Despite the environmental improvement, the relative contributions of S6 still dominate the global profile, with impacts between 79% and 99% for the best scenario (Sc 6). Other processes have relative contributions below 10% in all categories, except for FEP from S2 (13%). Regarding bioremediation, the filtering capacity per $cm^3$ sponge and year should be at least doubled to give a reduction higher than 1% in Sc 6.

❖ **Effect of vessel operations**

The environmental impacts of the materials required and emissions derived from vessel operation have a secondary contribution below 6% in all categories when considering the baseline scenario. This scenario consisted in the use of an inflatable boat for S1 and S2. However, this boat could also be used for other tasks (such as the preparation of mariculture systems in other locations). In this case, the production of the materials for the boat cannot be exclusively allocated to prenylhydroquinone production, but only a fraction of them is associated with the process. Sc 7 is proposed to evaluate the effect of a shared use of the boat, assuming an average use of 2 h per week each year. In addition, the effect of the substitution of the inflatable boat by a polyester fiberglass boat (with more maintenance requirements – painting and antifouling required –, but a longer life span) was considered in Sc 8. The same annual sharing conditions of Sc 7 were assumed.

The baseline scenario is based on the assumption that no boat and scuba equipment are needed for stages S4 (monitoring) and S5 (sponge harvesting). However, depending on the distance of the mariculture system to the shore, this assumption may be inaccurate. To take into account the possible need for boat and diving in these stages, Sc 9 and Sc 10 are also evaluated. Sc 9 corresponds to a 30 min sailing for each monitoring session (4 sessions per year), a 30 min sailing for the annual harvesting and the corresponding scuba equipment, with an exclusive use of the boat for the analyzed process. Sc 10 evaluates the same conditions with a shared boat (2 h of use per week).

The results, depicted in **Figure 6.5**, show that the assumptions considered to obtain the LCI data associated with the vessel operations have a minor effect on the final results. Sc 7 and Sc 8 have a slightly better performance than the baseline case, with impact reductions between 2% and 6% except for ODP and HTP. Since the improvement is similar for both cases, the changes in the environmental profile for Sc 8 may be linked to the effect of the shared use rather than to the substitution of materials itself. Thus, the results suggest that the higher impacts of a boat with a larger need of materials and chemicals for maintenance are compensated by the benefits of a longer life span of the vessel.

Furthermore, the use of boat and scuba equipment in the monitoring and harvesting stages (S4 and S5) involves a limited increase in the environmental impacts. The worst scenario (Sc 9) has contributions between 2% and 8% higher than Sc 1 for categories such as ADP, AP, EP or GWP. In other categories with a lower relative contribution of vessel operations, the change has virtually no effect. In the case of Sc 10, some categories such as ADP, FEP, MEP or TEP show a slight reduction of impact with respect to the baseline scenario, which is linked to the lower amount of material associated with the process under assessment, due to the shared use of the vessel.

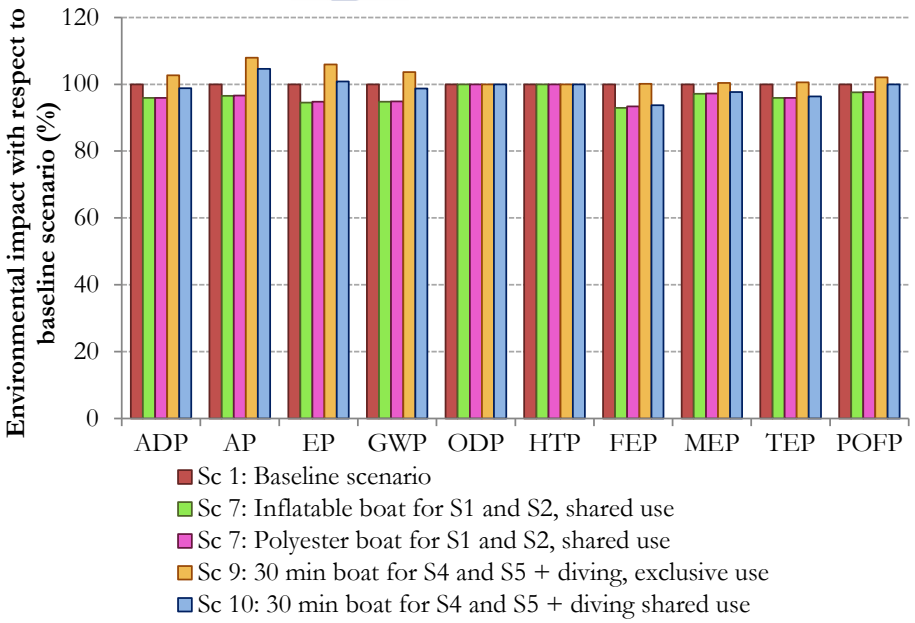



**Figure 6.5.** Effect of assumptions for the LCI of vessel operations on the environmental profile of the production of prenylhydroquinones by *S. spinsulus*.

The limited effect of the assumptions considered to obtain the inventory data for the stages involved in the cultivation of sponges in the mariculture system results from the low relative contribution of these stages compared to the downstream processing. In an optimized process with lower requirements for the extraction of compounds, the selection of appropriate procedures for the collection and monitoring of the cultivation system may affect significantly to the global environmental profile.

❖ **Effect of changes in survival rates**

In this case, an average survival rate of 80% was assumed, according to the values reported by Ledda et al. (2014) for mariculture in Mediterranean. As previously highlighted, a wide range of values for this parameter can be found in the literature, depending on several factors that include the species, location and other surrounding conditions (Duckworth, 2009; Schippers et al., 2012).

In order to evaluate changes in the environmental performance associated with possible variations in the survival rate, a sensitivity assessment is shown in **Figure 6.6**. The variations in the growth rate are not evaluated here, since this parameter is interrelated with the survival rate, and thus, the effect would be equivalent. Both parameters are jointly used to calculate the total amount of biomass harvested after one year of growth.

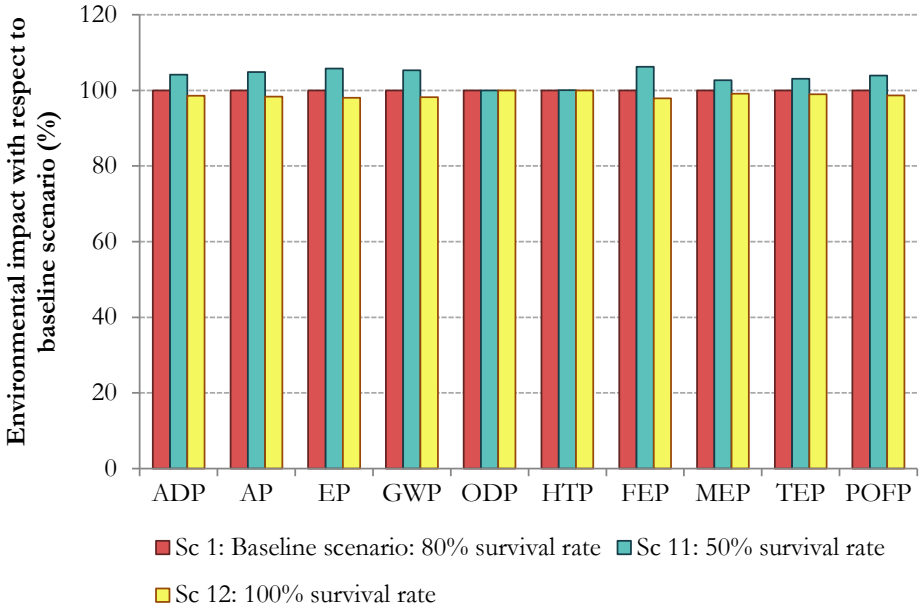



**Figure 6.6.** Effect of variations in the survival rate on the environmental profile of the production of prenylhydroquinones by *S. spinosulus*.

As in the case of vessel operations, the influence of the survival rate on the environmental impacts of the process is limited due to the low relative contributions of the mariculture stages compared to the downstream processing stages. Thus, the increase of the contributions to all impact categories due to a lower survival rate (50%) does not exceed 7%, whereas the improvement is below 3% for the maximum survival rate (100%). As expected, the lowest changes correspond to the categories of ODP and HTP, in which more than 99% of the impact is associated with the production of solvents.

Even if the solvent consumption is reduced by 50% (Sc 6), the changes in the environmental profile for ODP and HTP are lower than 1%. In this case, the impacts when considering 50% survival rate are between 5% and 11% higher than the reference scenario Sc 6, whereas the impact reductions for 100% survival range between 2% and 4% for the 8 remaining categories.

❖ **General remarks from the sensitivity assessment**

As well as highlighting again the importance of solvent optimization, the conducted assessment demonstrates the validity of the assumptions for the LCI. Thus, changes in the analyzed key parameters (i.e. different conditions for the use of the boat in the growth stages and variability of survival rates) result in limited variations of the obtained environmental profile with respect to the baseline scenario.

Moreover, the results suggest that, opposite to other processes in the field of marine biotechnology (Pérez-López et al., 2014b; Pérez-López et al., 2014c), the mariculture of sponges allows the continuous growth of the organisms with relatively low input requirements. This advantage is related to the use of natural resources (e.g. seawater and dissolved nutrients) as substitutes of raw materials from previous production processes (e.g. chemicals used to prepare artificial culture media).

## 6.3. Potential antitumor alkaloids from sponge *Crambe crambe* in *ex situ* culture systems

*Crambe crambe* (Schmidt, 1862) is a red encrusting sponge widely found in the Western Mediterranean Sea as well as in the Macaronesian that produces two families of valuable guanidine alkaloids: crambescins and crambescidins (Duran et al., 2004). Crambescidins were first isolated in the early 90s and patented several years later due to their cytotoxic and antiviral activities (Bondu et al., 2012; Rinehart and Jares-Erijman, 1998). More recently, crambescins have also revealed significant pharmacological properties (Bondu et al., 2012; Olszewski et al., 2004). As a result of these findings, these compounds are currently considered as potential anticancer drugs (Bondu et al., 2012; Laville et al., 2009; Martín et al., 2013).

In this section, a novel process for the combined production of crambescins and crambescidins by *C. crambe* in a controlled closed system is evaluated from an environmental perspective, according to the LCA standardized methodology (ISO 14040, 2006). The process consists in the periodic extraction of these biocompounds while maintaining the organisms alive. This option may allow a steady and prolonged production of antitumoral compounds as a basis for a commercial application. Although other production processes involving marine organisms, such as microalgae or macroalgae, have already been addressed through a life cycle approach for the production of both biofuels (Aresta et al., 2005; Brentner et al., 2011) and biocompounds (Pérez-López et al., 2014b; Pérez-López et al., 2014d), there are not available LCA studies focused specifically on the production of high value added molecules from sponges.

Therefore, this study develops for the first time a detailed life cycle inventory (LCI) and quantification of the environmental impacts associated with the production of bioactive compounds by sponges. Moreover, it presents a novel method to obtain the product while maintaining the organism alive. This approach is an alternative to the unsustainable exploitation of sponges by wild harvest of specimens in natural environments, where the growth of new individuals to replace those used to extract the target compounds and maintain the ecological balance would take such a long time that their production would be unfeasible.

### 6.3.1. Goal and scope

The main goal of this study is to identify the environmental impacts associated with the sustainable production of two potential antitumor molecules, specifically crambescin A1 and crambescidin 816, from the Mediterranean sponge *C. crambe*. The production process was developed in the Institut de Chimie de Nice at the Université Nice Sophie-Antipolis (France). After determining the major hot spots (or most problematic issues), alternative scenarios are simulated and evaluated from an environmental point of view in order to suggest feasible improvement measures that reduce impacts to obtain a more sustainable process. Moreover, a final comparison of the novel *ex situ* process with an *in situ* culture system is presented.

The study takes into account the production of the different mass and energy flows to the system, as well as the growth of the sponge in indoor aquarium and further periodic extraction and purification of the bioactive compounds. Although only crambescidins have been patented for their cytotoxic and antiviral activities (Rinehart and Jares-Erijman, 1998), recent studies suggest that also crambescins may have interesting biological properties (Bondu et al., 2012; Martín et al., 2013). Therefore, both families of guanidine alkaloids were considered as target products.

In this case, the selected functional unit was 100 mg of total bioactive fraction, including 50 mg of pure crambescin A1 and 50 mg crambescidin 816, which corresponds to the production during one year of operation for the baseline scenario. It should be pointed out that both products are obtained as pure compounds and they could be directly applied for pharmaceutical purposes. The total economic value of this production is estimated in roughly 7000 €, according to a price of 70 €·mg$^{-1}$ for a similar biocompound: halichondrin B (Sipkema et al., 2005b).

The system boundaries for the baseline scenario of the production of crambescins and crambescidins by *C. crambe* are shown in **Figure 6.7**. The stages or subsystems of the process included within the system boundaries are also described with reference to the extraction frequency and yield of the baseline scenario.

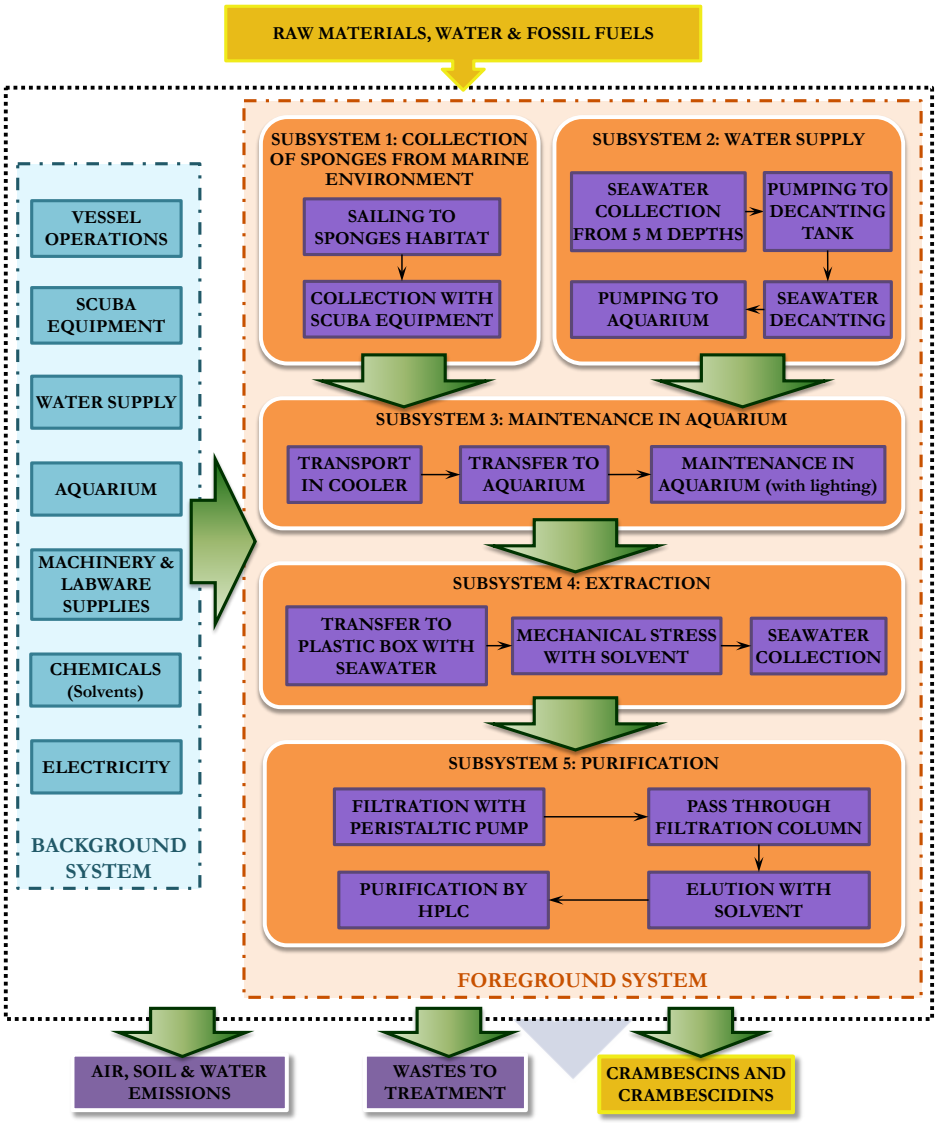



**Figure 6.7.** Process chain and system boundaries of the production of pure crambescin A1 and crambescidin 816 from *C. crambe* grown in indoor aquaria.

i) S1. Collection of sponges from marine environment: Specimens of the thin encrusting sponge *C. crambe* were collected with their substrate (hammer) at 25 m depth by scuba diving and transported in a cooler filled with seawater (18 L) in a 7 m length polyester vessel. The inventory data are based in experiments with model samples of 50 $cm^2$.

ii) S2. Water supply: Three aquaria (20 L volume, 12x15x120 cm) were sustained by an open seawater circuit which pumped water from a depth of 5 m. Seawater was fed at a flow of 2.5 $m^3 \cdot h^{-1}$ and then decanted in a tank of 10 $m^3$, which also served as water supply for other units in the facilities. Once decanted, seawater was transferred to the aquarium at a flow rate of 2 $L \cdot min^{-1}$.

iii) S3. Maintenance in aquarium: Ten individuals on their substrates, with an approximate surface of 50 $cm^2$ each, were transferred in each aquarium, which was illuminated by conventional fluorescent lamps. Since the aquaria were fed with seawater, operational conditions changed depending on the period of the year. The temperature was kept below 20ºC during summer with a control system, whereas it fell to 10ºC in winter. Although other parameters also fluctuated depending on the season, they exhibited values around 36.8 $kg \cdot dm^{-3}$ for salinity, 8 for pH and 6.5-7 $mg \cdot L^{-1}$ for oxygen demand. Detailed data can be accessed online (SOMLIT, 2015). After two days of acclimatization in these aquaria (**Figure 6.8**), which could be observed by the presence of open canals and oscules on the surface of the sponge, the specimens were ready for extraction.

iv) S4. Extraction: Each individual was transferred alive into a closed plastic box (700 mL) with 475 mL seawater and 25 mL of ethanol 96%. Mechanical stress was applied using a "snail fork" and scratching with 5 $cm^2$ intervals, avoiding canals. Half of the volume was collected for filtration in a second closed plastic box and the stressed sponge was replaced in the aquarium for 7 days of recovering. After this period, canals and oscules were opened again in the same way as before stressing the sponge.

**Figure 6.8.** Aquaria for the indoor cultivation of *C. crambe* in the facilities of the University of Nice Sophia-Antipolis.

v) S5. Purification: The resulting 250 mL solution from the previous stage was filtered with a peristaltic pump through 0.22 μm filter in order to remove all insoluble particles at 50 $mL \cdot min^{-1}$. After the filtration stage, the bioactive compounds were extracted from the seawater and purified by HPLC using water and methanol as solvents.

The obtained fractions contained around 0.1 mg of pure crambescin A1 and 0.1 mg of crambescidin 816 (0.2 mg of bioactive compounds obtained from each individual). Both extraction and purification were repeated weekly on the same specimens. Despite the periodical scratching of the sponge surface, individuals placed in the aquarium that were not covering all the substrate were observed to grow at a rate of c.a. 20% a year (area measurement) with or without milking. No comparison was done with culture in the sea but this result evidenced that the sponges placed in aquarium were in relatively good health.

### 6.3.2. Life cycle inventory, data quality and assumptions

As in previous case studies, the LCI data for the foreground system (including chemicals, water and electricity consumption for sponge aquaculture and extraction of bioactive compounds) consisted of average data obtained by on-site measurements. Water and air emissions were calculated on the basis that the chemicals which were not consumed during the process and gases from fuel combustion in the boat engine were directly discharged. The inventory data of the process are shown in **Table 6.4**.

Concerning the background system, the corresponding inventory data for the production of all the inputs to the system were taken from Ecoinvent database. A detailed description of the corresponding sources is shown in **Table 6.5**. The inputs include the production of the different chemicals required for the extraction and purification stages, the electricity used in the different stages, the materials for the equipment (vessel and scuba equipment, water supply system, aquarium, fluorescent tubes, electronic devices) and waste disposal.

In the case of the vessel, a shared use of the boat was considered for the baseline scenario. Thus, 1600 hours of annual operation were assumed, corresponding to 200 days of operation for 8 $h \cdot d^{-1}$. The amount of materials associated with the collection of sponges itself was estimated considering that

this stage only requires 2 h of sailing within the whole year. The effect of this assumption will be further discussed in the following sections. Emissions from fuel combustion were quantified according to the methodology described by the EMEP/EEA (2009). Chemicals related to vessel operations (i.e. paint, anti-fouling paint, marine lubricant oil) were inventoried according to Vázquez-Rowe et al. (2010), considering manufacturers' specifications. For paint and anti-fouling emitted to marine environment, a loss of two thirds of the total amount was considered (Hospido and Tyedmers, 2005). Solid wastes were disposed of in sanitary or inert landfills, except for synthetic rubber, which was sent to incineration.

Regarding water supply, the design of the pumping and decanting system was estimated from mass balances. As the output from the decanting tank was shared with other processes of the facility, the corresponding amount of material was calculated from the ratio between the flow to *C. crambe* aquarium and the total flow to the decanting tank. The quantification of the polymethyl metacrylate (PMMA) of the aquarium was also calculated according to the dimensions of the tank and the density of the material, considering a wall thickness of 4 mm. As the inventory is associated with a hypothetical facility placed in shore, transport of equipment and chemicals was neglected. The materials needed for the lab equipment, as well as for the vessel and scuba equipment, were estimated as average values from manufacturers' specifications. For the equipment, the life spans and assumptions are specified in **Table 6.6**.

*Allocation procedures*

Two target pure products were obtained from *C. crambe*: crambescin A1 and crambescidin 816. As both compounds have comparable activities, they may have similar market prices; accordingly, mass allocation was considered. Each alkaloid involves 50% of the total bioactive fraction, so the environmental burdens associated with each would be half of the total impacts. However, other fractions of guanidine alkaloids may be obtained as by-products. Although these fractions were neglected in the study, further research could provide additional potential uses. In such case, a fraction of the environmental impacts would be allocated to the by-products and, thus, the environmental burdens for the target compounds would decrease with respect to the results here presented.

**Table 6.4.** Inventory data for the production of pure crambescin and crambescidin by *C. crambe* in indoor aquaria (FU=100 mg bioactive fraction, consisting of 50 mg pure crambescin and 50 mg pure crambescidin)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Collection of sponges from marine environment* | | | |
| Polyester (vessel hull) | 0.111 kg | Steel (scuba tank) | 0.277 kg |
| Steel (engine) | 0.028 kg | Compressed air (200 bar) | 8.951 kg |
| Anti-fouling paint | 0.039 kg | Neoprene (scuba equipment) | 0.016 kg |
| Paint | 0.010 kg | | |
| Lubricant oil | 0.117 kg | PP (cooler) | 0.016 kg |
| *S2. Water supply* | | | |
| Steel | 0.961 kg | Concrete | 51.543 kg |
| PVC | 2.451 kg | | |
| *S3. Maintenance in aquarium* | | | |
| PMMA | 0.515 kg | Lamps | 0.059 kg |
| *S4. Extraction* | | | |
| Ethanol | 19.725 kg | PP | 0.160 kg |
| Distilled water | 1.316 kg | | |
| *S5. Purification* | | | |
| Steel | 1.539 kg | Methanol | 791.8 kg |
| Acetonitrile | 7.860 kg | Trifluoroacetic acid (TFA) | 1.489 kg |
| Mili-Q water | 1000 kg | | |
| **Energy** | | | |
| Electricity from French grid | | | |
| *S1. Collection of sponges from marine environment* | | *S4. Extraction* | |
| Diesel | 3.591 kg | Filtration (peristaltic pump) | 2.08 kWh |
| *S2. Water supply* | | Flow through column | 71.6 kWh |
| Pumping from sea to facilities | 234.8 kWh | *S5. Purification* | |
| Pumping from decanting tank to aquaria | 144.7 kWh | Purification with HPLC | 71.6 kWh |
| *S3. Maintenance in aquarium* | | | |
| Lighting | 1512 kWh | | |
| **INPUTS from ENVIRONMENT** | | | |
| **Materials** | | | |
| Sponge biomass | 159.6 g | Seawater | 50875 L |
| Substrate | 957.4 g | | |

**Table 6.4.** Inventory data for the production of pure crambescin and crambescidin by *C. crambe* in indoor aquaria (FU=100 mg bioactive fraction, consisting of 50 mg pure crambescin and 50 mg pure crambescidin) (*Cont.*)

| | | | |
|---|---|---|---|
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Product** | | | |
| Crambescin | 50 mg | Crambescidin | 50 mg |
| **Waste treatment** | | | |
| *S1. Collection of sponges from marine environment* | | *S3. Maintenance in aquarium* | |
| Polyester | 0.111 kg | PMMA | 0.515 kg |
| Steel | 0.305 kg | PP | 0.559 kg |
| *S2. Water supply* | | Lamps | 58.800 g |
| Steel | 0.961 kg | *S4. Extraction* | |
| PVC | 2.451 kg | PP | 0.160 kg |
| Concrete | 51.543 kg | *S5. Purification* | |
| | | Steel | 1.539 kg |
| **Wastes to municipal incineration** | | | |
| *S1. Collection of sponges from marine environment* | | | |
| Neoprene | 0.016 kg | | |
| **OUTPUTS to ENVIRONMENT** | | | |
| **Air emissions** | | | |
| *S1. Collection of sponges from marine environment* | | | |
| $CO_2$ | 11.218 kg | $NO_x$ | 0.125 kg |
| $SO_2$ | 0.007 kg | CO | 0.027 kg |
| NMVOC | 0.023 kg | PM | 0.013 kg |
| $CH_4$ | 0.646 g | | |
| **Water emissions** | | | |
| *S1. Collection of sponges from marine environment* | | | |
| Xylene | 3.496 g | Ethylbenzene | 0.914 g |
| Cobalt | 0.001 g | Sea Nine 211 | 0.392 g |
| Copper | 8.122 g | Ethanol | 0.392 g |
| Zinc | 3.673 g | 4-methylpentan-2-one | 0.392 g |
| *S3. Maintenance in aquarium* | | | |
| Wastewater | 50400 L | | |
| *S4. Extraction* | | | |
| Wastewater | 238.16 L | Ethanol | 9.860 kg |
| *S5. Purification* | | | |
| Wastewater | 1238 L | Methanol | 791.8 kg |
| Ethanol | 9.860 kg | Trifluoroacetic acid | 1.489 kg |
| Acetonitrile | 7.860 kg | | |

**Table 6.5.** Summary of data sources for the background system of the production of pure crambescin and crambescidin by *C. crambe* in indoor aquaria

| Type of involved process | Raw material | Data source |
|---|---|---|
| Energy | Diesel | Ecoinvent database (Jungbluth, 2007) |
| | Electricity (French electricity profile) | Ecoinvent database (Dones et al., 2007) |
| Chemicals related to vessel operation | Anti-fouling | Vázquez-Rowe et al. (2010) |
| | Boat paint | |
| | Marine lubricant oil | |
| Materials | Glass fiber reinforced plastic, polyester resin | Ecoinvent database (Kellenberger et al., 2007) |
| | Concrete | |
| | Steel | Ecoinvent database (Classen et al., 2007) |
| | Synthetic rubber | Ecoinvent database (Hischier, 2007) |
| | PVC | |
| | PMMA | |
| | PP | |
| | Lamps | Ecoinvent database (Hischier et al., 2007) |
| Air for scuba equipment | Compressed air | Ecoinvent database (Steiner and Frischknecht, 2007) |
| Water supply | Tap water | Ecoinvent database (Althaus et al., 2007) |
| | Distilled water | |
| Chemicals (solvents) | Methanol | Ecoinvent database (Althaus et al., 2007) |
| | Ethanol | Ecoinvent database (Sutter, 2007) |
| | Acetonitrile | |
| | Trifluoroacetic acid[1] | |
| Waste treatment | Inert landfill | Ecoinvent database (Doka, 2007) |
| | Sanitary landfill | |
| | Municipal incineration | |

[1]Assimilated to acetic acid.

**Table 6.6.** Life spans and assumptions for materials' quantification

| Equipment | Raw material | Life span | Assumptions |
|---|---|---|---|
| Vessel | Hull (polyester) | 30 years | Calculated material increased by 25% to account for vessel repairs and maintenance (Hospido and Tyedmers, 2005). |
| | Diesel engine (steel) | 15 years | Average weight estimated from manufacturers. Estimated weight increased by 50% to account for vessel repairs and maintenance (Hospido and Tyedmers, 2005). Life span estimated from EMEP/EEA (2009). |
| | Anti-fouling | 1 year | 2 coats per year assumed, according to manufacturers. |
| | Paint | 1 year | 1 coat per year assumed, according to manufacturers. |
| Scuba | Diving cylinder (steel) | 15 years | 10 uses·year$^{-1}$ with 1 use related to *C. crambe* process. |
| | Diving suit (neoprene) | 10 years | |
| Water supply | Pumps, water to decanting tank (steel) | 20 years | Designed for 10 m$^3$ decanting tank with 2.5 m$^3$·h$^{-1}$ flow rate. 14% of water pumped associated with *C. crambe* cultivation. |
| | Pipes, water to decanting tank (PVC) | | |
| | Decanting tank (concrete) | | |
| | Pumps, water to aquarium (steel) | 20 years | Designed to feed three aquaria with 2 L·min$^{-1}$ flow rate each. |
| | Pipes, water to aquarium (PVC) | | |
| Cooler | PP | 20 years | According to manufacturers' specifications. |
| Aquarium | PMMA tank | 10 years | Weight calculated from on-site direct measurement of dimensions. |
| | Lights | 30000 | According to manufacturers' specifications. |
| Purification system | Plastic boxes (PP) | 20 years | According to manufacturers' specifications. |
| | Pump (steel) | | |
| | Separation columns (steel) | | |

### 6.3.3. Environmental impact assessment

The environmental profile of the described system was again assessed according to the CML 2001 methodology (Guinée et al., 2002). The same impact categories used for the evaluation of *S. spinosulus* were analyzed: ADP, AP, EP, GWP, ODP, HTP, FEP, MEP, TEP and POFP. The software SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008).

❖ **Identification of hot spots**

The characterization results associated with the potential environmental impacts of the production of *C. crambe* biomolecules in the analyzed categories are detailed in **Table 6.7**.

**Table 6.7.** Impact assessment results (characterization step) associated with the baseline scenario of the production of pure crambescin and crambescidin by *C. crambe* in indoor aquaria (FU: 100 mg bioactive fraction)

| Impact category | Unit | Value |
|---|---|---|
| ADP | kg Sb eq | 17.27 |
| AP | kg $SO_2$ eq | 3.44 |
| EP | kg $PO_4^{-3}$ eq | 1.23 |
| GWP | kg $CO_2$ eq | 952.73 |
| ODP | kg CFC-11 eq | $0.13 \cdot 10^{-3}$ |
| HTP | kg 1,4-DB eq | 640.36 |
| FEP | kg 1,4-DB eq | 260.47 |
| MEP | kg 1,4-DB eq | 171.02 |
| TEP | kg 1,4-DB eq | 0.06 |
| POFP | kg $C_2H_4$ eq | 0.32 |

As shown in **Figure 6.9**, most of the environmental impacts are dominated by the purification stage, with contributions ranging from 34% (for TEP) to 90% (for ODP). The maintenance in aquarium is also a significant stage, especially in terms of toxicity potentials, which present values between 40% and 49%. Among the secondary subsystems, water supply is the only stage that has a relevant contribution related to toxicity potentials, with impacts ranging from 14% (MEP) to 22% (HTP).

Regarding the involved processes, the production of the chemicals required in the extraction and purification stages constitutes the major impact in the categories of ADP (89%), AP (51%), GWP (71%), ODP (90%) and POFP (79%). Electricity is the other significant contributor to most of the environmental impacts. Indeed, this process accounts for more than 40% in six of the assessed categories, being the main cause of EP (49%), HTP (54%), FEP (53%), MEP (51%) and TEP (64%). The main processes responsible for the environmental impacts are further analyzed below.

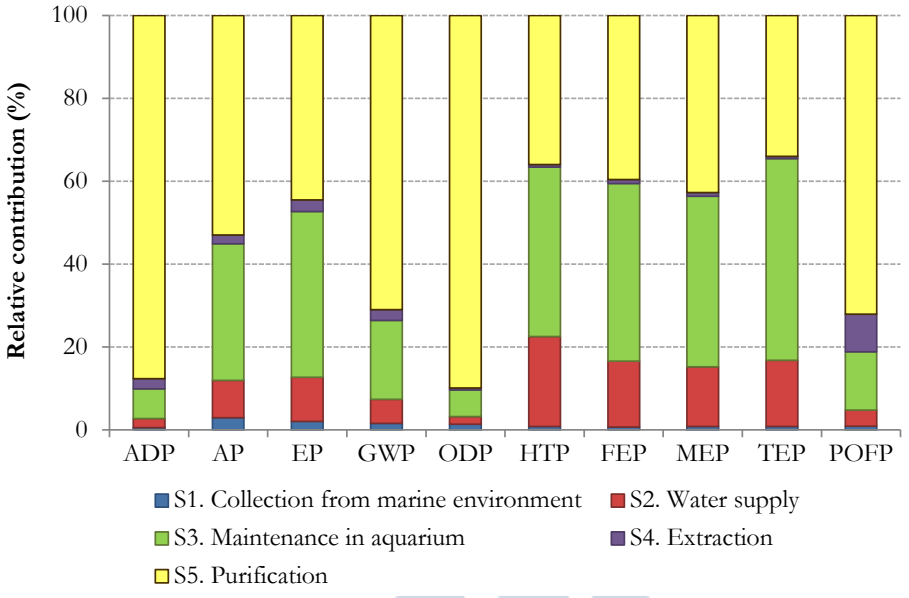
❖ **Major contributors among chemicals**

Since the production of chemicals is the main issue related to the environmental impacts of the production of crambescin and crambescidin by *C. crambe*, the breakdown of the contributions of these processes in all the assessed categories is depicted in **Figure 6.10**.

According to the graph, the production of methanol needed for the purification is the main cause of the environmental impacts related to chemicals, with more than 80% of the contributions to all the categories. Although the environmental impact of methanol per mass unit is remarkably lower than other involved chemicals such as acetonitrile or trifluoroacetic acid, the large amount of methanol that is consumed in the process results in the observed high impact.

The influence of the production of methanol is particularly significant in the categories ADP (in which it represents nearly 85% of the total impact of the whole production process), GWP (66% of total impact), ODP (88%) and POFP (68%). The contributions to ADP and ODP are mainly due to the transport and use of natural gas throughout the life cycle of methanol production, whereas GWP is linked to $CO_2$ emissions and POFP is related to the released $SO_2$.

**a) Relative contributions of guanidine alkaloids from *C. crambe* per stage**




**b) Relative contributions of guanidine alkaloids from *C. crambe* per involved process**

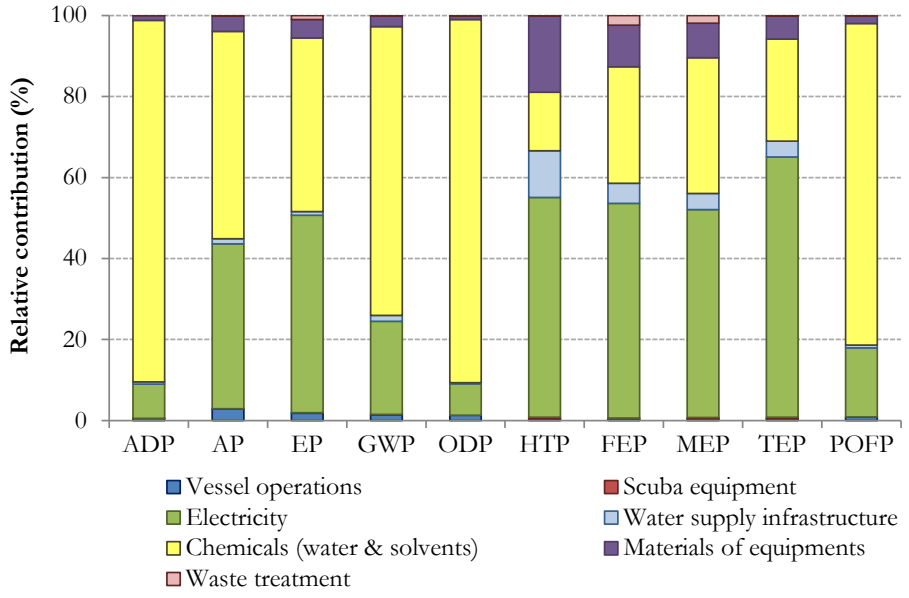



**Figure 6.9.** Relative contributions of the production of pure crambescin A1 and crambescidin 816 by *C. crambe* in the baseline scenario to each impact category per a) stage and b) involved process.

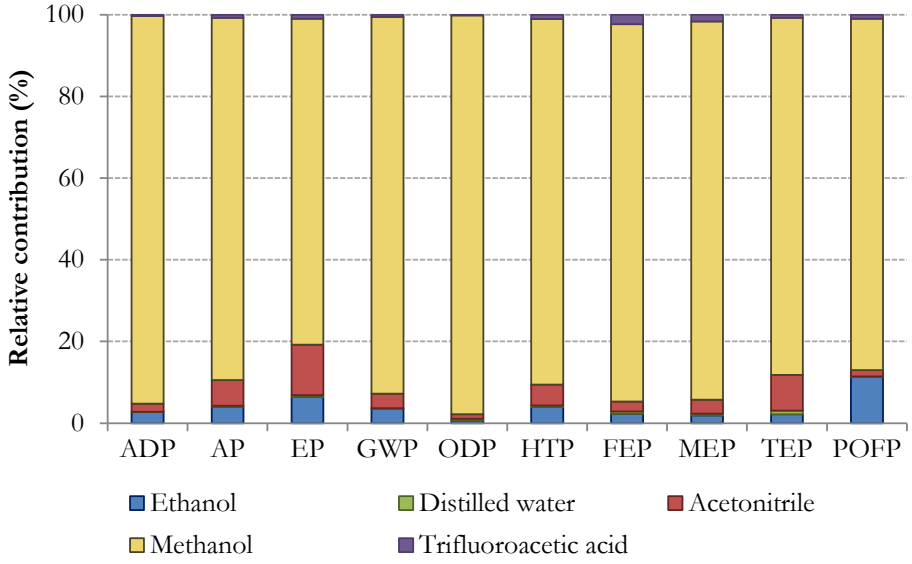



**Figure 6.10.** Relative contribution of the production of the different chemicals involved in the production of pure crambescin A1 and crambescidin 816 by *C. crambe* in the baseline scenario.

❖ **Major contributors among electricity**

The second hot spot found in the analysis corresponds to the production of electricity in the different stages of the process. This process especially contributes to EP and toxicity categories. The impacts to EP are mainly due to the emissions of phosphate and nitrogen oxides whereas the contributions to toxicity categories are mostly related to the emissions of metals to air and water. Particularly, HTP is highly affected by emissions of selenium, arsenic and chromium VI, while the major responsible for FEP and MEP are emissions of nickel, vanadium and beryllium. Finally, the environmental impacts to TEP principally come from emissions of mercury derived from the use of coal for electricity generation and chromium VI from the distribution network.

In order to identify the stages with higher electricity requirements, the contributions are depicted in **Figure 6.11**. Nearly three fourths of the electricity consumption comes from lighting during the maintenance of *C. crambe* in the aquarium. This finding is consistent with the experience from previous works, which point out the importance of artificial lighting in the total energy cost of

the cultivation of other marine organisms (Das and Obbard, 2011; Pérez-López et al., 2014b; Pulz and Scheibenbogen, 1998). Therefore, the optimization in terms of electricity consumption should be focused on the reduction of lighting. Among the secondary stages, water supply has the highest consumption, with 62% due to water pumping. This result suggests that recycling the seawater may help to reduce the environmental impacts associated with this stage.

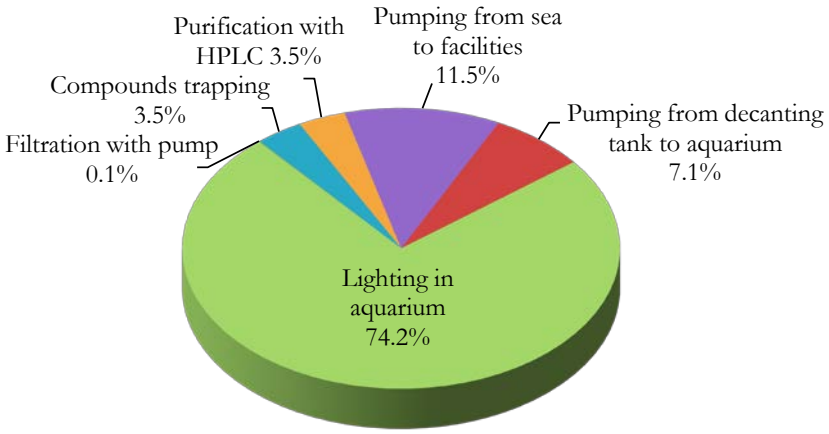



**Figure 6.11.** Relative contribution of the different steps to the total electricity requirements of the production of pure crambescin and crambescidin by *C. crambe* in the baseline scenario.

❖ **Effect of vessel operations**

According to the results, the collection of the sponges from the environment is a minor contributor to all impact categories. This contribution is mainly related to the vessel operations, including fuel consumption but also material inputs for vessel construction. Despite the limited effect found for this subsystem, the results are based on the assumption that the boat is also used for fishing. Thus, only a slight fraction of the total environmental impacts associated with vessel operations were allocated to *C. crambe* process.

Nevertheless, energy and material inputs in fishing vessels can affect the environmental profile significantly, not only due to fuel consumption but also derived from other materials, such as anti-fouling agents or paints (Hospido and

Tyedmers, 2005; Vázquez-Rowe et al., 2010). Moreover, the assumptions considered to allocate the impacts from the vessel may considerably affect the global results. For this reason, a sensitivity analysis is shown in **Figure 6.12**.

Three alternative situations were compared to the baseline scenario. In the first of them (Sc 2), all the impacts associated with the vessel operations were allocated to the production process of crambescins and crambescidins, assuming that the boat used for the collection was a recreational vessel with no additional function (in terms of other material products obtained). However, it may be argued that a recreational use is associated with an immaterial function that should be taken into account. For this reason, the second scenario (Sc 3) allocates the impact of the collection of sponges according to the ratio between the number of hours associated with this process (2 h per year) and the total number of sailed hours within the year, assuming 2 h sailed per week with 52 weeks per year. The third scenario (Sc 4) is based on the findings of previous works, which suggest that the inputs to vessel construction and maintenance have limited contributions to the total impacts of seafood products (Hospido and Tyedmers, 2005). In this case, a fishing vessel is again considered, and building materials are excluded from the system boundaries.

**Figure 6.12** shows that the assumptions considered to determine the impacts from the vessel considerably influence the global environmental profile of the production of crambescins and crambescidins. Indeed, when considering that the vessel use is only associated with sponge collection (Sc 2), the contributions to most impact categories vary between 1.5 and 3.5 times those of the baseline scenario. Despite these remarkable differences, it should be pointed out that this is the most unlikely scenario, as the collection stage requires the vessel use for a very short period of time. Therefore, a combined use of the boat for other purposes, such as fishing or collection of other marine specimens for product exploitation, is expected. Regarding the other analyzed scenarios, the effect of vessel operations seems rather restricted, with deviations lower than 4.5% in all the impact categories. Thus, Sc 3 results in impact increases between 0.4% (ADP) and 4.4% (HTP), whereas reductions observed in Sc 4 range from 0.03% (ADP) to 0.3% (HTP) with respect to the baseline scenario.

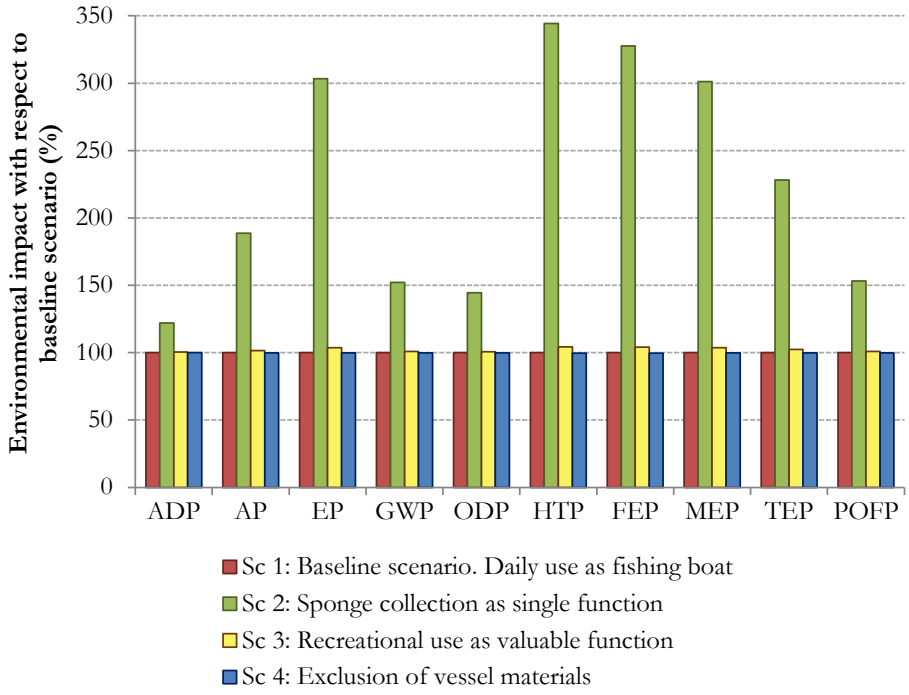



**Figure 6.12.** Effect of vessel operations on the environmental profile of the production of pure crambescin A1 and crambescidin 816 by *C. crambe*.

### 6.3.4. Discussion and recommendations

LCA methodology has allowed identifying the stages and processes with the greatest influence in the environmental profile of the production of crambescin and crambescidin from the encrusting sponge *C. crambe*. According to the results, alternative scenarios were simulated to evaluate possible improvement measures with respect to the current process:

❖ **Solvent reuse**

The production of the chemicals required for the purification stage was the principal contributor to the environmental impacts in five categories (ADP, AP, GWP, ODP and POFP). More than 85% of the mentioned contributions were specifically derived from the production of methanol, due to the large use of this solvent for the purification. For this reason, an alternative scenario (Sc 5) is suggested, consisting of the reuse of 50% of the methanol required for

obtaining of pure crambescin and crambescidin. Although this assumption was not based on experimental work, several authors have already checked the feasibility of reusing methanol to extract other similar alkaloids (Blaicher et al., 1981; Harkrader and Jones, 1998).

According to **Figure 6.13**, the reuse of methanol constitutes a promising option to improve the environmental profile of the studied process. The evaluated scenario presents remarkable reductions in terms of ADP (42.4%), AP (22.6%), GWP (32.4%), ODP (43.8%) and POFP (33.7%). The effect on other categories, such as HTP (6.5%) and TEP (11.0%) is relatively limited, though the performance in all the considered categories is better than the base scenario.

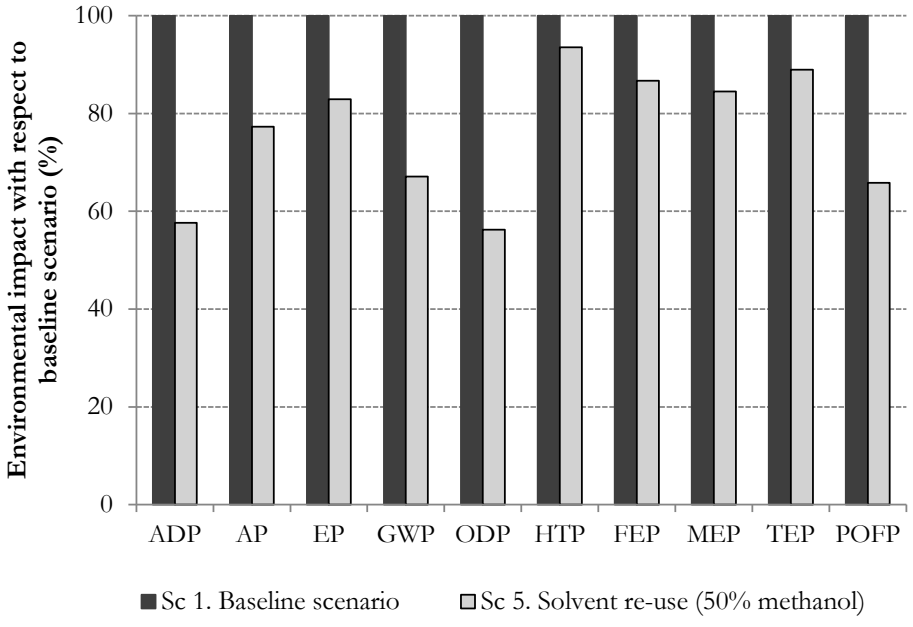



**Figure 6.13.** Effect of methanol reuse on the environmental profile of the production of pure crambescin A1 and crambescidin 816 by *C. crambe*.

❖ **Electricity optimization**

The production of electricity required throughout the whole *C. crambe* process was identified as a major concern in six of the ten impact categories under assessment (AP, EP, HTP, FEP, MEP and TEP). The main reason is the dependence on non-renewable sources due to the use of electricity that is directly taken from the French grid, which is characterized by a limited need of fossil fuels but a high reliance on nuclear energy (Dones et al., 2007). Hence, two possible scenarios are evaluated, concerning the use of solar (Sc 6) and wind (Sc 7) energy as alternative sources to the electricity taken from the grid.

In addition, the artificial lighting of the aquarium was identified as the main hot spot associated with electricity requirements, with 75% of the total electric consumption. This is due to the use of fluorescent lamps, which were switched on 24 $h \cdot day^{-1}$. However, the necessity of light for the sponge growth is lower than for other marine organisms such as microalgae or macroalgae (González-Rivero et al., 2012; Ogbonna and Tanaka, 2000; Yeh et al., 2010). Furthermore, in the evaluated process, the main goal is not maximizing the biomass production but maintaining the sponge in such healthy conditions that allow the periodical extraction of compounds from the specimens. Therefore, an alternative regime with less lighting seems a feasible strategy to reduce the total electricity consumption of the system. Thus, two additional improvement options were proposed: 16:8 regime scenario (Sc 8) and scenario with no lighting (Sc 9). Furthermore, an additional scenario (Sc 10) was evaluated, regarding the substitution of conventional fluorescent tubes by light-emitting diodes (LEDs). As well as having a longer life span (about three times higher) than fluorescent lamps, LEDs are also more efficient and can result in a 50% decrease in energy consumption (Chen et al., 2011).

According to **Figure 6.14**, all the proposed alternatives show remarkable reductions in the environmental impact for most categories, except from the solar scenario in ODP which had a higher contribution mainly due to the production of materials for the solar panels. As expected, the improvements were especially significant for toxicity categories, which were more affected by the electricity requirements, but also for AP and EP.

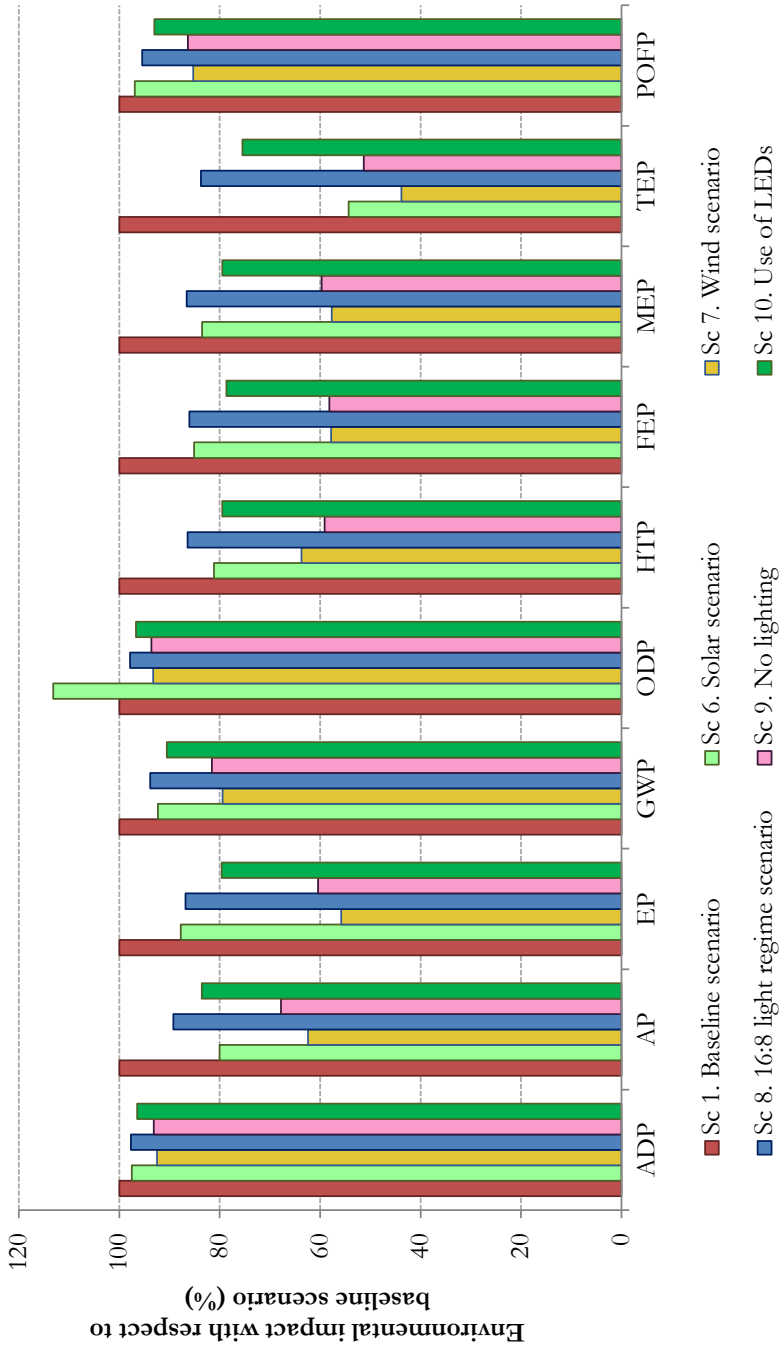



**Figure 6.14.** Sensitivity analysis of the environmental performance considering five improved alternatives for the reduction of electricity requirements in the production of pure crambescin A1 and crambescidin 816 by *C. crambe*.

In Sc 6 (solar energy), the reductions ranged from 2.5% (ADP) and 3.1% (POFP) up to 15% for HTP, FEP and MEP, and even 45.7% for TEP. Sc 7 (wind energy) showed the largest reductions, with more than 35% of improvement in six of the categories (AP, EP, HTP, FEP, MEP and TEP). Sc 9 (no lighting) had the second best performance, with reductions between 30% and 50% for the same categories. However, it should be highlighted that this scenario is based on the assumption that sponges can be maintained in the same conditions (comparable growth rate and equivalent amount of bioactive compounds obtained by extraction) as in the baseline scenario without lighting. As the verification of this assumption would require further research, the 16:8 regime (Sc 8) seems a more feasible strategy to be applied in the short-term. Despite the more restricted improvement, Sc 8 still showed significant reductions, ranging from 10% to 16% for those categories that are affected by the use of electricity within the process. Finally, the substitution of conventional fluorescent tubes by LEDs (Sc 10) allowed reductions between 3.5% and 24.5%.

❖ **Water recycling scenario**

As indicated in the previous section, water supply constitutes a secondary contributor that may have a relevant effect on some categories, due to the electricity consumption of the pumping system. Indeed, continuous pumping of water has already been identified as a significant issue in the cultivation of other marine organisms such as microalgae (Lam and Lee, 2012; Xu et al., 2011).

In the case of *C. crambe* process, this contribution is mostly associated with the seawater collection and pumping from the sea to the decanting tank. Therefore, an alternative scenario (Sc 11) where 50% seawater was recycled to the aquarium instead of its direct discharge to the sea was assessed. However, the results indicate that the improvement achieved with this measure would be rather limited, with reductions of impact between 0.7% and 4.3%. The highest reductions are found in the toxicity categories, which were those with a significant contribution of electricity. The improvements related to these categories would range from 3.5% for HTP to 4.3% for TEP.

❖ **Improved waste treatment**

Although the environmental burdens associated with waste treatment are rather slight in comparison with other subsystems of the process, an alternative option was proposed, regarding the final disposal of the waste. In the present study, the assumption that the materials of the equipment and infrastructure were finally sent to landfill was considered. Nevertheless, previous LCA studies of related processes proposed other approaches, such as sending these materials to recycling (Collet et al., 2011). In this case (Sc 12), the final disposal of steel, plastic materials and concrete to landfill was substituted by the recycling of these materials. However, the improvement observed with this measure is very limited and the highest reductions were between 1% and 2.4% for the categories of EP, FEP and MEP.

❖ **Best performance versus most feasible scenario**

Several of the simulated scenarios can be simultaneously applied, allowing higher reductions of impact. Therefore, the compatible improvement alternatives were combined in two hypothetical scenarios:

- **Sc 13. Best performance scenario**. In this case, the maintenance in aquarium with no lighting is considered together with the wind electricity supply for the other electricity requirements. Additional, a methanol reuse of 50% is taken into account, along with a 50% seawater reuse and a recycling scenario.
- **Sc 14. Most feasible scenario**. Despite having the lowest environmental burdens, the best performance scenario is based on the assumptions that the reduction of lighting and the re-use of the solvent neither affect the yield of the process nor the purity of the two produced compounds. As further research should be needed to prove the accuracy of these assumptions, another alternative scenario that seems more feasible in a short period of time was proposed. In this case, a 16:8 regime was considered jointly with solar electricity supply and the use of LEDs, as well as a methanol re-use of 25%, was assumed. Seawater recirculation and recycling of materials were also considered.

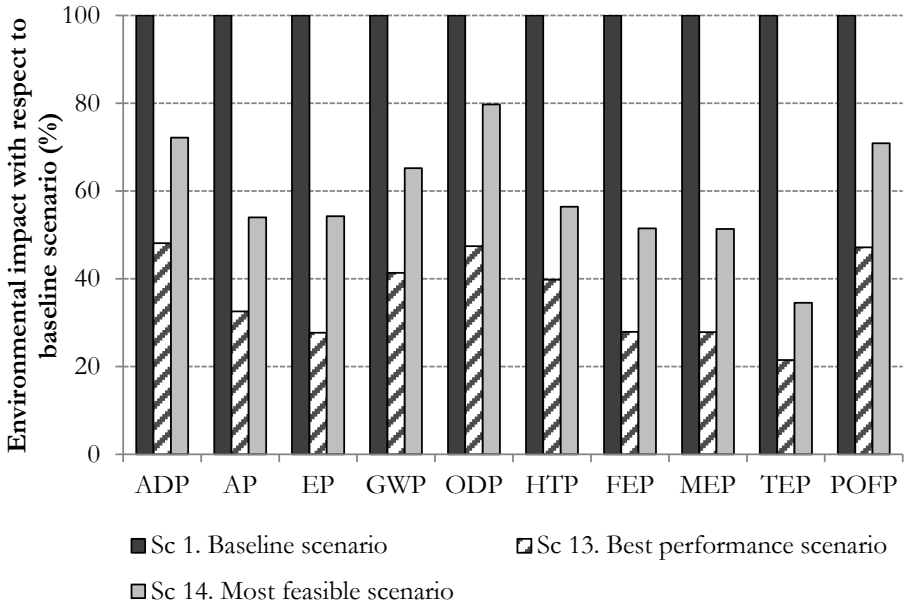



**Figure 6.15.** Comparative environmental profiles of the baseline scenario, the best performance scenario and the most feasible scenario for the production of crambescin A1 and crambescidin 816 by *C. crambe*.

The improvement that may be achieved by the combined implementation of the alternative scenarios is shown in **Figure 6.15**. According to the results, the environmental profile of the production of pure crambescin and crambescidin can be significantly enhanced, with reductions of impact for the best performance scenario between 52 and 78% depending on the category. Even if a more conservative approach is considered, the most feasible scenario in a short term period would allow improvements up to 40% for the categories of AP, EP, HTP, FEP and MEP, and as high as 65% for TEP.

### 6.3.5. Comparative assessment of *in situ* and *ex situ* cultivation systems for *C. crambe*

Although the main objective of the work presented in this section is the environmental evaluation of a novel *ex situ* culture system, an alternative *in situ* scheme is here proposed, similar to the sea-based farming system proposed in section 6.2 for the growth of *S. spinosulus*. The system boundaries are presented in **Figure 6.16**.

The description of the subsystems S1 to S5 is analogous to the *in situ* process for *S. spinosulus*. The main differences are:

- The same boat used for the collection of *C. crambe* from natural environment in the *ex situ* process is considered to obtain the LCI of the *in situ* cultivation. Therefore, the boat consists of a polyester resin hull and a 100 HP diesel engine instead of the inflatable boat used for *S. spinosulus* process.
- The monitoring (S4) and harvesting (S5) stages require the use of boat and scuba equipment. Four monitoring sessions per year are considered, with a total sailing time of 30 min each. For harvesting, 2 h per year of boat use is estimated, for a total of three farming structures containing 300 specimens in total. A survival rate of 80% is assumed, according to Ledda et al. (2014).
- A shared use of the vessel is considered, with a total of 1600 sailed hours per year (according to the baseline scenario for the *ex situ* process).

The changes in the inventory are presented in **Table 6.8**. Data for downstream processes (stages S6 and S7) are not included, since their values are the same as those listed for S4 and S5 in **Table 6.4** (The FU of 100 mg of bioactive fraction is maintained).

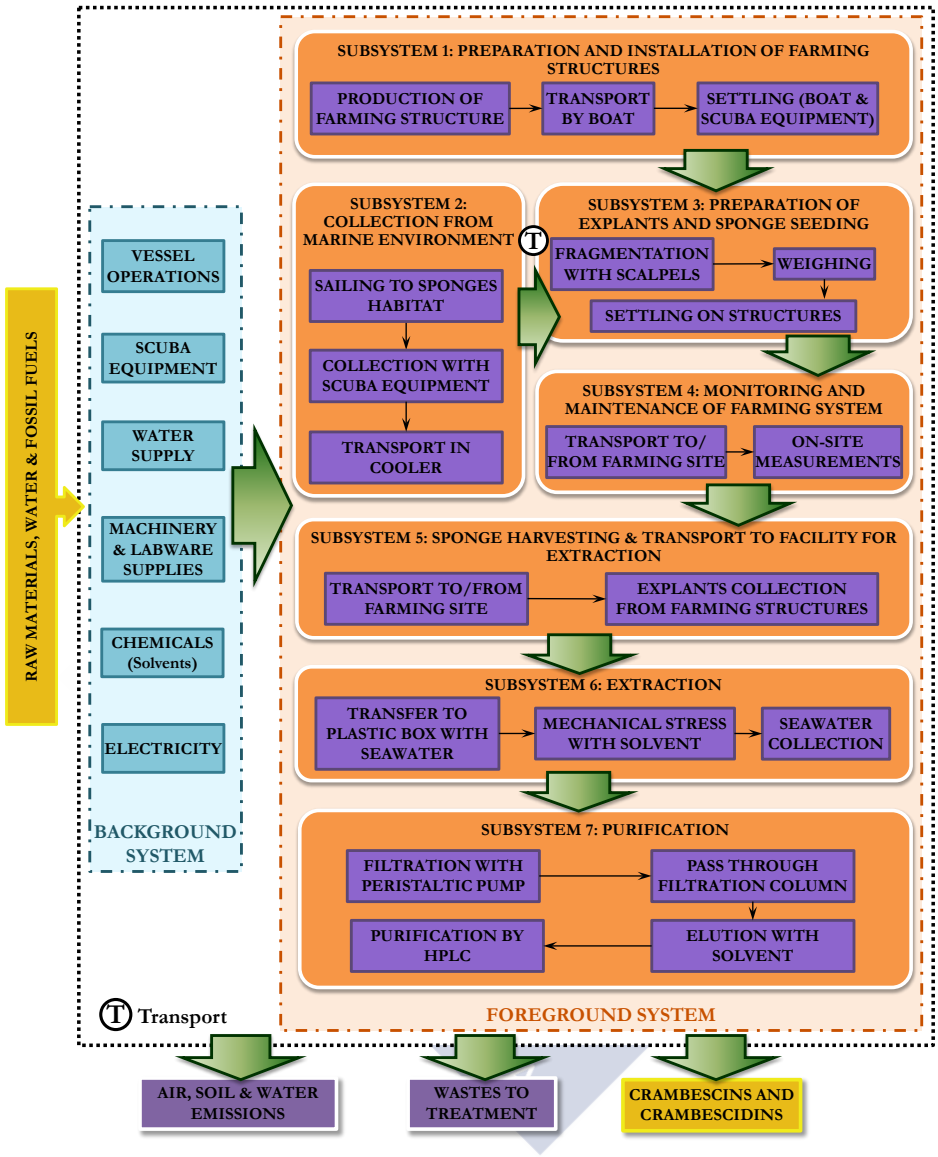



**Figure 6.16.** Process chain and system boundaries of the production of pure crambescin A1 and crambescidin 816 from *C. crambe* grown in a sea-based farming system.

**Table 6.8.** Inventory data for *in situ* production of *C. crambe* biomass for the subsequent extraction of crambescin A1 and crambescidin 816 (FU=100 mg bioactive fraction)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Preparation and installation of farming structures* | | | |
| Polyester (hull) | 0.40 g | Synthetic rubber | 0.39 g |
| Steel (engine and scuba equip.) | 5.36 g | Nylon (farming structures) | 28.72 g |
| Lubricant oil | 1.78 g | PVC (farming structures) | 28.72 g |
| Antifouling | 0.11 g | Steel (farming structures) | 14.36 g |
| Paint | 0.03 g | LDPE (farming structures) | 14.36 g |
| Compressed air (200 bar) | 0.11 kg | | |
| *S2. Collection of sponge seeds from natural habitat* | | | |
| Polyester | 5.98 g | Paint | 0.41 g |
| Steel | 80.44 g | Compressed air (200 bar) | 1.68 kg |
| Lubricant oil | 26.41 g | Synthetic rubber | 5.92 g |
| Antifouling | 1.63 g | PP | 0.42 kg |
| *S3. Preparation of explants and sponge seeding* | | | |
| Steel | 5.70 g | Electric battery | 0.28 g |
| *S4. Monitoring and maintenance of farming system* | | | |
| Polyester | 11.96 g | Paint | 0.82 g |
| Steel (engine and scuba equip.) | 0.32 kg | Synthetic rubber | 23.67 g |
| Lubricant oil | 52.82 g | Steel (lab equipment) | 21.06 g |
| Antifouling | 3.26 g | Electric battery | 1.10 g |
| *S5. Annual harvesting* | | | |
| Polyester | 5.98 g | Paint | 1.72 g |
| Steel (engine and scuba equip.) | 80.44 g | Compressed air (200 bar) | 1.68 kg |
| Lubricant oil | 26.41 g | Synthetic rubber | 5.92 g |
| Antifouling | 6.83 g | | |
| **Energy** | | | |
| *S1. Preparation and installation of farming structures* | | *S4. Monitoring and maintenance* | |
| Diesel | 0.54 kg | Diesel | 16.16 kg |
| *S2. Collection of sponge seeds from natural habitat* | | *S5. Sponge harvesting* | |
| Diesel | 8.08 kg | Diesel | 8.08 kg |

**Table 6.8.** Inventory data for *in situ* production of *C. crambe* biomass for the subsequent extraction of crambescin A1 and crambescidin 816 (FU=100 mg bioactive fraction) (*Cont.*)

| | | | |
|---|---|---|---|
| **INPUTS from TECHNOSPHERE** | | | |
| **Transport** | | | |
| Truck 3.5-7.5 t (materials) | 0.19 tkm | Passenger car | 169.79 pkm |
| Truck 3.5-7.5 t (wastes) | 0.05 tkm | | |
| **INPUTS from ENVIRONMENT** | | | |
| **Materials** | | | |
| Sponge biomass (wet weight) | 0.24 kg | Seawater | 14.12 L |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Product** | | | |
| Bioactive fraction containing crambescin and crambescidin | 100 mg (contained in 191 g harvested sponge, to extraction stages) | | |
| **Avoided product** | | | |
| Electricity (UV filter) | 6.46 kWh | | |
| **Waste treatment** | | | |
| *S1. Preparation and installation of farming structures* | | | |
| Polyester | 0.40 g | Steel | 5.36 g |
| Synthetic rubber | 0.39 g | | |
| *S2. Collection of sponge seeds from natural habitat* | | | |
| Polyester | 5.98 g | Synthetic rubber | 5.92 g |
| Steel | 80.44 g | PP | 0.41 kg |
| *S3. Preparation of explants and sponge seeding* | | | |
| Steel | 5.70 g | Electric battery | 0.28 g |
| *S4. Monitoring and maintenance of farming system* | | | |
| Polyester | 11.96 g | Synthetic rubber | 23.67 g |
| Steel | 0.34 kg | Electric battery | 1.10 g |
| *S5. Annual harvesting* | | | |
| Polyester | 5.98 g | Nylon (farming struct.) | 28.72 g |
| Steel (engine and scuba equip.) | 80.44 g | PVC (farming struct.) | 28.72 g |
| Synthetic rubber | 5.92 g | LDPE (farming struct.) | 14.36 g |
| | | Steel (farming struct.) | 14.36 g |

**Table 6.8.** Inventory data for *in situ* production of *C. crambe* biomass for the subsequent extraction of crambescin A1 and crambescidin 816 (FU=100 mg bioactive fraction) (*Cont.*)

| **OUTPUTS to ENVIRONMENT** | | | |
|---|---|---|---|
| **Air emissions** | | | |
| *Engine combustion in S1, S2, S4 and S5* | | | |
| $CO_2$ | 103.07 kg | $CH_4$ | 0.01 kg |
| $SO_2$ | 0.07 kg | $NO_x$ | 1.15 kg |
| NMVOC | 0.21 kg | CO | 0.24 kg |
| | | PM | 0.12 kg |
| **Water emissions** | | | |
| *Emissions from paint and antifouling in S1, S2, S4 and S5* | | | |
| Wastewater | 11.97 L | Ethylbenzene | 0.28 g |
| Xylene | 1.06 g | Sea Nine 211 | 0.12 g |
| Cobalt | 0.001 g | Ethanol | 0.12 g |
| Copper | 2.45 g | 4-methylpentan-2-one | 0.12 g |
| Zinc | 1.11 g | | |

The comparative results are presented in **Figure 6.17**. The production process based on the substitution of *ex situ* growth by the mariculture system (Sc 15) shows significant reductions of impact in all categories except for ODP, ranging from a 5% improvement (for ADP) to 60% reduction (for HTP or TEP). This improvement is mainly due to the removal of the most energy-intensive stage of the process: the growth in aquaria with artificial lights.

Although the sea-based farming option is clearly more efficient than the baseline system, other scenarios analyzed in section 6.3.4 must also be taken into account. Indeed, the *ex situ* option (Sc 13) considering the combined implementation of energy optimization, together with solvent and water reuse, as well as waste recycling has a remarkably better environmental performance than the sea-based farming process. Thus, the contributions of Sc 15 are between 1.5 and 2.5 times higher than the environmental burdens of Sc 13, except for the category of ODP. This category is mainly associated with the solvents used for extraction and purification, and therefore, no change in the cultivation stages affects the result significantly.

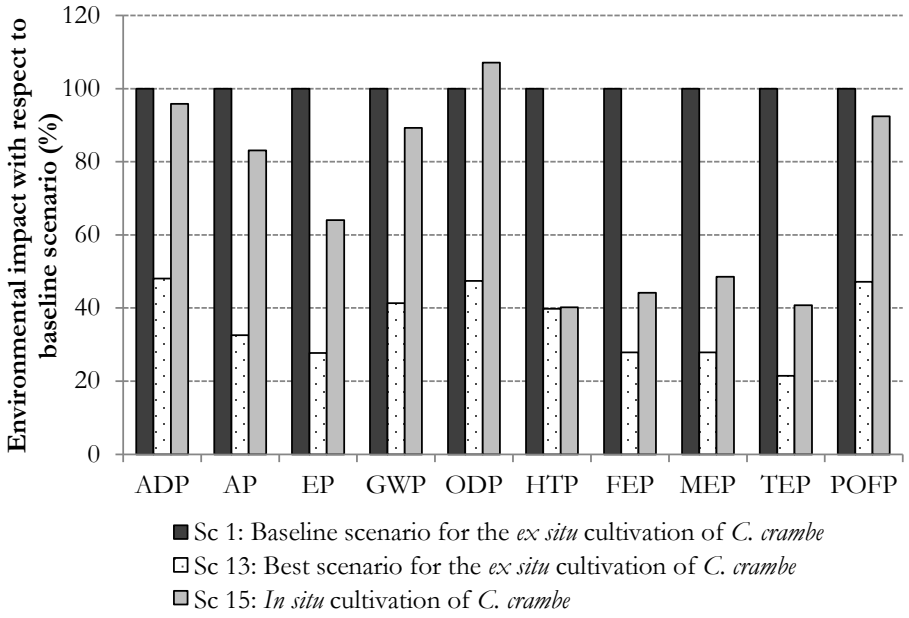
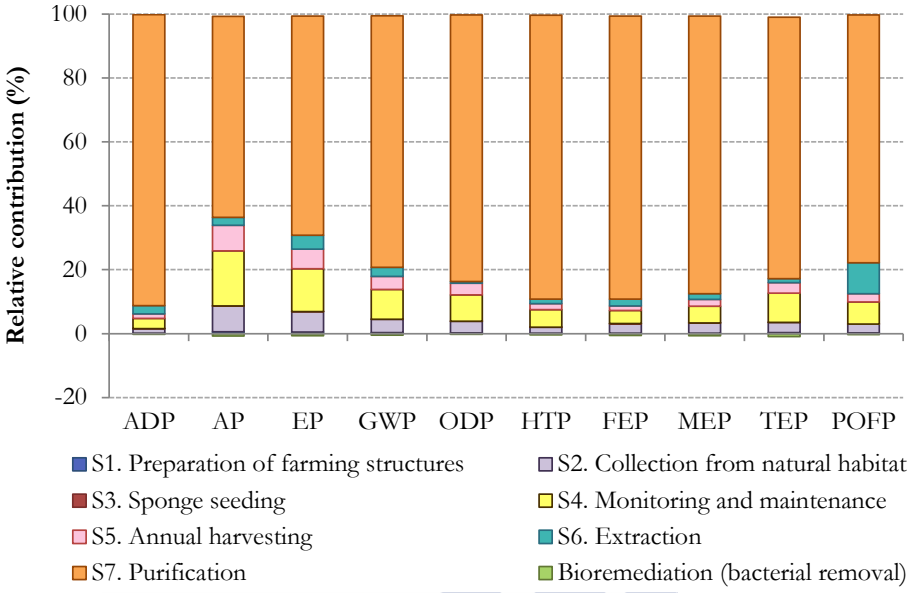



**Figure 6.17.** Comparative environmental profiles of the production of crambescin A1 and crambescidin 816 by *C. crambe* cultured in *ex situ* aquaria or *in situ* farming structures.

The relative contributions of the different stages and involved processes when considering the production of *C. crambe* in a sea-based farming system are depicted in **Figure 6.18**. As expected, the main stage responsible for the environmental impact in all the categories is the purification (S7). This result is in accordance with the findings described in section 6.3.3. In this case, the relative values are higher due to a lower total impact of other stages of the process. Thus, the production of electricity (which was one of the main hot spots in the baseline scenario) has contributions below 1%, regardless of the considered category. This is due to the reduction of the total electricity consumption linked to the removal of the cultivation stage, with high energy requirements related to lighting and pumping. Since no changes for the downstream processes were considered in Sc 15, the production of solvents for purification is again the main hot spot, with contributions between 34 and 90%.

**a) Relative contributions of guanidine alkaloids from *C. crambe* per stage**




**b) Relative contributions of guanidine alkaloids from *C. crambe* per involved process**

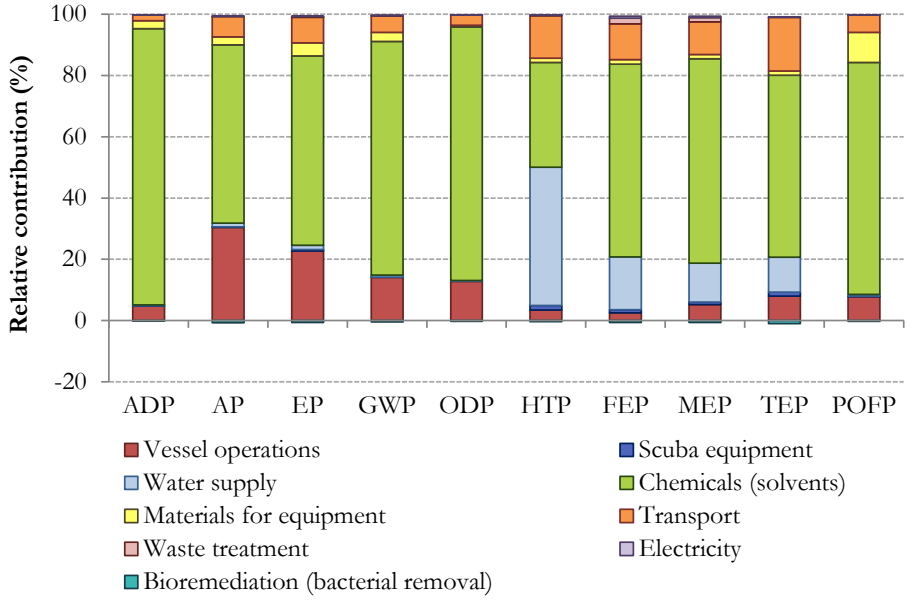



**Figure 6.18.** Relative contributions of the production of pure crambescin A1 and crambescidin 816 by *C. crambe* cultured *in situ* to each impact category per a) stage and b) involved process.

## 6.4. Conclusions

The aim of this chapter was to present the best available techniques and the environmental profile of the production of bioactive molecules with potential applications in the pharmaceutical sector from marine sponges. The current approaches can be divided in two main groups: *in situ* mariculture in farming structures or *ex situ* controlled growth in closed systems.

The first case study was an example of *in situ* systems, used for the extraction of biologically active prenylhydroquinones from the blackish sponge *S. spinosulus*. In this process, the extraction stages (in particular the preparation of the crude extract before the individual extraction of the target compound) were identified as the most problematic issues in environmental terms. The impact was associated with the large amounts of solvents required for the extraction. Even for optimized scenarios, the emissions derived from the production of chemicals had a significantly higher contribution than all the stages involved in the growth, which indicates that the developed mariculture system can already be considered an efficient process.

In the case of *ex situ* techniques, a novel process was evaluated, consisting in the maintenance of specimens of the sponge *C. crambe* in indoor aquaria illuminated by artificial lights. Some findings of this case study are common to the previous example. Thus, the production of solvents associated with the purification stage represented a significant contribution to the total impacts. However, the growth stage also had a remarkable effect on several categories, due to the high energy requirements. Although the substitution of the *ex situ* growth by a sea-based farming system can be seen as the most suitable measure to improve the profile, the conducted sensitivity assessment reveals that other options may allow higher reductions of impact by the combined optimization of key processes (including solvent reuse, medium recycling and lighting regime coupled to environmentally friendly energy sources).

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# Chapter 7

# Biomolecules from marine bacteria, chromists and fungi

*Summary*

Despite the limited research on other marine sources of bioactive compounds, many species of kingdoms such as Bacteria, Chromista and Fungi produce valuable compounds with high biological activities. Due to the lack of commercial processes, three novel systems for the lab-scale production of biomolecules by bacteria, chromists and marine fungi are inventoried and analyzed from an environmental perspective.

The outcome of the three LCA studies agrees with the findings of previous chapters. Thus, the production of organic solvents linked to the extraction stages and the high energy consumption, especially associated with the cultivation phases, are the main reasons for the environmental impact of the processes under assessment.

The conducted analyses highlight the importance of process optimization for the development of efficient large-scale routes. According to the identified hot spots, the improvement measures with higher potential involve changes in extraction methods (including solvent reuse or substitution by cleaner chemicals) and electricity reduction. The electricity optimization can be performed by modifying key steps of the process (such as the temperature control system) or implementing continuous processes that avoid stages that are only performed at the beginning of the operation.

# Index

## 7.1. Marine producers of bioactive compounds in Bacteria, Chromista and Fungi kingdoms

To date, the available LCA studies analyzing products from marine origin is restricted to microalgae, seaweed and sponges. However, other organisms have already been identified as potential producers of natural bioactive compounds (Blunt et al., 2015; Murray et al., 2013). According to Blunt et al. (2015), 6.6% of the marine natural compounds identified between 1963 and 2013 are produced by organisms from the kingdom Chromista, 6.6% are associated with Bacteria and 8.8% have been isolated from Fungi.

Several authors claim that many marine metabolites are produced by symbiotic associations of animals or plants with bacteria or fungi (König et al., 2006). Although the actual producer within the symbiotic system is still uncertain in most cases, some studies have proved the ability of microorganisms as sources of biomolecules (König et al., 2006; Murray et al., 2013). For example, the antimalarial alkaloid manzamine was originally considered a metabolite of the sponge *Acanthostronglylophora ingens* and it is now known to be produced by the bacterial symbiont *Micromonospora* sp. (Murray et al., 2013). *Prochloron didemni* is another bacterial symbiont identified as producer of compounds initially attributed to the ascidian *Lissoclinum patella* (König et al., 2006; Schmidt et al., 2005). Biocompounds with antibiotic activity have been isolated from marine fungi. Among them, the chlorinated benzophenone pestalone is produced by a fungus of the genus *Pestalotia* isolated from the surface of the brown alga *Rosenvingea* sp (Cueto et al., 2001). Chromists can also be involved in symbiotic relationships that lead to the production of biomolecules. Thus, the dinoflagellate *Symbiodinium* sp., associated with the gorgonian coral *Pseudopterogorgia elisabethae*, was identified as the producer of the analgesic and anti-inflammatory pseudopterosins (König et al., 2006; Mydlarz et al., 2003).

The environmental effects related to the potential use of marine microorganisms from the kingdoms Bacteria, Chromista and Fungi are examined in this chapter. Although no commercial processes have been developed to date, the following sections provide the first life cycle inventories and impact assessment results for bioactive compounds produced by epiphytic bacteria, the chromist *Ulkenia visurgensis* and marine fungi at lab scale.

## 7.2. Coenzyme $Q_{10}$ production by epiphytic bacteria

Coenzyme Q, also known as ubiquinone, is an isoprenylated benzoquinone found in diverse groups of organisms including microorganisms, plants and animals (Overvad et al., 1999). It plays a key role in the process that converts the energy contained in carbohydrates and fatty acids into Adenosine Triphosphate (ATP) to drive cellular synthesis and metabolism (Crane, 2001; Overvad et al., 1999).

The 1,4-benzoquinone coenzyme $Q_{10}$, present in human cells and other animals, has several therapeutic functions and its deficiency is associated with certain diseases, including cardiovascular and neurological disorders (Crane, 2001; Potgieter et al., 2013). Thus, several studies and clinical trials suggest that the supplementation of coenzyme $Q_{10}$ may help in the prevention and treatment of hypertension, hyperlipidemia and coronary artery disease, as well as Parkinson, Huntington and Alzheimer disease, among others (Hickey et al., 2012; Potgieter et al., 2013; Sarter, 2002; Shults et al., 2002).

Epiphytic bacteria are currently considered a potential source of coenzyme $Q_{10}$ (Ren and Ren, 2013). Marine macroscopic organisms such as macroalgae or sponges can host epiphytic bacteria according to a symbiotic relationship (Armstrong et al., 2001; Murray et al., 2013). In this work, the production of coenzyme $Q_{10}$ by epiphytic bacteria from macroalgae *Fucus spiralis* and *Sphaerococcus coronopifolius* is evaluated from a LCA perspective.

### 7.2.1. Goal and scope definition

The aim of this study is to determine the main environmental burdens and identify the hot spots associated with the cultivation of epiphytic bacteria isolated from macroalgae based on a novel process at lab scale. In this process, the epiphytic bacteria living on macroalgae *F. spiralis* and *S. coronopifolius* were isolated and cultivation in 1 L aerated flasks for three days to obtain a final concentration of 1.5-2.5 $g \cdot L^{-1}$ (in dry weight, $g_{DW}$), containing about 50 mg of coenzyme $Q_{10}$ per kg of harvested bacteria. The process was developed by the Marine Resources Group at the Polytechnic Institute of Leiria (Portugal).

According to the described operation, the chosen functional unit (FU) for the lab-scale cultivation of epiphytic bacteria from macroalgae was 1 mg of coenzyme $Q_{10}$, equivalent to 11 batches (approximately 1 month) of production in 1 L flask. The system was divided into five main stages, which are described below: i) algae collection and cleaning, ii) isolation and purification of epiphytic bacteria, iii) growth of isolated bacteria in 1 L flasks, iv) biomass separation from culture medium and v) extraction and purification of coenzyme $Q_{10}$. shows the different stages and processes that were included in the system boundaries:

i) S1. Algae collection and cleaning: Specimens of the marine algae *F. spiralis* (1 kg) and *S. coronopifolius* (500 g) were collected from the Peniche coast, namely from Marques Neves beach and Papôa respectively during the low tide. The collected biomass was transported to the lab (distance of 1.5 km for *F. spiralis* and 3.5 km for *S. coronopifolius*) by car in plastic bags (6 g) or buckets (200 g). Portions of the plant were then rinsed thoroughly with sterile seawater to remove loosely attached bacteria.

ii) S2. Isolation and purification of epiphytic bacteria: Cleaned algae were swabbed with sterile cotton tipped swab. The swab was then used to directly inoculate plates with marine-agar which were incubated at room temperature. The colonies were purified by the streak technique.

iii) S3. Growth of isolated bacteria in flasks: Isolated bacteria were grown in 1 L flasks containing marine broth with aeration for three days. The medium consisted of marine broth with the following composition: 19.40 $g \cdot L^{-1}$ sodium chloride (NaCl), 0.08 $g \cdot L^{-1}$ potassium bromide (KBr), 8.80 $g \cdot L^{-1}$ magnesium chloride ($MgCl_2$), 0.034 $g \cdot L^{-1}$ strontium chloride ($SrCl_2$), 5 $g \cdot L^{-1}$ bacteriological peptone, 0.022 $g \cdot L^{-1}$ boric acid ($H_3BO_3$), 3.24 $g \cdot L^{-1}$ sodium sulfate ($Na_2SO_4$), 0.008 $g \cdot L^{-1}$ sodium phosphate dibasic ($Na_2HPO_4$), 1.80 $g \cdot L^{-1}$ calcium chloride ($CaCl_2$), 0.004 $g \cdot L^{-1}$ sodium metasilicate ($Na_2SiO_3$), 1 $g \cdot L^{-1}$ yeast extract, 0.0024 $g \cdot L^{-1}$ sodium fluoride (NaF), 0.55 $g \cdot L^{-1}$ potassium chloride (KCl), 0.0016 $g \cdot L^{-1}$ ammonium nitrate ($NH_4NO_3$), 0.16 $g \cdot L^{-1}$ sodium bicarbonate ($NaHCO_3$) and 0.10 $g \cdot L^{-1}$ ferric citrate.

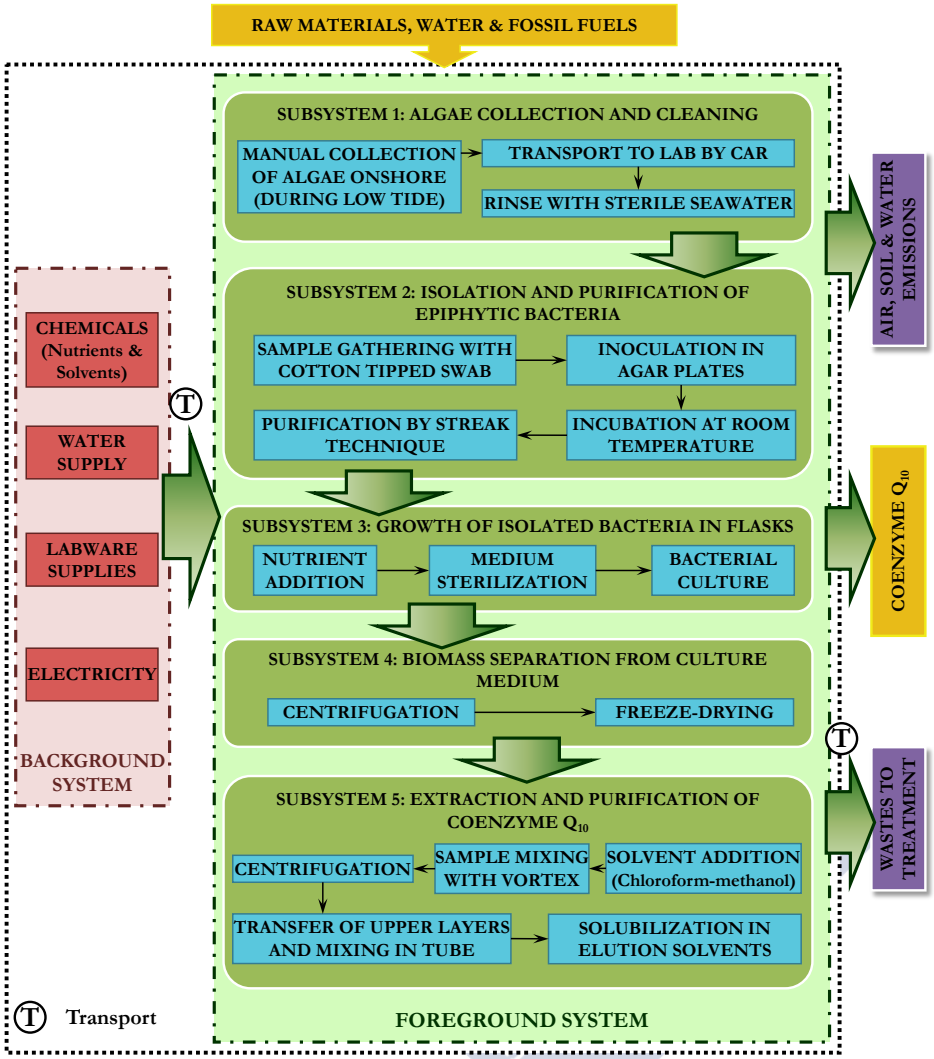



**Figure 7.1.** Process chain and system boundaries of the lab-scale production of coenzyme $Q_{10}$ by epiphytic bacteria.

iv) S4. Biomass separation from culture medium: After the growth in 1 L flasks, bacteria were collected in tubes and centrifuged to separate the biomass paste. The culture medium was removed and the biomass (approximately 1.5-2.5 $g_{DW} \cdot L^{-1}$ after three days of growth) was freeze-dried.

v) S5. Extraction and purification of coenzyme $Q_{10}$: For the extraction, 50 mg of biomass from each sample was added to 500 µl mixture of chloroform:methanol (2:1) and shaken for 30 min. The mixture was centrifuged for 3 min at 12000 rpm and the upper layer was transferred to a new tube. The extraction was repeated twice and the three collected layers (approximately 1.5 mL) were combined and dried in a vacuum chamber. Finally, the extract was solubilized in elution solvents: acetonitrile (400 mL), formic acid (750 mL) and isopropanol (350 mL).

### 7.2.2. Life cycle inventory, data quality and assumptions

The LCI data for the foreground system (i.e. chemicals and electricity consumptions as well as transport distances) consisted of average data obtained by on-site measurements. Regarding water emissions derived from the different production stages, they were calculated assuming that the nutrients not totally depleted during the growth, are directly discharged to the environment. The same assumption was taken for air emissions. The inventory data for the lab-scale process are shown in **Table 7.1**.

The processes of the background system include the production of chemicals and water required for the culture medium and the extraction, the electricity and the distribution of the inputs up to the lab gate and labware supplies (plastic containers for algae transport, flask) and waste disposal. The production of materials for large equipments (i.e. flow chamber, steam sterilizer, etc.) was excluded because these equipments have a long life span, which implies negligible environmental contribution to the total impact associated to the production of the enzyme. An average transport distance of 800 and 600 km within continental Europe was considered for chemicals and materials, respectively. Waste transport distance was estimated around 50 km. Plastic and glass wastes were assumed to be disposed in sanitary or inert landfills. Textile materials were sent to incineration. The data for the background processes were taken from Ecoinvent database, according to the reports listed in **Table 7.2**.

*Allocation procedures*

Since the analyzed process is focused on a single product (coenzyme $Q_{10}$), no allocation procedure was needed. The residual biomass might contain other

biomolecules. If further research allowed the development of a process for the combined production of coenzyme $Q_{10}$ and other bioactive fractions, the environmental burdens would be distributed among the different co-products. Thus, the environmental profile per product could significantly improve, although this analysis is out of the goal and scope of the current study.

**Table 7.1.** Inventory data for the lab-scale production of coenzyme $Q_{10}$ by epiphytic bacteria from *F. spiralis and S. coronopifolius* (FU=1 mg coenzyme $Q_{10}$)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Algae collection and cleaning* | | | |
| Polypropylene (PP) | 0.23 kg | | |
| *S2. Isolation and purification of epiphytic bacteria* | | | |
| Cotton | 2.00 g | Potato extract | 0.70 g |
| Polystyrene | 0.19 kg | Dextrose | 3.49 g |
| Distilled water | 0.17 L | Agar | 0.17 g |
| *S3. Growth of isolated bacteria in flasks* | | | |
| Glass | 58.10 g | $Na_2HPO_4$ | 0.09 g |
| Distilled water | 11.62 L | $CaCl_2$ | 20.92 g |
| NaCl | 225.44 g | $Na_2SiO_3$ | 0.05 g |
| KBr | 0.93 g | Yeast extract | 11.62 g |
| $MgCl_2$ | 102.26 g | NaF | 0.03 g |
| $SrCl_2$ | 0.40 g | KCl | 6.39 g |
| Bacterial peptone | 58.10 g | $NH_4NO_3$ | 0.02 g |
| $H_3BO_3$ | 0.26 g | $NaHCO_3$ | 1.86 g |
| $Na_2SO_4$ | 37.65 g | Ferric citrate | 1.16 g |
| *S5. Extraction and purification* | | | |
| Chloroform | 0.60 kg | Formic acid | 369.70 kg |
| Methanol | 0.16 kg | Isopropanol | 111.15 kg |
| Acetonitrile | 127.03 kg | | |
| **Energy** | | | |
| Electricity from Portuguese grid | | | |
| *S2. Isolation and purification of epiphytic bacteria* | | | |
| Inoculation | 0.10 kWh | Incubation | 4.82 kWh |
| *S3. Growth of isolated bacteria in flasks* | | | |
| Medium sterilization | 7.75 kWh | Maintenance in culture chamber | 144.74 kWh |
| Aeration | 54.38 kWh | | |

**Table 7.1.** Inventory data for the lab-scale production of coenzyme $Q_{10}$ by epiphytic bacteria from *F. spiralis and S. coronopifolius* (FU=1 mg coenzyme $Q_{10}$)

| | | | |
|---|---|---|---|
| **INPUTS from TECHNOSPHERE** | | | |
| **Energy** | | | |
| Electricity from Portuguese grid | | | |
| *S4. Biomass separation from culture medium* | | | |
| Centrifugation | 3.20 kWh | Freeze-drying | 43.58 kWh |
| *S5. Extraction and purification of coenzyme $Q_{10}$* | | | |
| Sample mixing | 27.27 kWh | Vacuum drying | 9.30 kWh |
| Centrifugation | 25.00 kWh | Purification of $Q_{10}$ | 57.91 kWh |
| **Transport** | | | |
| *S1. Algae collection and cleaning* | | | |
| Passenger car | 9.30 pkm | Truck, 3.5-7.5 t (wastes) | 11.62 kg·km |
| Truck, 3.5-7.5 t (materials) | 139.44 kg·km | | |
| *S2. Isolation and purification of epiphytic bacteria* | | | |
| Truck, 3.5-7.5 t (materials) | 113.39 kg·km | Truck, 3.5-7.5 t (wastes) | 9.67 kg·km |
| Truck, 3.5-7.5 t (chemicals) | 3.49 kg·km | | |
| *S3. Growth of isolated bacteria in flasks* | | | |
| Truck, 3.5-7.5 t (materials) | 7.25 tkm | Truck, 3.5-7.5 t (wastes) | 2.91 kg·km |
| *S5. Extraction and purification of coenzyme $Q_{10}$* | | | |
| Truck, 3.5-7.5 t (chemicals) | 365.18 tkm | | |
| **INPUTS from ENVIRONMENT** | | | |
| Macroalgal biomass | 1.74 kg | Seawater | 3.49 L |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Product** | | | |
| Coenzyme $Q_{10}$ | 1 mg | | |
| **Wastes to landfill** | | | |
| *S1. Algae collection and cleaning* | | | |
| PP | 0.23 kg | | |
| *S2. Isolation and purification of epiphytic bacteria* | | | |
| Polystyrene | 0.19 kg | Marine agar | 4.36 g |
| *S3. Growth of isolated bacteria in flasks* | | | |
| Glass | 58.10 g | | |
| **Wastes to municipal incineration** | | | |
| *S2. Isolation and purification of epiphytic bacteria* | | | |
| Cotton | 2.00 g | | |

**Table 7.1.** Inventory data for the lab-scale production of coenzyme $Q_{10}$ by epiphytic bacteria from *F. spiralis and S. coronopifolius* (FU=1 mg coenzyme $Q_{10}$)

| **OUTPUTS to ENVIRONMENT** | | | |
|---|---|---|---|
| **Water emissions** | | | |
| *S1. Algae collection and cleaning* | | | |
| Wastewater | 3.486 L | | |
| *S2. Isolation and purification of epiphytic bacteria* | | | |
| Wastewater | 0.17 L | | |
| *S4. Biomass separation from culture medium* | | | |
| Wastewater | 11.62 L | $CaCl_2$ | 2.09 g |
| NaCl | 22.54 g | $Na_2SiO_3$ | 4.65 mg |
| KBr | 0.09 g | Yeast extract | 1.16 g |
| $MgCl_2$ | 10.23 g | NaF | 2.79 mg |
| $SrCl_2$ | 0.04 g | KCl | 0.64 g |
| Bacterial peptone | 5.81 g | $NH_4NO_3$ | 1.86 mg |
| $H_3BO_3$ | 0.03 g | $NaHCO_3$ | 0.19 g |
| $Na_2SO_4$ | 3.77 g | Ferric citrate | 0.12 g |
| $Na_2HPO_4$ | 0.01 g | | |
| *S5. Extraction and purification of coenzyme $Q_{10}$* | | | |
| Chloroform | 0.60 kg | Formic acid | 369.70 kg |
| Methanol | 0.16 kg | Isopropanol | 111.15 kg |
| Acetonitrile | 127.03 kg | | |

**Table 7.2.** Summary of data sources for the background system of the lab-scale production of coenzyme $Q_{10}$ by epiphytic bacteria

| Involved process | Raw material | Reference |
|---|---|---|
| Energy | Electricity (from the Portuguese grid) | Ecoinvent database (Dones et al., 2007) |
| Materials | PP | Ecoinvent database (Hischier, 2007) |
| | Polystyrene | |
| | Glass | |
| | Cotton | Ecoinvent database (Nemecek and Kägi, 2007) |
| Chemicals (nutrients) | NaCl | Ecoinvent database (Althaus et al., 2007) |
| | KBr | |
| | $MgCl_2$ | |
| | $SrCl_2$ | |
| | Bacterial peptone | |
| | $H_3BO_3$ | |
| | $Na_2SO_4$ | |
| | $Na_2HPO_4$ | |
| | $CaCl_2$ | |
| | $Na_2SiO_3$ | |
| | Yeast extract | |
| | NaF | |
| | KCl | |
| | $NH_4NO_3$ | |
| | $NaHCO_3$ | |
| | Ferric citrate | |
| Chemicals (solvents) | Chloroform | Ecoinvent database (Althaus et al., 2007) |
| | Methanol | |
| | Isopropanol | |
| | Acetonitrile | Ecoinvent database (Sutter, 2007) |
| | Formic acid | |
| Water supply | Tap water | Ecoinvent database (Althaus et al., 2007) |
| | Distilled water | |
| Waste treatment | Inert landfill | Ecoinvent database (Doka, 2007) |
| | Sanitary landfill | |
| | Municipal incineration | |

### 7.2.3. Environmental impact assessment

The environmental profile for the production of coenzyme $Q_{10}$ from epiphytic bacteria isolated from macroalgae *F. spiralis* and *S. coronopifolius* was evaluated according to the classification and characterization stages of the LCA methodology (ISO 14040, 2006). The CML 2001 characterization factors were used (Guinée et al., 2002). The evaluated impact categories were abiotic depletion (ADP), acidification (AP), eutrophication (EP), global warming (GWP), ozone layer depletion (ODP), human toxicity (HTP), freshwater aquatic ecotoxicity (FEP), marine aquatic ecotoxicity (MEP), terrestrial ecotoxicity (TEP) and photochemical oxidants formation (POFP). The software SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008). The characterization results are shown in **Table 7.3** and the relative contributions of the different stages and processes are depicted in **Figure 7.2**.

**Table 7.3.** Environmental impact assessment results (characterization step) associated with the lab-scale production of 1 mg coenzyme $Q_{10}$ by epiphytic bacteria from macroalgae *F. spiralis* and *S. coronopifolius*

| Impact category | Unit | Value |
|---|---|---|
| ADP | kg Sb eq | 21.07 |
| AP | kg $SO_2$ eq | 8.86 |
| EP | kg $PO_4^{-3}$ eq | 3.36 |
| GWP | kg $CO_2$ eq | 1865.59 |
| ODP | kg CFC-11 eq | 0.01 |
| HTP | kg 1,4-DB eq | 458.55 |
| FEP | kg 1,4-DB eq | 320.04 |
| MEP | kg 1,4-DB eq | 240.01 |
| TEP | kg 1,4-DB eq | 0.09 |
| POFP | kg $C_2H_4$ eq | 0.68 |

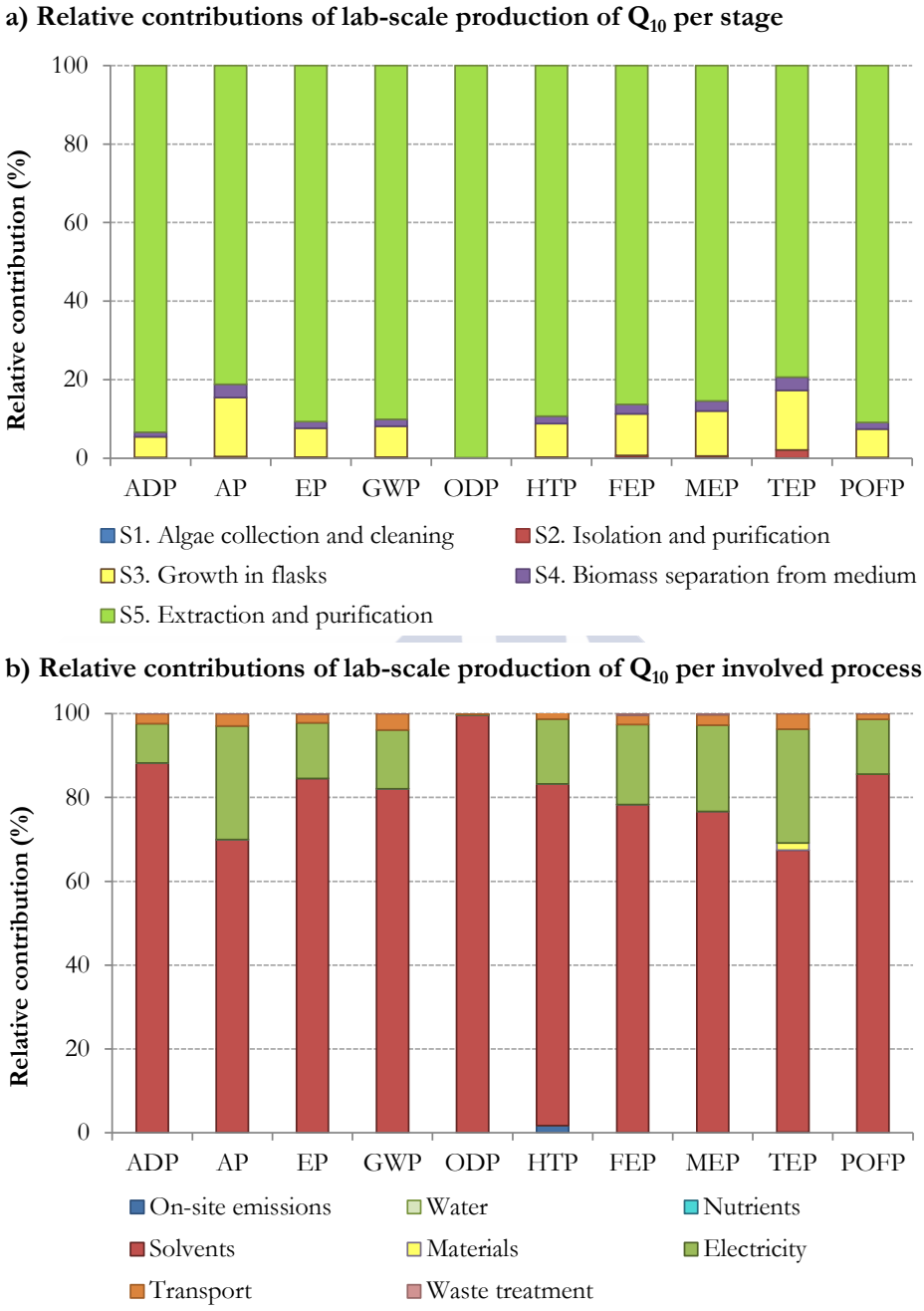



**Figure 7.2.** Relative contributions of the lab-scale production of coenzyme $Q_{10}$ by epiphytic bacteria from macroalgae *F. spiralis* and *S. coronopifolius* to each impact category per a) stage and b) involved process.

According to **Figure 7.2**, the extraction and purification stage (S5) is the major contributor to the environmental burdens derived from the production of coenzyme $Q_{10}$, with more than 75% of the impact in all the assessed categories. Among the other stages, only the growth of isolated bacteria has a significant contribution that ranges between 5% and 15% in most of the categories (except from ODP, in which extraction is responsible for more than 99% of the impact).

The production of chemicals used as solvents in S5 is the main factor responsible for the high impacts associated with the stages. It involves between 67% (for TEP) up to 99% (for ODP) of the contributions. The production of electricity required throughout the whole process is the only secondary process that shows relative contributions above 10% in most categories. The main contributions are related to the categories of AP (27%) and ecotoxicity categories (19% of FEP, 21% of MEP and 27% of TEP).

### 7.2.4. Discussion and recommendations

The conducted work provided the first life cycle inventory and environmental assessment of the production of coenzyme $Q_{10}$ by epiphytic bacteria isolated from marine macroalgae. The results allowed identifying the extraction and purification stage as the main contributor (hot spot) of the process for all the evaluated categories. The main cause of this impact is the production of the solvents required to separate the coenzyme $Q_{10}$ from the biomass.

**Figure 7.3** shows a breakdown of the different solvents used for the extraction and purification of the analyzed bioactive compound. Formic acid is the solvent with the highest contribution (between 45% and 79% of the total contribution of solvents) in all categories except for ODP, which is due to the production of chloroform (97% of total contribution). The production of acetonitrile has contributions ranging between 25% and 37% in categories such as ADP, AP, EP or TEP, whereas the influence of isopropanol only exceeds 15% of the total impact of solvents for ADP and EP. Methanol is a minor contributor in all the evaluated categories and has contributions below 1% in all impact categories.

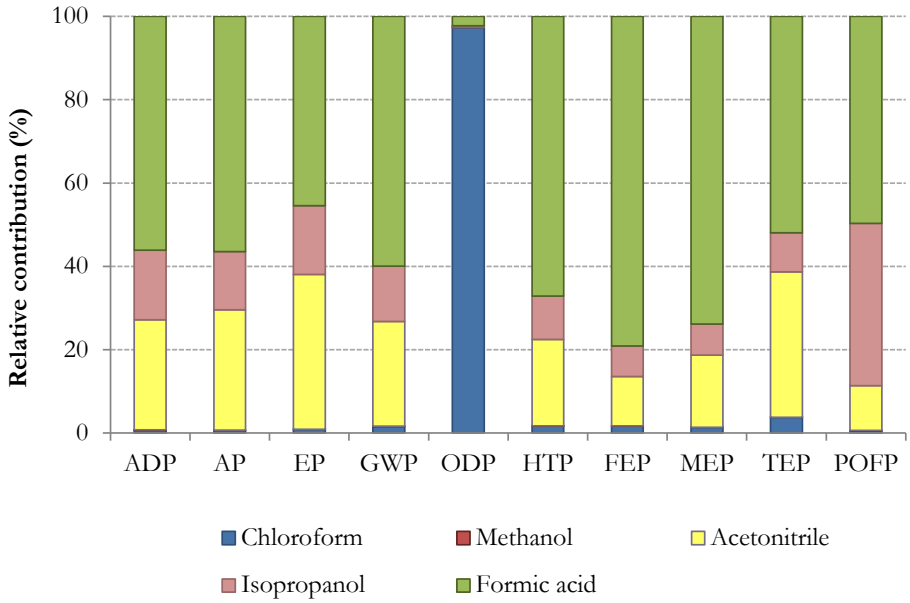



**Figure 7.3.** Relative contribution of the solvents required for the extraction and purification of coenzyme $Q_{10}$ produced by epiphytic bacteria at lab scale.

According to the identified hot spots, the most promising option to reduce the environmental burdens of the process would be the development of an alternative extraction route with lower amounts of acetonitrile and formic acid or their substitution by other cleaner solvents. If this is not possible, the addition of a solvent recovery stage could also reduce the consumption of organic chemicals significantly, and therefore improve the environmental performance of the process.

Regarding the electricity requirements, the impacts are not related to a specific stage but to several procedures throughout the process. However, as all the consumptions are calculated for lab-scale equipments, it is likely that the scale-up of the system would result in a much more efficient use of energy and a subsequent reduction of the impacts. Other measures to limit the electricity consumption and improve the environmental profile would be to reduce the use of the culture chamber and optimize the aeration system, which currently account for 33% and 12% of the total energy required, respectively.

## 7.3. Docosahexaenoic acid by *Ulkenia visurgensis*

As explained in Chapter 3, polyunsaturated fatty acids (PUFAs) are valuable bioactive compounds with potential uses in health and food industries (Murray et al., 2013; Yen et al., 2013). In particular, docosahexaenoic acid (DHA, C22:6ω3) is essential for the nervous system and retinal development (Stough et al., 2012). Moreover, several studies suggest the beneficial effects of DHA in the prevention of cardiovascular disease, obesity and diabetes, among others (Arnoldussen and Kiliaan, 2014; Singhal et al., 2013).

Marine organisms such as microalgae have already been proposed as a rich natural source of fatty acids that include DHA, together with eicosapentaenoic acid (EPA) (Chauton et al., 2015). Other marine sources are currently considered alternative producers of fatty acids. Thus, the thraustochytrid chromist *Ulkenia visurgensis* was found to accumulate significant amounts of DHA as well as other PUFAs (Huang et al., 2003; WoRMS Editorial Board, 2015). The environmental aspects of the production and extraction of DHA from *U. visurgensis* are here evaluated in a lab-scale process.

### 7.3.1. Goal and scope definition

This study aims to quantify the environmental burdens and identify the major stages and processes responsible for the impacts associated with the production of DHA by *U. visurgensis* at lab scale. The production was carried out by the State Research Institute of Genetics and Breeding of Industrial Microorganisms, Genetika (Russia). The cultivation was performed in a 3 L fermenter for 6 days. At the end of the batch, 12.3 $g_{DW}$ of biomass was produced. The lipids within the obtained biomass were then extracted and transformed into fatty acids. After the winterization of the free fatty acids, a total amount of 289.8 mg docosahexaenoic acid (DHA) was obtained.

According to the described operation, 16 g DHA equivalent to 1 year of production (330 days of operation, 6 days per batch) was selected as the FU. The system for the cultivation of *U. visurgensis* was divided into eight main stages including: i) preparation and sterilization of culture media, ii) cultivation, iii) harvesting and freeze-drying, iv) oil extraction, v) saponification; vi) soap extraction, vii) transformation of soaps to free fatty acids, and viii) winterization.

**Figure 7.4** shows the different stages and processes that were included in the system boundaries.

i) S1. Preparation and sterilization of culture media: Two culture media were prepared and sterilized. Medium A was used for the growth of *Ulkenia visurgensis* in glass tubes (12 mL medium per tube) and flasks (100 mL medium per flask). This culture medium was composed of 10 $g \cdot L^{-1}$ yeast extract, 2 $g \cdot L^{-1}$ peptone and 30 $g \cdot L^{-1}$ glycerol). For the culture in fermenter (800 mL), medium F was prepared, consisting of 30 $g \cdot L^{-1}$ sunflower oil and 3 $g \cdot L^{-1}$ corn steep liquor. Additionally, 60 mL solution containing 10 $g \cdot L^{-1}$ yeast extract and 20 $g \cdot L^{-1}$ peptone was fed into the fermenter in two pulses, on days 2 and 4 of cultivation. Deionized water was used to prepare all culture media. The media were autoclaved for 60 min at 120°C.

ii) S2. Cultivation: To prepare the inoculum a small piece of biomass from a Petri dish was inoculated into the glass tube containing 12 ml of medium A and then the tube was incubated for 2 days at 25°C and 200 rpm. Afterwards, 10 mL of grown culture was added to 100 mL of medium A in 750 ml flask and incubated for 2 days at 200 rpm and 25°C. The grown inoculum (5%) was added into a 3 L fermenter (Prointech KF103/4, Russia) containing 800 mL of medium F and cultivation was performed for 6 days with two feedings (60 mL containing 10 $g \cdot L^{-1}$ of yeast extract and 20 $g \cdot L^{-1}$ of peptone) on the $2^{nd}$ and $4^{th}$ days. The parameters for cultivation in the fermenter were: stirring rate=370 rpm, air delivery rate=1 $L \cdot L^{-1} \cdot min^{-1}$, pH=6.5 and temperature=25ºC.

iii) S3. Harvesting and freeze-drying: After six days, centrifugation was performed in the Eppendorf 5810R centrifuge (F-34-6-38 rotor) for 2 min to separate biomass from the culture medium. This operation was performed 3 times in six tubes filled with 50 mL of liquid. After centrifugation, the cells (12.3 $g_{DW}$ biomass from 900 mL culture) were washed with 50 mL sodium chloride (NaCl) solution (2%). Then, the biomass was frozen in ultra-low freezer Sanyo for 2 h at -70°C and

freeze-dried with Labconco Freezone 6 for 8 h at -80°C and residual pressure of 0.006 mmHg.

iv) S4. Oil extraction: The freeze-dried cells were suspended in a glass bottle with 100 mL Folch solution (chloroform:methanol mixture, $CHCl_3$:$CH_3OH$ 2:1 v/v) containing 5 mg butylated hydroxyanisol. The mixture was vigorously shaken for 30 min in an orbital shaker and then centrifuged in the Eppendorf 5810R centrifuge at 10000 rpm for 2 min. The upper layer was transferred to a clean bottle. The extraction procedure was repeated twice and the three layers were combined and filtered through a glass wool filter. The liquid was evaporated in an orbital evaporator for 2 h. The resulting oil was washed with 50 mL hexane containing 2.5 mg butylated hydroxyanisol and then evaporated for 40 min to obtain the crude oil.

v) S5. Saponification: The crude oil from the previous stage was mixed with 20 mL saponifying solution, which consisted of 2 g KOH, 10 mL $H_2O$ and 10 mL ethanol (96% v/v) containing 1 mg butylated hydroxyanisol. The reaction was performed in the orbital shaker.

vi) S6. Soap extraction: The impurities present in the saponified mixture were extracted using 50 mL hexane with 10 mg butylated hydroxyanisol. The mixture was placed on the orbital shaker for 10 min and then centrifuged at 10000 rpm for 2 min to separate the hexane phase from the soap. The extraction procedure was repeated once more.

vii) S7. Transformation of soaps to free fatty acids (FFA): In order to transform the soap into free fatty acids, 48 mL hydrochloric acid (HCl, 23% v/v) was added to the soap solution and both phases were mixed thoroughly on the orbital shaker for 30 min. The FFA in the final mixture were extracted with 200 mL hexane, containing 10 mg butylated hydroxyanisol using the orbital shaker for 10 min. The mixture was centrifuged in the Eppendorf 5810R centrifuge for 2 min and the hexane phase was collected. This phase was dried with anhydrous sodium sulphate and the solvent was removed in the orbital evaporator at 40°C for 90 min.

viii) S8. Winterization: In the last stage, the FFA were dissolved in 100 mL acetone containing 5 mg butylated hydroxyanisol and mixed in the orbital shaker for 5 min. The solution was stored at -20°C for 24 h in refrigerator. After 10 min centrifugation at 13000 rpm, the liquid fraction was transferred to a clean tube. The acetone was evaporated for 1 h to obtain FFA enriched with DHA.

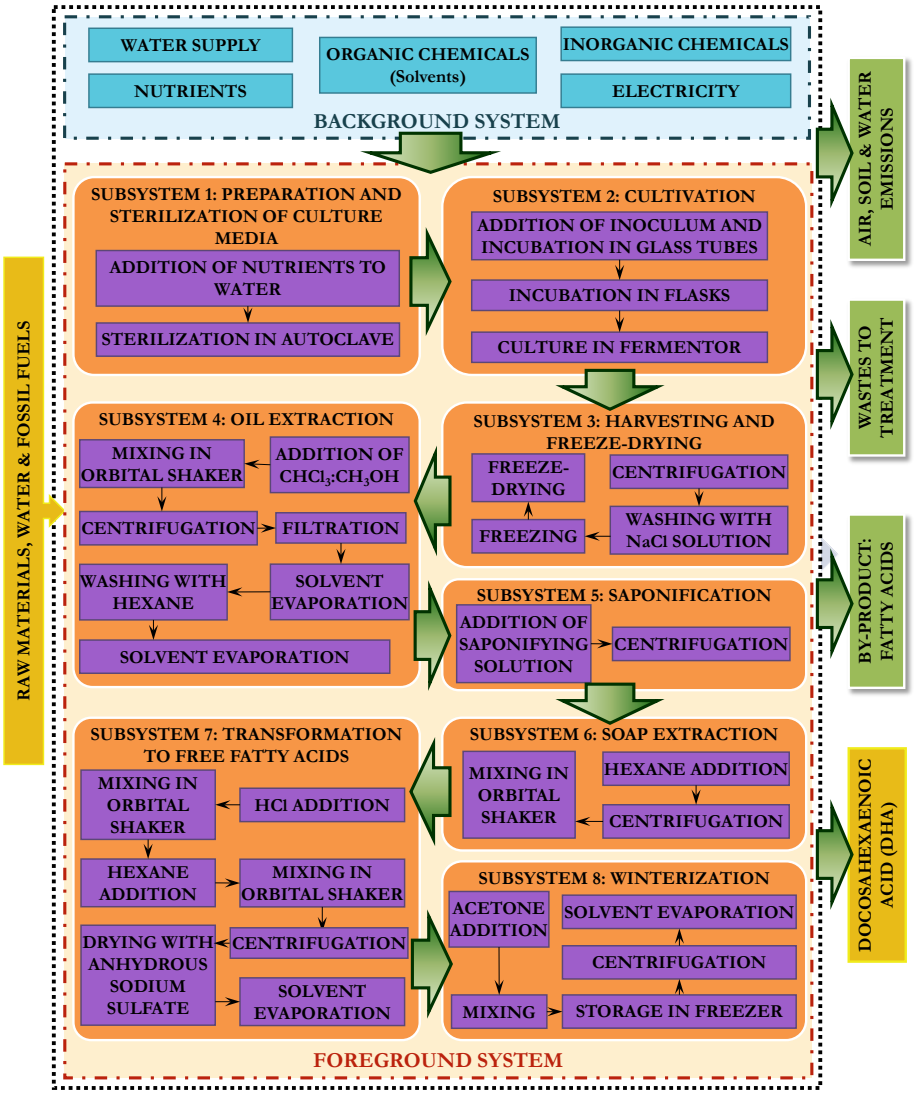



**Figure 7.4.** Process chain and system boundaries of the lab-scale production of DHA by *U. visurgensis*.

### 7.3.2. Life cycle inventory, data quality and assumptions

The LCI data for the foreground system (i.e. nutrients, water supply, organic and inorganic chemicals for the extraction and purification stages and electricity consumption) consisted of average data obtained by on-site measurements. Similarly to the previous case study, water emissions included non depleted nutrients and residual solvents. Air emissions from the process were considered negligible. The inventory data for the lab-scale process for one year of operation are shown in **Table 7.4**.

Concerning the background system, the corresponding inventory data for the production of all the inputs were taken from Ecoinvent database. These inputs included the production of the different chemicals required for the preparation of the culture media and the extraction stages, the electricity used in the different production stages and the waste disposal. The corresponding Ecoinvent reports for the different processes are listed in **Table 7.5**.

The materials for the equipments were excluded from the system boundaries, as previous works showed that these inputs have a very limited effect in the environmental profile, due to their long life span. Regarding the distribution of inputs, the distance from the supplier to the lab was only 10 km and therefore, the impact of this transport was considered negligible. Specific wastewater treatments were selected depending on the compositon of the effluent from each stage. Sodium sulfate used as a drying agent was considered to be sent to landfill.

*Allocation procedures*

The evaluated process is focused on the production of DHA. However, an additional fraction of mixed fatty acids is produced together with DHA. Since this fraction has a market value for applications in industries such as cosmetics or food sector, a system expansion approach is here considered to include the credits from its use.

**Table 7.4.** Inventory data for the lab-scale production of DHA by *U. visurgensis* (FU=16 g DHA)

| **INPUTS from TECHNOSPHERE** | | | |
|---|---|---|---|
| **Materials** | | | |
| *S1. Preparation and sterilization of culture media* | | | |
| Deionized water | 164.07 L | Glycerol | 185.49 g |
| Yeast extract | 94.95 g | Sunflower oil | 1.32 kg |
| Peptone | 78.61 g | Corn steep liquor | 0.13 kg |
| *S3. Harvesting and freeze-drying* | | | |
| Deionized water | 2.74 L | NaCl | 55.82 g |
| *S4. Oil extraction* | | | |
| Chloroform | 16.37 kg | Butylated hydroxyanisol | 0.97 g |
| Methanol | 4.37 kg | Hexane | 1.81 kg |
| *S5. Saponification* | | | |
| Deionized water | 0.57 L | Ethanol | 0.42 kg |
| Potassium hydroxide | 0.11 kg | Butylated hydroxyanisol | 0.06 g |
| *S6. Soap extraction* | | | |
| Hexane | 3.61 kg | Butylated hydroxyanisol | 1.10 g |
| *S7. Transformation of soaps to FFA* | | | |
| HCl | 0.23 kg | Butylated hydroxyanisol | 0.14 g |
| Deionized water | 0.51 L | Anhydrous sodium sulfate | 11.04 kg |
| Hexane | 1.81 kg | | |
| *S8. Winterization* | | | |
| Acetone | 4.37 kg | Butylated hydroxyanisol | 0.28 g |
| **Energy** | | | |
| Electricity from Russian grid | | | |
| *S1. Preparation and sterilization of culture media* | | | |
| Autoclaving | 165.61 kWh | | |
| *S2. Cultivation* | | | |
| Incubation | 21.55 kWh | Pumping | 1430.89 kWh |
| Growth in fermenter | 2384.81 kWh | Heat exchange | 3179.75 kWh |
| Air compression | 805.98 kWh | | |
| *S3. Harvesting and freeze-drying* | | | |
| Centrifugation | 9.11 kWh | Freeze-drying | 883.26 kWh |
| Freezing | 88.33 kWh | | |

**Table 7.4.** Inventory data for the lab-scale production of DHA by *U. visurgensis* (FU=16 g DHA) (*Cont.*)

| | | | |
|---|---|---|---|
| **INPUTS from TECHNOSPHERE** | | | |
| **Energy** | | | |
| Electricity from Russian grid | | | |
| *S4. Oil extraction* | | | |
| Mixing | 6.62 kWh | Solvent evaporation | 206.10 kWh |
| Centrifugation | 9.11 kWh | | |
| *S5. Saponification* | | | |
| Mixing | 4.42 kWh | | |
| *S6. Soap extraction* | | | |
| Mixing | 1.47 kWh | Centrifugation | 6.07 kWh |
| *S7. Transformation of soaps into FFA* | | | |
| Mixing | 2.94 kWh | Solvent evaporation | 115.93 kWh |
| Centrifugation | 3.04 kWh | | |
| *S8. Winterization* | | | |
| Mixing | 0.37 kWh | Centrifugation | 15.18 kWh |
| Freezing | 198.73 kWh | Solvent evaporation | 77.29 kWh |
| **INPUTS from ENVIRONMENT** | | | |
| *U. visurgensis* biomass | 1.79 g | | |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Product** | | **By-product** | |
| DHA | 16 g | Other fatty acids | 11.04 g |
| **Wastes to landfill** | | | |
| *S7. Transformation of soaps to FFA* | | | |
| Sodium sulfate | 11.04 kg | | |

**Table 7.4.** Inventory data for the lab-scale production of DHA by *U. visurgensis* (FU=16 g DHA) (*Cont.*)

| **OUTPUTS to TECHNOSPHERE** | | | |
|---|---|---|---|
| **Wastewater to treatment plant** | | | |
| *S1. Preparation and sterilization of culture media* | | | |
| Wastewater | 110.41 L | Yeast extract | 61.83 g |
| *S2. Cultivation* | | | |
| Residual culture medium comprising | | | |
| Wastewater | 3.86 L | Peptone | 0.39 g |
| Yeast extract | 1.93 g | Glycerol | 5.79 g |
| *S3. Harvesting and freeze-drying* | | | |
| Residual culture medium comprising | | | |
| Wastewater | 49.80 L | Glycerol | 2.04 g |
| Yeast extract | 20.07 g | Sunflower oil | 775.65 g |
| Peptone | 38.92 g | Corn steep liquor | 77.56 g |
| Wastewater from washing containing | | | |
| Wastewater | 2.74 L | NaCl | 55.82 g |
| *S5. Saponification* | | | |
| Chloroform | 16.37 kg | Butylated hydroxyanisol | 0.97 g |
| Methanol | 4.37 kg | Hexane | 1.81 kg |
| *S6. Soap extraction* | | | |
| Butylated hydroxyanisol | 1.16 g | Hexane | 3.61 kg |
| *S7. Transformation of soaps to FFA* | | | |
| Wastewater | 1.81 kg | Ethanol | 0.42 kg |
| Hexane | 1.01 kg | HCl | 0.19 kg |
| Potassium hydroxide | 0.09 kg | Potassium chloride | 0.03 kg |
| *S8. Winterization* | | | |
| Wastewater | 0.07 L | Acetone | 4.37 kg |
| Solid wastes to landfill: | | | |
| *S7. Transformation of soaps to FFA* | | | |
| Sodium sulfate | 11.04 kg | | |

**Table 7.5.** Summary of data sources for the background system of the lab-scale production of DHA by *U. visurgensis*

| Involved process | Raw material | Reference |
|---|---|---|
| Energy | Electricity (from the Russian grid) | Ecoinvent database (Dones et al., 2007; Moreno Ruiz et al., 2013). |
| Chemicals | Yeast extract | Ecoinvent database (Althaus et al., 2007) |
| | Peptone | |
| | Glycerol | |
| | Sunflower oil | |
| | Corn steep liquor | |
| | NaCl | |
| | Chloroform | |
| | Methanol | |
| | Butylated hydroxyanisol | |
| | HCl | |
| | Sodium sulfate | |
| | Acetone | |
| | Hexane | Ecoinvent database (Jungbluth et al., 2007) |
| Water supply | Tap water | Ecoinvent database (Althaus et al., 2007) |
| | Distilled water | |
| Waste treatment | Inert landfill | Ecoinvent database (Doka, 2007) |
| | Wastewater treatment plant | |

### 7.3.3. Environmental impact assessment

As in the previous study, the environmental results for the production of DHA by *U. visurgensis* at lab scale were quantified by conducting the classification and characterization stages of the standardized LCA framework, using the CML 2001 methodology (Guinée et al., 2002; ISO 14040, 2006). The environmental profile with respect to the same impact categories is presented: ADP, AP, EP, GWP, ODP, POFP and toxicity related impact categories: HTP, FEP, MEP and TEP. The software SimaPro 8 was used for the computational implementation of the inventories (Goedkoop et al., 2013). The characterization results for the process are presented in **Table 7.6**.

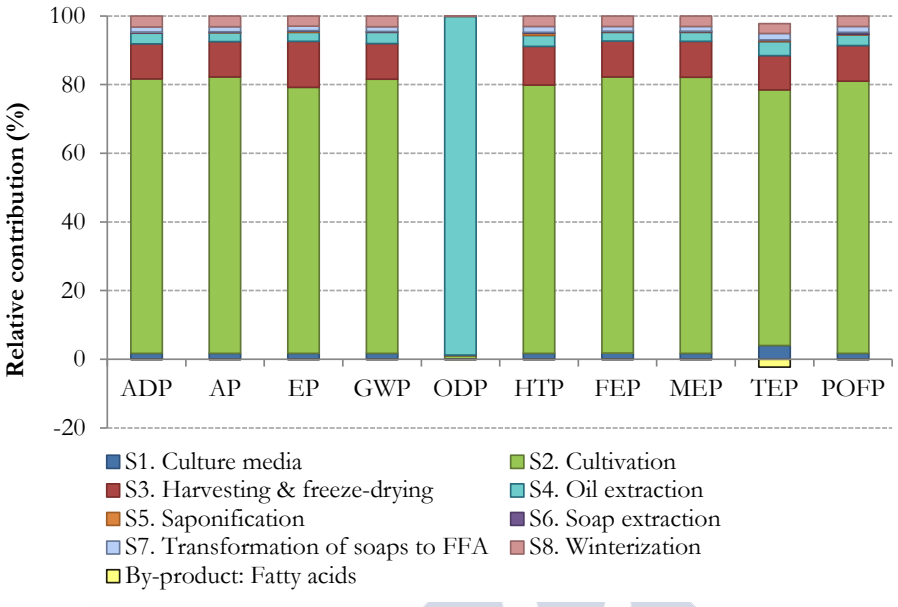
**Table 7.6.** Environmental impact assessment results (characterization step) associated with the lab-scale production of 16 g DHA by *U. visurgensis*

| Impact category | Unit | Value |
|---|---|---|
| ADP | kg Sb eq | 55.54 |
| AP | kg $SO_2$ eq | 42.23 |
| EP | kg $PO_4^{-3}$ eq | 16.05 |
| GWP | kg $CO_2$ eq | 7350.52 |
| ODP | kg CFC-11 eq | 0.02 |
| HTP | kg 1,4-DB eq | 1810.76 |
| FEP | kg 1,4-DB eq | 2537.61 |
| MEP | kg 1,4-DB eq | 1521.04 |
| TEP | kg 1,4-DB eq | 0.32 |
| POFP | kg $C_2H_4$ eq | 1.74 |

**Figure 7.5a** shows the contributions of the subsystems involved in the production of DHA by *U. visurgensis*. According to the results, the cultivation stage is the main contributor to the environmental burdens derived from the production of DHA, being responsible for more than 75% to all the assessed categories except for ODP. The contribution to ODP is dominated by the oil extraction (99%). Although the production of by-products (other fatty acids) was considered, the results show that they lead to a limited reduction of impact that is only noticeable in the case of TEP (2% reduction).

With respect to contributing activities (**Figure 7.5b**), the production of electricity required in the different stages of the process is the main hot spot of the production of DHA. This activity is associated with more than 90% of the environmental burdens in most impact categories. ODP is the only category with low contribution from electricity. The main cause of ODP is the production of chemicals. In particular, the production of organic solvents is responsible for 98% of the environmental impact to ODP. The different contributors to the impact from electricity and chemicals are identified in the next section.

**a) Relative contributions of lab-scale production of DHA per stage**




**b) Relative contributions of lab-scale production of DHA per involved process**

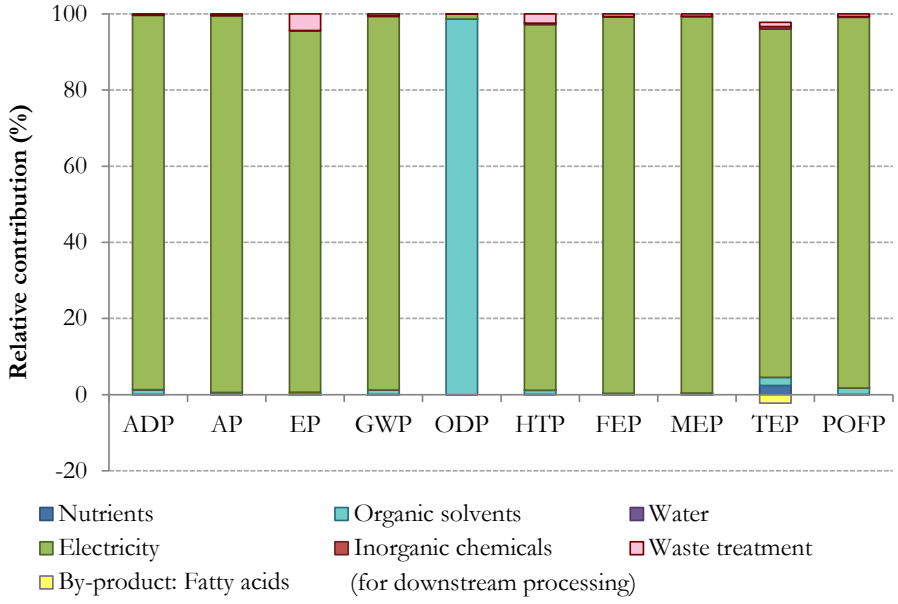



**Figure 7.5.** Relative contributions of the lab-scale production of DHA by *U. visurgensis* to each impact category per a) stage and b) involved process.

### 7.3.4. Discussion and recommendations

According to the results, the cultivation (S2) is the main contributor to most impact categories, especially related to the high electricity requirements. Among the other stages, only oil extraction had a significant contribution in the category of ODP. The specific steps responsible for the environmental burdens are discussed below.

❖ **Contributing steps to electricity consumption**

The production of electricity is clearly the main hot spot in the lab-scale production of DHA by *U. visurgensis*. Therefore, **Figure 7.6** shows a breakdown of the electricity consumptions throughout the process.

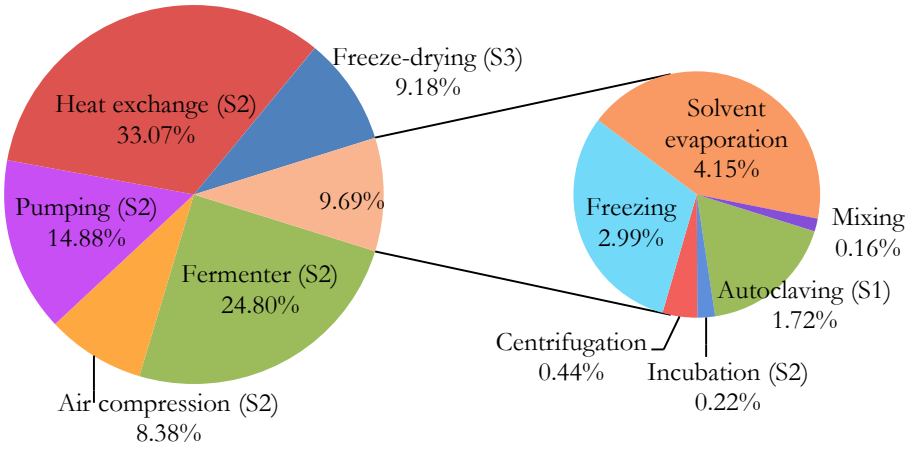



**Figure 7.6.** Relative contribution (%) of the electricity requirements to the potential environmental impacts of the lab-scale production of DHA by *U. visurgensis*.

As shown in **Figure 7.6**, the cultivation (S2) requires up to 80% of the electricity consumed in the process, mainly due to the needs of the heat exchanger and the fermenter. Pumping and air supply, also related to S2, as well as freeze-drying after harvesting of the biomass (S3) are the main secondary contributors. The electricity required to extract the DHA has a limited effect in comparison with the above-mentioned stages.

❖ **Effect of organic solvents on ODP**

The category of is affected up to 98% by the production of organic solvents used for the downstream processing, and especially linked to the oil extraction stage (S4). The breakdown of the different contributions is shown in **Figure 7.7**.

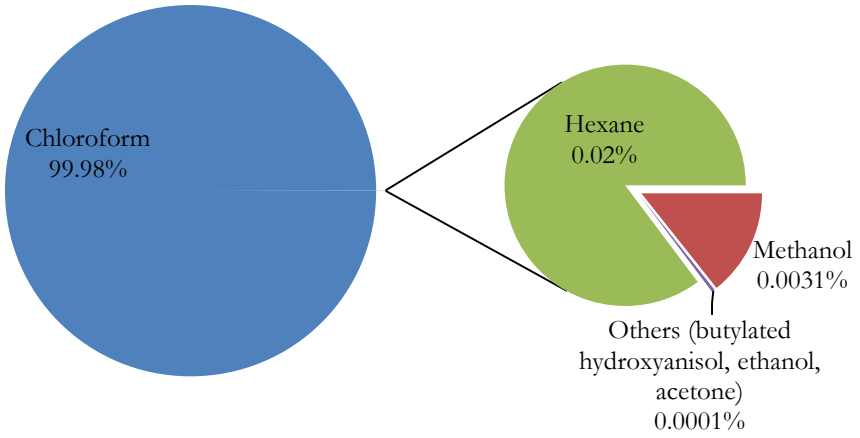



**Figure 7.7.** Relative contribution (%) of the organic solvents to the potential environmental impacts of the lab-scale production of DHA by *U. visurgensis*

According to the results, nearly all the contribution to the environmental impacts related to the production of organic chemicals is due to the extensive use of chloroform (component of the Folch solution) as extraction solvent in stage S4 (oil extraction). The main substance emitted to the environment during the production of chloroform that has a high impact in the analyzed category is CFC-10, which is responsible for more than 98% of the total impact in ODP.

❖ **Recommendations for process optimization**

According to the stages and processes identified as key contributors to the environmental burdens, one of the most suitable options to improve the process would be the optimization of the cultivation system (especially the temperature control system) in order to reduce the associated electricity consumption. The feasibility of reusing organic solvents (particularly chloroform) should also be check to improve the environmental response of the system in terms of ODP.

## 7.4. Lipase enzymes by marine fungi

Marine fungi are receiving increasing attention as a rich natural source of bioactive compounds. Their ability to grow in extreme habitats may allow the production of unique secondary metabolites with anticancer, antibacterial, anti-inflammatory and antiviral properties, among others (Bhadury et al., 2006). In addition, several species of marine fungi produce a wide variety of enzymes with biotechnological applications, including the use for decolorization of effluents from paper and pulp industries, textile and dye-making industries and alcohol distilleries (Raghukumar, 2008).

The marine fungi *Cryptococcus laurentii* and *Geomyces pannorum* are producers of lipases, a group of enzymes that catalyze the hydrolysis and synthesis of long chain acylglycerols. The activity of these enzymes is especially suitable for processes that are developed at low or moderate temperatures, such as the production of detergents for cold washing, as well as food, chemical, pharmaceutical or agricultural applications. *C. laurentii* is derived from sea urchins, while *Geomyces* sp. is found in association with Antartic macroalgae (Murray et al., 2013). Both fungi are common of cold marine and soil environments and usually grow under physchrophilic conditions (Hayes, 2012; Murray et al., 2013).

In this section, the production of lipases by *C. laurentii* and *G. pannorum* is evaluated from a life cycle perspective. The process was modeled according to a lab-scale scheme conducted in 2.5 L fermenters. The life cycle inventory and environmental performance are discussed below.

### 7.4.1. Goal and scope definition

The main objective of this study is to evaluate the environmental profile of the production of the enzyme lipase by two Antartic marine fungi: *C. laurentii* and *G. pannorum*. For the LCA, the proces was modeled according to the lab-scale system optimized by the Limerick Institute of Technology (Ireland). In order to allow comparisons between both species, a total production of 1 g of protein fraction (with a specific activity of lipase of 7.9 $U \cdot mg^{-1}$ protein) was selected as the FU.

The system was divided into the following four stages: i) inoculation of the fungus on agar plates, ii) preparation of starter liquid culture, iii) cultivation in fermenter using fine-tuned optimized growth conditions and iv) protein concentration. The steps included within the system boundaries were analogous for the two species of marine fungi and are shown in **Figure 7.8**.

i) S1. Inoculation of marine fungi on agar plate: The inoculum to be further cultured in liquid media was obtained from grown fungus on Petri dishes with Potato Dextrose Agar (PDA) medium incubated for seven days at 15°C.

ii) S2. Preparation of starter liquid culture: The prepared inoculum was transferred to Erlenmeyer flasks and incubated under the conditions described below for each species.

   For *C. laurentii*, 2 mL of cell suspension prepared to a concentration of $1x10^7$ cells/mL were transferred to Erlenmeyer flasks (125 mL) containing 50 mL of optimized medium with a composition of 30 $g \cdot L^{-1}$ of yeast extract, 0.02 $L \cdot L^{-1}$ of sunflower oil (equivalent to 2% v/v) and 10 $g \cdot L^{-1}$ glucose. The flasks were maintained at pH 8.0 and incubated at 20°C and 180 rpm for 120 h.

   For *G. pannorum*, five plugs were taken from the edge of the colony in the agar plates and transferred to Erlenmeyer flasks containing 50 mL of basal medium (pH 8) with the following composition: 7.0 $g \cdot L^{-1}$ potassium phosphate monobasic ($KH_2PO_4$), 2.0 $g \cdot L^{-1}$ sodium phosphate dibasic ($Na_2HPO_4$), 1.5 $g \cdot L^{-1}$ magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), 0.1 $g \cdot L^{-1}$ calcium chloride dihydrate ($CaCl_2 \cdot 2H_2O$), 0.008 $g \cdot L^{-1}$ iron (II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$), 0.0001 $g \cdot L^{-1}$ zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), 1.5 $g \cdot L^{-1}$ diammonium tartrate, 0.02 $L \cdot L^{-1}$ of soybean oil (equivalent to 2.21% v/v), 0.03 $L \cdot L^{-1}$ of sunflower oil (2.79% v/v) and 37.9 $g \cdot L^{-1}$ yeast extract. The flasks were incubated at 15°C and 150 rpm for seven days.

iii) S3. Cultivation: An inoculum of 200 mL from S2 was added and the fungi were cultured in 1.2 L of culture media at optimal temperature (15°C) and 150 rpm with the supply of filtered air to the fermenter at a rate of 0.4 $L \cdot L^{-1} \cdot min^{-1}$ until the maximum enzyme productivity was achieved. For both species, the optimal growth period was 5 days. The final protein concentration was 0.10 $g \cdot L^{-1}$ for *C. laurentii* and 0.06 $g \cdot L^{-1}$ for *G. pannorum*, with an approximate enzyme activity of 7.9 $U \cdot mg^{-1}$.

iv) S4. Protein concentration: The final culture medium was centrifuged at 10.000 rpm for 10 min to obtain the broth with lipase activity. The protein fraction was freeze-dried for concentration.

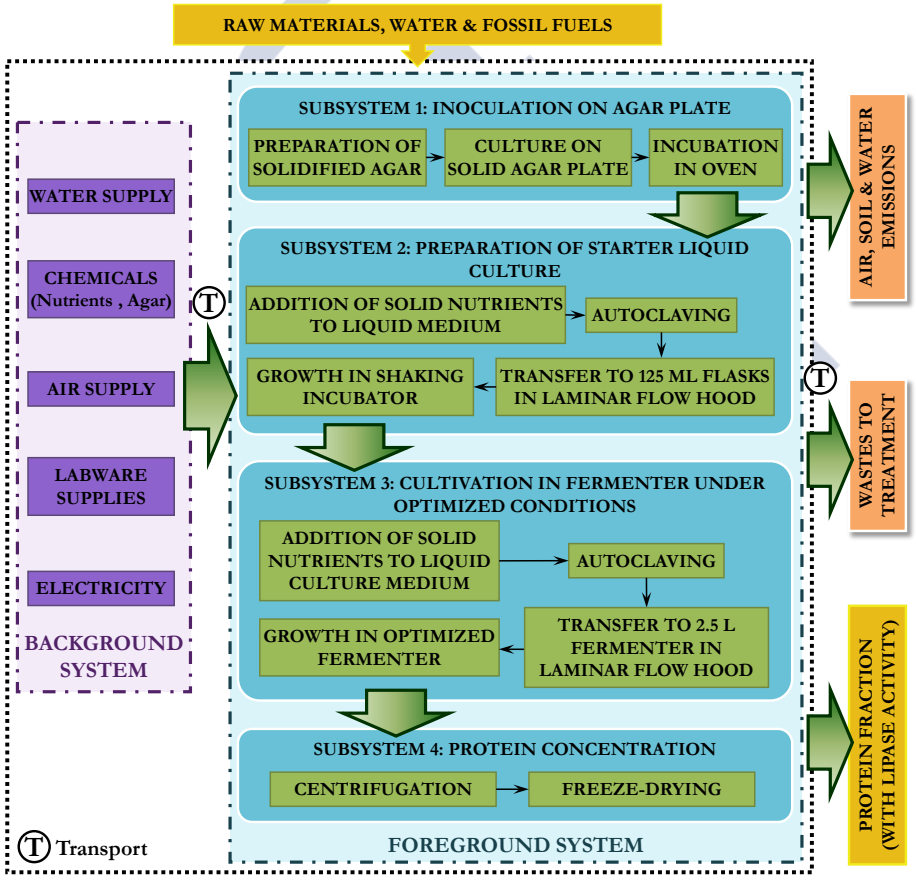



**Figure 7.8.** Process chain and system boundaries of the production of lipases at lab scale by marine fungi *C. laurentii* and *G. pannorum*.

### 7.4.2. Life cycle inventory, data quality and assumptions

The LCI data for the foreground system (i.e. organic and inorganic chemicals used as nutrients in the growth stages, electricity consumption throughout the stages of the process and transport distances) consisted of average data obtained by on-site measurements. Wastewater derived from the different production stages was discharged to the municipal sewage network and assumed to be treated in a medium-sized treatment plant. The inventory data for the production of lipase enzyme by the marine fungi *C. laurentii* and *G. pannorum* grown in 2.5 L fermenters are shown in **Table 7.7**.

Concerning the background system, the required inventory data include the production of the different chemicals used as nutrients for the preparation of the culture medium, the production of the lab materials (Petri dishes, Erlenmeyer flasks, and 2.5 L fermenter), the production of the electricity requirements, the distribution of the different inputs to the lab (average distance of 197 km) and solid wastes to the disposal facilities (average distance of 50 km), and the waste treatment in inert landfills, sanitary landfills and wastewater treatment plant.

The background activities were inventoried according to the same Ecoinvent processes listed in **Tables 7.2** and **7.5** for the production of bioactive compounds by epiphytic bacteria and *U. visurgensis*. The production of materials for large equipment was excluded from the system boundaries due to its minimal contribution.

*Allocation procedures*

This production process is focused on a single product (protein fraction with lipase activity). Thus, all the environmental burdens are assigned to the protein fraction and no allocation procedure is required in the assessment.

**Table 7.7.** Inventory data for the lab-scale production of lipase enzyme by marine fungi *C. laurentii* and *G. pannorum* (FU=1 g protein fraction)

| **INPUTS from TECHNOSPHERE** | | |
|---|---|---|
| | Production by *C. laurentii* | Production by *G. pannorum* |
| **Materials** | | |
| *S1. Inoculation of fungi on agar plate* | | |
| Polystyrene | 132.76 g | 213.04 g |
| Distilled water | 0.13 L | 0.21 L |
| Potato extract | 0.52 g | 0.84 g |
| Dextrose | 2.60 g | 4.18 g |
| Agar | 0.13 g | 0.21 g |
| *S2. Preparation of starter liquid culture* | | |
| Glass | 48.93 g | 78.53 g |
| Distilled water | 1.70 L | 2.65 L |
| $KH_2PO_4$ | 0 | 19.49 g |
| $Na_2HPO_4$ | 0 | 5.57 g |
| $MgSO_4 \cdot 7H_2O$ | 0 | 4.18 g |
| $CaCl_2 \cdot 2H_2O$ | 0 | 0.28 g |
| $FeCl_3 \cdot 4H_2O$ | 0 | 0.02 g |
| $ZnSO_4 \cdot 7H_2O$ | 0 | 0.28 mg |
| Ammonium tartrate | 0 | 4.18 g |
| Glucose | 0.17 g | 0 |
| Soybean oil | 0 | 61.55 mL |
| Sunflower oil | 34.71 mL | 77.70 mL |
| Yeast extract | 52.06 g | 105.55 g |
| *S3. Cultivation in fermenter* | | |
| Glass | 13.07 g | 20.97 g |
| Steel | 38.13 g | 61.18 g |
| Distilled water | 8.50 L | 13.23 L |
| $KH_2PO_4$ | 0 | 115.01 g |
| $Na_2HPO_4$ | 0 | 32.86 g |
| $MgSO_4 \cdot 7H_2O$ | 0 | 24.65 g |
| $CaCl_2 \cdot 2H_2O$ | 0 | 1.64 g |
| $FeCl_3 \cdot 4H_2O$ | 0 | 0.13 g |
| $ZnSO_4 \cdot 7H_2O$ | 0 | 1.64 mg |
| Ammonium tartrate | 0 | 24.65 g |
| Glucose | 102.39 g | 0 |
| Soybean oil | 0 | 0.31 L |

**Table 7.7.** Inventory data for the lab-scale production of lipase enzyme by marine fungi *C. laurentii* and *G. pannorum* (FU=1 g protein fraction) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | |
|---|---|---|
| | Production by *C. laurentii* | Production by *G. pannorum* |
| **Materials** | | |
| *S3. Cultivation in fermenter* | | |
| Sunflower oil | 0.17 L | 0.39 L |
| Yeast extract | 277.67 g | 527.73 g |
| Compressed air (at 1.5 bar) | 24.99 $m^3$ | 40.10 $m^3$ |
| **Energy** | | |
| Electricity from Irish grid: | | |
| *S1. Inoculation of fungi on agar plate* | | |
| Laminar flow hood | 1.42 kWh | 2.27 kWh |
| Incubation | 5.12 kWh | 19.18 kWh |
| *S2. Preparation of starter liquid culture* | | |
| Weighing | 0.01 kWh | 0.02 kWh |
| Autoclaving | 22.78 kWh | 36.55 kWh |
| Laminar flow hood | 4.25 kWh | 6.82 kWh |
| Incubation in shaker | 178.50 kWh | 401.02 kWh |
| *S3. Cultivation in fermenter* | | |
| Weighing | 0.01 kWh | 0.02 kWh |
| Autoclaving | 22.78 kWh | 36.55 kWh |
| Laminar flow hood | 4.25 kWh | 6.82 kWh |
| Fermenter monitoring and control system | 187.43 kWh | 300.76 kWh |
| *S4. Protein concentration* | | |
| Centrifugation | 3.37 kWh | 5.41 kWh |
| Freeze-drying | 26.66 kWh | 42.78 kWh |
| **Transport** | | |
| Truck, 3.5-7.5 t: | | |
| *S1. Inoculation of fungi on agar plate* | | |
| Materials | 26.28 kg·km | 42.17 kg·km |
| Chemicals | 26.15 kg·km | 41.97 kg·km |
| Wastes | 6.80 kg·km | 10.91 kg·km |
| *S2. Preparation of starter liquid culture* | | |
| Materials | 355.00 kg·km | 569.96 kg·km |
| Chemicals | 9.64 kg·km | 15.47 kg·km |

**Table 7.7.** Inventory data for the lab-scale production of lipase enzyme by marine fungi *C. laurentii* and *G. pannorum* (FU=1 g protein fraction) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | |
|---|---|---|
| | Production by *C. laurentii* | Production by *G. pannorum* |
| **Transport** | | |
| Truck, 3.5-7.5 t: | | |
| *S3. Cultivation in fermenter* | | |
| Materials | 1.78 tkm | 2.87 tkm |
| Chemicals | 10.09 kg·km | 16.18 kg·km |
| Wastes | 5.01 kg·km | 8.03 kg·km |
| **INPUTS from ENVIRONMENT** | | |
| Fungal sample | 8.68 mL | 13.92 mL |
| **OUTPUTS to TECHNOSPHERE** | | |
| **Product** | | |
| Protein fraction with lipase activity | 1 g | 1 g |
| **Wastes to landfill** | | |
| *S1. Inoculation of fungi on agar plate* | | |
| Polystyrene | 132.76 g | 213.04 g |
| PDA medium | 3.25 g | 5.22 g |
| *S3. Cultivation in fermenter* | | |
| Glass | 62.00 g | 99.50 g |
| Steel | 38.13 g | 61.18 g |
| **Wastewater to treatment plant** | | |
| *S1. Inoculation of fungi on agar plate* | | |
| Wastewater | 0.14 L | 0.22 L |
| *S3. Protein concentration* | | |
| Wastewater | 10.20 L | 15.87 L |
| $KH_2PO_4$ | 0 | 11.70 g |
| $Na_2HPO_4$ | 0 | 3.34 g |
| $MgSO_4 \cdot 7H_2O$ | 0 | 2.50 g |
| $CaCl_2 \cdot 2H_2O$ | 0 | 0.17 g |
| $FeCl_3 \cdot 4H_2O$ | 0 | 13.37 mg |
| $ZnSO_4 \cdot 7H_2O$ | 0 | 0.17 mg |
| Ammonium tartrate | 0 | 2.51 g |
| Glucose | 10.41 g | 0 |
| Soybean oil | 0 | 0.37 L |
| Sunflower oil | 0.21 L | 0.47 L |
| Yeast extract | 282.87 g | 527.83 g |

### 7.4.3. Environmental impact assessment

Again, the CML 2001 methodology was used to obtain the environmental profile of the process according to the classification and characterization steps of the ISO 14040 LCA framework (Guinée et al., 2002; ISO 14040, 2006).

The environmental performance was evaluated for the following impact categories: ADP, AP, EP, GWP, ODP, HTP, FEP, MEP, TEP and POFP. The software SimaPro 8 was used for the computational implementation of the inventories (Goedkoop et al., 2013). The characterization results for the process are presented in **Table 7.8**.

**Table 7.8.** Environmental impact assessment results (characterization step) associated with the lab-scale production of 1 g of protein fraction (with lipase activity) by marine fungi *C. laurentii* and *G. pannorum*

| Impact category | Unit | Production by *C. laurentii* | Production by *G. pannorum* |
|---|---|---|---|
| ADP | kg Sb eq | 2.94 | 5.51 |
| AP | kg $SO_2$ eq | 2.09 | 3.93 |
| EP | kg $PO_4^{-3}$ eq | 0.37 | 0.70 |
| GWP | kg $CO_2$ eq | 406.02 | 763.25 |
| ODP | g CFC-11 eq | 0.03 | 0.05 |
| HTP | kg 1,4-DB eq | 71.74 | 133.85 |
| FEP | kg 1,4-DB eq | 55.67 | 105.01 |
| MEP | kg 1,4-DB eq | 40.35 | 75.72 |
| TEP | kg 1,4-DB eq | 0.02 | 0.04 |
| POFP | kg $C_2H_4$ eq | 0.09 | 0.16 |

❖ **Comparative environmental performance of the production of lipase enzyme by *C. laurentii* and *G. pannorum***

According to the results indicated in **Table 7.8** and the corresponding environmental profiles depicted in **Figure 7.8**, the production of lipase enzyme by marine fungus *C. laurentii* has significantly lower environmental burdens than the equivalent process carried out by *G. pannorum* for all the evaluated impact categories. Thus, the contributions of *C. laurentii* process range between 50% and 54% of the total impact of *G. pannorum* production.

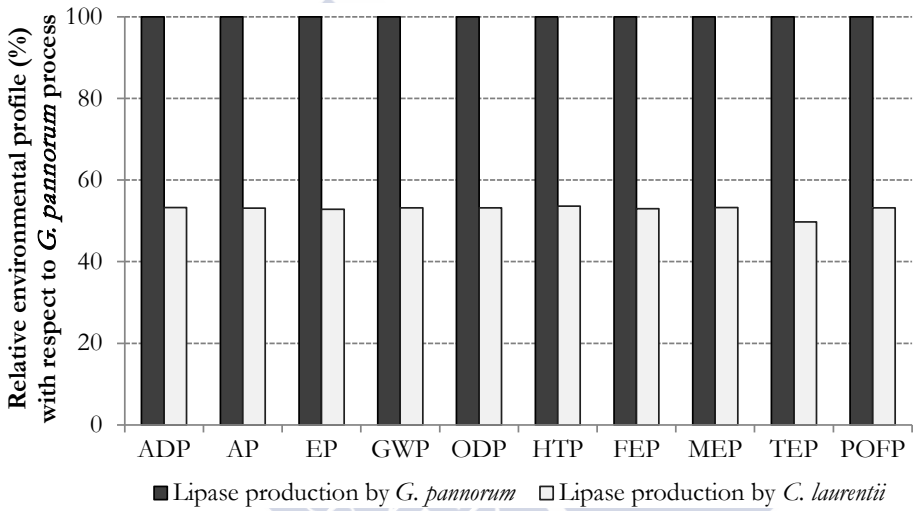



**Figure 7.9.** Comparative environmental profiles of the production of protein fraction with lipase activity by marine fungi *C. laurentii* and *G. pannorum*.

Although the main stages and associated activities are analogous for both processes, there are several factors that differ. Firstly, *G. pannorum* requires a higher quantity and variety of nutrients for growth in stages S2 (preparation of starter liquid culture) and S3 (cultivation in fermenter), as well as a longer period of incubation in S2. The incubation is carried out for five days in the case of *C. laurentii* and seven days for *G. pannorum*, and is an energy-intensive step of S2.

Moreover, the final protein concentration achieved in *C. laurentii* culture is 38% higher than in the case of *G. pannorum*. The higher yield of *C. laurentii* allows the same level of protein production with lower consumption of energy and raw materials, which results in a more efficient environmental profile.

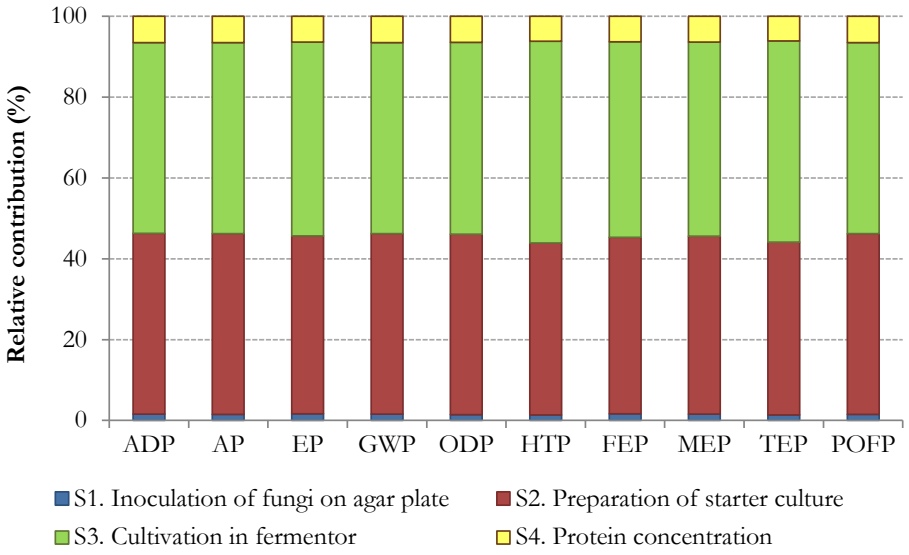
❖ **Identification of hot spots for the production of lipase enzyme by *C. laurentii* and *G. pannorum***

**Figures 7.10** and **7.11** show similar distributions of environmental impacts among the different stages and activities associated with the production of lipases by marine fungi. According to these results, the preparation of the starter liquid culture (S2) and the cultivation in fermenter (S3) are the most problematic stages of the process and they sum between 91% and 93% of the total contribution to each category. For both production systems, the inoculation of fungal samples on agar plates (S1) has secondary contributions below 3% for all the categories, whereas the protein concentration stage (including the centrifugation of the liquid culture and the freeze-drying of the protein fraction) only exceeds 6% of the total contributions for the production by *C. laurentii*.

S3 is the main contributor to the environmental impacts of *C. laurentii* process, with values ranging from 47% (for categories such as ADP or GWP) to 50% (for TEP). The contributions of S2 constitute between 42% and 45% of the total impact. In the case of *G. pannorum*, S2 has higher environmental burdens than S3 that range between 47% (for TEP) and 51% (for ADP, AP or GWP). S3 has relative contributions from 40% to 46%. The larger influence of S2 in *G. pannorum* process is mainly linked to the longer period of incubation (already pointed out in the previous section), which results in higher energy consumption in this stage.

Indeed, the production of electricity to meet the requirements of the different stages of the process is the main hot spot of the activities involved in the production of lipase enzyme by marine fungi. This activity is responsible for 92% up to 99% of the relative contributions for the production by *C. laurentii* and 87% to 99% of the impacts of *G. pannorum* process. Among secondary processes, the only activities that exceed 1% of the impact of *C. laurentii* process are the production of organic chemicals in the category of TEP and the air supply in the categories of EP, HTP, FEP and MEP. The production by *G. pannorum* has relative contributions above 1% for the production of organic chemicals in the categories of EP, FEP and especially TEP (with a relative contribution of 12%).

**a) Relative contributions of lab-scale production of lipases by *C. laurentii* per stage**




**b) Relative contributions of lab-scale production of lipases by *C. laurentii* per involved process**

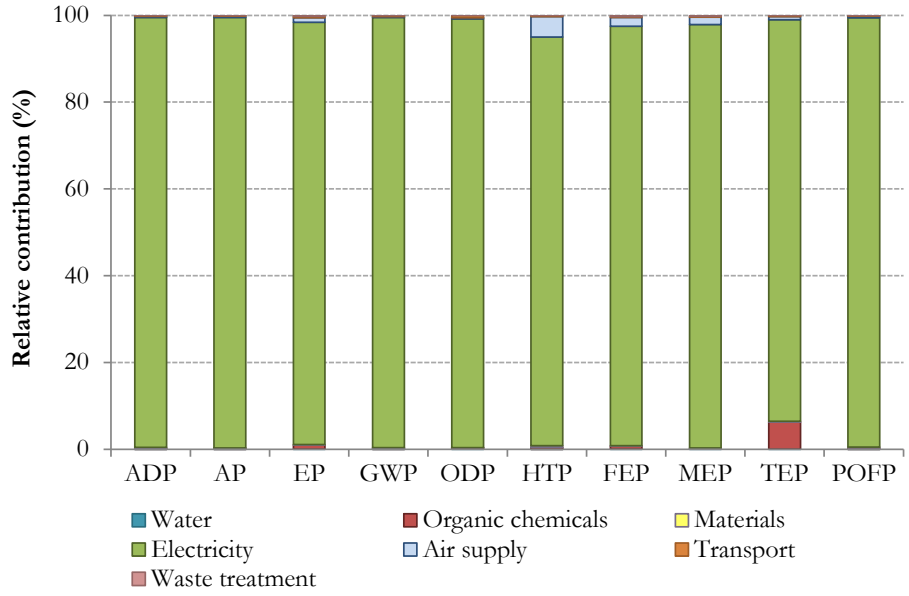
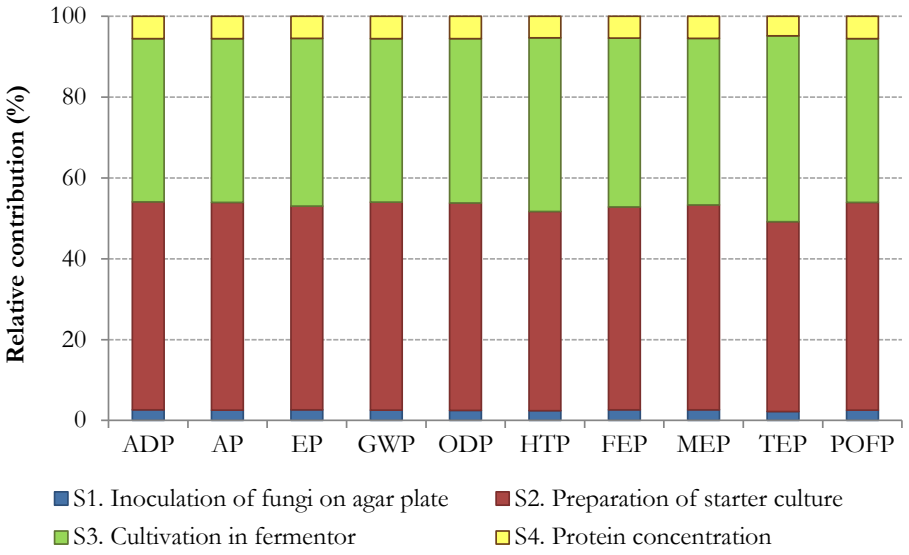



**Figure 7.10.** Relative contributions of the production of lipase enzyme by *C. laurentii* to each impact category per a) stage and b) involved process.

**a) Relative contributions of lab-scale production of lipases by *G. pannorum* per stage**




**b) Relative contributions of lab-scale production of lipases by *G. pannorum* per involved process**

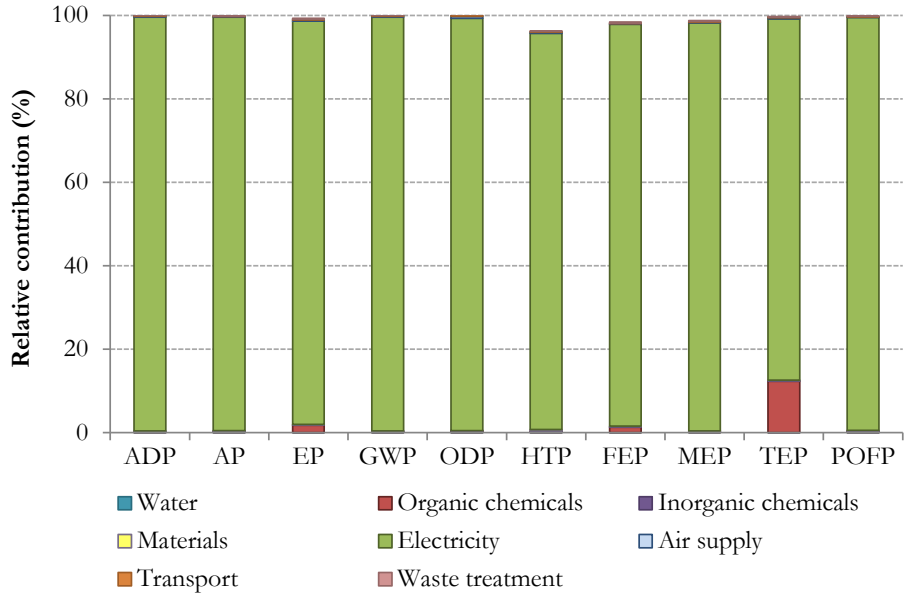



**Figure 7.11.** Relative contributions of the production of lipase enzyme by *G. pannorum* to each impact category per a) stage and b) involved process.

### 7.4.4. Discussion and recommendations

The conducted LCA has allowed the identification of S2 and S3 subsystems as the most contributing stages of the production of lipase enzyme by marine fungi. The rationale behind this is the high electricity consumption associated with these stages, especially inefficient in a batch. However, the inventoried process consists of a discontinuous lab-scale process. The different steps of the process that are responsible for the electricity requirements and the effect of the implementation of a continuous process to increase the efficiency of the process are discussed in this section.

❖ **Contributing steps to electricity consumption**

The results shown in **Figure 7.12** are consistent with the findings of section 7.4.3. The highest electricity requirements are associated with steps that are carried out in stages S2 and S3. For *C. laurentii*, 45% of the total electricity needs are related to processes of S2 and 47% are due to S3. In total, they include 92% of the electricity consumed during the process, which is close to the combined contributions of S2 and S3 indicated in the previous section. With respect to *G. pannorum*, 52% of the required electricity is linked to steps of S2, whereas 40% is associated with S3.

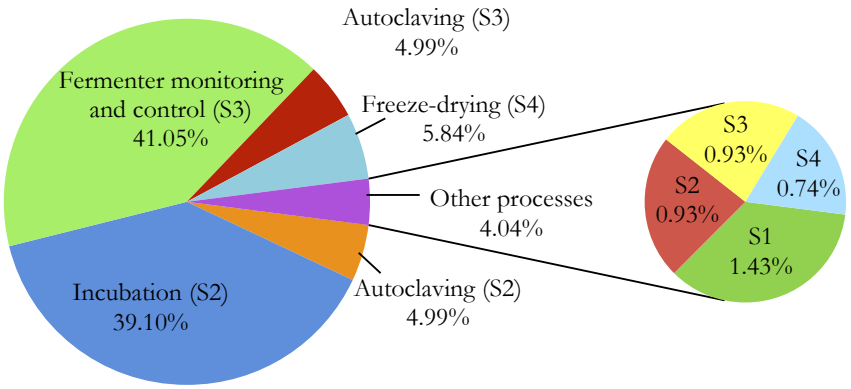
In particular, the incubation of the starter liquid culture in shaker (S2) and the monitoring and control system of the fermenter (S3) have the highest electricity consumption of all the steps. As expected, the relative contribution of the electricity required by the shaker incubator is slightly higher for the lipase production by *G. pannorum* (nearly 46%) than for *C. laurentii* (39%). This is due to the longer incubation period, previously discussed in 7.4.3.

The lower effect of incubation when considering *C. laurentii* process leads to a higher contribution of electricity for the monitoring and control system in relative terms. Although this process is responsible for 41% of the energy requirements of *C. laurentii* process, compared to 35% for *G. pannorum*, the total electricity consumed by the first system per FU (1 g protein fraction with lipase activity of 7.9 U/mg) is 44% lower than the requirements of the latter. This means that the total electricity consumed by the monitoring and control system

in *C. laurentii* process involves only 53% of the total energy required by the same system to produce an equivalent amount of product from *G. pannorum*.

Among the steps of the secondary stages, only the freeze-drying of the product associated with S4 involves a noticeable contribution to the energy requirements, which is close to 6% for *C. laurentii* and to 5% for *G. pannorum*. The electricity consumed in S1 constitutes less than 3% of the total energy requirements for both systems.

**a) Electricity requirements of *C. laurentii* process**




**b) Electricity requirements of *G. pannorum* process**

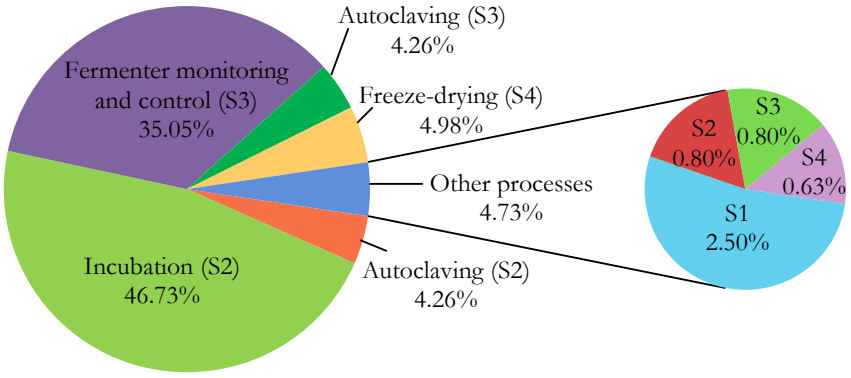



**Figure 7.12.** Relative contribution (%) of the electricity requirements to the potential environmental impacts of the production of lipase enzyme by marine fungi a) *C. laurentii* and b) *G. pannorum*.

❖ **Effect of continuous operation**

The preparation of the starter liquid culture (S2) was identified as one of the main hot spots of the production of lipase enzyme by marine fungi. The reason for the high impact is the fact that the process is operated on a discontinuous mode and the inoculum has to be prepared for each cultivation. In a pilot or large scale process, the inoculum for each batch would probably be obtained by recycling a small biomass fraction from the previous culture. This effect is depicted in **Figure 7.13** for a continuous process maintained during one year.

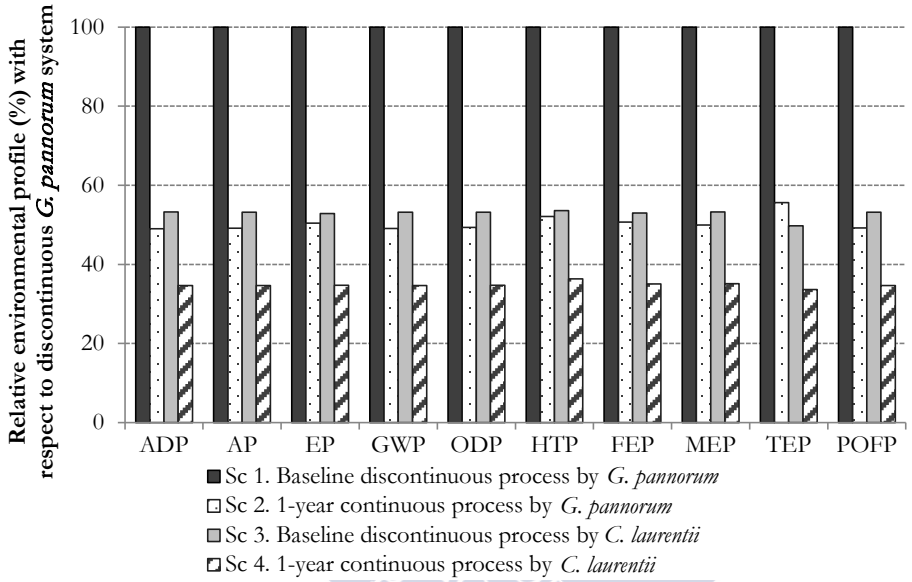



**Figure 7.13.** Effect of continuous operation on the environmental profile of lipase production by marine fungi.

According to the results, the implementation of the continuous process would lead to significant reductions of impact between 44% and 51% for *G. pannorum* system and 32% to 35% for *C. laurentii* process. The more remarkable improvement of the lipase production by *G. pannorum* is due to the higher relative contribution of S2 in the initial scenario. Thus, the reduction of impact for this process results in lower environmental impacts for Sc 2 (*G. pannorum* continuous process) than for the baseline *C. laurentii* process (Sc 3), although the potential improvement of the equivalent continuous process for the second fungal species is significantly higher.

## 7.5. Conclusions

Nowadays, the available literature related to the products from marine origin is limited to processes involving algae and sponges. Recent research highlights the potential of other marine organisms as sources of a wide variety of bioactive molecules. These organisms include bacteria, chromists and fungi. Three novel processes based on the production of high value products by selected species belonging to the aforementioned kingdoms were analyzed from a life cycle thinking approach. The main findings of the conducted LCA studies are consistent with previous work on biocompounds from microalgae, seaweed and sponges.

Thus, the main hot spot of the production of coenzyme $Q_{10}$ by epiphytic bacteria isolated from macroalgae was the production of the chemicals consumed in the process, especially organic solvents such as formic acid or chloroform, used for the extraction and purification of the target compound.

In the cases of the thraustochytrid *U. visurgensis* (which belongs to kingdom Chromista) and the marine fungi *C. laurentii* and *G. pannorum*, the high electricity consumption, mainly linked to the cultivation stages, was the main factor responsible for the environmental burdens of the processes. For this reason, a careful optimization of the energy-intensive stages was identified as an essential step for the design of environmentally sustainable process with an efficient balance between electricity consumption and global yield of the system.

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# SECTION III

## MICROALGAL BIOREFINERIES

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# Chapter 8

# Comparative LCA of microalgal cultivation systems[1]


*Summary*

Despite the economic potential of microalgae as natural sources for biofuels, food, and high-value product, the environmental sustainability must be examined. Cultivation is a major stage with an environmental impact that strongly depends on reactor selection and operating conditions. This study provides a comparative life cycle assessment of the most common cultivation systems: open raceway ponds (ORP), horizontal and vertical tubular photobioreactors (PBRs) and flat-panel reactors. The aim is to analyze the productivity and environmental performance of the systemss based on experimental pilot-plant data of systems operated at AlgaePARC pilot facility (Bennekom, The Netherlands). Moreover, the influence of weather conditions on ORP and tubular PBRs is discussed in detail. The energy consumption during microalgal cultivation, especially related to temperature regulation, presents the highest environmental burdens. The production of nutrients is a secondary contributor affecting some categories. Despite limited differences with the vertical system, the horizontal PBR was found the most efficient in terms of productivity and environmental impact. The ORP is, given the climatic conditions, only feasible under favorable summer conditions.


[1] Van Boxtel AJB, **Pérez-López P**, Breitmayer E, Slegers PM. The potential of optimized process design to advance LCA performance of algae production systems. Applied Energy 2015, 154:1122-1127.

# Index

## 8.1. Role of cultivation in microalgal processes

The scarcity of natural resources and particularly the exhaustion of fossil fuels involve a global challenge that needs to be addressed by developing alternative processes to satisfy the increasing demand (Costa and de Morais, 2011; Draaisma et al., 2013; Kirrolia et al., 2013; Singh et al., 2014). Microalgae show a great potential for the production of biofuels and mid- and high-value compounds with a wide variety of applications in chemical, food or pharmaceutical industries (Draaisma et al., 2013).

Although microalgae have significantly lower environmental burdens compared to other feedstocks, in categories such as land competition or eutrophication, other aspects, such as greenhouse gas (GHG) emissions or energy balance, need to be further optimized in order to develop efficient processes at commercial scale (Clarens et al., 2010; Lam and Lee, 2012).

Algae cultivation has been identified as a major contributor to the energy consumption of microalgal processes (Brentner et al., 2011; Lam and Lee, 2012; Stephenson et al., 2010). The energy requirements of the cultivation stage strongly depend on the type of reactor configuration and are linked to the pumping and mixing operations during the addition of nutrients and $CO_2$ in the reactor, as well as the previous manufacturing processes of synthetic fertilizers (Brentner et al., 2011; Clarens et al., 2010; Draaisma et al., 2013; Jorquera et al., 2010). Moreover, algae are temperature sensitive and thus, heating and cooling are required to operate close to the optimal temperature of the algal species.

Temperature regulation allows operating the reactor at high productivities while avoiding growth inhibition (Bosma et al., 2014; Slade and Bauen, 2013). Although temperature control may increase the energy demand of the process, integration of options such as the use of waste heat from power generation or cold water resources contributes to the optimization of the cultivation stage (Slade and Bauen, 2013; Taelman et al., 2013). Furthermore, climatic data including irradiation and temperature depend on the geographic location. Therefore the heating and cooling needs of the system vary between locations (Moody et al., 2014; Slegers et al., 2013). Hence, the selection of an appropriate location combined with available resources and the optimal algal strain may

serve to maintain the optimal temperature with low heating and cooling requirements so that the energy consumption is minimized.

Due to the influence of the reactor configuration in the energy requirements, the reactor selection is a key issue affecting the environmental profile. As explained in previous chapters, open ponds and closed photobioreactors (PBR) are the two groups of systems for microalgal cultivation. The main benefits and limitations of the most common types of reactor are summarized in **Table 8.1**.

**Table 8.1.** Benefits and limitations of microalgal cultivation systems (Borowitzka, 1999; Brennan and Owende, 2010; Ugwu et al., 2008)

| Production system | Benefits | Limitations |
|---|---|---|
| Open raceway ponds (ORP) | Low operational costs | Low productivity |
| | Low aeration and mixing costs | Large area of land required |
| | Easy cleaning and maintenance | Limited use to specific strains |
| | | Poor temperature control |
| | | Poor mixing, light and $CO_2$ utilization |
| | | Difficult focus on target products due to poor control |
| Tubular PBRs | Large illumination surface area | Fouling, cell adherence to wall |
| | Moderate biomass productivity | Relatively large land required |
| | Moderate operational cost | Gradients of operational parameters (pH, $CO_2$, oxygen) |
| | Good temperature control | |
| Flat-panel PBRs | High biomass productivity | Difficult scale-up |
| | Easy sterilization | Cell adherence to wall |
| | Good light pattern | Moderate hydrodynamic stress |
| | Large illumination surface area | |
| | Good temperature control | |
| | Low oxygen accumulation | |

To date, numerous studies dealing with the environmental performance of different reactor designs for microalgae cultivation have been published; (Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2010; 2011; Collet et al., 2014; Draaisma et al., 2013; Jorquera et al., 2010; Stephenson et al., 2010; Taelman et al., 2013; Woertz et al., 2014). However, most of these works consider hypothetical simulated scenarios or extrapolations from lab-scale data rather than existing pilot or commercial systems (Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2010; 2011; Draaisma et al., 2013; Jorquera et al., 2010; Stephenson et al., 2010; Woertz et al., 2014). Moreover, few studies make a comparison between different configurations and they are restricted to a very limited set of indicators that mainly take into account energy requirements and GHG emissions (Brentner et al., 2011; Draaisma et al., 2013; Jorquera et al., 2010; Stephenson et al., 2010).

For this reason, a more detailed assessment is here proposed, including the comparison of ORP, tubular and flat panel PBRs operated in favorable conditions (summer period). Additionally, the variability of the environmental behavior of the two most common systems, ORP and tubular PBRs is further analyzed, according to surrounding conditions in different seasons of the year. All the results refer to real pilot-plant data for the cultivation of the eustigmatophyte *Nannochloropsis* sp. at AlgaePARC pilot research facility (Wageningen University and Research Center, Bennekom, The Netherlands).

*Nannochloropsis* has a high capacity to produce storage triacylglycerols (TAG), mainly saturated and monounsaturated C16 fatty acids, which makes it a good feedstock for biodiesel (Pal et al., 2011). Moreover, the microalga contains significant amounts of the essential ω-3 long-chain polyunsaturated fatty acid eicosapentaenoic acid (EPA, 20:5ω-3) as well as several carotenoids (especially violaxanthin). This composition reflects the potential application for human consumption and as algal feed for aquaculture (Sukenik et al., 2009; Volkman et al., 1993). Since growth rate and fatty acids composition are strongly affected by operational conditions such as light intensity, nutrient limitation, salinity or temperature and the optimal conditions are not necessarily the same for both parameters, an optimization process is needed to determine the most appropriate conditions for the cultivation (Pal et al., 2011).

## 8.2. AlgaePARC

AlgaePARC aims to develop and compare new reactor alternatives together with process control strategies for microalgae cultivation and processing. The main objective is to design systems with low production costs and energy requirement that can serve as a basis for the improvement of large-scale microalgae plants. AlgaePARC outdoor facilities comprise several pilot-scale reactors, including an ORP, horizontal and vertical tubular PBRs and a flat-panel PBR (Proviapt). These systems were monitored throughout the year 2013. The layout of each system is depicted in **Figure 8.1**, and described in detail in Bosma et al. (2014).

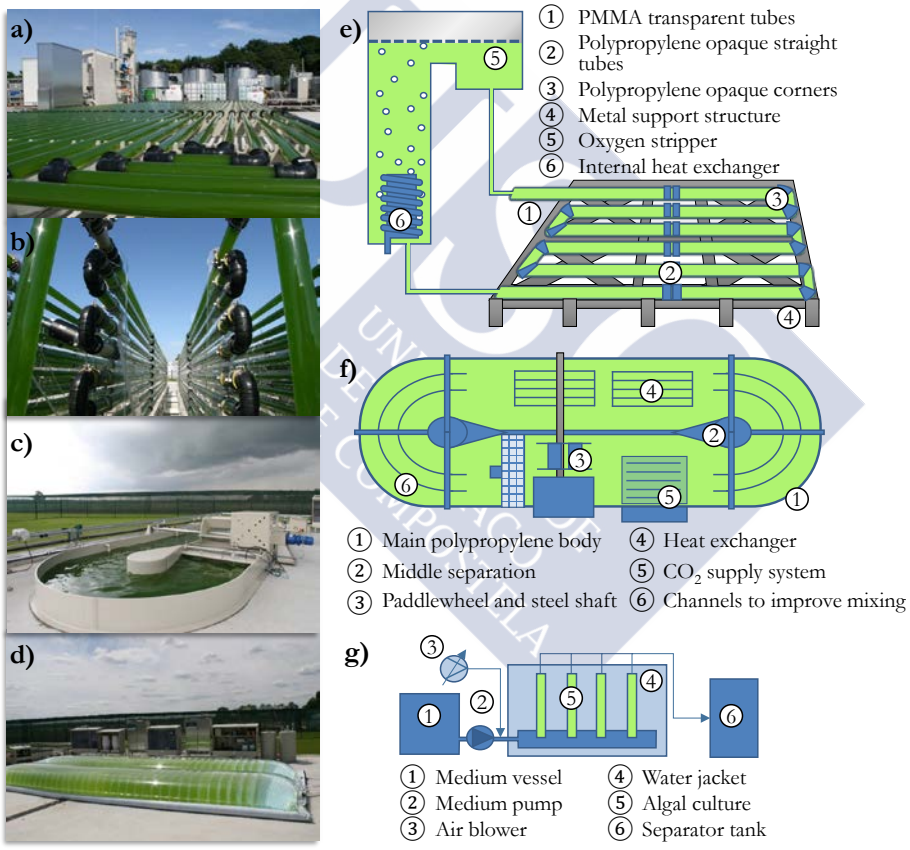



**Figure 8.1.** AlgaePARC pilot systems: a) horizontal tubular PBR, b) vertical tubular PBR, c) ORP, d) Proviapt, e) schematic drawing of tubular PBRs, f) schematic drawing of ORP and g) schematic drawing of Proviapt.
Source: Photos from Bosma et al. (2014) and Wijffels et al. (2013).

## 8.3. Comparative assessment of open ponds, tubular PBRs and flat-panel reactors operated in summer

The first stage of this LCA study presents the comparison of the environmental performance of the four largest reactors available in AlgaePARC. The design specifications for each reactor are listed in **Table 8.2**.

**Table 8.2.** Specifications of AlgaePARC analyzed reactors (Bosma et al., 2014)

| Parameter | ORP | Horizontal PBR | Vertical PBR | Flat-panel PBR (Proviapt) |
|---|---|---|---|---|
| Volume ($m^3$) | 4.73 | 0.56 | 1.06 | 0.39 |
| Expected biomass concentration ($g \cdot L^{-1}$) | 0.2-1 | 2-4 | 1-3 | 2-5 |
| Occupied ground area ($m^2$) | 25.4 | 27.0 | 31.0 | 26.9 |
| Optical path (m) | 0.20 | 0.046 | 0.046 | 0.02 |
| Illumination surface A/V ratio ($m^2 \cdot m^{-3}$) | 5 | 63.7 | 61.6 | 100 |
| Illuminated volume | 100 | 73 | 71 | 100 |

The scenario for the assessment corresponds to the operation of the reactors during the summer 2013. In order to obtain a representative evaluation and a fair comparison between the systems, the reference period in which all the reactors showed the highest daily biomass productivity was selected. This productivity was associated with a different dilution rate in each reactor.

Since the large-scale Proviapt system was not operated during the period, the cultivation parameters were estimated from extrapolations based on a smaller flat-panel PBR available in AlgaePARC outdoor facilities. The energy consumption was calculated according to a similar period in which the large system was running. Despite the deviations affecting productivities and yields due to the different scale of the system, the obtained results may serve as a first approximation for a comparison with the other systems.

### 8.3.1. Goal and scope

The main focus of the operation in the systems was to optimize the culture conditions so as to maximize the biomass production. Thus, the quantified environmental impacts refer to 1 kg of final biomass after cultivation. The selected system boundaries are presented in **Figure 8.2** and described below:

i) S1. Cleaning and sterilization: In the first stage of the process, tap water was stored in a silo (6 $m^3$) and sterilized with sodium hypochlorite (NaClO, 2 $mg \cdot L^{-1}$). The sterilized water was passed through activated carbon filters to remove hypochlorite and pumped to the systems before inoculation. In the case of the tubular PBRs, 3% of a disinfection agent (Freebac Clearoxyl®, containing hydrogen peroxide, ethanol, glycerin and water) as well as 0.5 $g \cdot L^{-1}$ plastic beads (polymethyl methacrylate, PMMA) were added. For the ORP, tap water in a quantity equivalent to three times the usable volume of the reactor was required. After the last washing, a vacuum cleaning system was applied for 1-2 h to remove all the water. The Proviapt system could be either rinsed with water or substituted by new plastic bags.

ii) S2. Preparation of culture medium: The main source of nutrients for the cultivation of *Nannochloropsis* is natural seawater. The seawater was sterilized by adding NaClO (5 $mg \cdot L^{-1}$), which was later removed with activated carbon. Then, it was passed through a cascade filter (10 μm, 5 μm and 1 μm) and supplied to the systems.

Two artificial nutrient sources were supplemented to the culture medium: sodium bicarbonate ($NaHCO_3$) and a specific medium containing iron (II) sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$), manganese (II) chloride dihydrate ($MnCl_2 \cdot 2H_2O$), zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$), cobalt (II) nitrate hexahydrate $Co(NO_3)_2 \cdot 6H_2O$, copper (II) sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$), sodium molybdate dihydrate ($Na_2MoO_4 \cdot 2H_2O$), ethylendiaminetetraacetic acid disodium salt dihydrate (EDTA-$Na_2 \cdot 2H_2O$) and sodium hydroxide (NaOH). The nitrogen source could be added in the form of urea or nitrate. In this case, the urea-based medium was considered as nitrogen source, with potassium diphosphate ($KH_2PO_4$) as the main phosphorous source.

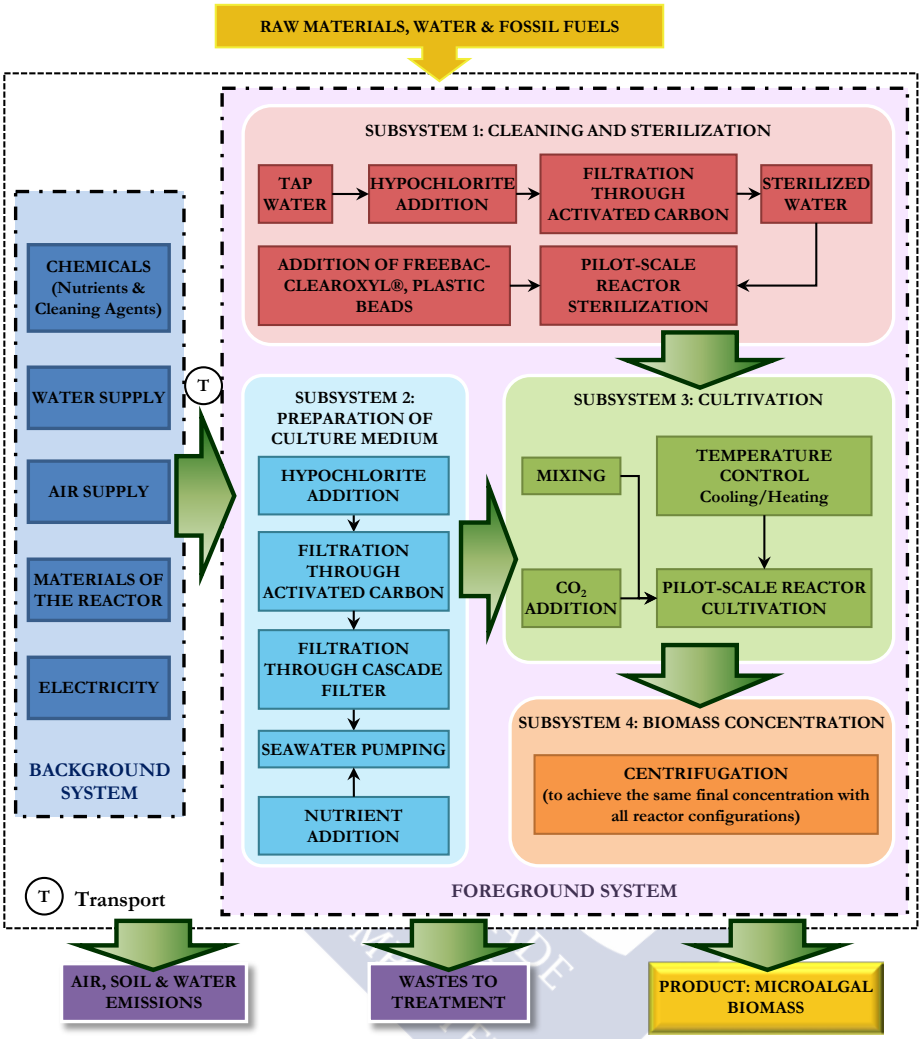



**Figure 8.2.** Process chain and system boundaries of the production of *Nannochloropsis* sp. in large pilot-scale reactors.

iii) S3. Cultivation: This stage consisted in a semi-continuous process operated in chemostat mode in which the biomass was daily harvested on variable dilution rates and conditions (light and temperature). As no source of artificial light was provided, light intensity only depended on weather conditions. To maintain the temperature close to the optimal temperature of the species, a central chiller and heater were used. Heating was applied to prevent temperatures below 20ºC and cooling

was used when culture temperature raised above 30°C. Apart from the culture medium supply from S2, the main inputs for S3 were the energy requirements for temperature control, mixing and aeration.

iv) S4. Biomass concentration: For the same operation conditions (light, temperature), the final biomass concentration depended on the cultivation system. Moreover, this concentration affects downstream processes related to harvesting and extraction of compounds from the microalgal biomass, which were excluded from the system boundaries in the study, according to the defined goal and scope. Thus, to make accurate comparisons between the behaviors of the different reactors, a final stage was taken into account, regarding the concentration of biomass coming from the reactors with lower microalgal concentration. To do so, a centrifugation step was considered to adjust the biomass concentration of all the systems to the highest value (3 $g \cdot L^{-1}$ in dry weight, DW), which was achieved by the Proviapt PBR.

### 8.3.2. Life cycle inventory, data quality and assumptions

The inventory data for the foreground system included the chemicals used for the culture medium, tap and deionized water, seawater, and electricity consumption. They consisted of primary data obtained by on-site measurements from the monitoring systems. Concerning the background system, inventory data for the production of chemicals (cleaning agents and nutrients), materials for the reactors, as well as electricity, waste treatment (sanitary and inert material landfills) and transport of the different inputs were taken from the Ecoinvent database (Frischknecht et al., 2007a). The inventory data are shown in **Table 8.3** and the Ecoinvent sources for background processes are listed in **Table 8.4**.

Tap water and chemicals for the cleaning stage were estimated by assuming a total number of three cleanings per year and four rinses per cleaning. Only two of the four rinses would require the addition of disinfection agents.

For the medium supply, seawater enriched with artificial nutrients was sterilized with hypochlorite and filtered through an activated carbon filter. The volume of culture medium was quantified according to the usable volume of each system and the dilution rate (i.e. percentage of culture volume that is daily harvested).

The materials for the equipment only included the components of the reactors. The materials for auxiliary equipment such as pumps, storage vessels and monitoring system were excluded from the system boundaries, since they have a long life span and are shared within the whole facility, so the corresponding ratio for each reactor when applying the appropriate allocation rules was considered negligible. The materials were estimated according to the dimensions of each component determined by direct measures. For the main structure of ORP and tubular PBRs a 10-year life span was considered, while 20 years were assumed for support elements (e.g. aluminum and steel components).

Regarding energy consumption, the active power used by each reactor for mixing and aeration was recorded in the monitoring software. The temperature control system (cooling and heating) was common to all the reactors, so the individual values were estimated from the cooling and heating flows that were regulated by valves. The energy for cooling and heating for each reactor was calculated by multiplying the total energy consumed by the temperature control system at each period of time by the ratio between the volume of water to the specific reactor and the total volume used for temperature control in the period.

Transport of raw materials and wastes was included in the system boundaries. For seawater, a transport distance of 180 km was considered (between Zeeland and Wageningen). For chemicals and materials, a distance of 200 km was assumed and for wastes to treatment, 50 km were considered.

Final disposal in sanitary landfill was considered for plastic materials, while other materials were disposed of in inert landfill. Wastewater was discharged to the municipal sewage network and assumed to be treated in a medium-sized treatment plant.

Due to the particular conditions of Proviapt system, the inventory for this configuration is subjected to a higher level of uncertainty than the ORP and tubular PBRs. This uncertainty is associated with the different approaches for the use and substitution of the individual bags that make up the system and the lack of energy data for the reference period. Three scenarios were evaluated:

i) Scenario 1 (Sc 1): Reuse of bags (1 year life span) and extrapolation of energy for aeration from smaller reactor in the reference period.

ii) Scenario 2 (Sc 2): Reuse of bags (1 year life span) and extrapolation of energy for aeration from the analyzed reactor in a different period.

iii) Scenario 3 (Sc 3): Substitution of bags and extrapolation of energy for aeration from the analyzed reactor in a different period.

In all the scenarios, the energy for heating and cooling was estimated from the requirements of the smaller reactor in the reference period, since the weather conditions in the other period were significantly different, so the heating and cooling requirements were not representative of the analyzed conditions.

*Allocation procedures*

In this study, no allocation procedure was required, since the assessment was only focused on biomass production in order to compare the operation of the different reactor configurations. Thus, all the environmental burdens were allocated to the total quantity of biomass harvested from each reactor.

**Table 8.3.** Inventory data for the production of *Nannochloropsis* sp. biomass in AlgaePARC pilot systems (FU=1 kg biomass produced)

| **INPUTS from TECHNOSPHERE** | | | | |
|---|---|---|---|---|
| | ORP | Horizontal tubular PBR | Vertical tubular PBR | Flat-panel PBR (Proviapt) |
| **Materials** | | | | |
| *S1. Cleaning and sterilization* | | | | |
| Tap water ($m^3$) | 1.186 | 0.159 | 0.205 | 0.090[1] |
| Bleach (kg) | 0.042 | 0.006 | 0.007 | 0.003[1] |
| Disinfectant (kg) | 0 | 2.900 | 3.749 | 0 |
| Plastic beads, PMMA (g) | 0 | 0.265 | 0.343 | 0 |
| *S2. Preparation of culture medium* | | | | |
| Bleach (kg) | 0.211 | 0.031 | 0.034 | 0.018 |
| Deionized water (L) | 8.802 | 3.258 | 3.546 | 1.847 |
| $FeSO_4 \cdot H_2O$ (g) | 30.572 | 11.317 | 12.317 | 6.415 |
| $MnCl_2 \cdot 2H_2O$ (g) | 1.743 | 0.645 | 0.702 | 0.366 |
| $ZnSO_4 \cdot 7H_2O$ (g) | 0.673 | 0.249 | 0.271 | 0.141 |
| $Co(NO_3)_2 \cdot 6H_2O$ (g) | 0.071 | 0.026 | 0.029 | 0.015 |
| $CuSO_4 \cdot 5H_2O$ (g) | 0.024 | 0.009 | 0.010 | 0.005 |
| $Na_2MoO_4 \cdot 2H_2O$ (g) | 0.247 | 0.091 | 0.099 | 0.052 |

[1] For scenarios 1 and 2 (1-year reuse of plastic bags, rinsed with water).

**Table 8.3.** Inventory data for the production of *Nannochloropsis* sp. biomass in AlgaePARC pilot systems (FU=1 kg biomass produced) (*Cont.*)

| **INPUTS from TECHNOSPHERE** | | | | |
|---|---|---|---|---|
| | ORP | Horizontal tubular PBR | Vertical tubular PBR | Flat-panel PBR (Proviapt) |
| *S2. Preparation of culture medium* | | | | |
| EDTA-$Na_2$·$2H_2O$ (kg) | 0.107 | 0.040 | 0.043 | 0.022 |
| Urea (kg) | 1.529 | 0.566 | 0.616 | 0.321 |
| $KH_2PO_4$ (kg) | 0.234 | 0.087 | 0.094 | 0.049 |
| NaOH (kg) | 0.037 | 0.014 | 0.015 | 0.008 |
| $NaHCO_3$ (kg) | 0.963 | 0.317 | 0.345 | 0.081 |
| *S3. Cultivation* | | | | |
| PMMA (kg) | 0.012 | 0.215 | 0.499 | 0 |
| Polypropylene (PP) (kg) | 1.488 | 0.135 | 0.112 | 0.375[1] (11.255[2]) |
| Steel (kg) | 0.137 | 0 | 0.025 | 0 |
| Aluminum (kg) | 0 | 0.336 | 0.226 | 0 |
| Synthetic rubber (kg) | 0.004 | 0 | 0.015 | 0 |
| Carbon dioxide, $CO_2$ ($m^3$) | 1.867 | 3.092 | 1.921 | 0 |
| **Energy** | | | | |
| Electricity from Dutch grid | | | | |
| *S1. Cleaning and sterilization* | | | | |
| Active carbon filtration (kWh) | 0.244 | 0.033 | 0.042 | 0.019[1] |
| Vacuum system (kWh) | 0.517 | 0 | 0 | 0 |
| *S2. Preparation of culture medium* | | | | |
| Filtration (kWh) | 1.661 | 0.246 | 0.268 | 0.139 |
| Culture medium mixing (kWh) | 0.095 | 0.035 | 0.038 | 0.020 |
| Culture medium pumping (kWh) | 1.401 | 0.207 | 0.226 | 0.118 |
| *S3. Cultivation* | | | | |
| Mixing (kWh) | 15.496 | 10.774 | 5.900 | 0 |
| Aeration (kWh) | 50.571 | 61.787 | 51.844 | 316.790[3] (98.718[4]) |
| Cooling (kWh) | 1.944 | 2.122 | 1.575 | 5.945 |
| Heating (kWh) | 0.467 | 0.629 | 0.499 | 0 |
| *S4. Biomass concentration* | | | | |
| Centrifugation (kWh) | 8.870 | 1.270 | 2.675 | 0 |

[1] For scenarios 1 and 2 (1-year reuse of plastic bags, rinsed with water).

[2] For scenario 3 (substitution of plastic bags after each batch).

[3] For scenario 1 (energy extrapolation from smaller Proviapt PBR in reference period).

[3] For scenarios 2 and 3 (energy extrapolation from the analyzed Proviapt PBR in a different period).

**Table 8.3.** Inventory data for the production of *Nannochloropsis* sp. biomass in AlgaePARC pilot systems (FU=1 kg biomass produced) (*Cont.*)

| | ORP | Horizontal tubular PBR | Vertical tubular PBR | Flat-panel PBR (Proviapt) |
|---|---|---|---|---|
| **INPUTS from TECHNOSPHERE** | | | | |
| **Transport** | | | | |
| *S1. Cleaning and sterilization* | | | | |
| Chemicals (tkm) | 0.008 | 0.581 | 0.751 | 0.001[1] |
| Materials (kg·km) | 0 | 0.053 | 0.069 | 0 |
| Wastes (kg·km) | 0 | 0.013 | 0.017 | 0 |
| *S2. Preparation of culture medium* | | | | |
| Seawater (tkm) | 917.151 | 135.803 | 147.809 | 76.984 |
| Chemicals (tkm) | 0.235 | 0.070 | 0.076 | 0.020 |
| *S3. Cultivation* | | | | |
| Materials (tkm) | 0.328 | 0.137 | 0.175 | 0.075[1] (2.251[2]) |
| Wastes (tkm) | 0.082 | 0.034 | 0.044 | 0.019[1] (0.563[2]) |
| **INPUTS from ENVIRONMENT** | | | | |
| Seawater ($m^3$) | 5.095 | 0.754 | 0.821 | 0.423 |
| **OUTPUTS to TECHNOSPHERE** | | | | |
| **Product** | | | | |
| Microalgal biomass (kg), in culture medium (3 $g \cdot L^{-1}$) | 1.000 | 1.000 | 1.000 | 1.000 |
| **Wastes to treatment** | | | | |
| *S1. Cleaning and sterilization* | | | | |
| Wastewater, including bleach and disinfectant ($m^3$) | 1.186 | 0.161 | 0.209 | 0.090[1] |
| Plastic beads (g) | 0 | 0.265 | 0.343 | 0 |
| *S3. Cultivation* | | | | |
| PMMA (kg) | 0.012 | 0.215 | 0.499 | 0 |
| PP (kg) | 1.488 | 0.135 | 0.112 | 0.375[1] (11.255[2]) |
| Steel (kg) | 0.137 | 0 | 0.025 | 0 |
| Aluminum (kg) | 0 | 0.336 | 0.226 | 0 |
| Synthetic rubber (kg) | 0.004 | 0 | 0.015 | 0 |

[1] For scenarios 1 and 2 (1-year reuse of plastic bags, rinsed with water).

[2] For scenario 3 (substitution of plastic bags after each batch).

**Table 8.4.** Summary of data sources for the background system of the production of *Nannochloropsis* sp. biomass in AlgaePARC pilot systems

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Energy | Electricity (Dutch electricity profile) | Ecoinvent database (Dones et al., 2007) |
| Water | Tap water<br>Deionized water | Ecoinvent database (Althaus et al., 2007) |
| Chemicals and materials for cleaning | Sodium hypochlorite<br>Hydrogen peroxide | Ecoinvent database (Althaus et al., 2007) |
| | Ethanol from ethylene | Ecoinvent database (Sutter, 2007b) |
| | Glycerine from vegetable oil | Ecoinvent database (Jungbluth et al., 2007) |
| | Plastic beads, PMMA | Ecoinvent database (Classen et al., 2007) |
| Chemicals for nutrient supply | $FeSO_4 \cdot H_2O$<br>$MnCl_2 \cdot 2H_2O$<br>$Co(NO_3)_2 \cdot 6H_2O$<br>$CuSO_4 \cdot 5H_2O$<br>$Na_2MoO_4 \cdot 2H_2O$<br>EDTA-$Na_2 \cdot 2H_2O$<br>$KH_2PO_4$ (kg)<br>NaOH (kg) | Ecoinvent database (Althaus et al., 2007) |
| | $ZnSO_4$ | Ecoinvent database (Hischier et al., 2007) |
| | Urea | Ecoinvent database (Nemecek and Kägi, 2007) |
| | $NaHCO_3$ | Ecoinvent database (Sutter, 2007a) |
| Aeration | $CO_2$ | Ecoinvent database (Althaus et al., 2007) |
| Materials for the reactor | PMMA<br>PP<br>Synthetic rubber | Ecoinvent database (Hischier, 2007) |
| | Steel<br>Aluminum | Ecoinvent database (Classen et al., 2007) |

**Table 8.4.** Summary of data sources for the background system of the production of *Nannochloropsis* sp. biomass in AlgaePARC pilot systems (*Cont.*)

| Type of involved process | Raw material/Energy | Data source |
|---|---|---|
| Transport | Truck 3.5-7.5 t | Ecoinvent database (Spielmann et al., 2007) |
| Waste treatment | Sanitary landfill<br>Inert landfill<br>Wastewater treatment plant | Ecoinvent database (Doka, 2007) |

### 8.3.3. Environmental impact assessment

The environmental profile of the analyzed systems was assessed by performing the classification and characterization stages of the LCA methodology (ISO 14040, 2006). Two methodologies were used: CML 2001, reported by the Centre of Environmental Science of Leiden University (Guinée et al., 2002) and Cumulative Energy Demand (CED) based on the method published in Ecoinvent version 2.0 (Frischknecht et al., 2007b).

The impact categories evaluated according to the CML 2001 methodology were: abiotic depletion (ADP), acidification (AP), eutrophication (EP), global warming (GWP), ozone layer depletion (ODP), human toxicity (HTP), freshwater, marine and terrestrial ecotoxicities (FEP, MEP and TEP respectively) and photochemical oxidants formation (POFP). CED methodology included three categories of non-renewable sources of energy and three of renewable sources:

- Non-renewable: fossil (NR-F, including hard coal, lignite, crude oil, etc.), nuclear (NR-N, with uranium as source), biomass (NR-B, including wood and biomass from primary forests).
- Renewable: biomass (R-B, including wood, food products, biomass from agriculture); wind, solar and geothermal (R-WSG); and water (R-HYD, including run-of river hydropower and reservoir hydropower).

The software SimaPro 7.3 was used for the computational implementation of the inventories (Goedkoop et al., 2008). The characterization results for the cultivation of *Nannochloropsis sp.* in the evaluated systems are listed in **Table 8.5** for CML 2001 impact categories and in **Table 8.6** for CED categories.

**Table 8.5.** Environmental impact assessment results (characterization step) for the production of *Nannochloropsis sp.* in AlgaePARC pilot reactors according to CML 2001 methodology

| Impact category | Unit | ORP | Horizontal PBR | Vertical PBR | Proviapt - Sc 1 | Proviapt - Sc 2 | Proviapt - Sc 3 |
|---|---|---|---|---|---|---|---|
| ADP | kg Sb eq | 3.566 | 0.957 | 0.922 | 2.093 | 0.867 | 1.229 |
| AP | kg $SO_2$ eq | 1.691 | 0.363 | 0.373 | 0.500 | 0.257 | 0.331 |
| EP | kg $PO_4^{-3}$ eq | 0.561 | 0.171 | 0.160 | 0.405 | 0.161 | 0.216 |
| GWP | kg $CO_2$ eq | 497.624 | 130.448 | 125.930 | 272.633 | 114.608 | 138.107 |
| ODP | g CFC-11 eq | 0.068 | 0.012 | 0.013 | 0.014 | 0.008 | 0.008 |
| HTP | kg 1,4-DB eq | 94.933 | 42.003 | 37.159 | 51.826 | 22.284 | 24.134 |
| FEP | kg 1,4-DB eq | 65.587 | 23.859 | 21.555 | 60.223 | 23.556 | 76.326 |
| MEP | kg 1,4-DB eq | 49.807 | 16.518 | 15.068 | 39.516 | 15.740 | 43.278 |
| TEP | kg 1,4-DB eq | 0.025 | 0.006 | 0.006 | 0.009 | 0.004 | 0.004 |
| POFP | kg $C_2H_4$ eq | 0.061 | 0.015 | 0.015 | 0.020 | 0.010 | 0.015 |

**Table 8.6.** Environmental impact assessment results (characterization step) for the production of *Nannochloropsis sp.* in AlgaePARC pilot reactors according to CED methodology (results for all categories in MJ)

| Impact category | ORP | Horizontal PBR | Vertical PBR | Proviapt - Sc 1 | Proviapt - Sc 2 | Proviapt - Sc 3 |
|---|---|---|---|---|---|---|
| NR-F | 7557 | 1901 | 1857 | 3875 | 1662 | 2450 |
| NR-N | 786 | 221 | 210 | 468 | 193 | 241 |
| NR-B | 0.032 | 0.007 | 0.007 | 0.005 | 0.004 | 0.004 |
| R-B | 50.4 | 32.8 | 27.3 | 122 | 41.1 | 43.1 |
| R-WSG | 12.4 | 8.37 | 6.92 | 31.6 | 10.6 | 10.6 |
| R-HYD | 126 | 30.9 | 29.4 | 24.3 | 15.2 | 18.7 |
| **TOTAL** | 8532 | 2195 | 2131 | 4521 | 1922 | 2764 |

According to **Figure 8.3a**, ORP is the production system with highest contributions to all the evaluated categories (CML 2001 methodology) except for FEP, which presents a higher impact when considering scenario C of Proviapt system.

Horizontal and vertical tubular PBRs have remarkably lower impacts than ORP in all categories (with reductions of impact between 60% and 80% with respect to the ORP). Both tubular PBRs have similar environmental profiles that differ in less than 10% for all categories except for HTP (12%). Although the performance of the vertical PBR seems slightly more efficient than the horizontal PBR, the results are not conclusive, as the assumptions and the short length of the period cause an important uncertainty that would require further sensitivity assessments. Moreover, in some categories the horizontal PBR has lower contributions than the vertical one (AP, ODP and POFP).

In the case of Proviapt reactor, the results strongly depend on the considered scenario. When analyzing scenario Sc 1 (energy extrapolated from pilot-scale data), the environmental performance is significantly less efficient than the profile observed for tubular reactors and nearly as high as the impacts obtained for the ORP. However, the calculated energy consumption related to mixing and aeration seems too high in comparison with the other systems, which suggests that the extrapolation from data of a smaller reactor (55 L reactor instead of the analyzed 350 L pilot-scale reactor) may be inaccurate in this case.

Therefore, two alternative scenarios were evaluated, substituting the estimated energy consumption by the value obtained according to an average active power for a different time period of the pilot-scale Proviapt with the same recirculation flow. For Proviapt - Sc 2, which considers the same approach as Sc 1 regarding the reuse of plastic bags, the Proviapt system presents the lowest environmental impacts in most categories except for FEP and MEP. Thus, characterization results of Sc 2 range between 12% and 36% of the impacts of ORP system. If plastic bags are replaced before starting the operation instead of cleaned and reused (Sc 3), the environmental profile is less favorable than in the previous scenario for all categories. Six of the ten evaluated categories also show higher impacts for Proviapt - Sc 3 than for the two tubular systems and its impact to FEP is even 16% higher than the value found for ORP cultivation system.

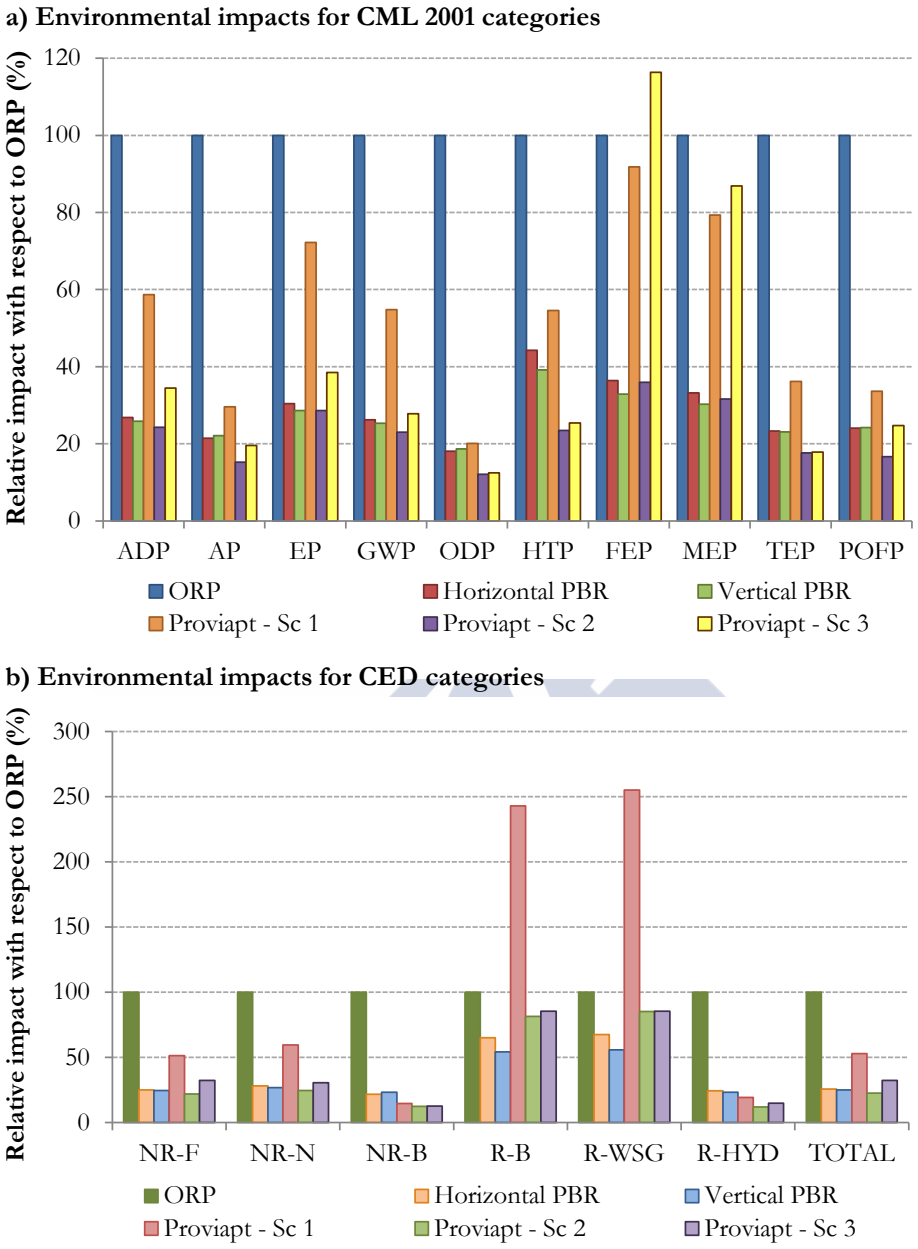



**Figure 8.3.** Relative environmental profile of the compared cultivation systems with respect to ORP (index = 100) for 1 kg *Nannochloropsis* sp. biomass as FU, according to a) CML 2001 and b) CED impact categories.

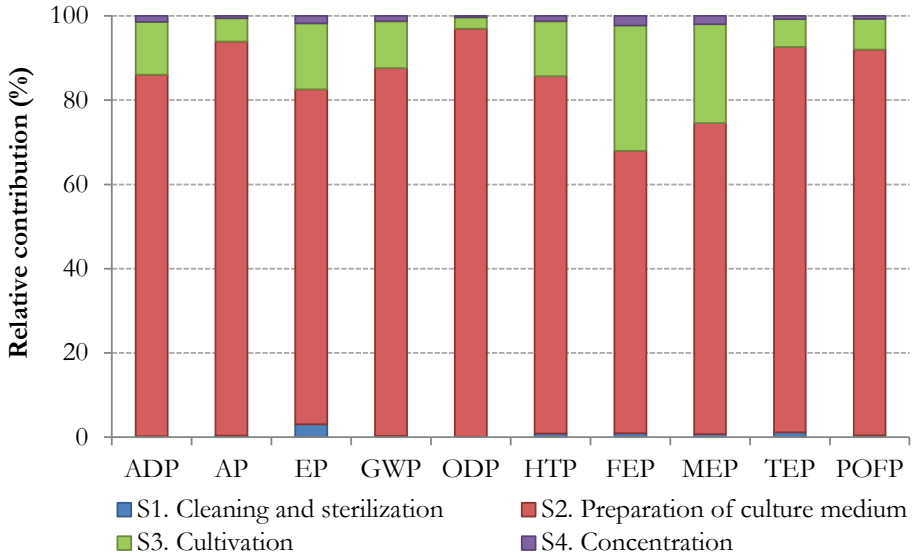
A similar behavior is observed when considering CED methodology (**Figure 8.3b**). Again, ORP is the system with the highest environmental impact in most categories. Nevertheless, Proviapt - Sc 1 has remarkably higher contributions in the categories of renewable energy from biomass and renewable solar, wind and geothermal energy. Both tubular PBRs have comparable energy requirements (deviation between the two systems lower than 20% in all categories). Their energy demand ranges between 22% and 68% of the values associated with the ORP depending on the category, while the total CED is about 25%. Proviapt system has again a very variable behavior that is determined by the considered approach. Proviapt - Sc 1 present the highest contributions of the three evaluated scenarios, especially in the case of renewable energies, although the global CED is 56% lower than the energy of the ORP. Proviapt - Sc 2 has the lowest energy demand of all the alternatives in most cases, including total CED, whereas Sc 3 has a worse environmental profile especially in terms of non-renewable energies.

❖ **Identification of hot spots for ORP**

According to **Figure 8.4a**, the preparation of the culture medium (S2) is the main responsible for the contributions to the evaluated impact categories (from 67% to 97%). Between 93 and 99% of this contribution (depending on the impact category) is due to transport, especially linked to seawater requirements for the culture medium. Thus, 99.97% of the total transport associated with this stage corresponds to seawater. Among the secondary contributions, only cultivation (S3) has a remarkable effect in two categories: FEP (30%) and MEP (24%). Cleaning (S1) and biomass concentration (S4) have minor contributions (below 3%) to all the assessed categories.

The breakdown of the contributions of the involved processes is depicted in **Figure 8.4b**. In accordance with the results per stage, transport is the main hot spot related to the process in ORP, with more than 70% of the contribution to all impact categories except for FEP (64%). Most of this impact is due to the transport of seawater for the culture medium from the coast (5 t seawater transported per kg biomass produced, distance of 180 km). Electricity was the only significant secondary process with 16% of EP, 21% of FEP and 18% of MEP.

**a) Relative contributions of production in ORP (CML 2001) per stage**




**b) Relative contributions of production in ORP (CML 2001) per involved process**

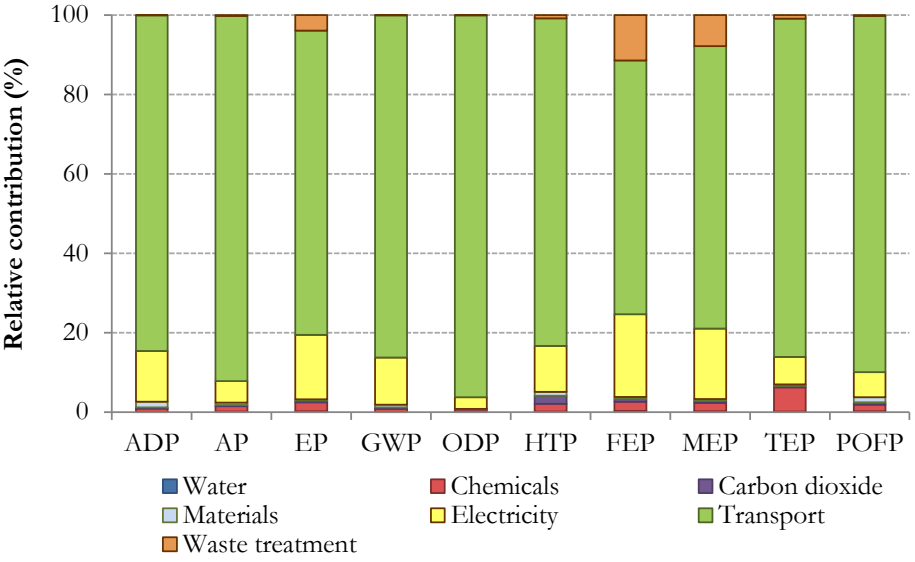



**Figure 8.4.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the ORP system to each impact category of CML 2001 methodology per a) stage and b) involved process.

Regarding CED methodology, **Figure 8.5** shows that preparation of the culture medium is also the main contributor to most CED categories (more than 85% to all non-renewable sources and renewable hydropower). Only S3 has a relevant effect associated with renewable sources (specifically biomass, and the combination of wind, solar and geothermal), which is linked to the production of electricity to meet the energy requirements. Again, S1 and S4 are secondary contributors with less than 7% of impact in all the categories.

Transport is the main activity associated with the consumption of non-renewable sources, including 87% energy from fossil fuels, 84% from nuclear sources, and 75% from biomass. The main consumption of renewable energy from biomass (60%), as well as energy from wind, solar and geothermal origin (63%) is related to the production of electricity, especially associated with cultivation (84%) and particularly to mixing and aeration (23% and 74% of the energy consumed in this stage, respectively). The main contributor to hydropower consumption is again transport (95%). Thus, the total energy demand is mainly associated with transport, which requires more than 80% of the total CED of the system. As in the case of CML 2001 indicators, this contribution is especially due to the large amount of seawater to be transported from the coast to the facility.

❖ **Identification of hot spots for tubular PBRs**

According to **Figures 8.6a** and **8.7a**, S2 and S3 are the main contributors to all the evaluated CML categories for both tubular reactors. In the case of the horizontal tubular PBR, S2 is the main stage affecting AP (66%), ODP (80%), TEP (64%) and POFP (58%), whereas S3 is the major cause of EP (56%), HTP (61%), FEP (68%) and MEP (63%). Both stages are responsible for nearly all the impact to ADP (48% of the contribution from S2 and 50% from S3).

For the vertical PBR, S2 is the main issue in terms of ADP (54%), AP (70%), GWP (56%), ODP (84%), TEP (70%) and POFP (63%). S3 dominates the impacts in EP (49%), HTP (50%), FEP (61%) and MEP (55%), and also presents significant contributions in AP (28%), GWP (41%), TEP (26%) and POFP (34%). As for the ORP system, cleaning and biomass concentration secondary stages with less than 10% of impact in all categories except for HTP in the vertical system, which has 13% impact related to cleaning.

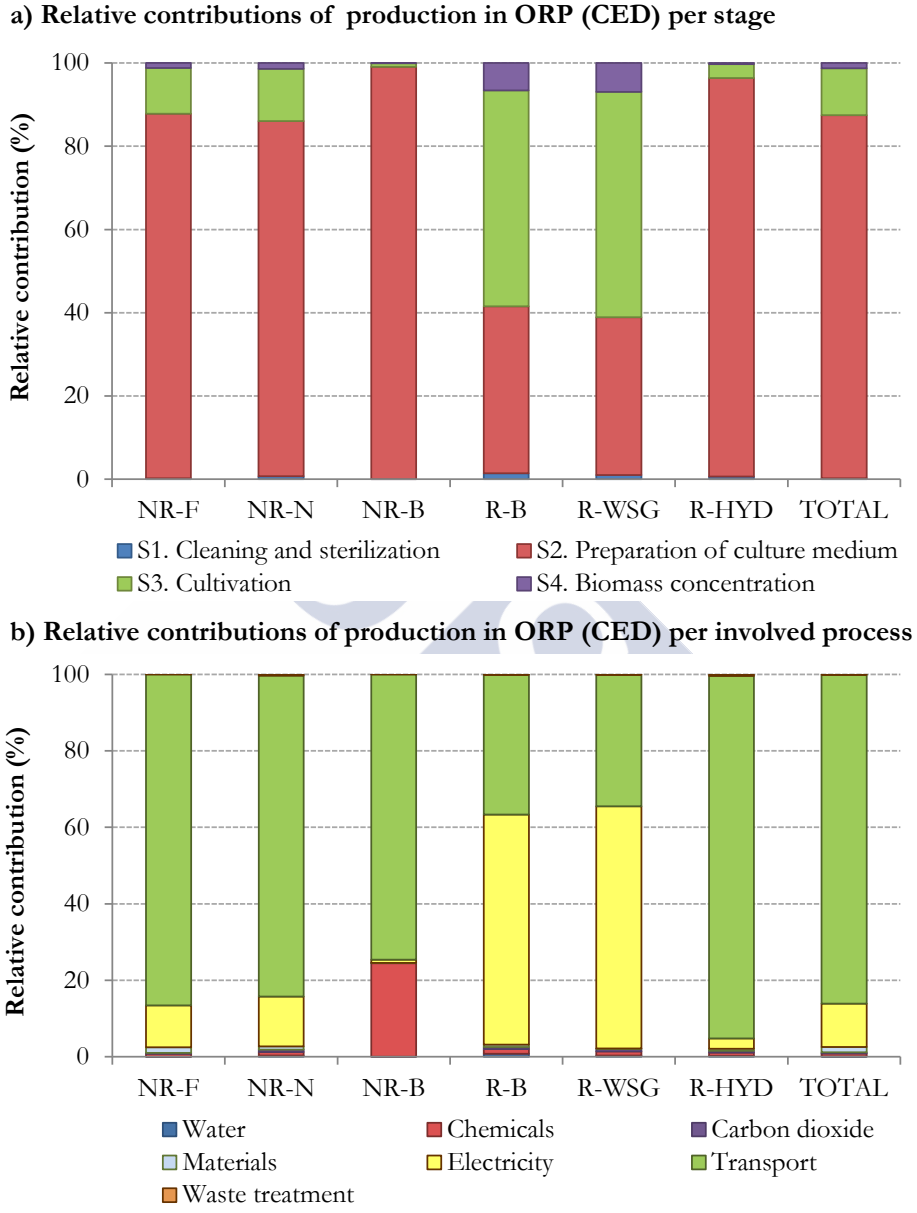



**Figure 8.5.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the ORP system to each impact category of CED methodology per a) stage and b) involved process.

The impacts to most CML categories are related to the production of electricity and the transport (**Figures 8.6b** and **8.7b**). For the horizontal system, the contributions of electricity range from 24% to 54% in all categories except for ODP (16%). Transport contributes between 26% and 80% depending on the considered category.

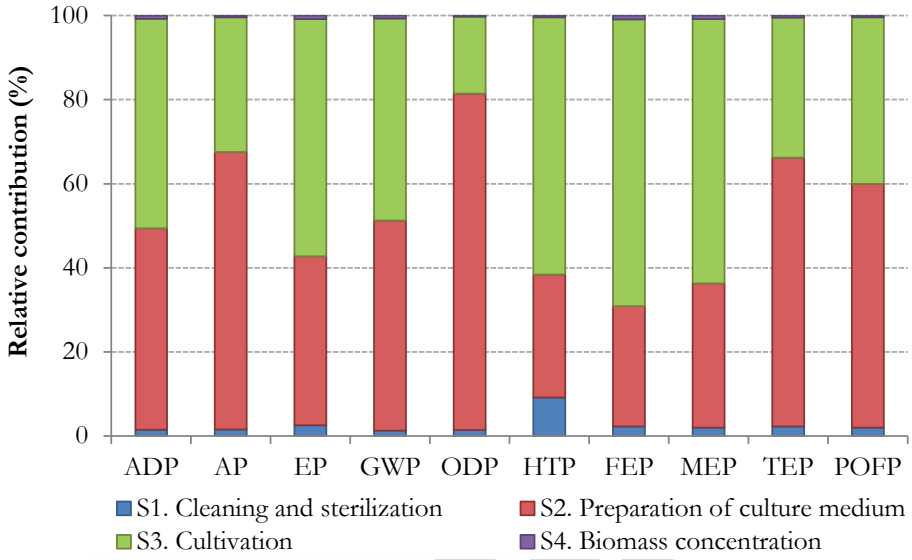
The high electricity consumption is specifically related to S3, which consumes 98% of the total electricity requirements. Mixing and aeration are the main reason for this consumption. They require 14% and 82% of the electricity consumed during *Nannochloropsis* sp. cultivation, respectively.

Concerning transport, as in the case of ORP system, the main contributor to the environmental impact is the large amount of seawater to be transported, which involves 99% of the contribution of transport. Among the secondary processes, the only activity with a significant contribution is the production of materials, which accounts for 29% of HTP. Most of this contribution (up to 99%) is due to the production of aluminum for the supporting structures.

Transport is the main hot spot of the production in the vertical PBR in all categories except for FEP and MEP. The contributions from transport range from 35% in HTP up to 80% in ODP, mainly associated with seawater transport for the preparation of culture medium in S2.

The production of electricity needed in the vertical system is responsible for 48% of the total impact to FEP and 45% of the impact to MEP. It is also associated with 38% of the impacts to ADP, 43% to EP, 36% to GWP and 23% to HTP and TEP. Among the secondary contributors, the most remarkable impacts are related to the production of materials for the reactor (23% HTP and 12% POFP) and chemicals (15% HTP, especially related to components of the disinfectant used for cleaning).

**a) Relative contributions of production in horizontal tubular PBR (CML 2001) per stage**




**b) Relative contributions of production in horizontal tubular PBR (CML 2001) per involved process**

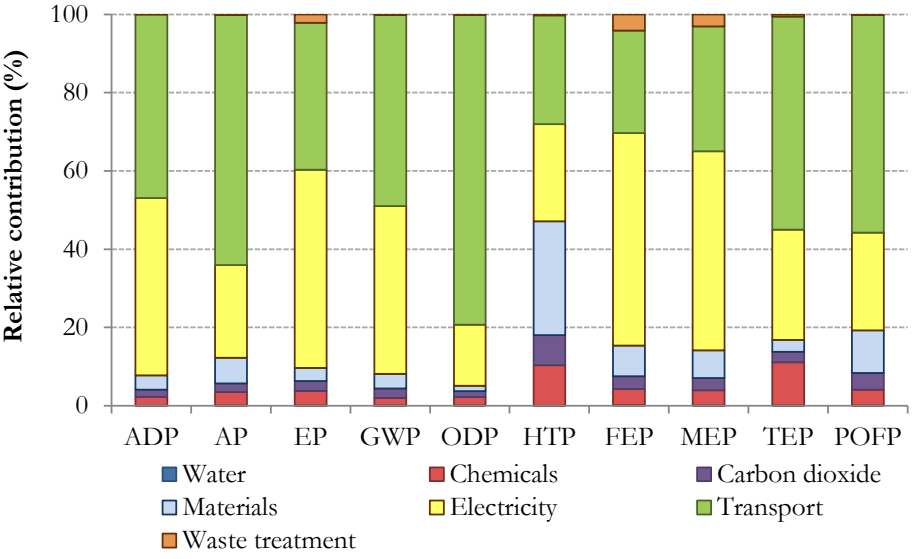
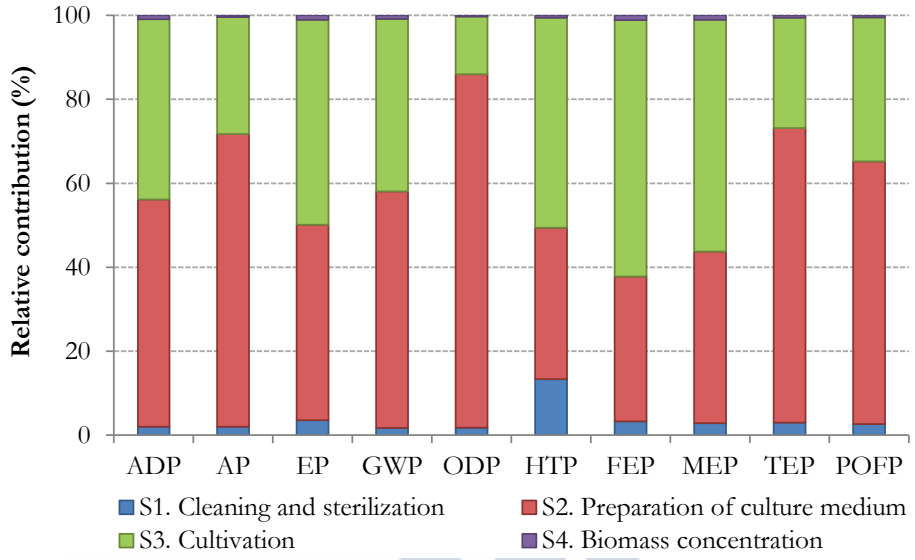



**Figure 8.6.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the horizontal tubular PBR to each impact category of CML 2001 methodology per a) stage and b) involved process.

**a) Relative contributions of production in vertical tubular PBR (CML 2001) per stage**




**b) Relative contributions of production in vertical tubular PBR (CML 2001) per involved process**

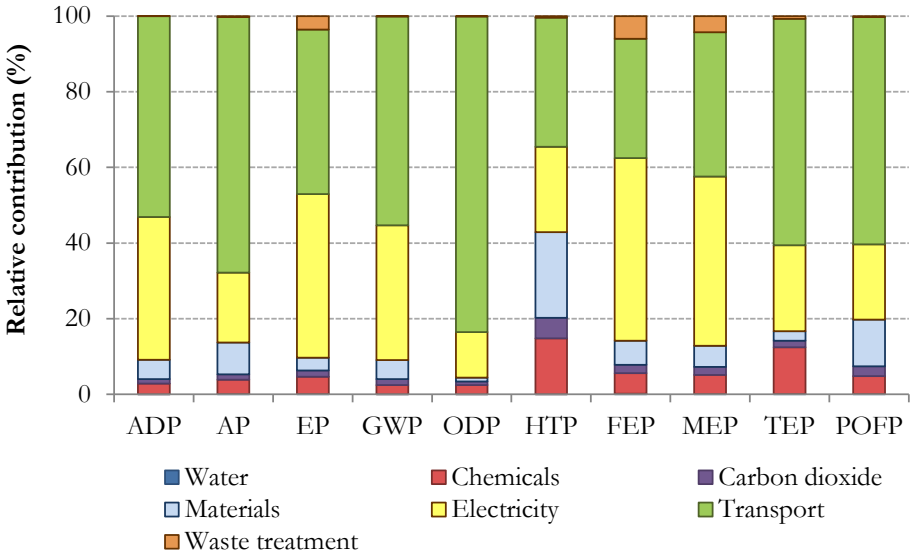



**Figure 8.7.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the vertical tubular PBR to each impact category of CML 2001 methodology per a) stage and b) involved process.

As shown in **Figures 8.8** and **8.9**, CED results match with the outcomes of CML methodology and follow the same trends in the two tubular reactors. Again, S2 and S3 are the main subsystems contributing to CED categories.

For the horizontal PBR, S2 is the main cause of the consumption of non-renewable biomass sources (93%), whereas S3 has the highest consumption of renewable energies from biomass, as well as wind, sun and geothermal sources (nearly 90%). In other categories, as well as for the total energy balance, both processes have contributions between 45% and 60% of the total CED.

In the vertical PBR, S2 dominates the contribution associated with non-renewable energies (58% fossil fuels, 52% nuclear and 94% biomass), as well as renewable hydropower (67%) and total CED (57%). With respect to renewable energy from biomass and from wind, solar and geothermal sources, S3 is the major factor responsible with 84% and 85% of the contributions respectively.

Regarding the involved processes, electricity and transport are the main causes of impact, due to the factors already reported for CML 2001 categories. Thus, transport involves consumption of 51% of the energy from fossil fuels required throughout the process, 44% of nuclear energy, as well as 58% of energy from hydropower facilities and 49% of the total energy demand. The highest contributions of electricity are related to the consumption of renewable energy from biomass (87%) and from wind, solar and geothermal sources (88%). Other remarkable contributions of electricity are linked to the consumption of energy from fossil fuels (41% of total NR-F) and from nuclear sources (44% of total NR-N), as well as the indicator of total CED (42%). The production of chemicals has a relevant contribution in terms of non-renewable energy from biomass, especially the production of potassium phosphate. The production of materials also involves consumption of renewable hydropower (25% of total R-HYD).

Regarding the vertical PBR, transport and related processes dominate the contribution to most categories, including all non-renewable sources (57% fossil fuels, 51% nuclear mainly and 52% biomass), as well as renewable hydropower (66%) and total CED (56%). The production of electricity also has a significant contribution, especially in terms of renewable energies (85% biomass and 86% wind, solar and geothermal, with a final contribution of 35% to total CED).

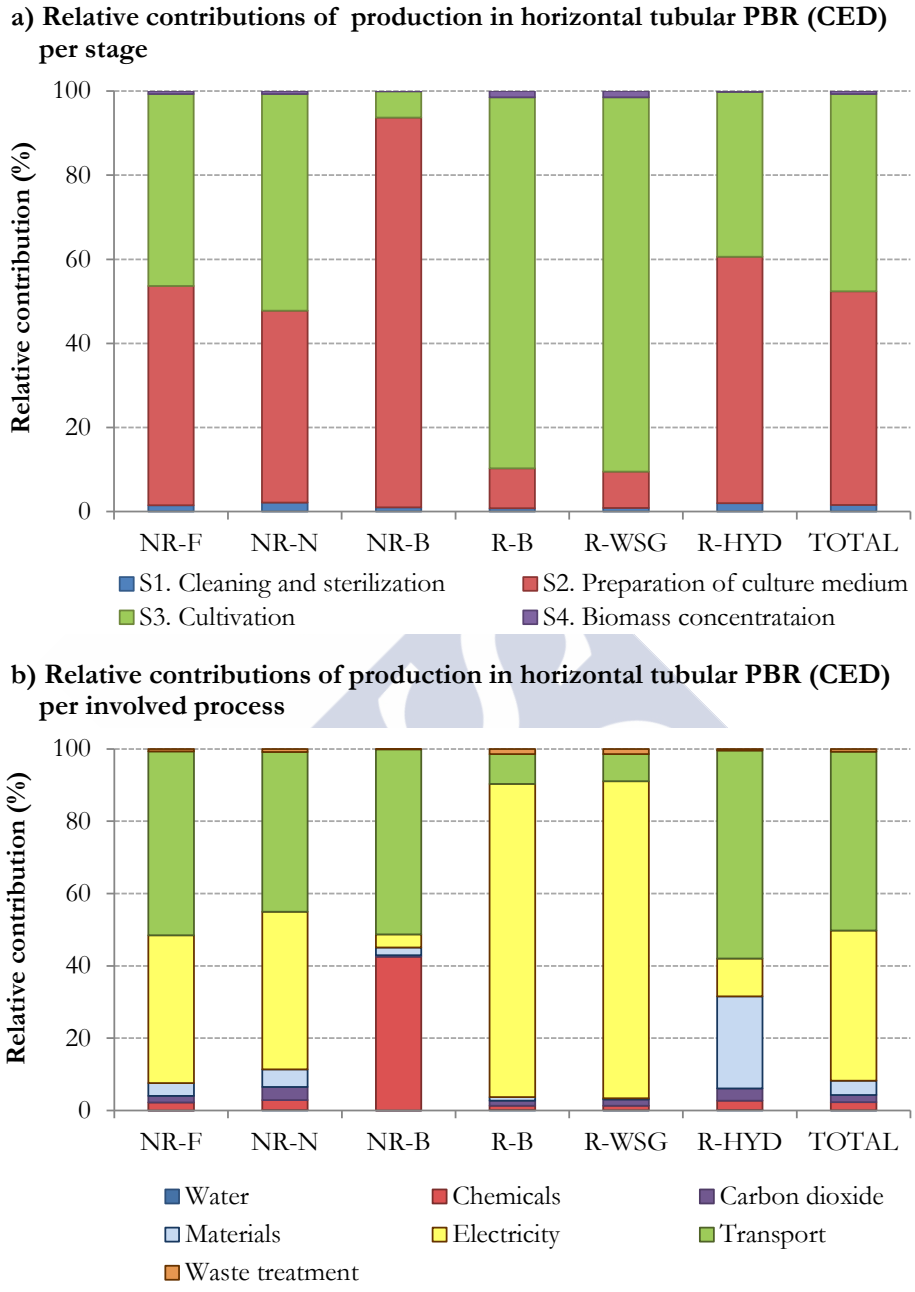
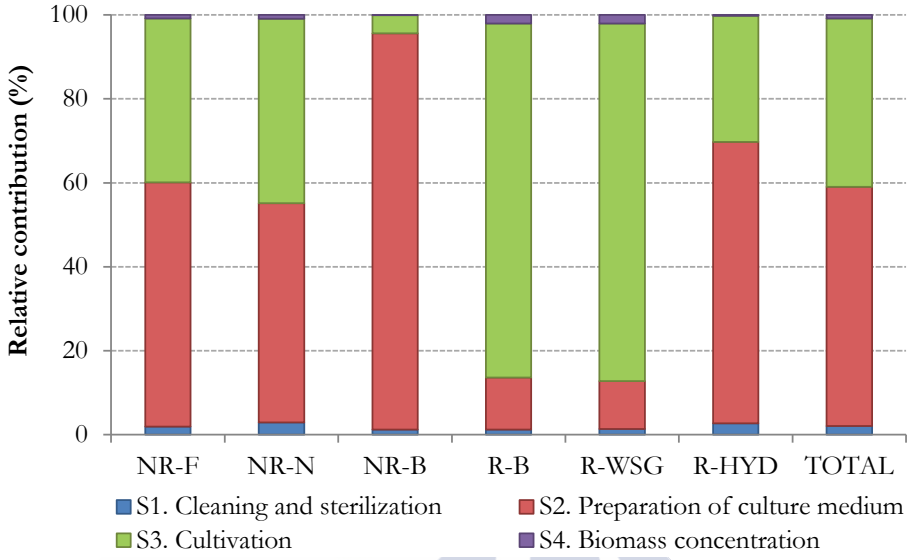



**Figure 8.8.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the horizontal tubular PBR to each impact category of CED methodology per a) stage and b) involved process.

**a) Relative contributions of production in vertical tubular PBR (CED) per stage**




**b) Relative contributions of production in vertical tubular PBR (CED) per involved process**

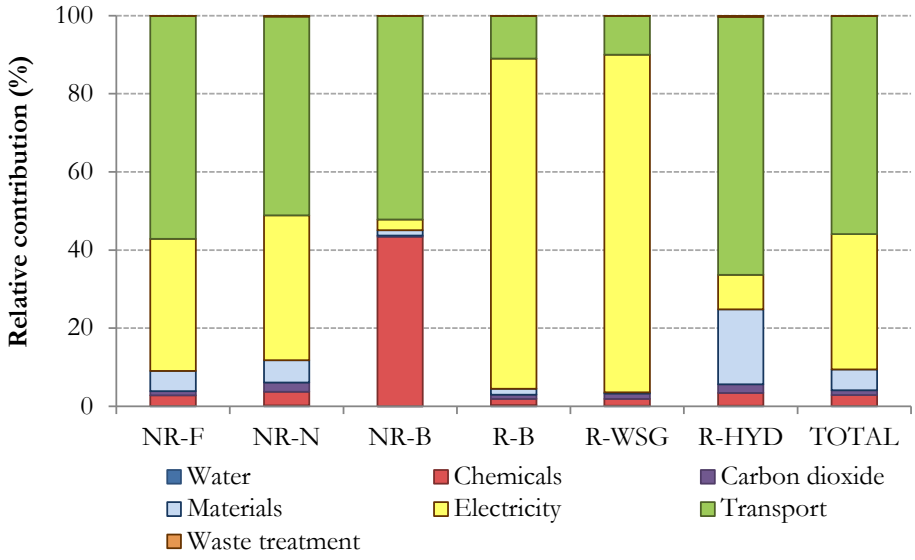



**Figure 8.9.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the vertical tubular PBR to each impact category of CED methodology per a) stage and b) involved process.

❖ **Identification of hot spots for the flat-panel (Proviapt) PBR**

The environmental performance of Proviapt system in the three evaluated scenarios is presented in **Figure 8.10** for CML 2001 methodology. The three scenarios show similar trends in most categories. S3 is the main hot spot, with contributions between 59% and 94% in Sc 1, 32% and 84% in Sc 2 and 34% and 95% in Sc 3. The effect of S2 depends on the considered scenario. In Sc 1, this stage constitutes a secondary contribution that is only relevant in terms of AP (27%), ODP (41%), TEP (23%) and POFP (23%). On the other hand, Sc 2 is strongly affected by S2, with effects ranging from 16% (FEP) up to 68% (ODP). This influence is also observed in Sc 3, with contributions of 41% (AP), 47% (TEP) and 66% (ODP), though other categories such as EP, FEP or MEP present rather limited effects (18%, 5% and 7% respectively). The impact of S1 is almost negligible in all scenarios (below 1%), due to the low amount of water required, which associated with the small volume of the Proviapt reactor compared to the ORP and tubular PBRs. S4 is not needed to compare the environmental performance of this reactor, since it corresponds to the highest biomass concentration, used as the reference value for all systems.

The production of electricity is the key issue to take into account in Sc 1 and Sc 2, with contributions between 59% and 90% for the first and 32% to 75% of impacts for the second. For Sc 1, transport constitutes a relevant secondary contribution only for the category of ODP (40%), whereas this activity clearly affects most impact categories in Sc 2 (e.g. 51% to AP, 67% to ODP, 41% to TEP or 45% to POFP). Although transport has virtually the same absolute impact in both scenarios, the lower energy consumption of Sc 2 leads to a more remarkable effect in relative terms for other processes such as transport. Sc 3 shows a quite irregular behavior, depending on the considered category. The production of electricity is the main hot spot in several categories, including ADP, EP, GWP, HTP and TEP, with contributions between 48% and 59%. The production of materials has significant contributions to ADP (30%), AP (21%) and POFP (32%), whereas waste treatment is responsible for 23% of EP, 71% of FEP and 65% of MEP. The increase of impacts from activities related to the production of materials and waste treatment is mainly due to the large amount of polypropylene needed to substitute the individual bags in each batch.

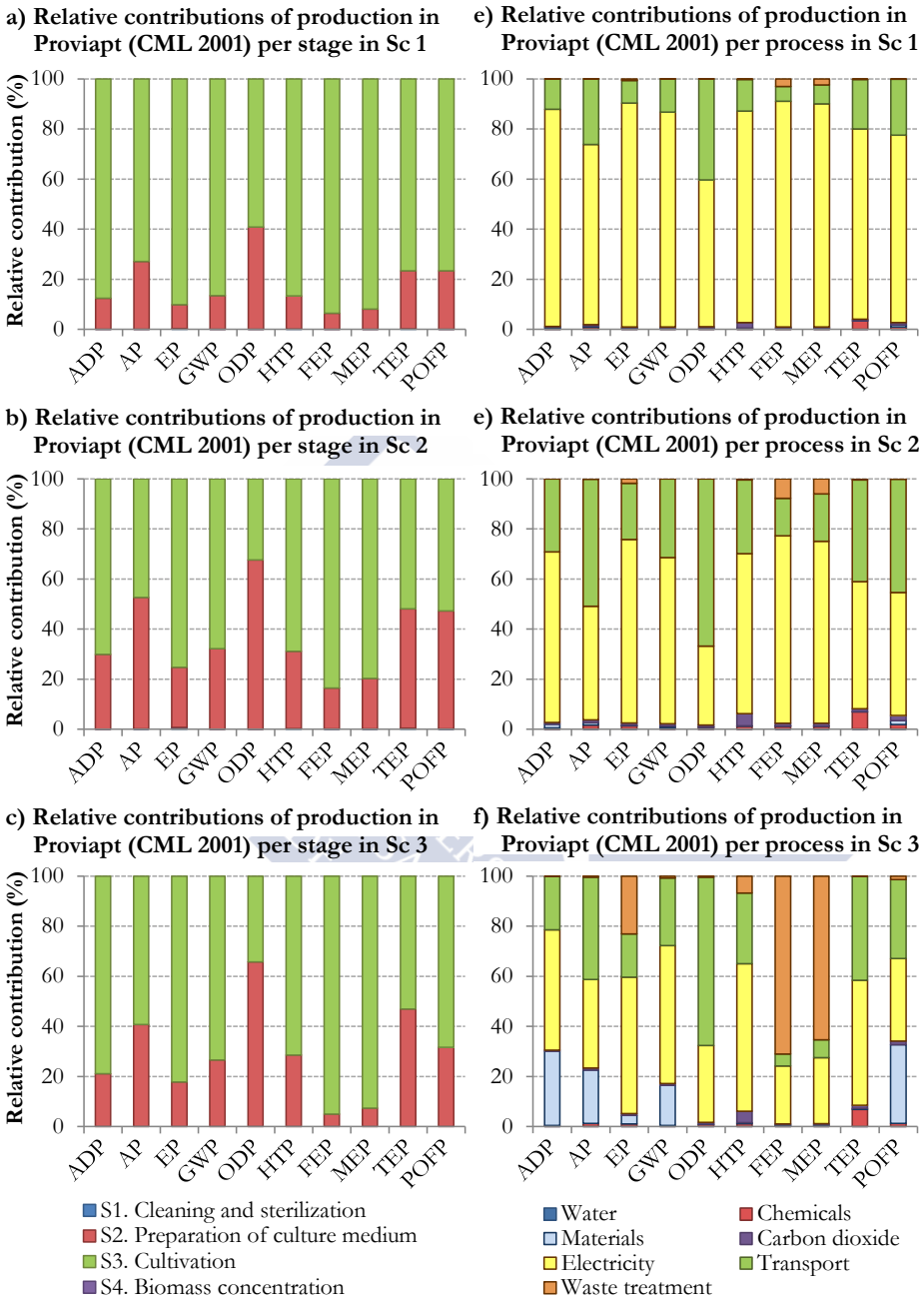



**Figure 8.10.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the flat-panel PBR (Proviapt) to each impact category of CML 2001 methodology per stage (a, b and c) and involved process (d, e and f).

As in the case of CML methodology, the environmental profiles of Proviapt scenarios when considering CED indicators are relatively similar in most categories (**Figure 8.11**). S3 (cultivation) is the major contributor to most categories, with more than 65% of CED related to non-renewable fossil energy, more than 70% of non-renewable nuclear, and more than 95% of renewable energies from biomass and from wind, solar and geothermal origin. Moreover, this subsystem is responsible for 86% of total CED in Sc 1, 67% in Sc 2 and 77% in Sc 3. Whereas contributions to renewable hydropower in Sc 1 are also dominated by cultivation (58%), S2 (preparation of culture medium) is responsible for more than 77% of the consumption of non-renewable energy from biomass in this scenario, and constitutes the main hot spot in the two mentioned categories for Sc 2 (91% of non-renewable energy from biomass and 67% of renewable hydropower) and Sc 3 (89% and 55% respectively).

Regarding the involved processes, the production of electricity is again the major factor for most contributions. All the categories except for non-renewable energy from biomass and renewable from water are dominated by this process (from 56% up to 98% for Sc 1, 54%-96% for Sc 2 and 44%-96% for Sc 3, depending on the considered category). In Sc 1, electricity is also the main contributor affecting renewable energy from hydropower facilities (56%), whereas non-renewable energy from biomass is primarily due to transport (43%). For Sc 2 and Sc 3, transport is the main cause of CED related to non-renewable energy from biomass (50% for Sc 2 and 51% for Sc 3), as well as renewable energy from water (66% for Sc 2 and 56% for Sc 3). The need for non-renewable energy from biomass for the production of chemicals (for the culture medium) also represents a significant contribution (35% for Sc 1, 41% for Sc 2 and 40% for Sc 3). In addition, the production of materials also has a relevant effect related to non-renewable fossil (32%) and nuclear (20%) sources, as well as total CED (31%).

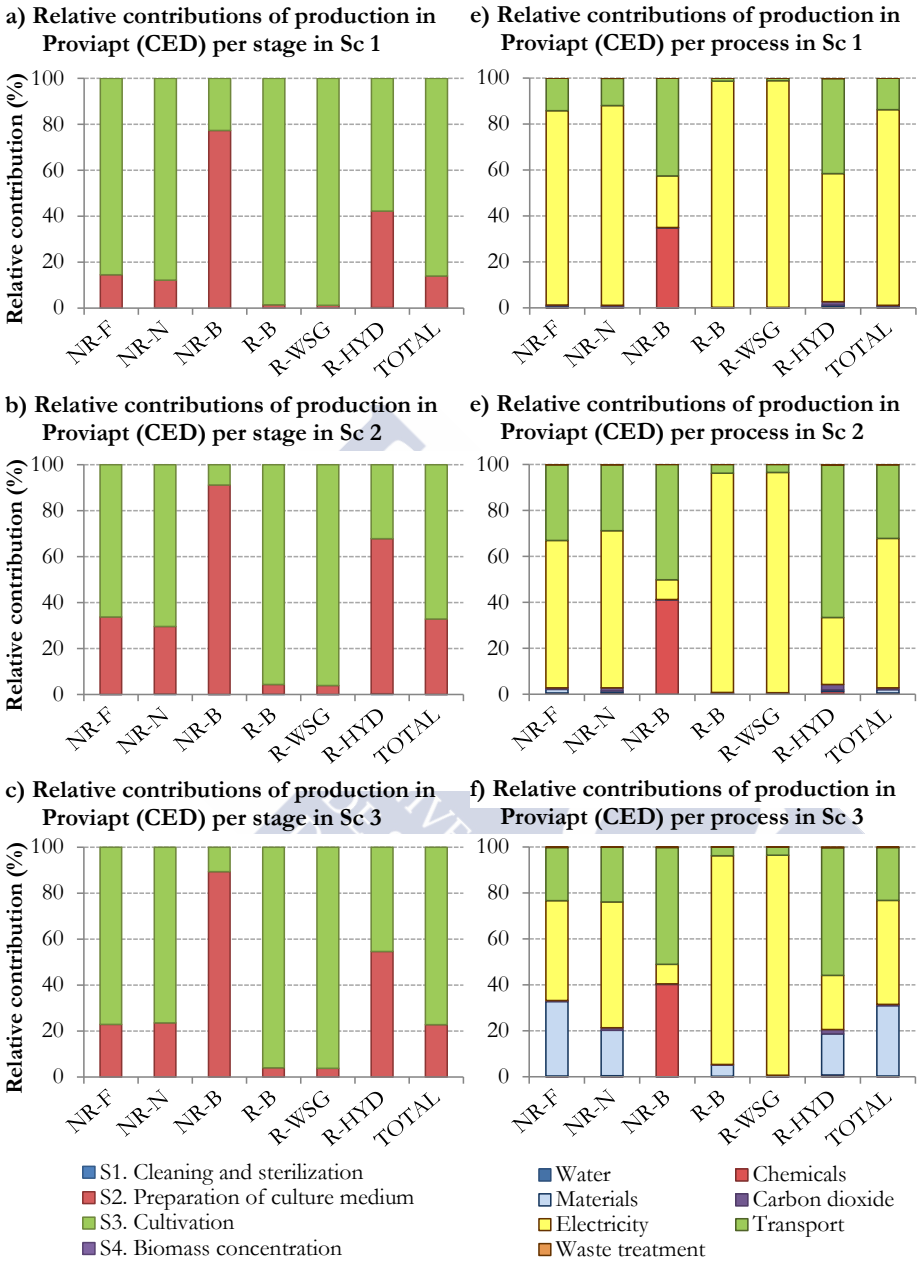



**Figure 8.11.** Relative contributions of the pilot-scale production of *Nannochloropsis* sp. in the flat-panel PBR (Proviapt) to each impact category of CED methodology per stage (a, b and c) and involved process (d, e and f).

### 8.3.4. Discussion and recommendations

The results from the previous section allowed the comparison between AlgaePARC pilot systems for the production of *Nannochloropsis* biomass and the identification of hot spots or main issues affecting the impact categories.

According to the environmental impact assessment, the ORP system was identified as the less efficient alternative for the process, with much higher impacts than other options such as cultivation in horizontal or vertical tubular PBR. This impact is mainly associated with the inputs for S2 (preparation of culture medium), especially due to the large amount of seawater and its transport from the coast. Seawater requirements are not only related to the large volume (ORP is the largest of the four assessed systems) but rather to the low concentration of biomass obtained in this reactor, which means that a large volume of culture medium is required to produce the same amount of biomass as other system with more concentrated biomass. Among the secondary contributions of ORP profile, electricity related to S3 (cultivation) was the most remarkable issue to take into account.

In the case of tubular PBRs, most of the impact is also distributed between S2 and S3, again related to transport and electricity. Among the activities with secondary contributions, only the production of chemicals for the medium and materials for the PBRs have relatively significant effects in some categories.

Regarding Proviapt system, three scenarios were proposed due to the uncertainty in the inventory data. The environmental profile of this strategy depends on the considered scenario and the contributions are mainly associated with the electricity consumption in S3, although other activities such as transport or production of materials remarkably affect some categories.

Thus, transport and electricity are the major hot spots common to all the production systems and are mainly related to subsystems S2 (culture medium) and S3 (cultivation). However, the inventory data for the assessment correspond to a very limited period of time with specific operational and weather conditions, so the results may be subjected to a considerable uncertainty. For this reason, sensitivity analyses concerning the key hot spots are presented below, in order to evaluate the effect of possible changes in these parameters.

❖ **Effect of seawater transport elimination**

The characterization results show that seawater transport from the coast to the facility has a remarkable contribution to the environmental profile for all the systems. However, the transport is unlikely to have a significant effect on a large-scale process for two reasons: i) the facility could be placed in a coastal location near the water source, and ii) most seawater could be recycled after biomass harvesting and fed back into the reactor after the addition of fresh nutrients.

This sensitivity assessment evaluates the change caused by the elimination of seawater transport in a hypothetical scenario that would be more representative of a large-scale microalgal process (**Figures 8.12** and **8.13**). According to the results, the elimination of seawater transport entails important reductions of impact in most impact categories for all the systems. However, the significance of the improvement depends on the system.

As expected, the highest impact reductions correspond to the ORP system, with a decrease ranging between 70% and 90% for all the categories except for FEP (64%), R-B (37%) and R-WSG (34%). This is due to the larger volume of culture medium required for cultivation in ORP system, compared to the other systems (mainly related to the low biomass concentration obtained in the ORP).

Regarding the tubular systems, the absence of seawater transport leads to 25%-80% lower impacts in all CML categories, together with around 50% less CED in terms of non-renewable energies as well as R-HYD and total CED. The reductions associated with R-B and R-WSG are rather limited (less than 11% for the two tubular PBRs).

Although all Proviapt scenarios show lower improvements than ORP and tubular PBR (as a result of lower volume of culture medium required to produce the same amount of biomass), the change in transport would allow reductions between 20% and 60% in most CML and CED categories, especially for Sc 2 and Sc 3. The limited reductions for Sc 1 (between 6% and 26% for all CML categories except for ODP, and below 15% for most CED indicators) are due to the less significant effect of transport in relative terms, compared to the high contributions of electricity (previously discussed in section 8.3.3).

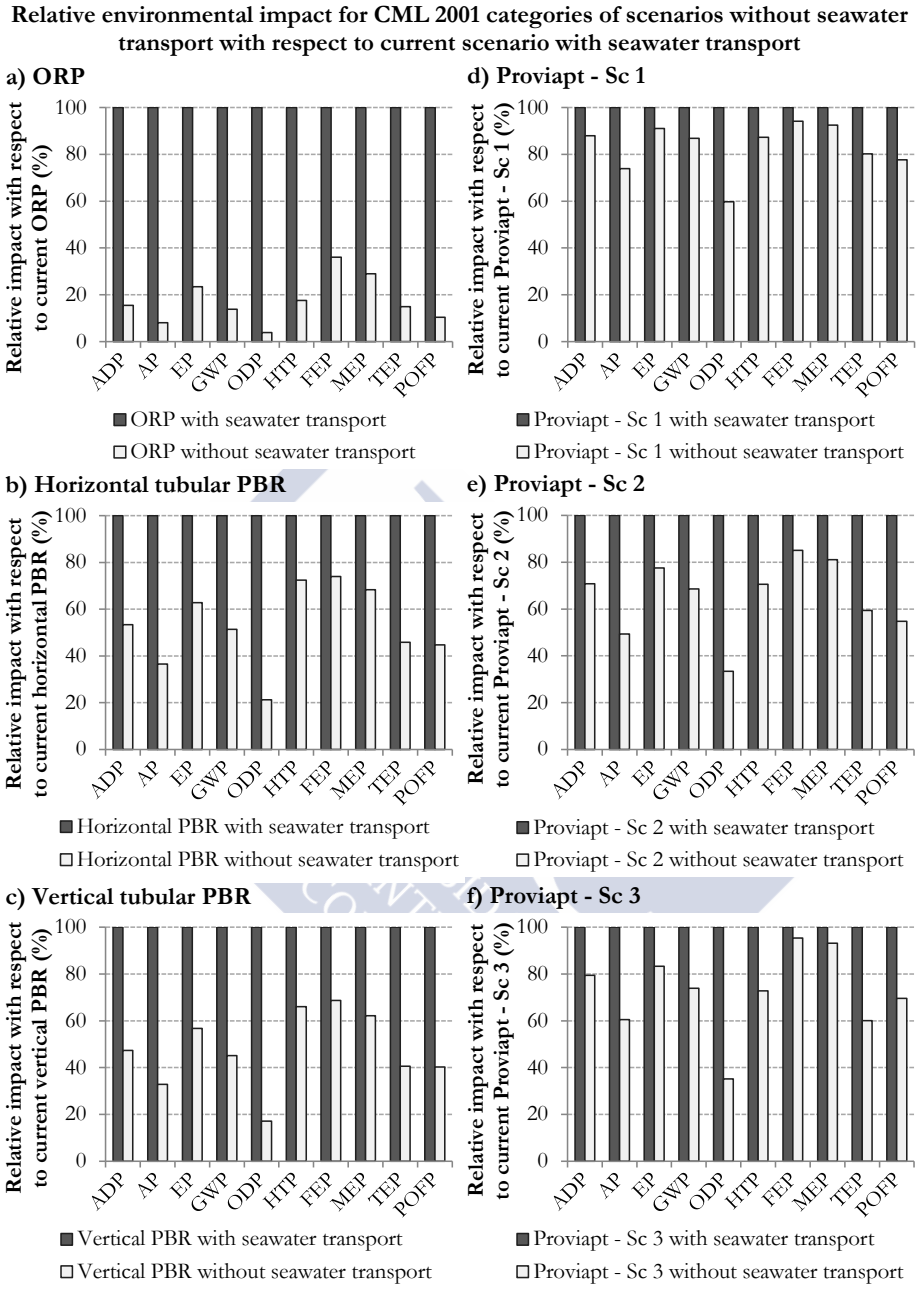



**Figure 8.12.** Relative contributions to CML 2001 categories with respect to current scenario (index=100) for the pilot-scale production of *Nannochloropsis* sp. without seawater transport for a) ORP, b) horizontal tubular PBR, c) vertical tubular PBR, d) Proviapt - Sc 1, e) Proviapt - Sc 2 and f) Proviapt - Sc 3.

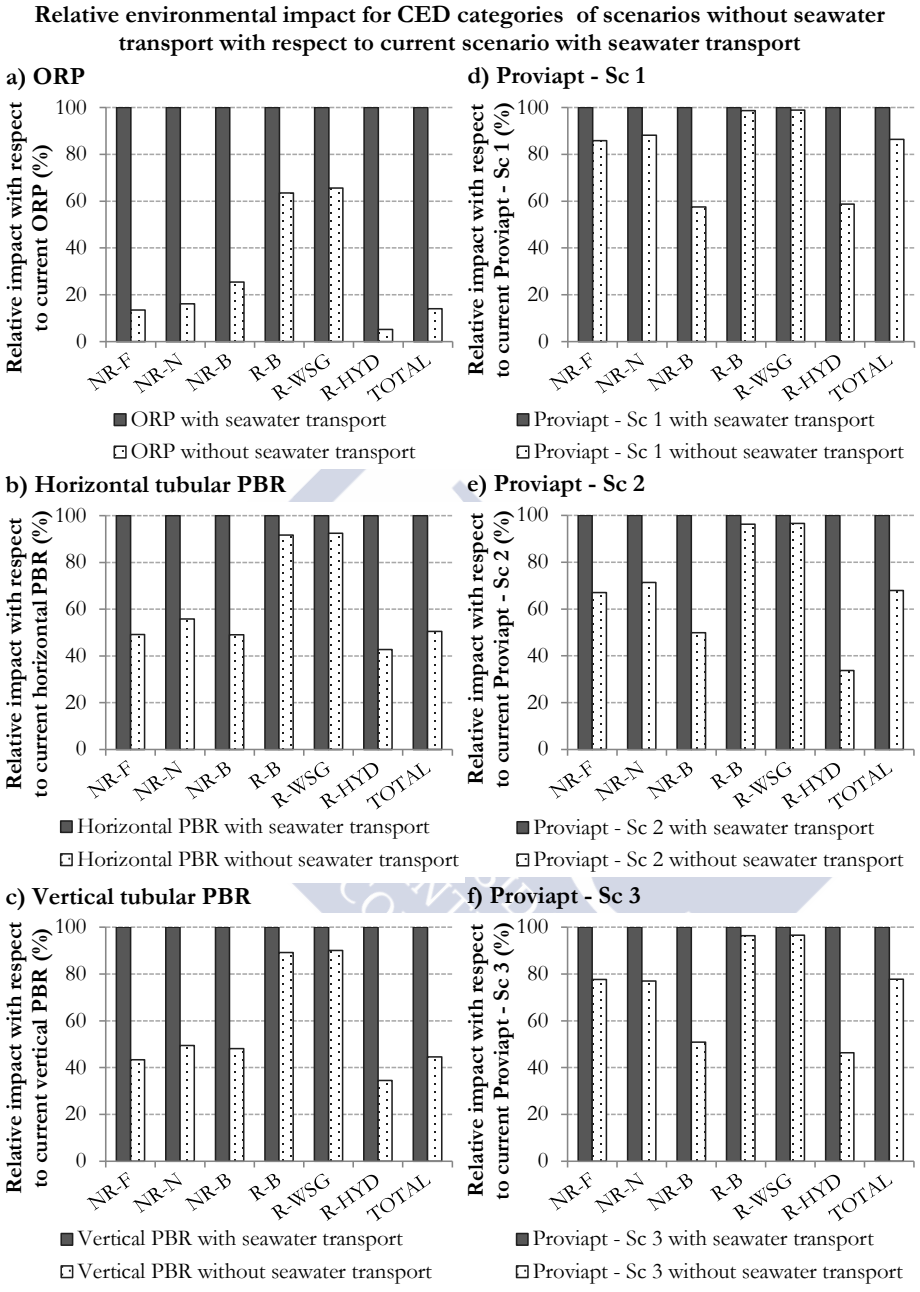



**Figure 8.13.** Relative contributions to CED categories with respect to current scenario (index=100) for the pilot-scale production of *Nannochloropsis* sp. without seawater transport for a) ORP, b) horizontal tubular PBR, c) vertical tubular PBR, d) Proviapt - Sc 1, e) Proviapt - Sc 2 and f) Proviapt - Sc 3.

The reductions of impact derived from the elimination of seawater transport in the system result in a noticeably different environmental performance of each option, which affects the comparative analysis of the alternative configurations. For this reason, a comparative representation of the six scenarios without seawater transport is depicted in **Figure 8.14**.

According to the results, ORP shows a remarkable improvement when no seawater transport is required and constitutes a much more competitive alternative with respect to other systems.

Tubular PBRs still present lower impacts in most of the evaluated categories, although the benefits in comparison with the ORP differ in less than 20% in most categories. For the category of HTP, the contribution is 82% higher for the horizontal PBR and 47% for the vertical PBR than the value associated with ORP.

Proviapt scenarios have a worse profile than ORP and tubular PBRs, with higher contributions to most categories considering both CML and CED methodologies. Among them, Proviapt system can only be an efficient option comparable to ORP and tubular systems if the assumptions considered for Sc 2 (bags reuse and energy extrapolated from a different period) are accurate. Thus, Sc 2 has environmental impacts between 5% and 15% lower than those of ORP for all CML categories except for ADP and ODP.

The reason for the limited impact reduction for the Proviapt system when excluding the seawater transport is the fact that this configuration requires a lower volume of culture medium for the same biomass production. Thus, transport has a lower relative contribution compared to other processes such as electricity of chemicals in the original scenario, and therefore, the reduction obtained by eliminating the seawater transport is more limited for the Proviapt scenarios than for other reactors, such as ORP, which present a high relative contribution related to transport.

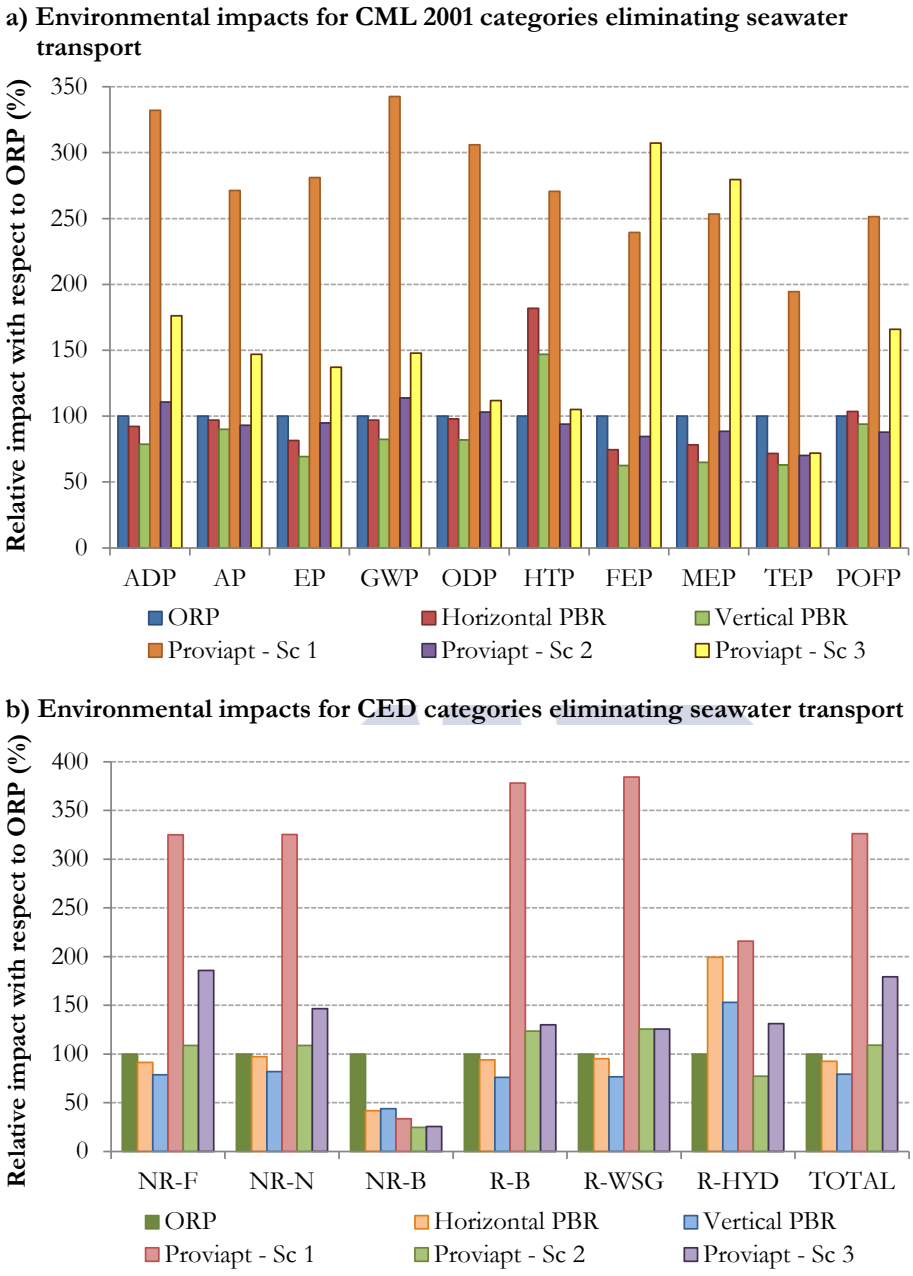



**Figure 8.14.** Relative environmental profile of the compared cultivation systems in absence of seawater transport with respect to ORP (index = 100) for the production of *Nannochloropsis* sp., according to a) CML 2001 and b) CED impact categories.

❖ **Effect of changes in electricity consumption**

Since the production of electricity, mainly consumed in the cultivation stage, was identified as the second hot spot in all the evaluated systems, a sensitivity assessment is shown in **Figures 8.15** to **8.18**.

The analysis considers a 25% reduction and increase of electricity consumption during the cultivation, which can be due to a variation in the electricity required for aeration and mixing, or a possible change on weather conditions in a different cultivation period that may affect cooling and heating requirements.

According to the results, a limited change in energy consumption during cultivation stage may lead to a significant variation in the environmental profile of most systems. The behavior for the current scenarios (which include seawater transport) depends on the considered reactor, as shown in **Figures 8.15** and **8.16**. Thus, ORP or tubular systems present changes lower than 10% in most categories, while the variation for Proviapt scenarios reaches values of 20% or higher in some impact categories. The cause of this trend is the lower contribution of transport found for Proviapt scenarios, linked to a lower need for seawater in these systems, which have higher productivity and achieve higher biomass concentrations in the culture medium. This is also the reason for the slighter difference between the same scenario when including and excluding transport.

As shown in **Figures 8.17** and **8.18**, the effect of changes in electricity when excluding seawater transport is similar for all the reactor configurations. The variation for both CML and CED environmental results with respect to the base scenarios ranges between 10% and 20% for most categories. These results reflect the high relative contribution of electricity when seawater transport is not considered. In these scenarios, the production of electricity associated with the cultivation is the main hot spot for most categories in all the evaluated configurations.

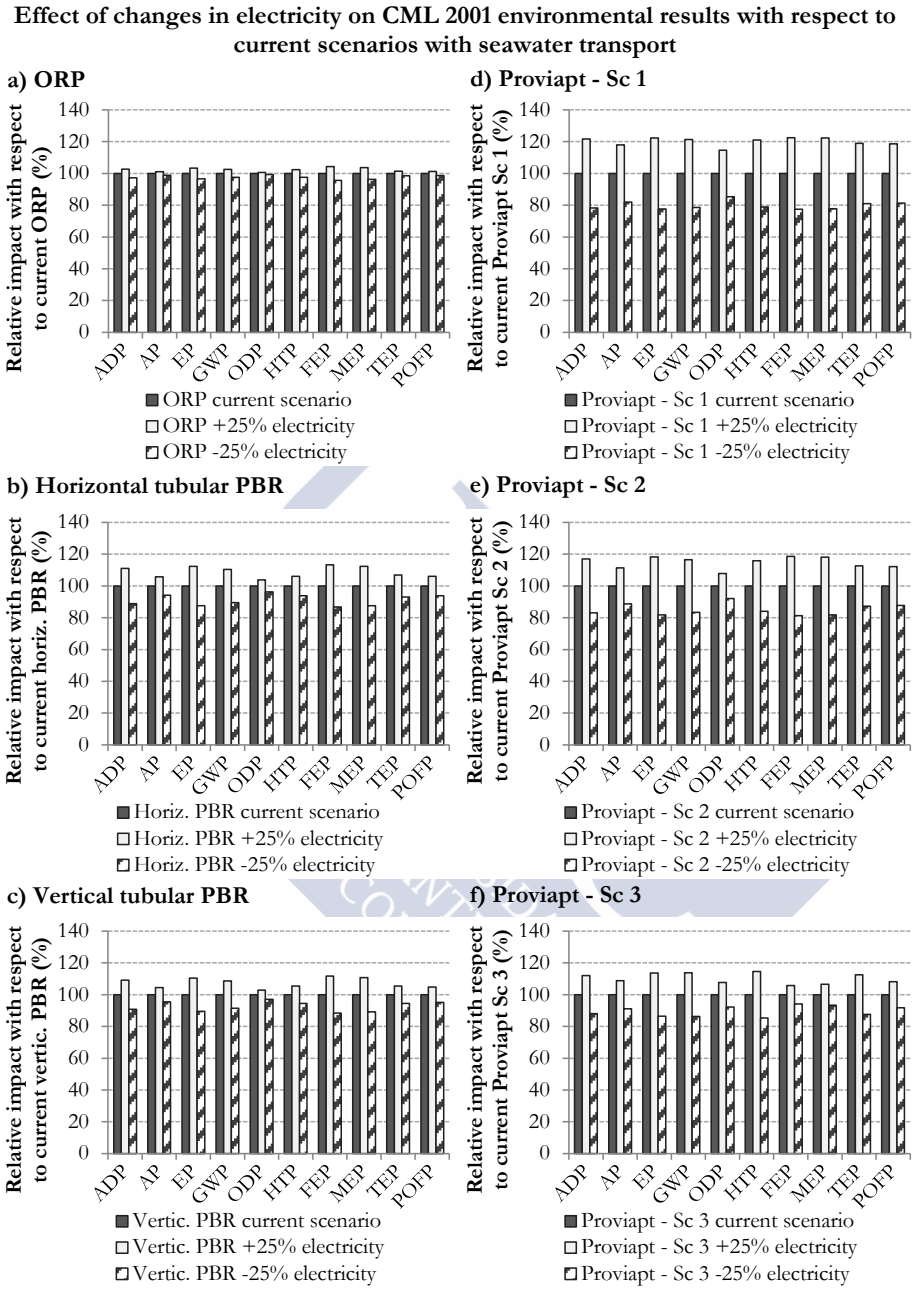



**Figure 8.15.** Effect of electricity in CML 2001 environmental results for the pilot-scale production of *Nannochloropsis* sp. including seawater transport for a) ORP, b) horizontal tubular PBR, c) vertical tubular PBR, d) Proviapt - Sc 1, e) Proviapt - Sc 2 and f) Proviapt - Sc 3.

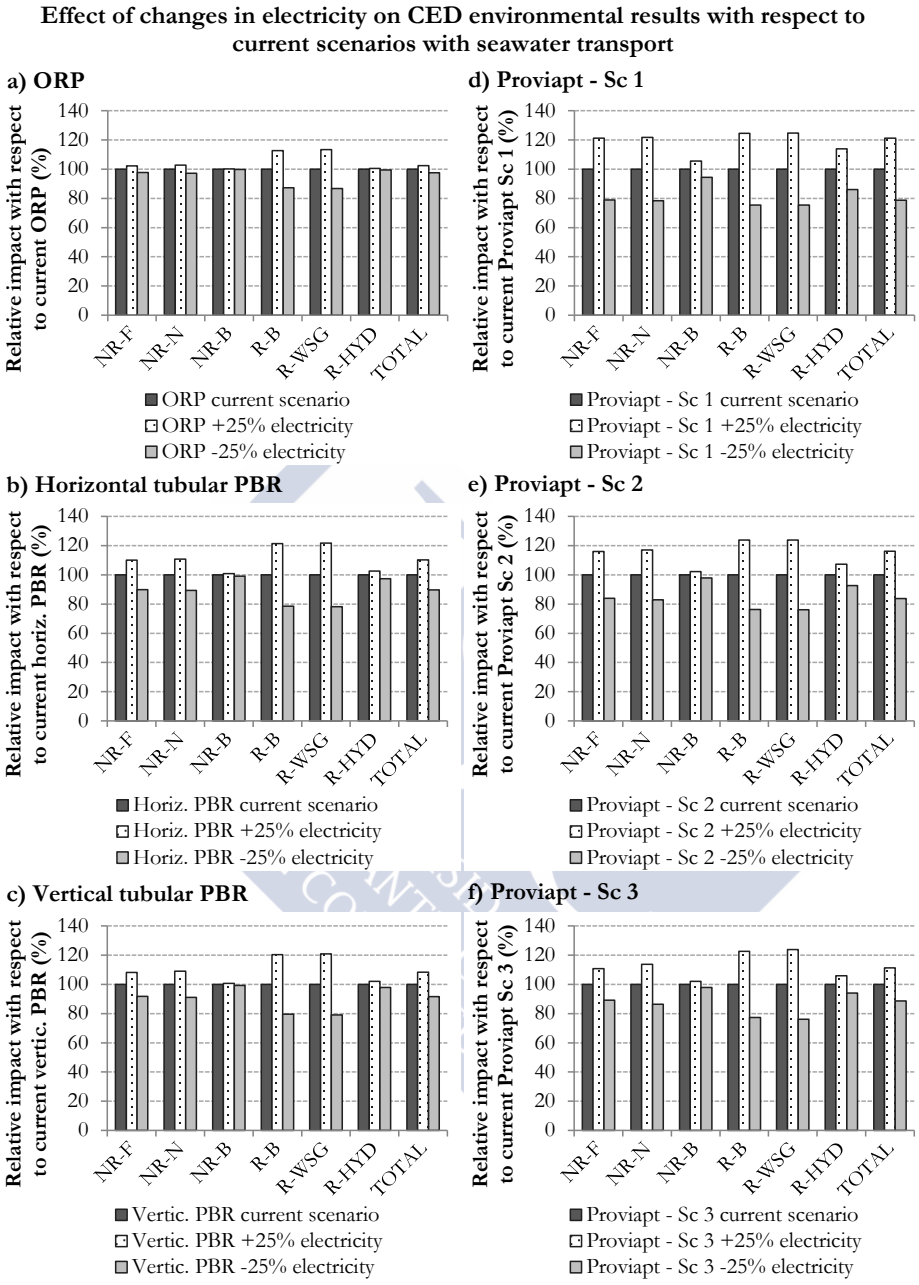



**Figure 8.16.** Effect of electricity in CED environmental results for the pilot-scale production of *Nannochloropsis* sp. including seawater transport for a) ORP, b) horizontal tubular PBR, c) vertical tubular PBR, d) Proviapt - Sc 1, e) Proviapt - Sc 2 and f) Proviapt - Sc 3.

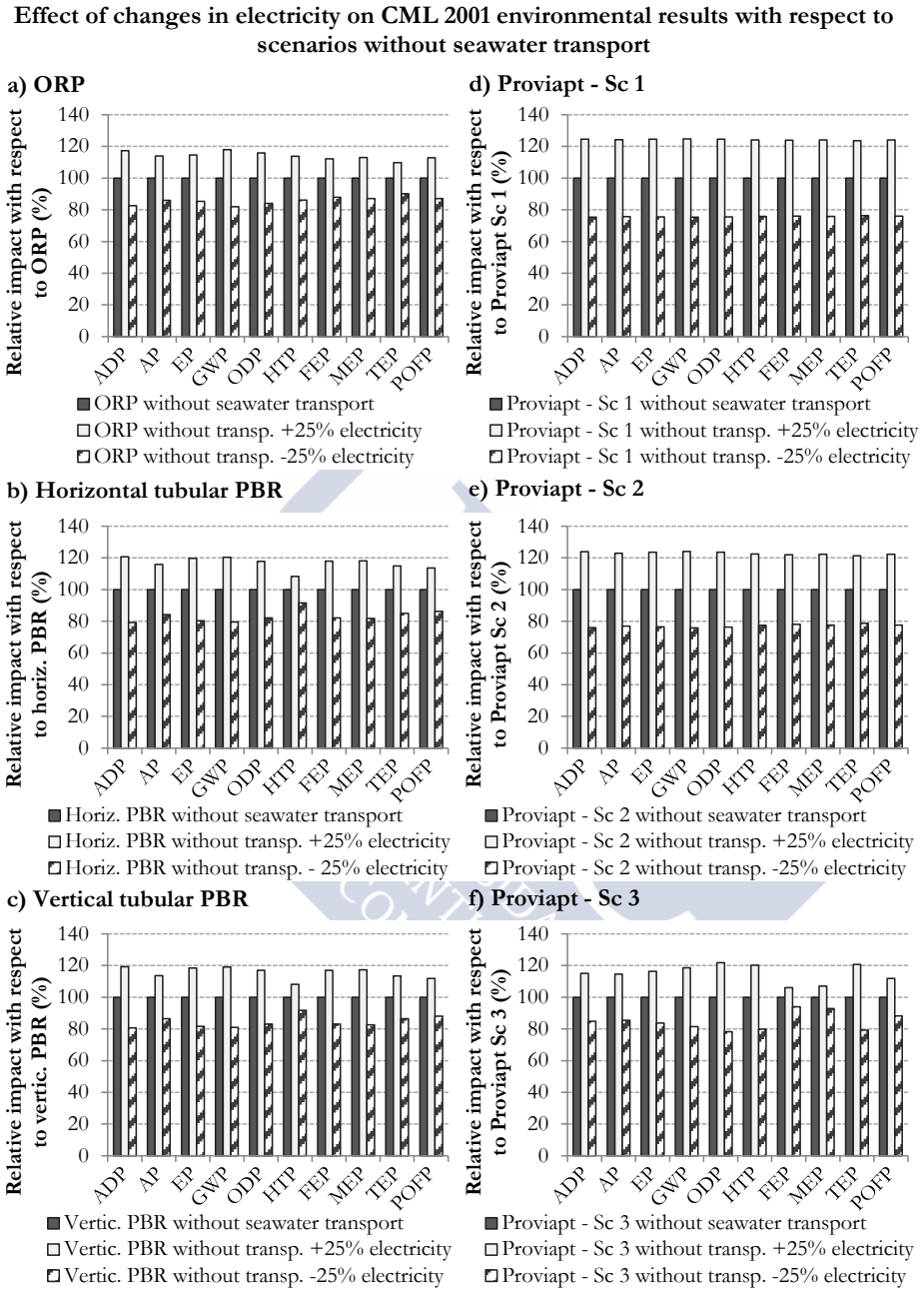



**Figure 8.17.** Effect of electricity in CML 2001 environmental results for the pilot-scale production of *Nannochloropsis* sp. excluding seawater transport for a) ORP, b) horizontal tubular PBR, c) vertical tubular PBR, d) Proviapt - Sc 1, e) Proviapt - Sc 2 and f) Proviapt - Sc 3.

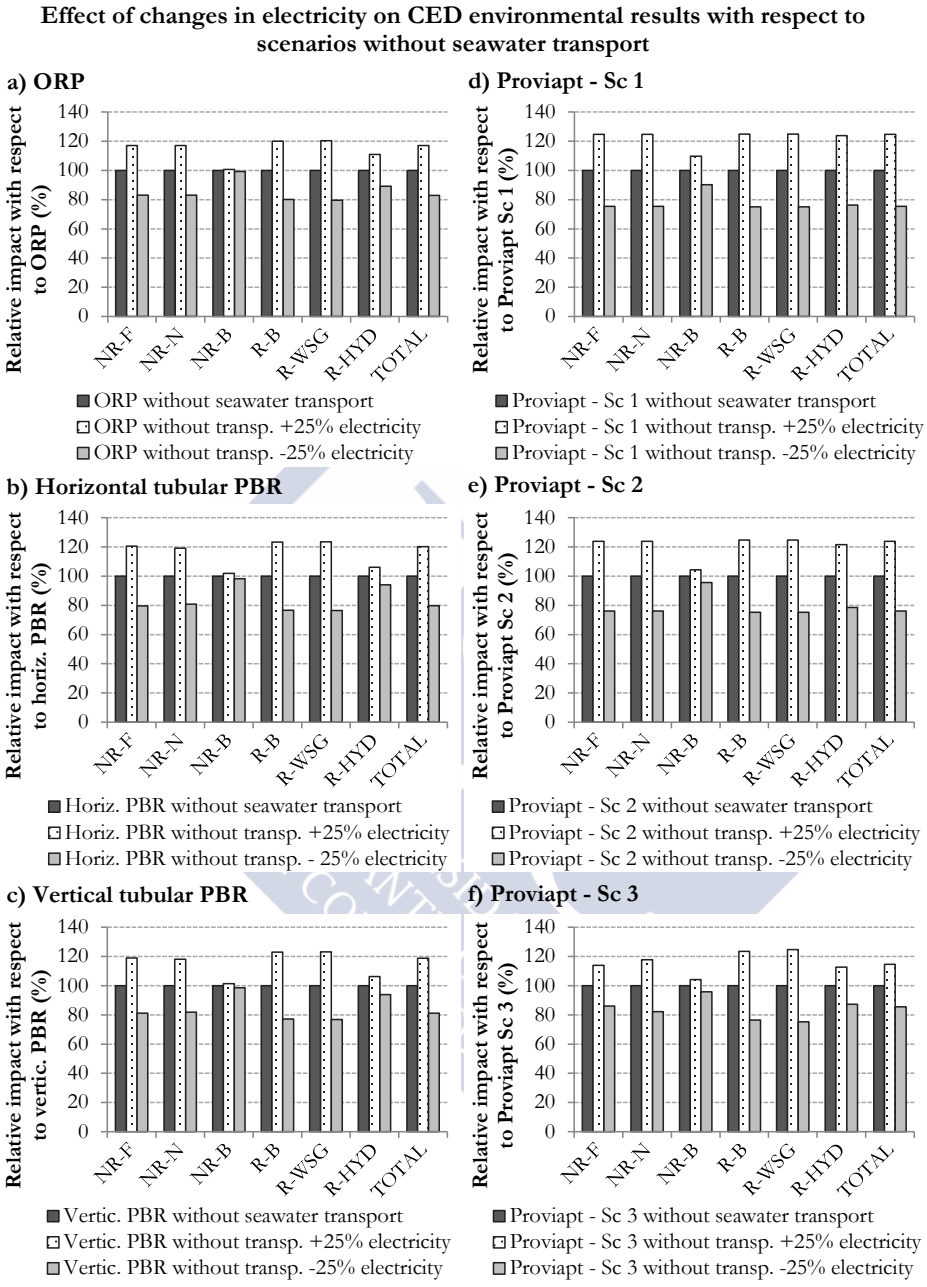



**Figure 8.18.** Effect of electricity in CED environmental results for the pilot-scale production of *Nannochloropsis* sp. excluding seawater transport for a) ORP, b) horizontal tubular PBR, c) vertical tubular PBR, d) Proviapt - Sc 1, e) Proviapt - Sc 2 and f) Proviapt - Sc 3.

❖ **Proviapt scenarios: Reuse vs. substitution of polypropylene bags**

Although the amount of propylene was calculated considering the replacement of bags after each batch, it should be pointed out that the duration of the evaluated batch is only 4 days. It is expected that the real duration of the batch in a commercial scale process is longer than the considered period, so the amount of material per kg of biomass produced would be significantly lower.

According to the sensitivity assessment presented in **Figure 8.19**, the substitution approach would require a total operation of at least 60 days (scenario Sc 4) in the considered conditions (productivity, energy consumption) with the same polypropylene bags in order to achieve an environmental performance as efficient as the profile found for Sc 2, in which the bags are rinsed with water and reused. Therefore, the results suggest that reusing plastic bags would be a more recommendable option from an environmental perspective unless the operation can be maintained with the same bags and operational conditions for more than 2 months.

❖ **Effect of biomass productivity**

The reported environmental results are based on the values of specific biomass productivity for each scenario. Since the productivity affects all the inputs and outputs collected in the inventory per FU, a sensitivity assessment was conducted to analyze the change in the environmental profile associated with increases of 25% and 50% of total biomass productivity and analogous reductions. However, since a change in this parameter has the same effect in all the scenarios and impact categories, individual charts for each system are not necessary.

According to **Figure 8.20**, if biomass productivity was doubled, while maintaining the same conditions (energy consumption, nutrients, final concentration...), the impact would be reduced in a 33% with respect to the environmental profile of the current scenario. On the contrary, if biomass production was reduced to 50%, the system would have 2 times higher impacts. According to this dependence, biomass productivity constitutes the key parameter that determines the environmental response of the system to a larger extent than any other factor.

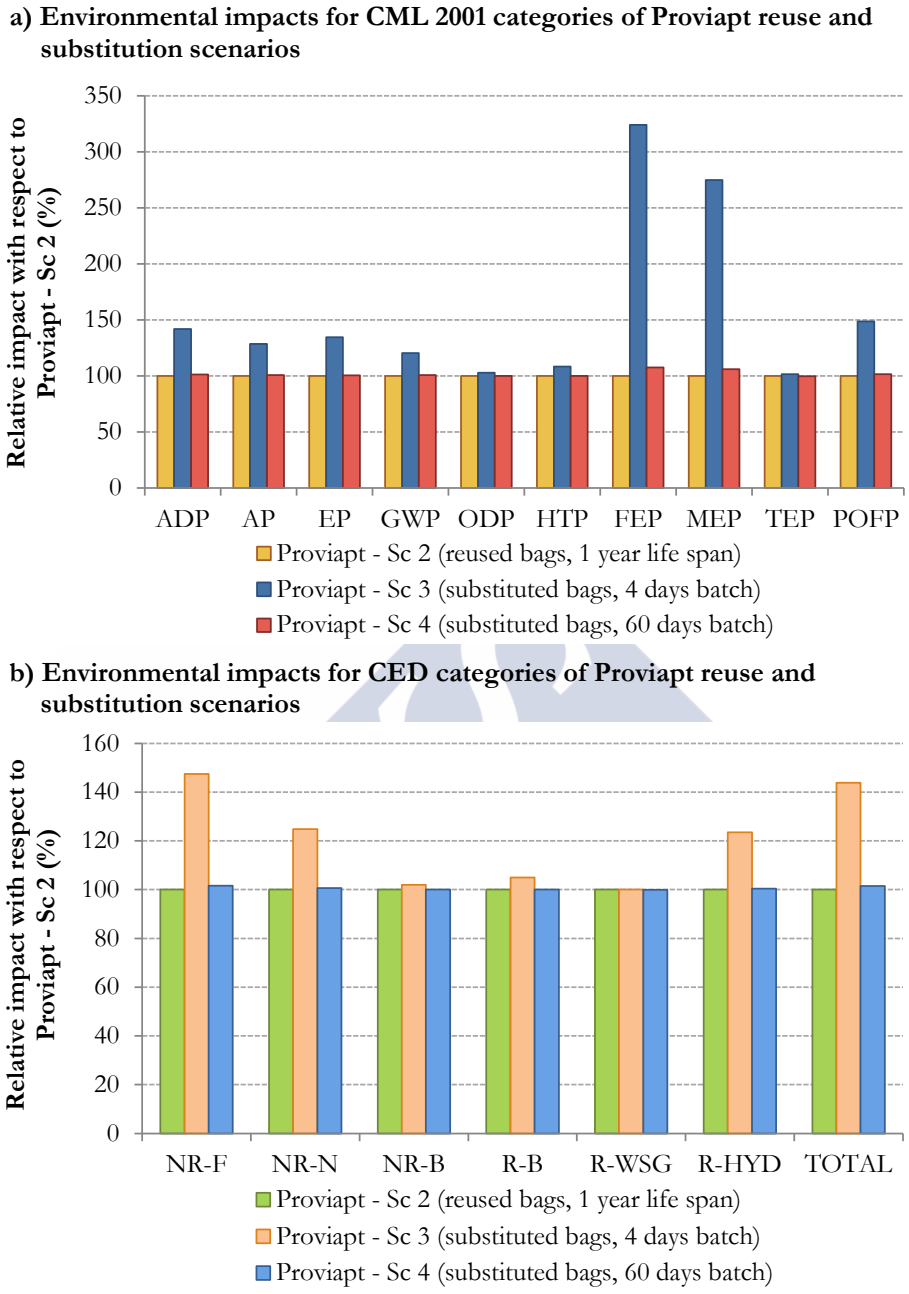



**Figure 8.19.** Relative environmental profile of the production of *Nannochloropsis* sp. in Proviapt systems with bag reuse and substitution approaches according to a) CML 2001 and b) CED impact categories.

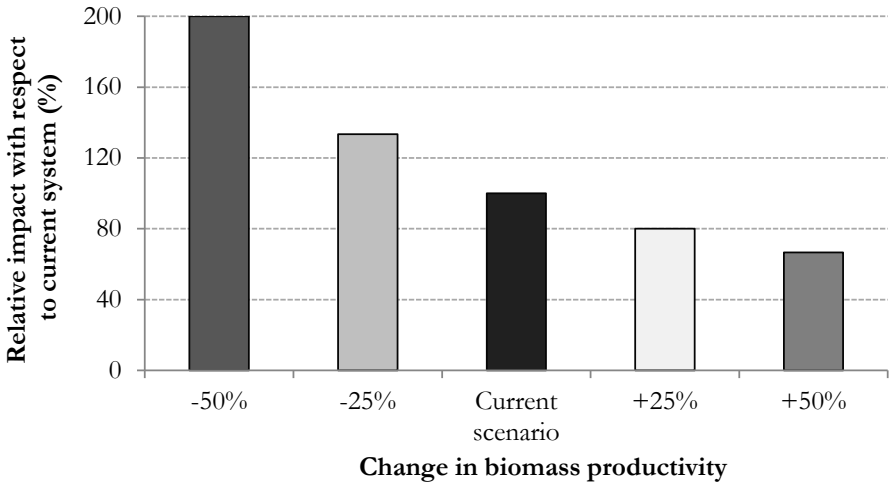



**Figure 8.20.** Effect of changes in biomass productivity on the environmental profile of the production of *Nannochloropsis* sp.

## 8.4. Seasonal variability of the environmental profile of ORPs and tubular PBRs

Section 8.3 presents the environmental results for all the reactors operated under the most favorable productivity conditions. However, algal growth parameters and requirements are strongly dependent on surrounding conditions. In this second stage, the effect of weather variability on the reactor performance was evaluated for the two most common reactor configurations: ORP and tubular PBRs. Proviapt was excluded from the analysis due to the lack of detailed data on the operation for the reference year. The evaluation includes the operation in three different seasons (summer, fall and winter).

### 8.4.1. Goal and scope

As aforementioned, the goal of the present study was to assess three reactor configurations (ORP, horizontal tubular PBR and vertical tubular PBR) for the pilot-scale production of the eustigmatophyte *Nannochloropsis* sp operated under different conditions throughout the year (summer, fall and winter). With this work, bottlenecks in environmental performance (referred to as hot spots) of the systems were identified.

The assessment allows a comparison between the environmental efficiency of the different reactor configurations, which is closely linked to the biomass production. The collected inventory and determined environmental impacts are refer to 1 kg of final biomass (in a slurry of 22% DW) obtained after the pilot-scale cultivation and harvesting.

Since the compared systems are the same as those presented in the previous section, the system boundaries coincide with the stages depicted in **Figure 8.2**. Thus, the process is divided into: i) cleaning and sterilization (Sc 1), ii) preparation of the culture medium (S2), iii) cultivation (S3) and iv) biomass concentration (S4).

The only differences with respect to the stages described in section 8.3.1 are the removal of $NaHCO_3$ and substitution of urea by $NaNO_3$ as nutrient source (linked to S2) and the addition of a microfiltration step before centrifugation in S4.

### 8.4.2. Life cycle inventory, data quality and assumptions

As in the previous section, the information for the foreground system mainly consisted of primary data collected in the facility. The inputs and outputs for the cleaning stage (S1) were quantified by assuming a consumption of water equal to three times the total volume of the corresponding reactor. The total quantities of chlorine and other cleaning agents (disinfectant and plastic beads used to clean the tubular PBRs) as well as energy consumptions were calculated with respect to the volume of water, according to the specified concentrations. Six cleanings per year were considered for the tubular PBRs, while ten cleanings were required for the ORP. The difference in the number of cleanings is due to the shortness of the periods in which the ORP can be continuously operated, in comparison with the tubular PBRs. The total quantity of inputs for each evaluated period was estimated according to the ratio between the duration of the period and the total feasible operation time per year (approximately 10 months).

The chemicals for the preparation of the culture medium (S2) were calculated by considering the average dilution rate of each period, which determined the volume of seawater and thus, the consumption of water, chlorine and nutrients

according to the selected concentrations. The energy requirements were estimated with respect to the total seawater and medium needed for each system, assuming that the equipment was operating at the maximum allowed capacity.

Regarding the cultivation stage (S3), the energy consumption for the different operations (base energy of monitoring system, mixing, aeration and temperature control) was directly obtained from the on-line monitoring system. The quantities of building materials for each reactor were calculated from measurements of the dimensions to determine the volume of each component and obtain the weight by multiplying by the corresponding density. Different life spans were considered for the building materials depending on their properties and function. For plastic components of the main body of the reactors, 10-year life span was considered. For the auxiliary and support elements, as well as for steel components, 20-year life span was estimated.

The single input for the biomass concentration (S4) was the energy consumption for the consecutive units of microfiltration and centrifuge, which was calculated according to the total volume of medium to separate from the biomass in order to achieve the final concentration of 22% DW.

In all subsystems, solid wastes were assumed to be disposed of in sanitary or inert landfills, whereas the resulting wastewater was collected in the general sewage system and treated in a conventional wastewater treatment plant. An average transport distance of 200 km was considered for chemicals and building materials and 50 km was estimated for wastes. No transport was considered for the seawater in this case, since it was assumed that a commercial scale facility would be placed close to the coast. The amounts of materials for auxiliary equipment used in the process (filters, pumps, centrifuge...) were neglected, since this equipment was shared between several systems and the corresponding quantities for each system after applying the appropriate allocation procedures would be very limited. Moreover, the equipment was common to the three analyzed reactors and thus, no additional information for the comparative purposes of the work would be obtained by taking the materials into account. The global inventory of the assessed scenarios is shown in **Table 8.7**.

**Table 8.7.** Global inventory for the production of *Nannochloropsis* sp. biomass in ORP and tubular pilot systems operated under different weather conditions (FU=1 kg biomass produced)

| **Reactor configuration** | **ORP** | | **Horizontal PBR** | | | **Vertical PBR** | | |
|---|---|---|---|---|---|---|---|---|
| **Season** | **Summer** | **Fall** | **Summer** | **Fall** | **Winter** | **Summer** | **Fall** | **Winter** |
| **INPUTS from TECHNOSPHERE** | | | | | | | | |
| **Materials** | | | | | | | | |
| *S1. Cleaning and sterilization* | | | | | | | | |
| Tap water ($m^3$) | 2.959 | 15.984 | 0.128 | 0.398 | 0.994 | 0.163 | 0.439 | 0.977 |
| Chlorine solution (NaClO, kg) | 0.104 | 0.559 | 0.004 | 0.014 | 0.035 | 0.006 | 0.015 | 0.034 |
| Disinfectant (kg) | 0 | 0 | 0.002 | 0.005 | 0.012 | 0.002 | 0.005 | 0.012 |
| Plastic beads (kg) | 0 | 0 | 0.021 | 0.066 | 0.166 | 0.027 | 0.073 | 0.163 |
| *S2. Preparation of culture medium* | | | | | | | | |
| Chlorine solution (NaClO, kg) | 0.145 | 0.649 | 0.018 | 0.043 | 0.089 | 0.022 | 0.048 | 0.090 |
| Deionized water (kg) | 6.286 | 27.986 | 3.845 | 9.328 | 19.099 | 4.709 | 10.328 | 19.415 |
| $FeSO_4 \cdot 7H_2O$ (g) | 10.521 | 46.977 | 6.455 | 15.649 | 32.060 | 7.905 | 17.337 | 32.590 |
| $MnCl_2 \cdot 2H_2O$ (g) | 0.600 | 2.678 | 0.368 | 0.893 | 1.827 | 0.451 | 0.988 | 1.858 |
| $ZnSO_4 \cdot 7H_2O$ (g) | 0.231 | 1.033 | 0.142 | 0.344 | 0.705 | 0.174 | 0.381 | 0.717 |
| $Co(NO_3)_2 \cdot 6H_2O$ (g) | 0.025 | 0.110 | 0.015 | 0.037 | 0.075 | 0.018 | 0.040 | 0.076 |
| $CuSO_4 \cdot 5H_2O$ (g) | 0.008 | 0.038 | 0.005 | 0.013 | 0.026 | 0.006 | 0.014 | 0.026 |
| $Na_2MoO_4 \cdot 2H_2O$ (g) | 0.085 | 0.379 | 0.052 | 0.126 | 0.259 | 0.064 | 0.140 | 0.263 |
| EDTA-$Na_2 \cdot 2H_2O$ (kg) | 0.037 | 0.164 | 0.023 | 0.055 | 0.112 | 0.028 | 0.061 | 0.114 |
| $NaNO_3$ (kg) | 1.487 | 6.639 | 0.912 | 2.213 | 4.531 | 1.117 | 2.450 | 4.606 |
| $KH_2PO_4$ (kg) | 0.081 | 0.360 | 0.050 | 0.120 | 0.246 | 0.061 | 0.133 | 0.250 |
| NaOH (kg) | 0.022 | 0.100 | 0.014 | 0.033 | 0.068 | 0.017 | 0.037 | 0.070 |

**Table 8.7.** Global inventory for the production of *Nannochloropsis* sp. biomass in ORP and tubular pilot systems operated in different seasons (FU=1 kg biomass produced) (*Cont.*)

| Reactor configuration | ORP | | Horizontal PBR | | | Vertical PBR | | |
|---|---|---|---|---|---|---|---|---|
| **Season** | **Summer** | **Fall** | **Summer** | **Fall** | **Winter** | **Summer** | **Fall** | **Winter** |
| **INPUTS from TECHNOSPHERE** | | | | | | | | |
| **Materials** | | | | | | | | |
| *S3. Cultivation* | | | | | | | | |
| Biomass (kg) | 0.399 | 3.045 | 0.084 | 0.197 | 0.210 | 0.100 | 0.189 | 0.206 |
| PMMA (kg) | 0.012 | 0.066 | 0.122 | 0.380 | 0.951 | 0.301 | 0.813 | 1.809 |
| PP (kg) | 1.486 | 8.024 | 0.077 | 0.239 | 0.598 | 0.068 | 0.182 | 0.406 |
| Steel (kg) | 0.136 | 0.737 | 0 | 0 | 0 | 0.015 | 0.040 | 0.089 |
| Aluminum (kg) | 0 | 0 | 0.192 | 0.594 | 1.485 | 0.136 | 0.368 | 0.819 |
| Synthetic rubber (kg) | 0.004 | 0.023 | 0 | 0 | 0 | 0.009 | 0.024 | 0.053 |
| Compressed air ($m^3$) | 0 | 0 | 480 | 1363 | 4653 | 503 | 1125 | 2940 |
| $CO_2$ ($m^3$) | 3.433 | 22.720 | 3.439 | 4.833 | 6.815 | 2.308 | 2.942 | 4.247 |
| **Energy (electricity from Dutch grid)** | | | | | | | | |
| *S1. Cleaning and sterilization* | | | | | | | | |
| Filtration (kWh) | 0.608 | 3.286 | 0.026 | 0.082 | 0.204 | 0.033 | 0.090 | 0.201 |
| Vacuum system (kWh) | 1.720 | 9.291 | 0 | 0 | 0 | 0 | 0 | 0 |
| *S2. Preparation of culture medium* | | | | | | | | |
| Water pumping (kWh) | 0.964 | 4.306 | 0.118 | 0.287 | 0.588 | 0.145 | 0.318 | 0.597 |
| Filtration (kWh) | 1.143 | 5.104 | 0.140 | 0.340 | 0.697 | 0.172 | 0.377 | 0.708 |
| Mixing (kWh) | 0.047 | 0.209 | 0.029 | 0.070 | 0.143 | 0.035 | 0.077 | 0.145 |

**Table 8.7.** Global inventory for the production of *Nannochloropsis* sp. biomass in ORP and tubular pilot systems operated in different seasons (FU=1 kg biomass produced) (*Cont.*)

| **Reactor configuration** | **ORP** | | **Horizontal PBR** | | | **Vertical PBR** | | |
|---|---|---|---|---|---|---|---|---|
| **Season** | **Summer** | **Fall** | **Summer** | **Fall** | **Winter** | **Summer** | **Fall** | **Winter** |
| **INPUTS from TECHNOSPHERE** | | | | | | | | |
| **Energy (electricity from Dutch grid)** | | | | | | | | |
| *S3. Cultivation* | | | | | | | | |
| Base energy (kWh) | 5.671 | 37.539 | 14.597 | 38.618 | 131.949 | 8.216 | 21.453 | 57.786 |
| Aeration and $CO_2$ (kWh) | 26.089 | 172.680 | 51.520 | 136.299 | 465.703 | 20.323 | 53.068 | 149.945 |
| Mixing (kWh) | 89.517 | 618.714 | 21.686 | 60.352 | 139.005 | 33.255 | 89.429 | 119.596 |
| Heating (kWh) | 198.440 | 4873.194 | 35.309 | 286.464 | 2495.885 | 60.996 | 548.654 | 3267.089 |
| Cooling (kWh) | 0 | 0 | 156.872 | 46.654 | 0 | 151.705 | 34.518 | 0 |
| *S4.Biomass concentration* | | | | | | | | |
| Microfiltration (kWh) | 1.438 | 6.419 | 0.176 | 0.428 | 0.875 | 0.216 | 0.473 | 0.890 |
| Centrifugation (kWh) | 1.627 | 7.264 | 0.199 | 0.484 | 0.991 | 0.244 | 0.536 | 1.007 |
| **Transport** | | | | | | | | |
| *S1. Cleaning* | | | | | | | | |
| Chemicals (tkm) | 0.021 | 0.111 | 0.313 | 0.970 | 2.425 | 0.397 | 1.072 | 2.384 |
| Materials (tkm) | 0 | 0 | 0.004 | 0.013 | 0.033 | 0.005 | 0.015 | 0.033 |
| Wastes (tkm) | 0 | 0 | 0.001 | 0.003 | 0.008 | 0.001 | 0.004 | 0.008 |
| *S2. Preparation of culture medium* | | | | | | | | |
| Chemicals (tkm) | 0.357 | 1.593 | 0.205 | 0.496 | 1.016 | 0.251 | 0.550 | 1.033 |
| *S3. Cultivation* | | | | | | | | |
| Materials (tkm) | 0.328 | 1.770 | 0.078 | 0.243 | 0.606 | 0.106 | 0.286 | 0.635 |
| Wastes (tkm) | 0.082 | 0.442 | 0.020 | 0.061 | 0.152 | 0.026 | 0.071 | 0.159 |

**Table 8.7.** Global inventory for the production of *Nannochloropsis* sp. biomass in ORP and tubular pilot systems operated in different seasons (FU=1 kg biomass produced) (*Cont.*)

| Reactor configuration | ORP | | Horizontal PBR | | | Vertical PBR | | |
|---|---|---|---|---|---|---|---|---|
| **Season** | **Summer** | **Fall** | **Summer** | **Fall** | **Winter** | **Summer** | **Fall** | **Winter** |
| **INPUTS from ENVIRONMENT** | | | | | | | | |
| *S2. Preparation of culture medium* | | | | | | | | |
| Seawater ($m^3$) | 3.500 | 15.628 | 0.426 | 1.033 | 2.116 | 0.521 | 1.144 | 2.151 |
| *S3. Cultivation* | | | | | | | | |
| Occupation, land ($m^2$·year) | 0.529 | 2.857 | 0.385 | 1.193 | 2.984 | 0.265 | 0.714 | 1.587 |
| **OUTPUTS to TECHNOSPHERE** | | | | | | | | |
| **Product** | | | | | | | | |
| Microalgal biomass (22% DW) | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **Wastes to treatment** | | | | | | | | |
| *S1. Cleaning and sterilization* | | | | | | | | |
| Plastic beads to landfill (kg) | 0 | 0 | 0.021 | 0.066 | 0.166 | 0.027 | 0.073 | 0.163 |
| Wastewater to treatment plant ($m^3$) | 2.959 | 15.984 | 0.130 | 0.402 | 1.004 | 0.165 | 0.444 | 0.987 |
| *S3. Cultivation* | | | | | | | | |
| PMMA (kg) | 0.012 | 0.066 | 0.123 | 0.380 | 0.951 | 0.302 | 0.813 | 1.809 |
| PP (kg) | 1.486 | 8.024 | 0.077 | 0.239 | 0.598 | 0.068 | 0.182 | 0.406 |
| Steel (kg) | 0.136 | 0.737 | 0 | 0 | 0 | 0.015 | 0.040 | 0.089 |
| Aluminum (kg) | 0 | 0 | 0.192 | 0.594 | 1.485 | 0.136 | 0.368 | 0.819 |
| Synthetic rubber (kg) | 0.004 | 0.023 | 0 | 0 | 0 | 0.009 | 0.024 | 0.053 |
| *S4. Biomass concentration* | | | | | | | | |
| Wastewater to treatment plant ($m^3$) | 3.502 | 15.652 | 0.425 | 1.038 | 2.130 | 0.522 | 1.150 | 2.166 |

The inventory data related to the background system were obtained from Ecoinvent database (Frischknecht et al., 2007a), according to the reports listed in **Table 8.4**. These inputs include the production of the chemicals required for the cleaning and the nutrients for the culture medium, as well as the production of electricity used throughout the stages of the processes, the manufacture of the building materials for each reactor and the waste disposal. With regard to $NaNO_3$ production, this process is not defined in the Ecoinvent database. Therefore, the considered inventory data correspond to the synthetic process as described by Bhat et al. (1994) and UNIDO/IFDC (1998).

### 8.4.3. Environmental impact assessment

Again, classification and characterization stages of the LCA methodology (ISO 14040, 2006) were conducted for the comparative assessment. The same impact categories of CML 2001 (i.e. ADP, AP, EP, GWP, ODP, HTP, FEP, MEP, TEP and POFP) and CED (i.e. NR-F, NR-N, NR-B, R-B, R-WSG, R-HYD, TOTAL CED) methodologies were evaluated. Additionally, the category of land competition (LC) from CML 2001 methodology was included in the assessment, since one of the potential uses of microalgal biomass is the production of fuels, for which the land requirement is a key parameter to compare the efficiency with alternative energy feedstocks. The inventory data were implemented in SimaPro 8 (Goedkoop et al., 2013). The characterization results are listed in **Tables 8.8** and **8.9**.

**Table 8.8.** Environmental impact assessment results (characterization step) for the production of *Nannochloropsis sp.* in ORP and tubular PBR pilot reactors according to CML 2001 methodology

| Impact category | Unit | ORP | | Horizontal PBR | | | Vertical PBR | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Summer | Fall | Summer | Fall | Winter | Summer | Fall | Winter |
| ADP | kg Sb eq | 1.983 | 33.012 | 1.660 | 3.404 | 18.628 | 1.642 | 4.427 | 20.630 |
| AP | kg $SO_2$ eq | 0.605 | 7.512 | 0.460 | 0.996 | 4.361 | 0.485 | 1.232 | 4.769 |
| EP | kg $PO_4^{-3}$ eq | 0.514 | 7.149 | 0.362 | 0.753 | 3.868 | 0.363 | 0.961 | 4.262 |
| GWP | kg $CO_2$ eq | 256-120 | 4255.601 | 216.119 | 443.029 | 2408.580 | 213.663 | 574.258 | 2664.979 |
| ODP | g CFC-11 eq | 0.009 | 0.147 | 0.008 | 0.016 | 0.080 | 0.008 | 0.020 | 0.092 |
| HTP | kg 1,4-DB eq | 55.199 | 836.350 | 52.460 | 114.688 | 524.592 | 49.652 | 130.263 | 546.485 |
| FEP | kg 1,4-DB eq | 66.778 | 1027.342 | 50.844 | 105.152 | 565.127 | 49.916 | 134.367 | 621.195 |
| MEP | kg 1,4-DB eq | 42.393 | 661.139 | 33.009 | 68.184 | 366.267 | 32.392 | 87.089 | 402.574 |
| TEP | kg 1,4-DB eq | 0.009 | 0.134 | 0.007 | 0.014 | 0.073 | 0.007 | 0.018 | 0.080 |
| POFP | kg $C_2H_4$ eq | 0.020 | 0.294 | 0.016 | 0.035 | 0.169 | 0.016 | 0.043 | 0.185 |
| LC | $m^2$year | 5.288 | 81.182 | 4.322 | 9.230 | 46.894 | 4.126 | 11.088 | 50.078 |

**Table 8.9.** Environmental impact assessment results (characterization step) for the production of *Nannochloropsis sp.* in ORP and tubular PBR pilot reactors according to CED methodology (results for all categories in MJ)

| Impact category | ORP | | Horizontal PBR | | | Vertical PBR | | |
|---|---|---|---|---|---|---|---|---|
| | Summer | Fall | Summer | Fall | Winter | Summer | Fall | Winter |
| NR-F | 3610 | 59727 | 3010 | 6179 | 33692 | 2983 | 8032 | 37315 |
| NR-N | 455 | 7449 | 377 | 771 | 4187 | 370 | 994 | 4622 |
| NR-B | 0.004 | 0.031 | 0.003 | 0.006 | 0.020 | 0.003 | 0.007 | 0.021 |
| R-B | 125 | 2156 | 106 | 215 | 1213 | 104 | 282 | 1345 |
| R-WSG | 32.1 | 557 | 27.4 | 55.6 | 314 | 26.9 | 72.9 | 348 |
| R-HYD | 20.1 | 275 | 18.7 | 42.7 | 180 | 17.3 | 45.2 | 180 |
| **TOTAL** | 4242 | 70164 | 3539 | 7263 | 39586 | 3501 | 9427 | 43810 |

❖ **Identification of hot spots**

The average distribution of impacts for each reactor configuration, classified per subsystem, is given in **Figure 8.21a**. This figure shows that, the cultivation stage (S3) is the main hot spot for all the reactors in all the analyzed categories (both CML and CED) except for NR-B, with 80% or more impact. This result confirms for an operational system the findings of previous studies based on hypothetical scenarios with extrapolated data (Brentner et al., 2011; Lam and Lee, 2012; Stephenson et al., 2010). Brentner et al. (2011) proposed a set of scenarios, including cultivation and downstream processing, for which the influence of cultivation to CED varied from 20% up to 90% of the total consumption. According to Stephenson et al. (2010), the energy requirements and GWP of algal cultivation represented more than 90% of the total contribution for tubular PBRs and approximately 55% in the case of ORP. The main contributions to the total CED are those of NR-F (85% of total CED) and NR-N (10% total CED). These are two important categories which can be reduced by minimizing the energy requirements for algal products.

Concerning the preparation of the culture medium (S2), only the CML categories of AP, EP and POFP, as well as the CED category of NR-B show significant contributions related to this stage. The stages of cleaning (S1) and biomass concentration (S4) have very limited contributions to all categories. For the tubular PBRs, the highest contribution from S1 is associated with the category of HTP, with only 4-6%, while the contribution from S4 only exceeds 1% for the summer and fall periods in the categories of EP, TEP and R-HYD.

**Figure 8.21b** shows the breakdown of the contributions per involved production process. The main reason for the environmental burden of S3 is the impact of the electricity production for cultivation. The figure shows that electricity for cultivation has contributions between 80-95% in most of the categories. The main reason for the high energy consumption is the use of an electrical heater and chiller in this pilot plant. These units, used for temperature control, can be substituted by more efficient alternatives, such as ground water cooling and using waste heat. Nevertheless, these findings are consistent with other studies in which the electricity for cultivation has been identified as the main hot spot, but with slightly lower relative contributions than that of

AlgaePARC pilot systems (Lardon et al., 2009; Stephenson et al., 2010; Taelman et al., 2013). Stephenson et al. (2010) reported that electrical power during cultivation in ORPs has a contribution of 74% to fossil energy requirement and 65% to GWP. Similarly, energy was identified by Lardon et al. (2009) as one of the main causes of impact for a raceway pond (with contributions between 42-75% to CED and 18-36% to GWP), together with fuel combustion and use of fertilizers. Energy also influences the performance of alternative reactor configurations, such as the ProviAPT system analyzed by Taelman et al. (2013) with a total contribution to resource impact ranging between 49% and 78%, and to carbon footprint (analogous to GWP) between 60% and 76%.

The different elements with electricity requirements during cultivation and the causes of the higher contribution in this work compared to the results in the literature are further discussed in section 8.4.4. It should be remarked that the absolute values in **Tables 8.8** and **8.9** are based on pilot-scale systems, and may change significantly after scaling up to commercial scale. Electrical efficiencies of the equipment are expected to improve in commercial systems. However, in this work we don't extrapolate the inventory to large scale to avoid assumptions that can favor one of the systems. Main trends for up-scaling are also discussed in section 8.4.4. The production of building materials for the reactors (plastics, steel and aluminum grouped as "infrastructure" in **Figure 8.21b**) or the compressed carbon dioxide, also included in S3, have low contributions. The infrastructure is responsible for more than 5% of the impacts in six of the eight assessed scenarios: the categories of HTP, POFP, LC and R-HYD. The contribution of this production process only exceeds 16% for the category of R-HYD. However, this category represented less than 1% of total CED in all scenarios. Most of the impacts from infrastructure are associated with the production of metals, specifically aluminum for the tubular PBRs (used for the supporting structure) and steel for the ORP (among others used for the shaft of the paddle wheel and the tubes of the heat exchanger).

Among other processes, the production of nutrients has the highest impact, although it is restricted to the categories AP (between 14-30% depending on the season), NR-B (from 40% to 65%) and to a lesser extent to EP, TEP and POFP (from 4 to 10%). Sodium nitrate, which comprises more than 90% of the

nutrients, has the highest contribution. The limited effect of nutrients on the total environmental impacts of the analyzed systems differs with previous research that mention a noticeable influence of the production of fertilizers in the environmental profile (Clarens et al., 2010; Draaisma et al., 2013; Lardon et al., 2009). This limited effect is caused by the higher contribution of electricity of the pilot-scale reactors, which attenuated the relative contribution of the other processes. In addition, the use of assumptions and extrapolated laboratory data for productivity used in life cycle and techno-economic studies of algal biofuels may lead to underestimation of energy requirements due to overestimation of the productivity potential (Moody et al., 2014).

The relative impact of nutrient production in the environmental profile depends on the season of cultivation. The lowest contributions were found for the winter operation in tubular reactors and fall operation in ORP (notice that the winter operation for this system failed). This is related to the lower dilution rates due to low productivity, together with higher electricity consumption in fall and especially winter. On the contrary, higher productivities in summer, due to high light intensities, allowed higher dilution rates. This large harvested volume has to be replaced by an equal volume of fresh medium and thus, large quantities of nutrients were needed.

Waste treatment has a moderate contribution (around 10%) for ORP scenarios to EP and toxicity categories (FEP, MEP, TEP) and to R-HYD. This impact was linked to the treatment of wastewater from S1 and S4. The influence of waste treatment for tubular systems, however, was below 3% in all operating conditions. The difference is mainly result of the wastewater from S1. Due to the larger volume, the ORP needed a significant larger volume of water for cleaning than the tubular reactors. Although the tubular PBRs required the addition of chemicals (disinfectant) and materials (plastic beads) with higher impacts, they needed a lower number of cleanings per year than ORP. A minor difference in waste treatment between the ORP and tubular reactor results from S4. The harvested biomass from the ORP has a lower concentration than the biomass from the tubular PBRs, and therefore a higher amount of wastewater was generated to achieve the same biomass concentration by first microfiltration followed by centrifugation.

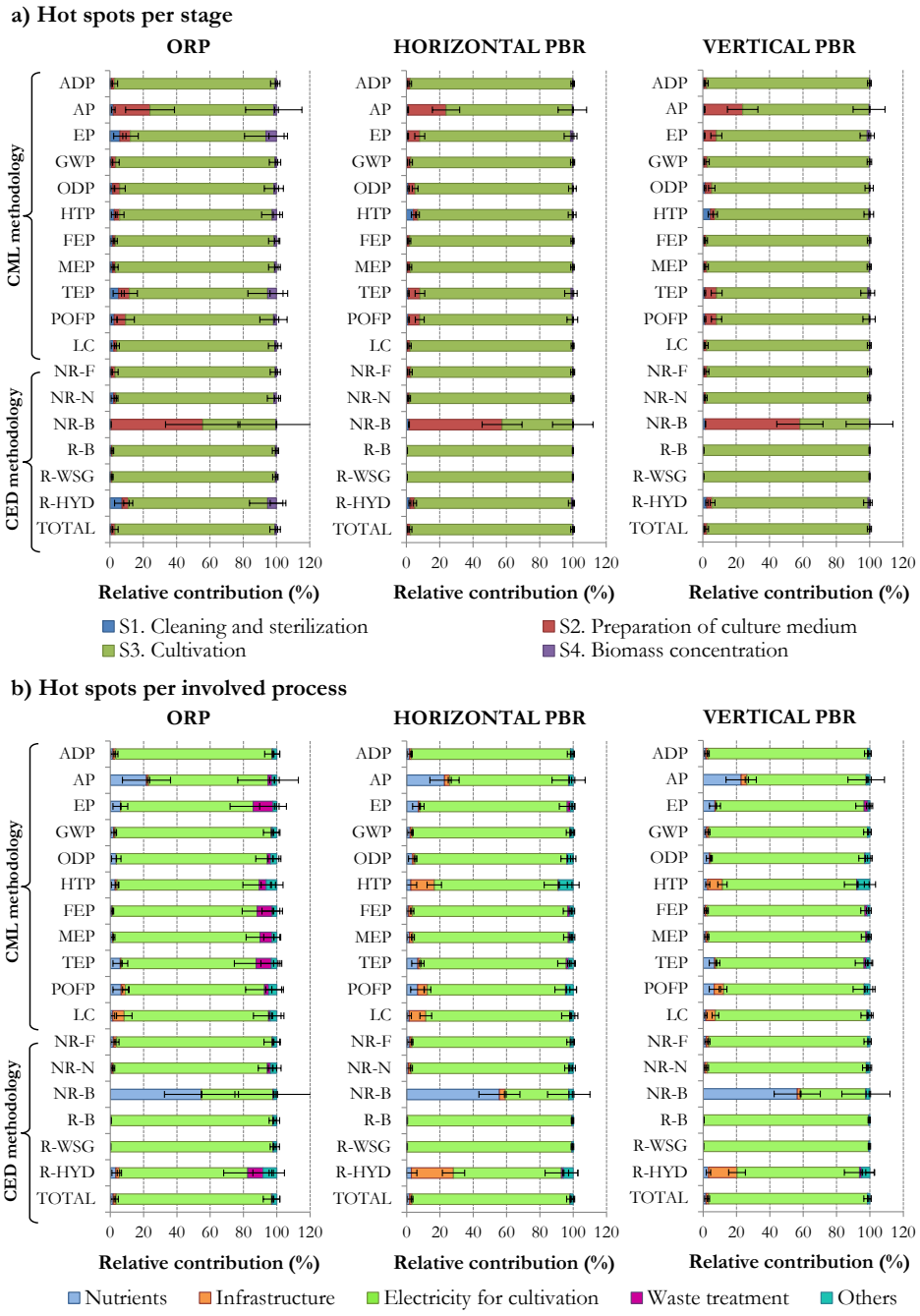



**Figure 8.21.** Relative contributions of the compared reactor configurations for the production of *Nannochloropsis* sp. to the environmental profile grouped by a) subsystem, b) production process.

❖ **Comparative environmental assessment of cultivation scenarios**

The comparison between the analyzed scenarios is depicted in **Figure 8.22**. According to the results, the cultivation period is a key factor affecting the environmental response of the systems. The operation under summer conditions shows low impacts and the three assessed reactors are relatively efficient in comparison to the environmental performance during fall and winter. This is mainly linked to the significantly lower energy requirements in the cultivation stage (S3) during summer compared to fall and winter, which compensate other higher relative contributions for this period (e.g. higher nutrient consumption for summer than for fall and winter due to higher dilution rates). The contributions of ORP operated in summer are 5% (for categories such as HTP, or R-HYD) to 25% (for FEP, MEP or TEP) above those of the tubular PBRs. The contributions of the horizontal PBR are slightly higher than those of the vertical system, but the deviations are too small for a significant distinction between both tubular systems.

For the operation in fall and winter, the difference between configurations is critical. While the environmental burdens of the horizontal PBR operated in fall approximately double compared to the summer period, the effects nearly triple for the vertical tubular system. For the operation in fall, the horizontal PBR presents between 15% and 30% lower impacts than the vertical configuration; mainly due to a 30% lower electricity consumption for cultivation. The difference between the summer and fall scenarios is much more pronounced in the case of ORP, for which the impacts in fall are between 12 and 17 times higher than for the summer operation. Hence, the fall performance of the ORP is 90% worse than any of the tubular systems under the same conditions and even exceeds the environmental profile of both PBRs operated in winter (with significantly colder conditions and less irradiation) with 40%. This finding is in agreement with the experimental difficulties that prevented the operation of ORP in winter and supports the unfeasibility of ponds except for locations with very favorable thermal and solar conditions.

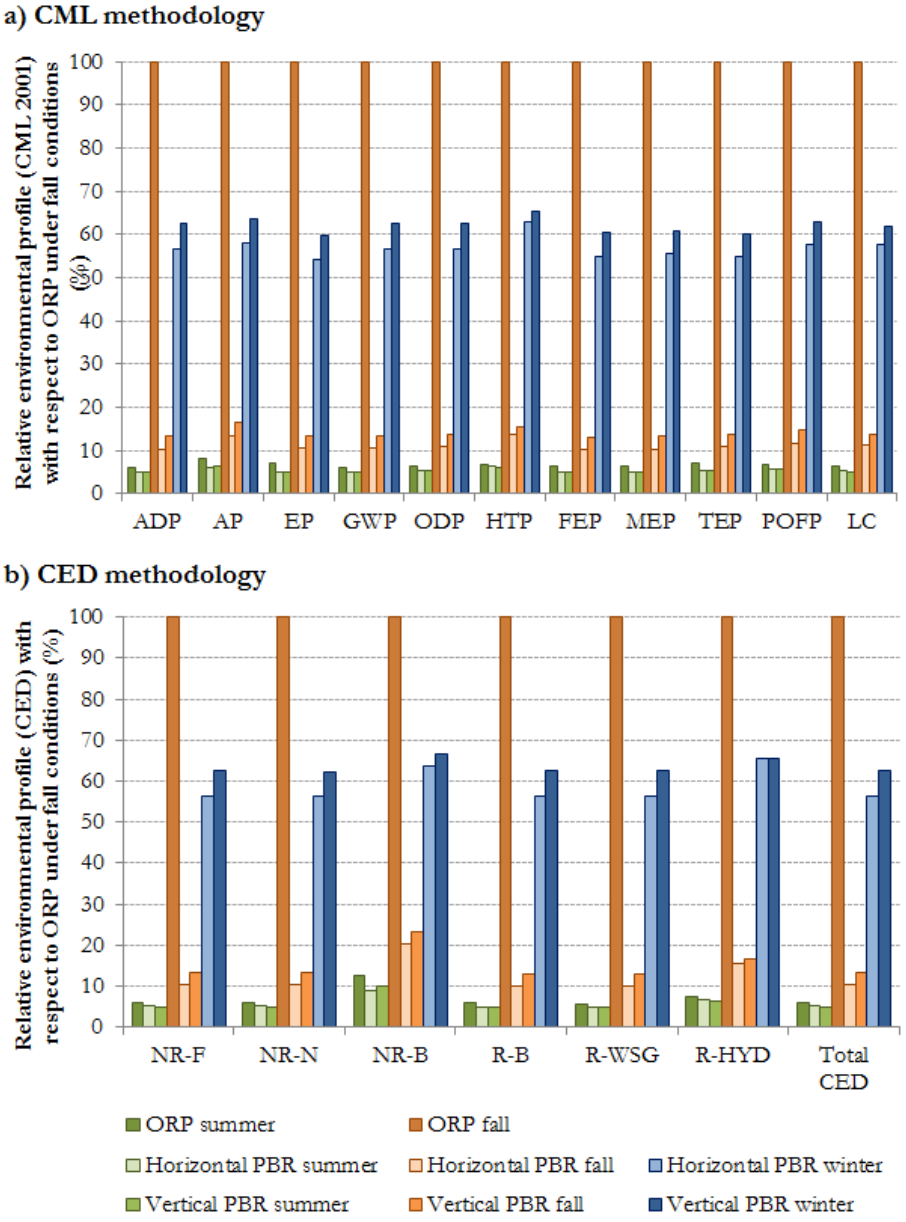



**Figure 8.22.** Relative environmental profile of the compared reactor configurations with respect to ORP in fall conditions for 1 $kg_{DW}$ *Nannochloropsis* sp. biomass as functional unit, according to the impact categories of a) CML methodology and b) CED methodology.

Although tubular PBRs were operated under winter conditions and present a better behavior than ORP in fall, the environmental burdens are significantly higher, compared to the relatively efficient performance during summer and fall periods. The contributions of the horizontal PBR during winter are about 5 times higher than those of fall, and up to 10 times above those of summer. Similarly, the vertical PBR in winter conditions has an average of 4 times the impacts of fall and more than 12 times the impacts of summer. The environmental impact of the horizontal PBR in fall is between 5-10% lower than that of the vertical PBR.

### 8.4.4. Discussion and recommendations

The comparative assessment has identified the ORP as the less efficient system among the evaluated options. In addition, the results reveal that the production of electricity during the cultivation of *Nannochloropsis* sp. is the main contributor to the environmental impact for all the reactor designs and weather conditions. In this section, the key issues associated with the environmental impact of the process, as well as the applicability of the results to a large-scale process are discussed.

❖ **Comparative environmental assessment of cultivation scenarios**

The energy requirements from the cultivation arise from four activities: 1) temperature regulation (including heating and cooling of the culture medium, 2) mixing, 3) aeration and 4) base energy of monitoring system. To determine the relevance of each activity, the distribution of electricity consumption is depicted in **Figure 8.23**.

According to the results, temperature regulation is the main consumer of electricity during cultivation in all evaluated scenarios, with total requirements ranging between 60% and 90%. The relative contributions of heating and cooling for the tubular systems strongly depend on the season. While cooling requires about 55% of the electricity consumption for cultivation in the summer for both tubular PBRs, it takes less than 10% of the requirements during fall and, as expected, it has no contribution at all for the winter period. The ORP needs no cooling regardless of the weather conditions because it cools by evaporation of water. All systems need additional heating to maintain the

temperature above the set point, even in the summer period, due to the surrounding temperature decrease during the night. While the electricity for heating the tubular PBRs in summer is moderate (13% and 22% respectively for horizontal and vertical systems), the consumption is up to 62% for the ORP. Heating accounts for the highest energy consumption during fall and exceeds 75% of the total requirements for the two tubular PBRs operated in winter.

Despite the significant effect of temperature regulation system on the environmental impacts, no previous LCA study discussed this key issue. Most studies exclude this activity from the system boundaries. In some cases, this seems a realistic assumption, since the operating conditions to estimate the inventory data are based on locations with warm temperatures and sunlight intensities (Draaisma et al., 2013; Lardon et al., 2009), but for these locations the contribution of cooling will increase severely. For other studies that consider less favorable locations (Clarens et al., 2010; Stephenson et al., 2010), the effect of temperature regulation is expected to be relevant for the total impacts. To date, only Taelman et al. (2013) specified the use of waste heat to maintain temperature in winter. This input had no relevant contribution to the analyzed impacts compared to other processes within the system.

When comparing both tubular systems, the electricity consumption per functional unit (kWh per $kg_{DW}$ biomass produced) for the heating in the vertical PBR was between 1.3 and 1.9 times higher than for the horizontal PBR. This is linked to the larger tube area of the vertical system compared to the horizontal system (about two times larger); consequently more heating is needed to maintain minimum set temperature of 20°C. In addition, during daytime, less light is absorbed per loop in the vertical system compared to the horizontal system due to its design, light dilution effect and shading of the tubes. In fall/winter this effect is even more pronounced, because the lower tubes in the loops almost receive less light due to low inclination of the sun and shading.

Aeration and $CO_2$ addition had a contribution to the cultivation in the horizontal system of approximately 20% (similar in the three scenarios), but was below 10% for all vertical PBR and ORP scenarios. The high impact of the aeration/$CO_2$ is due to the back pressure of the stripper on pilot-scale. Small electrical blowers are not designed to overcome this pressure and for that an

oversized blower was needed; on large scale an air compressor would be used and electricity consumption would decrease largely. The impact of mixing (pumping and paddle wheel) was higher for the vertical PBR and ORP contributions than for the horizontal PBR due to higher volumes that needed to be mixed. For the ORP, the electricity consumption of the paddle wheel has already been pointed out as a relevant contributor by other authors (Draaisma et al., 2013; Lardon et al., 2009). Although the impact of the paddle wheel in fall is seven times higher than in summer (due to lower productivities), the relative contribution with respect to the total energy requirements is significantly higher for summer due to the lower consumption of other components (e.g. the heating system).

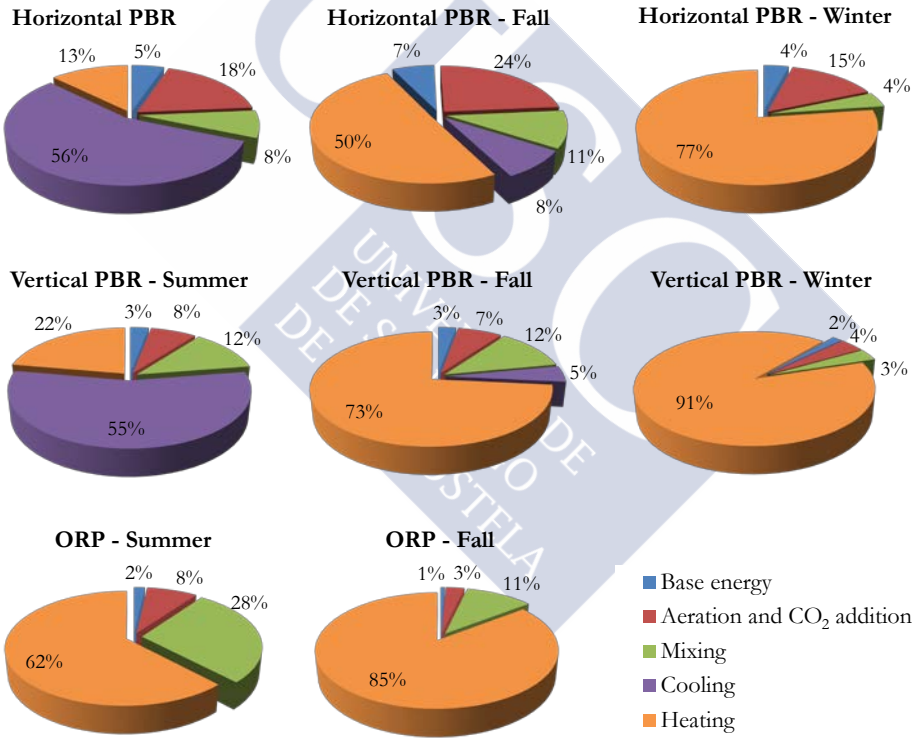



**Figure 8.23.** Distribution of electricity requirements during the cultivation stage for the production of *Nannochloropsis* sp. in the evaluated pilot reactors and weather conditions.

❖ **Scalability of the results**

The systems at AlgaePARC pilot facility are pilot-scale reactors built to reflect industrial scale systems as close as possible. However, at any pilot scale size, there are limitations with regard to e.g. efficiencies of circulation pumps, air blowers and especially temperature control, which are largely improved when scaling up. This effect influences significantly the absolute values measured at pilot scale. Thus, Taelman et al. (2013) indicated that upscaling from pilot to large scale may increase the efficiency of circulation pumps from 11% to 80%.

As mentioned before, culture temperature was controlled by a central electrical chiller and electrical heater. Choice for electrical cooling/heating is easily installable for a pilot plant meant for research, but due to their low efficiency and consequently very high energy demand, this should not be used at industrial scale plants. For large scale applications the use of ground water for cooling and waste heat from a biorefinery or power generation are much more convenient. If these heat sources are not available, the use of direct burning of fuels instead of electrical heating will reduce the impact of heating with a factor 2-2.5 (Slegers et al., 2014). Therefore, the values for heating and cooling reported here are much higher than what is expected at large scale.

Further improvements on the environmental impact can be obtained by either moving the production facility to a warmer climate with a higher sunlight intensity (Norsker et al., 2011; Taelman et al., 2013), by using a waste heat stream from e.g. power generation (Slade and Bauen, 2013) or by choosing a microalgal species that can grow at a wider range of temperatures, and therefore decreasing the need for heating or cooling. In addition, heating could be turned off during the night (only frost protection). However, before sunrise, the culture temperature should be above 20°C to prevent low productivities.

Since the inefficiencies of electrical equipment (e.g. the circulation pumps for the tubular systems) and the temperature regulation at AlgaePARC pilot facilites are analogous for all systems, comparison between systems is still valid. Consequently, these data should not be used to calculate absolute impacts for microalgae cultivation at industrial scale, but they serve well for analysis and comparison of the environmental performance of various process designs and to help debottlenecking these configurations.

## 8.5. Conclusions

The main aim of this work was to compare the environmental performance of the different alternatives for the cultivation of *Nannochloropsis* sp. Four types of reactors were evaluated, namely ORP, horizontal tubular PBR, vertical tubular PBR and flat-panel system. The effect of variable weather conditions on the environmental profiles was analyzed in detail for the most common reactor configurations: ORP and tubular PBRs. In addition, the major hot spots or problematic issues were identified.

The results show that the efficient environmental performance of an ORP system is extremely restricted to the weather conditions and this system may only be feasible during a limited period of the year, especially for locations with moderate to low temperatures and low sunlight intensities. This is due to the combination of higher electricity consumptions during cultivation stage for heating, together with a low volumetric productivity. Flat-panel reactors show a noticeable potential associated with the high biomass concentrations that can achieve, but their global efficiency is strongly affected by the chosen approaches to deal with specific factors of the process such as substitution or reuse of plastic bags. Tubular reactors have a good average performance during a longer period and are less dependent on the weather conditions.

The results from the outdoor installations deviate in several aspects from LCAs based on simulations and literature data, which highlighted the lower impacts of ORP compared to tubular PBRs due to a more simple operational strategy. The temperature regulation system and the variations in productivity during the seasons are the key factors for the results obtained in this study. Optimized temperature control strategies (e.g. integration of waste heat, using ground water for cooling or wider temperature ranges) are essential to maintain moderate energy consumption. Moreover, in the aforementioned studies, different algae productivities are used in the inventory analysis stage, due to more favorable locations for the considered facilities. The use of experimental data from pilot systems is essential to analyze and debottleneck the environmental impact for large scale cultivation.

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# Chapter 9

# Integrated economic and environmental assessment of algal multi-product systems[1]


*Summary*

The economic and environmental performance of microalgal processes has been widely analyzed in the last decades. Most of the evaluations focus on a single aspect and few examples propose an integrated approach to relate economic with environmental indicators. Biodiesel is usually the single product and the available LCA studies rarely discuss the effect of environmental benefits of co-products obtained in the same process. In addition, there is a wide variability in the results due to the different assumptions in the models and the limited knowledge about the processes.

In this study, two standardized models were combined to provide an integrated simulation tool including economic and environmental indicators. In the first stage, the developed model allowed assessing the performance of a harmonized scenario. For this case study, the findings were consistent with previous environmental and techno-economic assessments. In a second stage, the Monte Carlo simulation method was applied to quantify the influence of uncertain parameters in the economic and environmental results. Despite the wide range of possible values, the simulation showed a significant probability of achieving favorable environmental performance for all the evaluated categories and a minimum selling price in the same range reported in previous works.


[1] Research conducted in collaboration with the Department of Civil & Environmental Engineering, College of Engineering at Northeastern University (Boston, USA).

# Index

## 9.1. Measuring economic and environmental viability of microalgal biorefineries

The potential of microalgal products and particularly biofuels is widely recognized (Collet et al., 2014; Davis et al., 2011; Draaisma et al., 2013). However, the environmental feasibility is still subject to further optimization for the reduction of energy and fertilizer consumption, as well as to the development of novel technologies for algae processing that allow the decrease of the associated impacts (Collet et al., 2014). Moreover, there is currently a great controversy about the economic viability of large-scale algae production in the short-term (Davis et al., 2011; Richardson et al., 2012).

As already mentioned in previous chapters, life cycle assessment is probably the most widespread management tool addressing the environmental aspects of microalgal processes. Among the large number of LCA studies (Brentner et al., 2011; Campbell et al., 2011; Clarens et al., 2011; Collet et al., 2011; 2014; Draaisma et al., 2013; Jorquera et al., 2010; Sills et al., 2013; Soh et al., 2014; Taelman et al., 2013; Woertz et al., 2014), the production of bioenergy is the most common focus, especially in the form of biodiesel (Collet et al., 2015).

For this goal and functional unit (FU), most studies evaluate impact categories related to greenhouse gas emissions (GHG) and energy consumption (Brentner et al., 2011; Clarens et al., 2011; Collet et al., 2015; Draaisma et al., 2013; Jorquera et al., 2010; Sills et al., 2013; Woertz et al., 2014). Energy balance can be analyzed in terms of cumulative energy demand (CED, i.e. total energy consumed throughout the process) or energy return on investment (EROI, i.e. ratio between the total energy produced and the energy consumed in the process), also referred to as net energy ratio (Collet et al., 2015; Soh et al., 2014). Other common indicators include the eutrophication potential of the process, as well as land competition and water demand (Brentner et al., 2011; Clarens et al., 2011; Collet et al., 2015; Lardon et al., 2009; Soh et al., 2014).

Recent works highlight the multi-functional nature of microalgal processes and the importance of co-product exploitation coupled to biofuel production, which may allow significant environmental benefits (Collet et al., 2015; Soh et al., 2014). Soh et al. (2014) suggest that the optimal environmental performance of

biorefinery schemes is not necessarily associated with operating conditions that maximize lipid productivity (linked to the maximum biodiesel production), but with a balanced distribution of lipid and non-lipid fractions.

Techno-economic assessments of microalgal biorefineries are another essential element for the feasible implementation at large scale (Sun et al., 2011). Techno-economic models constitute key tools for the strategic planning and decision making process that help in the evaluation of project value (Borowitzka, 2013). Several studies on the economics of microalgal processes have been published in the last 30 years (Benemann and Oswald, 1996; Davis et al., 2011; 2014a; 2014b; Gong and You, 2014; Huntley and Redalje, 2007; Norsker et al., 2011; Richardson et al., 2012; Sun et al., 2011).

One of the first and more detailed economic evaluations was the analysis by Benemann and Oswald (1996). This study provides a comprehensive estimate of capital and operating costs (per barrel, bbl, of oil produced) of the main open pond designs and auxiliary elements for cultivation and downstream processing (including harvesting and extraction) that were available at the time. Despite its great value, the report lacks of information for closed photobioreactors (PBRs) and sensitivity or risk analysis. Moreover, the accurate evaluation of current technological advances requires an exhaustive update (Richardson et al., 2012).

More recent studies compare the economics of open ponds and other production systems including tubular and flat-panel PBRs (Davis et al., 2011; 2014b; Norsker et al., 2011), as well as hybrid configurations that combine the use of open and closed reactors (Huntley and Redalje, 2007). As in the report by Benemann and Oswald (1996), the results of most studies are expressed in economic units per barrel (Huntley and Redalje, 2007; Lundquist et al., 2010) or gallon (Richardson et al., 2012; Sun et al., 2011) of microalgal oil produced, before conversion into biodiesel or renewable diesel[2]. The reported values range

[2] As explained in Chapter 1, the term "biodiesel" is the mixture of mono-alkyl esters of long-chain fatty acids obtained by chemical reaction between crude oil (rich in triglycerides, TAGs) and alcohol in the presence of a catalyst, with glycerol as co-product while "renewable diesel" is the mixture of straight-chain and branched alkanes and aromatic compounds produced by hydroprocessing with no alcohol required (Hoekman et al., 2012).

between \$0.9-43 $gal^{-1}$, which correspond to \$28-1300 $bbl^{-1}$ (Sun et al., 2011). Some exceptions such as Norsker et al. (2011) evaluate the cost referred to biomass production, finding values between 4-6 €·$kg^{-1}$ biomass for the base scenarios that may decrease to 0.7 €·$kg^{-1}$ biomass after optimization. Davis et al. (2011; 2014b) introduce another indicator to express the economic performance which includes the conversion of algal oil to renewable diesel in order to estimate the final minimum selling price of the product. The values obtained by Davis et al. (2011) range between \$9.8-20.5 $gal^{-1}$ biodiesel, whereas Davis (2014b) reported minimum prices from \$5 $gal^{-1}$ up to \$22 $gal^{-1}$. Lundquist et al. (2010) also analyzed scenarios of biogas production. For these scenarios, the production costs are expressed in \$·$kWh^{-1}$ and range between \$0.17-0.89 $kWh^{-1}$.

Despite the previous efforts to measure environmental and economic behavior of microalgal systems, very few examples combine both aspects in an integrated analysis (Davis et al., 2014b; Gong and You, 2014). Since optimal economic and environmental results are not necessarily linked to the same operating conditions, the integrated evaluation is needed to ensure the design of processes that fulfill the requirements with respect to both criteria. Davis et al. (2014b) studied the variability of economic and environmental performance of renewable diesel obtained by hydrothermal liquefaction. However, the evaluation is restricted to a single parameter: productivity in different cultivation sites and seasons. Many other factors (e.g. oil content, co-product distribution, input and co-product prices, choices related to the varied alternatives for cultivation and downstream processing) affect the economic and environmental feasibility of microalgal biorefineries (Collet et al., 2015; Richardson et al., 2012; Sills et al., 2013). In order to take into account the effect of multiple parameters simultaneously, Gong and You (2014) present one of the first works on the integration of both economic and environmental criteria for the holistic optimization of the process using a multi-objective optimization approach. The combined study aims at determining the optimal technologies and operating conditions for a process focused on the carbon sequestration of coal-fired power plant emissions by algae according to a set of economic and environmental constraints. A similar multi-variable approach will be presented in this chapter to address simultaneously the economic and environmental aspects of a microalgal biorefinery producing biodiesel and several co-products.

## 9.2. Parameter uncertainty in algal processes

The results reported in the large number of economic and environmental assessments presented in the previous section show a high variability (Sills et al., 2013; Sun et al., 2011). The deviation is due to the wide range of alternatives for each production stage as well as the numerous assumptions for growth and operational parameters considered by the authors (Collet et al., 2015; Sills et al., 2013; Sun et al., 2011). The need of adopting assumptions and modeling choices is linked to the scarcity of real data on cultivation and processing technologies (Richardson et al., 2012; Sills et al., 2013). The lack of commercial facilities and the confidential nature of the existing information lead to large uncertainties in model parameters and resulting predictions (Sills et al., 2013).

Most available studies addressing either economic or environmental aspects consider one set of process and economic conditions at a time, according to a deterministic approach (Richardson et al., 2012; Sills et al., 2013). Thus, the outcomes consist of single-point results with minimal uncertainty that poorly reflect the inherent variability of the process models. To overcome this weakness, some authors conduct a sensitivity analysis for selected key parameters (Clarens et al., 2010; 2011; Davis et al., 2011; Liu et al., 2013). However, most of these analyses evaluate the changes associated with each variable separately rather than showing the combined effect of simultaneous changes in the entire set of parameters. Moreover, they usually establish a limited number of point values (e.g. effect of ±10% change in one input parameter) instead of considering the probability distributions for all the evaluated variables (Richardson et al., 2012; Sills et al., 2013).

Sills et al. (2013) highlight the suitability of the Monte Carlo simulation to conduct detailed risk assessments that provide more reliable environmental information for industrial stakeholders and policy-makers. Similarly, Richardson et al. (2012) apply the Monte Carlo method to carry out a financial feasibility study. The analysis differs from traditional deterministic techno-economic assessments in the incorporation of risk to estimate the probability of economic and financial success of a project instead of single-value results. The approach proposed in both studies is considered in this work to include parameter uncertainty in the integrated economic and environmental assessment.

## 9.3. Standardized tools for algal process modeling

Microalgal cultivation and downstream processing were simulated at commercial scale according to the baseline harmonized model described by Davis et al. (2012). This model was adopted as a result of the initiative launched by the U.S. Department of Energy's Biomass Program in 2011 to define a baseline scenario to simulate algal biofuel production at large scale. The harmonized scenario was established by adjusting the assumptions, proposed unit processes and values for key parameters that were previously considered in three separated models for addressing economic, environmental and resource aspects. The three models and associated approaches were discussed at the Harmonization Workshop, organized in 2011 in the framework of the Biomass Program, and a final set of technological options and consistent values for the main parameters in the three models were selected. The stages of algal biofuel production for the harmonized scenario are shown in **Figure 9.1**.

The environmental well-to-pump model applies a life cycle approach and is implemented in the Algae Process Description (APD) module. This module is associated with the Greenhouse Gases, Regulated Emissions and Energy Use in Transportation (GREET) model developed by Argonne National Laboratory (Frank et al., 2011a; b). The financial evaluation is based on the techno-economic analysis (TEA) model described by Davis et al. (2012). TEA methodology has already been used to evaluate the economic aspects of algal biofuels according to different growth conditions (Davis et al., 2014b). The resource assessment (RA) model consists of a national-scale resource and production evaluation that aimed at identifying suitable locations for open pond microalgae production (Davis et al., 2012). It involved the estimation of key parameters such as potential biomass and oil production, land resources and water consumption in open pond systems by modeling the major meteorological and physical processes influencing algal growth in a wide range of non-competitive areas within the United States (Davis et al., 2012; Wigmosta et al., 2011). However, the RA model was not required for the assessment presented in this chapter since the information provided by the model is out of the scope of the study, which comprises the economic and environmental issues.

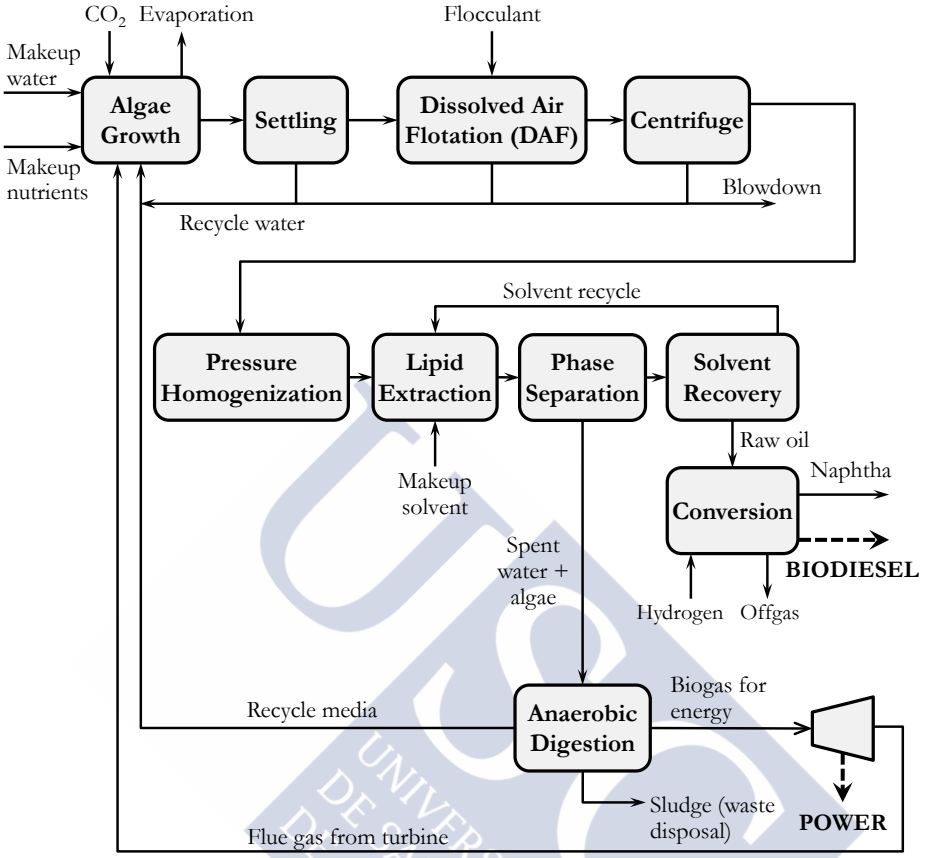


**Figure 9.1.** Schematic flow diagram of algal biofuel production according to the harmonized scenario implemented in APD and TEA models.
Source: Adapted from Davis et al. (2012) and Frank et al. (2011a).

In this work, the LCA and TEA models were combined in an integrated simulation tool that allowed the simultaneous evaluation of economic and environmental aspects of the process according to a set of input parameters. The integrated model was built in a single Excel file where both models were implemented. The input parameters were introduced in a general input sheet connected to the specific sections of the file that simulated each stage. The model was validated by determining the selected economic and environmental indicators for the harmonized scenario reported by Davis et al. (2012). The information for the selection of values to simulate the stages of cultivation, dewatering and bio-oil extraction was completed with data from the APD

module, whereas the conversion of bio-oil to renewable diesel[3] was simulated according to the full GREET model. The costs and other economic parameters were estimated according to the TEA model (Davis et al., 2012; 2014b). After the validation of the integrated model according to the harmonized parameters, probability distribution functions for the key input variables were estimated and implemented in a risk assessment tool to evaluate the effects of parameter uncertainty and identify the possible correlations between economic and environmental results.

### 9.3.1. GREET model: Algae Process Description tool

The GREET model is a spreadsheet-based tool implemented in Excel to evaluate fuel-cycle energy and emission impacts of available transportation fuels and vehicle technologies (Wang, 2001). The first version of GREET model was released in 1996 and has regularly been revised and updated to include new technologies and fuel types (Wang, 1996; 2001). According to Argonne National Laboratory (2010), the recent versions of GREET contain more than 100 fuel pathways including petroleum fuels, natural gas fuels, biofuels, hydrogen and electricity obtained from several energy feedstock sources. It includes three vehicle classes: passenger cars, light duty trucks with gross weight lower than 6000 lb and light duty trucks below 8500 lb. Given the available combinations of fuels and vehicles, GREET allows the simulation of more than 80 vehicle/fuel systems.

APD tool is a complement of GREET model that allows the complete simulation of the large-scale cultivation of algae for biofuel production (Frank et al., 2011a; b). In order to facilitate the integration of APD tool with GREET, this module is also implemented in Excel. It consists of several worksheets that contain the information required to determine the mass and energy inputs for each production stage, according to a set of growth and operational parameters.

[3] Renewable diesel was considered as the target biofuel according to TEA and harmonized models. Original APD model considered both conversion of algal oil to biodiesel by transesterification and conversion to renewable diesel via hydroprocessing, but focused on the results for biodiesel. This approach changed after the model harmonization (Davis et al., 2012).

The process is divided into the following five stages:

i) S1. Growth and first dewatering: This sheet contains the data and equations to calculate water and nutrient balances throughout the process, as well as biomass productivity and energy requirements of the medium pumping, aeration and other elements of the cultivation and the first steps of biomass harvesting. The simulating tool allows the user to simulate open pond and airlift photobioreactors. This study focuses on the performance of open pond systems, since they are currently the most common and inexpensive large-scale configuration (Brennan and Owende, 2010; Davis et al., 2011; Richardson et al., 2012). According to Frank et al. (2011a), the first dewatering step is also included in this sheet because it involves the movement of a large water volume (for the separation of the algal biomass from the culture medium and the recycling of medium to the pond) compared to the downstream operations.

ii) S2. Remaining dewatering: Several separation technologies can be selected for the biomass concentration simulated in this stage. The available methods include dissolved air flotation, centrifugation, flocculation and settling, use of belt filter press or Fournier rotary press, thermal drying and electrocoagulation. The key operating parameters (e.g. retention efficiency, final concentration) are specified for each technology and can be changed to simulate different conditions. According to the baseline harmonized model defined by Davis et al. (2011) a sequential separation based on dissolved air flotation (DAF) followed by a disk-stack centrifuge is here proposed.

iii) S3. Lipid extraction: As for the previous stage, this sheet allows the selection of different extraction routes such as hydrothermal liquefaction or hexane extraction. In this case, pressure homogenization and hexane extraction were considered.

iv) S4. Conversion to biofuel: The APD tool simulates the conversion of the extracted oil fraction by transesterification and hydroprocessing, according to the parameters for gasoline and biodiesel implemented in the complete GREET model (simulated in another Excel file).

v) S5. Recovery: Three possible routes for the use of the remaining biomass for energy generation are provided. The available processes are anaerobic digestion (AD) and two alternative gasification methods.

### 9.3.2. Techno-economic analysis model

The TEA model is based on the methodology described by Davis et al. (2011; 2014a; 2014b) and Humbird et al. (2011). The method consists of the estimation of direct and indirect capital costs as well as variable and fixed operating costs to determine the minimum diesel selling price by conducting a discounted cash flow rate of return analysis. In this type of analysis (already applied in Chapter 4 for astaxanthin production), the future cash flows throughout the plant life are calculated according to a set of financial parameters. These parameters include the internal rate of return and the tax rate as well as other ratios to estimate indirect costs and expenses that are subject to uncertainty. The minimum selling price is the price required to obtain a net present value (which represents the net benefit after subtracting the total costs) of zero.

The simulation tool quantified mass and energy balances to determine the required equipment and the corresponding direct costs. The total capital investment was obtained by applying estimated factors to calculate overhead costs that are not individually accounted for. Mass and energy balances were also required to calculate the operating costs per year, which depend on the consumption of raw materials and electricity. The credits associated with additional co-products were deducted from the calculated costs to obtain a final production cost. The minimum selling price was determined according to the total revenue required to compensate the calculated production cost (including the depreciation rate for the depreciable capital) as well as the desired return on investment. Several examples of Excel techno-economic models for other biomass products can be found in the website of the National Renewable Energy Laboratory (www.nrel.gov).

### 9.3.3. Sensitivity and uncertainty analysis: @RISK tool

Sensitivity and uncertainty analyses are two different approaches to evaluate the variability of a model. Despite the current lack of unified terminology, the term sensitivity analysis generally refers to the approach that aims at quantifying the impacts of possible variations in input data on the model outputs and performance indicators (Cacuci et al., 2008; Loucks et al., 2005). In most cases, the variability is measured in a localized region of the space of inputs and the potential changes are quantified separately for each parameter, assuming no changes in the other inputs (Loucks et al., 2005). Thus, sensitivity analysis allows estimating the relative importance of initial values assigned to uncertain parameters when using a model to describe a system (De Rocquigny, 2012; Van Asselt, 2000).

Uncertainty analysis is a broader group of methodologies that have the purpose of evaluating the entire set of possible performances of the modeled system (Loucks et al., 2005; Van Asselt, 2000). The selection of the method depends on the type of uncertainty to be analyzed. Uncertainty can be classified in two types or categories: i) epistemic and ii) stochastic. Epistemic uncertainty includes the variability that can be reduced by collecting a larger number of data (to reduce statistical uncertainty) or by developing more accurate models (to reduce model uncertainty, also known as structural uncertainty). Stochastic or aleatory uncertainty refers to the inherent natural variability of the system that cannot be reduced by an increase in data or knowledge (Cacuci et al., 2008; De Rocquigny, 2012; Faber, 2012).

Among the current techniques, probability-based methods are commonly used as a mathematical approach to express uncertainty that can be applied to measure both statistical (i.e. related to lack of information on the typical values of input parameters) and stochastic uncertainties (Van Asselt, 2000). These methods allow propagating the uncertainty through the model by considering probabilistic descriptions of variable input parameters so as to obtain the corresponding probability distribution of the outputs (Loucks et al., 2005). Hence, uncertainty analysis based on probabilistic approaches provides information of the likelihood of each response according to the likelihood associated with the uncertain input parameters of the model (Van Asselt, 2000).

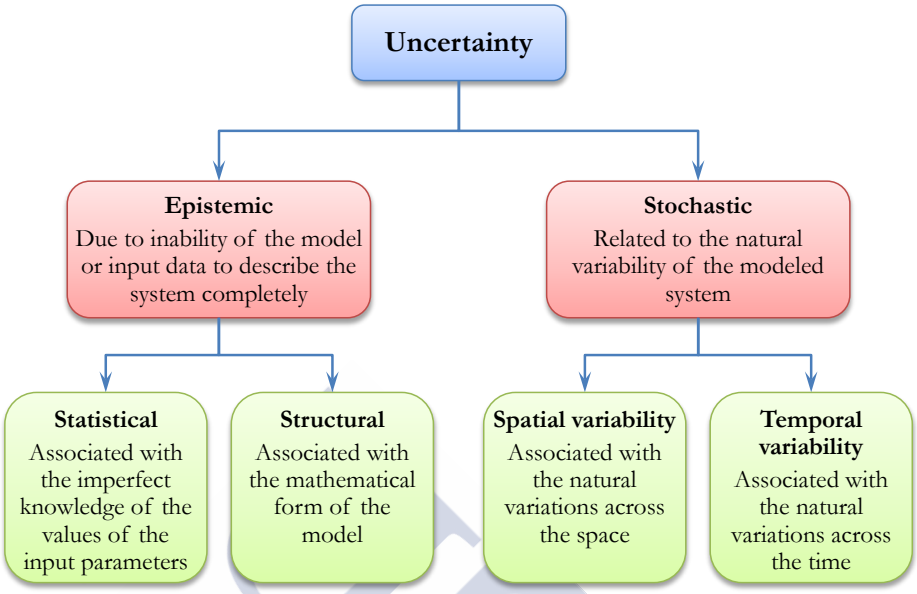


**Figure 9.2.** Classification of uncertainty sources.

One of the methods to conduct a probability-based uncertainty analysis is the Monte Carlo simulation (or Monte Carlo method). The Monte Carlo method serves to generate a large number of scenarios with random sets of input data that are calculated from their individual probability distribution functions by applying specific algorithms. It can be used to analyze both statistical and stochastic uncertainty and allows evaluating single uncertain parameters separately or a set of multiple parameters jointly. The random generation of values for the parameters may conduct to inconsistent scenarios, especially when working with complex multi-variable models. Therefore, correlations among input data need to be considered and validity checks may be required in these systems (Loucks et al., 2005).

The Monte Carlo simulation was the method selected in this case to analyze the effect of the uncertainty of input parameters on the economic and environmental results. To do so, the licensed software @RISK from Palisade Corporation was used (Palisade Corp., 2015). @RISK is a tool for conducting risk assessment that works as a complement of Excel and allows evaluating the variability of a set of output parameters from a spreadsheet model with respect to the uncertain input parameters.

The use of @RISK tool to analyze the uncertainty of a model once it has been built consists of three main steps:

i) Definition of input parameters

Firstly, the uncertain variables are defined as input parameters with the command "Define Distributions". To do so, the constant values previously specified in the corresponding cells are substituted by probability distribution functions that reflect the range of possible values and the probability of each value. The distribution functions can be obtained from several sources including available literature, experimental data fitting and estimations based on expert judgment. The @RISK tool itself has the specific command "Distribution Fitting" that allows obtaining the corresponding distribution function for a range of sample data.

ii) Definition of output parameters.

The mathematical relationships between input and output parameters have to be appropriately defined by Excel formulas in the original model. Each output parameter for which the uncertainty is evaluated has to be defined by using the @RISK command "Add Output".

iii) Simulation run

Once the input and output parameters as well as the corresponding distribution functions have been defined, the simulation is started after specifying the number of iterations to be performed. The simulation results provided by @RISK include the probability function of each output parameter, the list of individual scenarios evaluated during the simulation (with values for all input and output parameters), detailed statistical information for each input and output (e.g. mean value, minimum and maximum values, standard deviation, variance, percentiles) and graphical representations of the behavior of the different parameters and the correlations between parameters.

## 9.4. Integrated economic and environmental evaluation of a multi-product algal biorefinery scheme

As explained in Sections 9.1 and 9.2, the economic and environmental performance of microalgal biofuels have been widely analyzed in the last decades (Benemann and Oswald, 1996; Brentner et al., 2011; Clarens et al., 2011; Collet et al., 2015; Davis et al., 2011; Lundquist et al., 2010). However, there are very few examples that deal with both aspects (Davis et al., 2014b; Gong and You, 2014). The available integrated assessments are mainly focused on the production of biofuel (biodiesel or renewable diesel) as the single or main product of the system and provide scarce information on the potential benefits of co-products that can be obtained simultaneously. These benefits have already been pointed out in recent environmental assessments (Sills et al., 2013; Soh et al., 2014), but are usually underestimated or omitted in techno-economic results.

Moreover, the inherent variability of microalgal processes is rarely analyzed in detail. Most studies are based on deterministic models that provide single-point results (Richardson et al., 2012; Sills et al., 2013). Although some works include sensitivity assessments of key parameters subject to uncertainty, the approaches usually consist of the evaluation of a limited number of possible values rather than considering the probability distribution functions that describe the normal behavior of the parameters more accurately.

In this work, an integrated model for the combined study of economic and environmental criteria is presented for a microalgal biorefinery scheme. The system is simulated at large scale according to the harmonized model described by Davis et al. (2012). The assessment includes the potential benefits of process multi-functionality by taking into account economic and environmental credits of co-products. The evaluation incorporates the analysis of parameter uncertainty by applying a multi-variable approach that considers the probability functions of a wide range of operational and economic parameters simultaneously. The uncertainty assessment is performed according to the Monte Carlo simulation method.

### 9.4.1. Goal and scope

The aim of this study was to conduct a holistic evaluation of economic and environmental indicators of a multi-product microalgal system and integrate the effect of uncertainty inherent to the process. For the assessment, two standardized models were integrated in a single Excel spreadsheet and the parameters were initially validated according to the harmonized scenario for the production of renewable diesel from microalgae described by Davis et al. (2012). After model validation, the effect of uncertainty was evaluated by applying the Monte Carlo method with the tool @RISK.

As highlighted in previous chapters, the selection of the functional unit (FU) in a life cycle assessment is a critical issue that can affect the results (Pérez-López et al., 2014; Schau and Fet, 2008). For microalgal biodiesel, most of the available LCA studies express the results in terms of energy units (e.g. 1 MJ of biodiesel) or mass units (e.g. 1 kg of biodiesel), whereas most techno-economic assessments refer to monetary units per volume of biofuel produced (e.g. $\$\cdot gal^{-1}$ of biodiesel) (Collet et al., 2015; Davis et al., 2014b; Richardson et al., 2012). In the case of integrated economic and environmental assessments, there is no standardized approach to date regarding the selection of a common or two different FUs for economic and environmental indicators respectively. Thus, while some integrated assessments on biodiesel production (from either microalgae or other feedstocks) express each indicator in a specific reference unit (Davis et al., 2014b; Delrue et al., 2012), other authors give a clear definition of a fixed FU used to address both economic and environmental results (Campbell et al., 2011; Wang et al., 2011). In this work, the first approach was selected to allow comparisons of each group of indicators with previous studies. Thus, the environmental indicators are here calculated for a FU of 1 kg renewable diesel, whereas 1 gal of diesel was selected to refer the economic results in consistency with previous techno-economic assessments (Davis et al., 2011; 2014b; Richardson et al., 2012; Sun et al., 2011). The second FU (1 gal diesel) is equivalent to 2.95 kg of renewable diesel produced, according to the density specified in GREET model.

The system boundaries are divided into five stages according to the production scheme described in Section 9.3.1 for the GREET model (Frank et al., 2011a; b): i) cultivation (S1, including growth and first dewatering), ii) dewatering (S2), iii) oil extraction (S3), iv) oil conversion (S4) and v) co-product recovery (S5). For the analyzed biorefinery, an hydrotreating process was considered for oil conversion (S4) in order to obtain renewable diesel, as indicated for the harmonized scenario described by Davis et al. (2012). The co-product recovery stage (S5) included the separation of protein and fertilizer fractions as well as the AD of remaining biomass to obtain biogas that is then combusted for electricity and heat production. The complete scheme is presented in **Figure 9.3**.

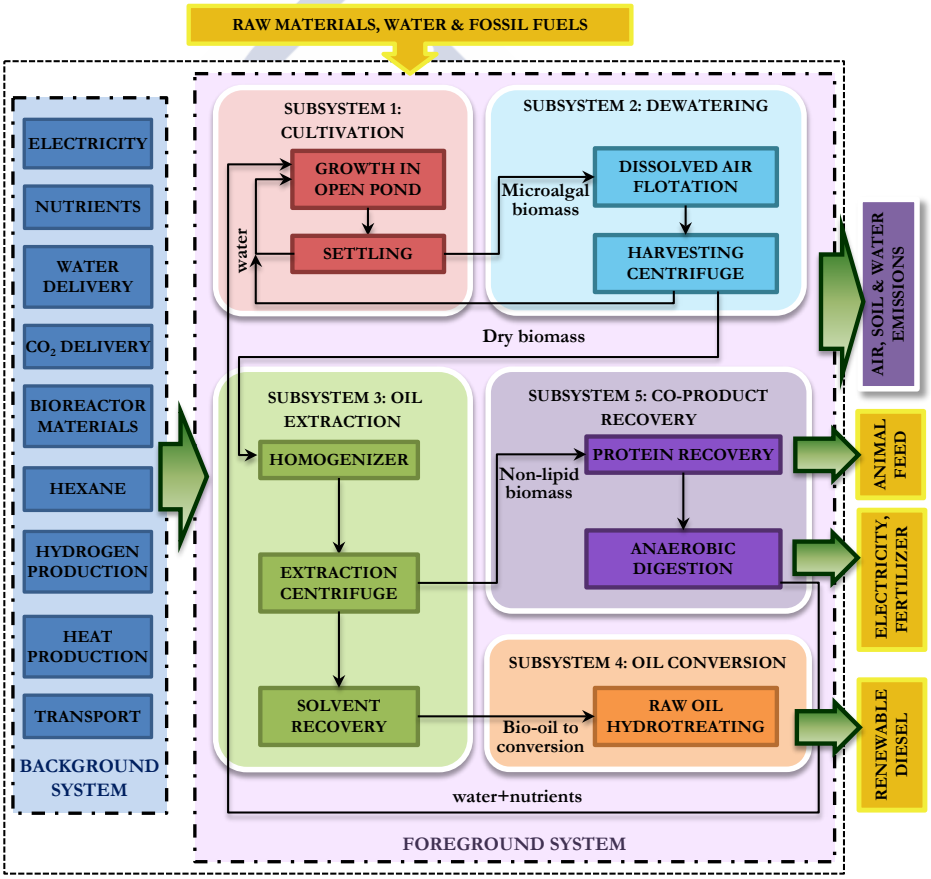


**Figure 9.3.** Process chain and system boundaries of the microalgal biorefinery for the simultaneous production of renewable diesel, animal feed and fertilizer fraction coupled to energy recovery by AD.

### 9.4.2. Data collection, sources and assumptions

As aforementioned, the integrated assessment was performed in two stages. In the first stage, a deterministic approach was used to validate the model according to a single set of parameters. In the second stage, the effect of uncertainty was evaluated by applying the Monte Carlo method to generate random scenarios. The data sources and assumptions considered in each stage are explained below.

❖ **Deterministic approach for model validation**

For model validation, both the life cycle inventory and the intermediate calculations of the economic model were determined by implementing the values of the harmonized scenario reported by Davis et al. (2012) for the input parameters. The main deviation from the original harmonized model was the introduction of a protein recovery step to obtain a fraction with potential uses as animal feed. The main process parameters are listed in **Table 9.1**.

**Table 9.1.** Values for the main process parameters according to the original APD and TEA models and new harmonized parameters

| Parameter | APD model | TEA model | Harmonized model |
|---|---|---|---|
| Lipid fraction (wt%) | 25% | 25% | 25% |
| Protein fraction (wt%) | 25% | NA | 47% |
| Carbohydrate fraction (wt%) | 50% | NA | 28% |
| Biomass productivity ($g \cdot m^{-2} \cdot d^{-1}$) | 25 | 25 | 13.2 |
| Water evaporative loss ($cm \cdot d^{-1}$) | 0.6 | 0.3 | 0.06 |
| Net harvesting efficiency | 85.5% | 99.0% | 95.0% |
| Net extraction efficiency | 85.5% | 85.5% | 85.5% |
| RD yield from raw oil (wt%) | 85% | 78% | 85% |
| Net nitrogen recovery to culture (N in effluent from total N into AD) | 76% | 75% | 76% |
| Net phosphorus recovery to culture (P in effluent from total P into AD) | 50% | 50% | 50% |

**Table 9.1.** Values for the main process parameters according to the original APD and TEA models and new harmonized parameters (*Cont.*)

| Parameter | APD model | TEA model | Harmonized model |
|---|---|---|---|
| Net N demand (mg·g$^{-1}$ algae) | 14 | 32 | 19 |
| Net P demand (mg·g$^{-1}$ algae) | 6.3 | 6.4 | 4.1 |
| Pond mixing (kWh·ha$^{-1}$·d$^{-1}$) | 48 | 48 | 48 |
| Recycle pump (kWh·m$^{-3}$) | 0.048 | 0.019 | 0.025 |
| Water pump from off-site (kWh·m$^{-3}$) | 0.048 | 0.3 | 0.123 |
| DAF output solids content (wt%) | 10% | 10% | 6% |
| Centrifuge power (kWh·kg$^{-1}$ out) | 0.0577 | 0.0101 | 0.0193 |
| Homogenizer power (kWh·kg$^{-1}$ homogenized) | 0.000204 | 0.00011 | 0.000204 |
| Solvent extraction heat (kWh·kg$^{-1}$ oil) | 1.38 | 4.48 | 3.09 |
| Solvent extraction electricity, (kWh·kg$^{-1}$ oil) | 0.54 | 0.05 | 0.069 |
| AD heat demand (kWh·kg$^{-1}$ total solids, TS) | 0.54 | NA | 0.22 |
| AD electricity demand (kWh·kg$^{-1}$ TS) | 0.136 | 0.027 | 0.085 |
| AD yield (L $CH_4$/g-TS) | 0.3 | 0.33 | 0.3 |
| Gross electricity demand (including all $CO_2$) (kWh·kg$^{-1}$ oil) | 5.7 | 3.7 | 5.1 |
| Net electricity imported (kWh·kg$^{-1}$ oil) | 1.4 | -1.8 | 1.32[1] |

[1] This value corresponds to the energy recovery in the harmonized scenario described by Davis et al. (2011), which considers all non-lipid biomass sent to AD and no protein recovery. In this chapter, the protein fraction is first separated, so the produced energy is lower, and the net electricity imported is 3.4 kWh·kg$^{-1}$ oil.

*Environmental LCA*

The inventory data for the foreground system included the consumption of nutrients for the culture medium, the chemicals used in the downstream processing (i.e. flocculant for dewatering, hexane for oil extraction and hydrogen for oil hydrotreating) and the energy requirements (electricity and natural gas). The estimation of the materials for the infrastructure (e.g. steel, concrete, polyethylene) was based upon a group of 10 hypothetical facilities of 405 ha each, with 30 years of life span. This group of facilities was equivalent to a total diesel production of approximately 10 million gallons per year, considering 13.2 $g \cdot m^{-2} \cdot d^{-1}$ biomass productivity and 25% lipid content (harmonized model). Each facility consisted of 100 individual ponds (4 ha each) as well as the equipment for downstream processing. The pond design was based on the technical features proposed by Lundquist et al. (2010).

The background processes (e.g. production of nutrients for cultivation and chemicals for downstream processing, manufacturing process of materials for infrastructure, electricity production) were inventoried according to unit processes from Ecoinvent 2.2 and US LCI databases. All Ecoinvent processes were adjusted to rely on national average U.S. energy system parameters using the US-EI database (EarthShift, 2013; Frischknecht et al., 2007).

Regarding allocation procedures, renewable diesel (RD) was considered the main product in this study according to the harmonized model used as the baseline scenario. However, four additional co-products were obtained in the system: protein fraction with potential applications as animal feed, naphtha separated from renewable diesel in the hydrotreating unit and fertilizer and energy from the biogas obtained in the AD. $CO_2$ sequestration potential of cultured algal biomass was also included in the assessment. The environmental benefits of the co-products and $CO_2$ sequestration were taken into account in the LCA study as environmental credits by applying a system expansion approach. For the economic assessment, the market value of the co-products was also accounted for to determine the total revenues of the process.

The global inventory was determined for the selected FU (1 kg renewable diesel) with the integrated simulation model. The inventory data calculated from the simulation of the harmonized scenario are shown in **Table 9.2**.

**Table 9.2.** Inventory data for the simulated microalgal biorefinery in the harmonized scenario (FU=1 kg renewable diesel)

| | | | |
|---|---|---|---|
| **INPUTS from TECHNOSPHERE** | | | |
| **Materials** | | | |
| *S1. Cultivation* | | | |
| N-fertilizer | 0.11 kg | Reinforcing steel | 0.96 g |
| P-fertilizer | 0.02 kg | Polyethylene | 0.07 kg |
| Concrete | 0.14 L | Cast iron | 0.02 g |
| *S2. Dewatering* | | | |
| Aluminum sulfate (flocculant) | 0.09 kg | | |
| *S3. Oil extraction* | | | |
| Hexane | 0.06 kg | | |
| *S4. Oil conversion* | | | |
| Hydrogen | 0.03 kg | | |
| **Energy** | | | |
| *S1. Cultivation* | | | |
| Electricity from US grid | 3.34 kWh | Diesel (for excavation) | 0.04 kg |
| *S2. Dewatering* | | | |
| Electricity from US grid | 1.33 kWh | | |
| *S3. Oil extraction* | | | |
| Electricity from US grid | 1.09 kWh | Natural gas | 3.62 kWh |
| *S4. Oil conversion* | | | |
| Electricity from US grid | 0.06 kWh | Natural gas | 0.05 kWh |
| *S5. Co-product recovery* | | | |
| Electricity from US grid | 0.18 kWh | Natural gas | 0.46 kWh |
| **OUTPUTS to TECHNOSPHERE** | | | |
| **Products** | | | |
| Renewable diesel | 1 kg | N-fertilizer | 22.78 g |
| Electricity | 1.99 kWh | P-fertilizer | 15.91 g |
| Natural gas | 2.58 kWh | Sequestered carbon | 20.38 g |
| Protein (use as animal feed) | 2.57 kg | | |

*Economic data*

For the economic analysis, a desired rate of return of 10% was assumed and 2011 was selected as the base year, according to previous techno-economic assessments (Davis et al., 2014a; 2014b).

The equipment costs were estimated from available literature (Benemann and Oswald, 1996; Davis et al., 2012; Lundquist et al., 2010) and updated to 2011-dollars according to the Chemical Engineering Plant Cost Index (CEPCI) from Chemical Engineering magazine (Chemical Engineering, 2012). Overhead cost factors were applied to include other facility costs in the calculation of total direct cost (TDC) and fixed capital investment (FCI).

Labor costs were estimated for 2011 according to Davis et al. (2014a). Prices for raw materials and co-products were obtained from the sources indicated in Appendix II (Table II.3). Prices of chemicals were adjusted to 2011-dollars when required according to the annual average producer price indexes from the U.S. Bureau of Labor Statistics (2015).

The main economic parameters (e.g. project lifetime, tax rate, depreciation model) for the first stage of the assessment (single-point analysis for model validation) were estimated from Davis et al. (2014a; 2014b). The considered values are summarized in **Table 9.3**.

❖ **Uncertainty analysis**

In the second stage of the assessment, the effect of uncertainty was evaluated with @RISK. In this case, the key parameters were classified in three groups: i) process parameters, ii) characterization factors and iii) economic parameters. The specific parameters included in each group are listed in **Table 9.4**. The probability distribution function reflecting the variability of each parameter was required. The estimated distribution functions and corresponding sources are indicated in Appendix II.

**Table 9.3.** Main economic parameters for model validation (Davis et al., 2014a; 2014b)

| Parameter | Value |
|---|---|
| Reference year | 2011 |
| Rate of return | 10% |
| Plant life | 30 years |
| Annual operating days | 330 |
| General plant depreciation | MACRS[1] 200% declining balance |
| Recovery period | 7 years |
| Tax rate | 35% |
| Indirect costs | |
| Site development | 9% of installed equipment cost |
| Warehouse | 4% of installed equipment cost |
| Prorateable Costs | 10% of TDC |
| Field Expenses | 10% of TDC |
| Home Office and Construction | 20% of TDC |
| Contingency | 10% of TDC |
| Other Costs | 10% of TDC |
| Working capital | 5% of FCI |
| Fixed operating costs | |
| Labor burden | 90% of labor cost |
| Maintenance | 3% of installed equipment cost |
| Property insurance and taxes | 0.7% of FCI |

[1] Modified Accelerated Cost Recovery System

Table II.1 presents the data for process parameters. The distribution functions for biomass composition and productivity were obtained with @RISK Distribution Fitting tool from experimental data for microalgae *Chlorella sorokiniana*, *Nannocloropsis oculata* and *Neochloris oleabundans* grown at lab-scale under nitrogen-deplete conditions using different cultivation periods. Due to the large variability of growth parameters depending on species and conditions, uniform distributions gave the best fit. Other parameters including nutrient excess, co-product substitution ratio (which measures the equivalency between the obtained co-product and the similar product for which the environmental credits are calculated) and AD yield were estimated from the literature assuming triangular distributions (Bryant et al., 2012; Mulbry et al., 2005; Sills et al., 2013).

In the case of characterization factors, the distribution function indicated in Table II.2 for each environmental indicator and Ecoinvent unit process was estimated by using the Monte Carlo Analysis tool available in SimaPro and @RISK Distribution Fitting tool (Goedkoop et al., 2013; Palisade Corp., 2015). Three types of distributions gave the best fitting results: normal distribution, lognormal distribution and loglogistic distribution.

Regarding the economic parameters (Table III.3), the rate of return and cost factors were adjusted to triangular distributions, considering the different assumptions from previous assessments related to algae (Davis et al., 2011; 2012; 2014a; 2014b; Lundquist et al., 2010) and other biomass sources (Aden et al., 2002; Humbird et al., 2011). The direct capital cost of ponds, pond liners and $CO_2$ system were introduced as normal distributions. The mean ($\mu$) was equal to the initial value used for model validation and standard deviation $\sigma$ was set as 10% (Mariano et al., 2013). Land cost function was obtained by adjusting average U.S. farm values per state (USDA, 2014) with @RISK Distribution Fitting tool. The price of electricity and flocculant were introduced as triangular distributions according to the variability in the period 2007-2014. Data for electricity were obtained from EIA (2015) whereas the initial price of flocculant was adjusted with the price index for chemicals (U.S. Bureau of Labor Statistics, 2015). The prices of hydrogen (raw material), naphtha and fertilizers (co-products) were also fitted to triangular distributions according to the minimum and maximum values from the sensitivity analysis by Davis et al. (2014a). The

price of protein was estimated according to a triangular function with the same range as soybean meal for the period between January 2011 and January 2012 (IndexMundi, 2015). The different quality of the algal protein was already taken into account with the process parameter "co-product substitution ratio".

**Table 9.4.** Classification of the variable parameters included in the uncertainty analysis

| Category | Analyzed effect | Parameter |
|---|---|---|
| Process parameters | Biomass composition | Lipid content |
| | | Protein content |
| | | Carbohydrate content |
| | Algal yield | Specific (aerial) productivity |
| | Fertilizer requirements | Excess of N and P |
| | Carbon sequestration in AD residue | C sequestered/C applied |
| | Biogas from AD | Methane yield |
| | Co-product substitution ratio | Microalgae to commercial protein ratio |
| | | N bioavailability |
| | | Algal extractable P |
| Characterization factors | Uncertainty in background processes (expressed in Ecoinvent according to probability functions) | Variability of GHG factors |
| | | Variability of eutrophication factors |
| | | Variability of CED factors |
| Economic parameters | Financial parameter and cost factors | Desired return on investment |
| | | % indirect contingency costs |
| | | % labor and overhead |
| | | % maintenance |
| | | % property insurance, taxes |
| | Direct capital costs | Base cost of ponds |
| | | Base cost of pond liners |
| | | Base cost of $CO_2$ system |
| | | Land cost |
| | Price of raw materials | Power |
| | | Hydrogen |
| | | Flocculant |
| | Price of byproducts | Price of naphtha |
| | | Price of protein |
| | | Price of N-fertilizer |
| | | Price of P-fertilizer |

### 9.4.3. Economic and environmental results for harmonized scenario

Before conducting the uncertainty assessment, the model was validated with the parameters of the harmonized scenario. As in previous chapters, the environmental profile was obtained by performing the classification and characterization stages of the LCA methodology (ISO 14040, 2006). Since the model and operational parameters used in this study correspond to the North American context, CML methodology was not applied. Instead, the impact assessment methodology from the U.S. Environmental Protection Agency (EPA) was considered. Thus, the characterization factors from the Tool for the Reduction and Assessment of Chemical and other environmental Impacts (TRACI) model were used to evaluate GHG emissions (in $CO_2$ eq), eutrophication (in N eq), and cumulative energy demand (in MJ). These categories were selected as the most common environmental indicators in LCA studies dealing with environmental aspects of microalgal biofuels (Brentner et al., 2011; Clarens et al., 2010; Soh et al., 2014). EROI was calculated based on a HHV of 36 MJ·kg$^{-1}$ of renewable diesel, obtained from the GREET model.

❖ **Environmental results**

The characterization results for the harmonized scenario are shown in **Table 9.5**. The obtained values are consistent with previous results in the literature. Although most of the previous studies refer to biodiesel rather than renewable diesel, the difference associated with the conversion stage is here considered to be sufficiently limited so as to allow straightforward comparisons for the two products. For comparative purposes, all the values from the literature are here expressed in terms of the selected FU (1 kg diesel).

Thus, the calculated GHG emissions are in the range of 2-4.5 kg $CO_2$ eq·kg$^{-1}$ of diesel reported by Campbell et al. (2011), 1.5-4 kg $CO_2$ eq·kg$^{-1}$ of diesel indicated by Davis et al. (2014b) and 0.5-2.5 kg $CO_2$ eq·kg$^{-1}$ of diesel for the nitrogen-deplete scenarios from Soh et al. (2014). For eutrophication potential, as explained below in more detail, the co-product credits totally compensate the environmental burdens and lead to a negative impact. This means that the protein and fertilizer obtained in the process substitute other products from alternative routes with higher environmental burdens, and therefore avoid these impacts. The results for eutrophication show a better profile than previous

findings, although the favorable performance of algae compared to other feedstocks (i.e. terrestrial crops) was already pointed out in previous works with low reported values between 0.0002 and 0.20 kg N eq·kg$^{-1}$ of diesel (Brentner et al., 2011; Clarens et al., 2010; Soh et al., 2014). The energy demand indicated in **Table 9.5** can be expressed as EROI by dividing the heating potential of 1 kg renewable diesel by the calculated CED. Assuming a standard HHV of 36 MJ·kg$^{-1}$, the harmonized scenario leads to a favorable EROI=1.67 MJ. This result is in the range of previous LCA studies (Clarens et al., 2011; Jorquera et al., 2010; Lardon et al., 2009; Sander and Murthy, 2010; Stephenson et al., 2010), including the EROI values between 1-4 MJ produced·MJ$^{-1}$ consumed for a high-productivity scenario obtained in the uncertainty assessment conducted by Sills et al. (2013).

**Table 9.5.** Environmental impact assessment results (characterization step) associated with the production of 1 kg renewable biodiesel with protein, fertilizer and electricity as co-products according to harmonized scenario

| Impact category | Unit | Value |
|---|---|---|
| GHG emissions | kg $CO_2$ eq | 2.46 |
| Eutrophication | kg N eq | -0.02 |
| CED | MJ | 21.51 |

The relative contributions per stage are depicted in **Figure 9.4**. Among the production stages, cultivation has the largest effect, regardless of the considered impact category. Its contribution exceeds 50% of the total environmental impact for each indicator. Most of the impact (between 70% and 90%) is due to the electricity consumption for media circulation. The production of nutrients for the culture media and polyethylene of the reactor constitute the highest secondary contributions. Oil extraction is the second stage affecting GHG emissions and CED. The main reason for this impact is the high electricity consumption in the pressure homogenizer. For eutrophication, dewatering has a higher impact than oil extraction, although both contributions are mainly associated with electricity.

The three categories show high reductions of impact related to the environmental benefits of the co-products. In the case of eutrophication, the credits from the production of protein fraction alone are higher than the total impact of the production stages. For this reason, the environmental impact has a negative value, which entails that the obtained co-products allow avoiding the production of alternative substances with higher impacts than the whole process analyzed here. The environmental benefits of GHG emissions are nearly 60% of the total impacts, whereas the credits for CED represent 81% of the total demand throughout the production stages. The final GHG emissions are therefore 55% lower than the total emissions that the process would have if no co-product was obtained, whereas the energy balance is 80% lower than the CED of the same process with no co-products.

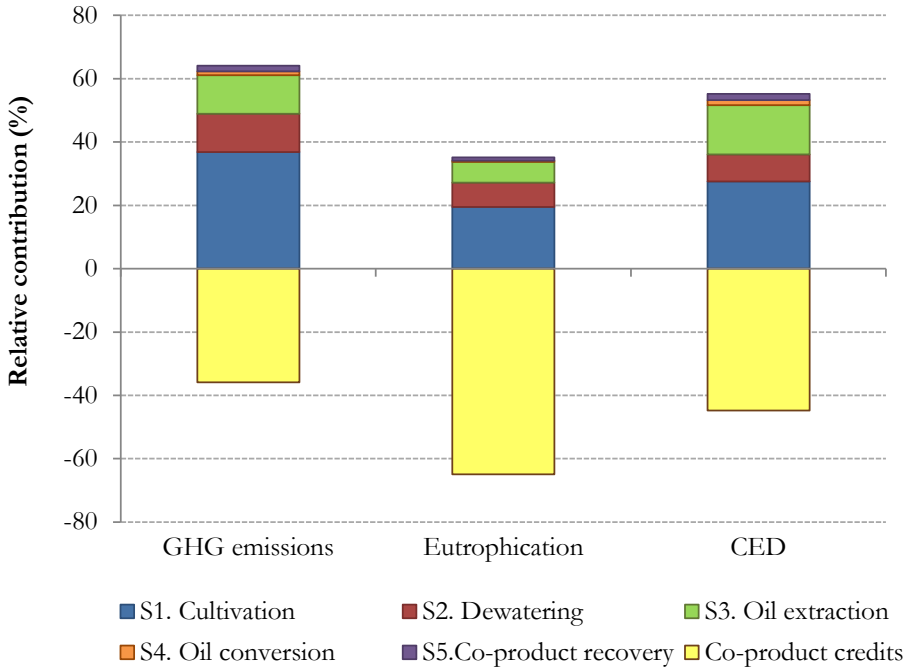


**Figure 9.4.** Relative contributions of microalgal production of renewable diesel, protein fraction and fertilizer coupled to energy recovery by AD per stage.

❖ **Economic results**

The economic results were calculated assuming a desired rate of return of 10% and using 2011 as the base year, following the assumptions of Davis et al. (2014b). For the operational parameters of the harmonized scenario, a total production cost of \$14.91 $gal^{-1}$ of raw oil is obtained when the production of renewable biodiesel is coupled to the production of protein and fertilizer fractions and the remaining biomass is subjected to an AD process to recover energy in the form of biogas (**Figure 9.5**). This production cost corresponds to a minimum selling price of \$16.18 $gal^{-1}$ of renewable diesel.

The obtained values are significantly higher than the results reported by Davis et al. (2011) for open ponds, although they are below the values given for tubular PBR in the same assessment. The main reason for the worse economic performance of the harmonized scenario is the lower biomass productivity. Thus, the productivity considered by Davis et al. (2011) corresponds to the value of 25 $g \cdot m^{-2} \cdot d^{-2}$ indicated in **Table 9.1** for the original TEA model. As explained in Davis et al. (2012), the application of the RA model (mentioned in Section 9.3) resulted in an estimate of the mean annual biomass productivity of 13.2 $g \cdot m^{-2} \cdot d^{-1}$, which was significantly lower than the value considered in the original APD and TEA models. Thus, Davis et al. (2012) indicate that the application of the new scenario led to remarkably higher costs and emissions than the previous estimates.

When comparing the results of the scheme analyzed in this chapter with the values from Davis et al. (2014b), the minimum selling price is close to the range of \$10-15 $gal^{-1}$ for biomass productivities between 10-14 $g \cdot m^{-2} \cdot d^{-1}$. The slightly higher value found for this study is mainly linked to the different approach considered for the oil conversion stage. The scenario evaluated by Davis et al. (2014b) includes a hydrothermal liquefaction step that is not considered in the current study. Other factors are related to the fluctuations in prices and economic parameters. Since these fluctuations are inherent of the system and cannot be avoided, the following uncertainty assessment will complete the information and give a wider view of the possible economic performances of the process.

According to **Figure 9.5**, the production costs for microalgal biodiesel are mainly associated with the capital costs. Thus, nearly 80% of the total cost is due to the capital investment required for the establishment of the facility, the construction of the production systems and other related costs. Operating costs involve less than 20% of the total, whereas land costs are below 5%. The low contribution of land to the final cost reflects one of the main advantages of microalgae: the possibility to cultivate algal biomass in marginal and non-competitive land with low value. Regarding operating costs, about 40% are expenses related to maintenance, taxes and insurance, while electricity is responsible for 16%, and nutrients, waste management and labor costs are in the range of 10-15% each. The sum of other operating costs is below 10%.

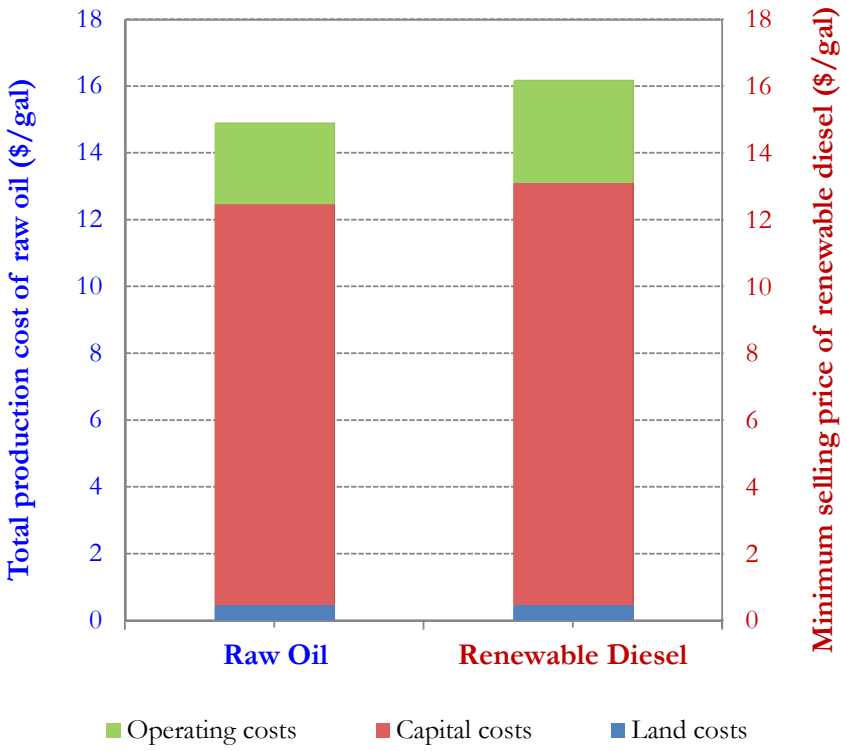


**Figure 9.5.** Breakdown of oil production costs (blue axis) and diesel minimum selling price (red axis), considering 10% internal rate of return and 2011 as the base year.

### 9.4.4. Uncertainty assessment with @RISK tool

In this stage, the key uncertain parameters were defined as @RISK inputs according to the probability distribution functions listed in Appendix II (Tables II.1 to II.5). The four performance indicators were defined as outputs together with the parameter "others". This parameter refers to the percentage of remaining biomass (mainly the mineral fraction of the biomass) after subtracting lipid, protein and carbohydrate content. It was included as an output in the simulation to ensure (by applying an @RISK filter to the parameter) that the sum of lipid, protein and carbohydrate fractions was below 100% in all the simulated scenarios. Once the inputs and outputs were defined, the Monte Carlo simulation was run for 5000 iterations.

❖ **Global variability and probability functions for the output parameters**

The main statistical results are listed in **Table 9.6**. The range of possible values for the measured economic and environmental indicators is depicted in **Figure 9.6**, which shows the probability density function and cumulative distribution function for each parameter. According to the results, GHG emissions in 90% of the evaluated scenarios range between 1.27 and 11.07 kg $CO_2$ eq·kg$^{-1}$ renewable diesel, whereas eutrophication varies from a negative impact of -0.013 kg N eq·kg$^{-1}$ diesel to 0.025 kg N eq·kg$^{-1}$ diesel and the energy demand is between 19 and 203 MJ·kg$^{-1}$. Although the highest values for GHG emissions and CED show a less favorable environmental profile than the best cases reported in the literature, the ranges are consistent with common average values (Campbell et al., 2011; Clarens et al., 2010; Sills et al., 2013; Soh et al., 2014). Thus, there is a 65% probability of GHG emissions in the range of 0.5-4.5 kg $CO_2$ eq·kg$^{-1}$ of diesel from previous LCA studies indicated in Section 9.4.3 (Campbell et al., 2011; Davis et al., 2014b; Soh et al., 2014). In the case of eutrophication, the results show the benefits related to the integration of co-products in the biorefinery scheme, especially associated with the credits from the protein fraction and, to a lesser extent, from the recovered fertilizer. CED shows a wide range of values. Despite the 5% probability of obtaining an EROI<0.18, 50% of the cases may have a CED between -31 and 52 MJ·kg$^{-1}$, which would lead to EROI values above 0.7. These results are consistent with the values reported by Sills et al. (2013) for best and worst production scenarios.

**Table 9.6.** Statistical parameters of the probability distributions for the four evaluated indicators

| Statistical parameter | GHG emissions (kg $CO_2$ eq) | Eutrophication (kg N eq) | CED (MJ) | Minimum selling price ($/gal) |
|---|---|---|---|---|
| Minimum | -1.875 | -0.072 | -31.418 | 7.942 |
| Maximum | 38.482 | 0.238 | 2495.274 | 336.453 |
| Mean | 4.165 | 0.003 | 74.538 | 38.863 |
| Mode | 1.948 | -0.001 | 31.485 | 13.595 |
| Standard deviation | 3.727 | 0.016 | 84.651 | 35.075 |
| Variance | 13.887 | 0.0002 | 7165.864 | 1230.270 |
| Skewness[1] | 3.171 | 4.144 | 9.160 | 2.822 |
| Kurtosis[2] | 17.278 | 39.573 | 185.369 | 14.201 |
| Percentiles: | | | | |
| 5% | 1.269 | -0.013 | 19.913 | 11.175 |
| 25% | 1.993 | -0.003 | 34.648 | 17.122 |
| 50% (median) | 3.001 | 0.000 | 51.706 | 27.079 |
| 75% | 4.868 | 0.005 | 85.787 | 46.644 |
| 95% | 11.070 | 0.025 | 202.713 | 105.639 |

[1] Measure of the symmetry of the probability distribution. For symmetrical distributions, skewness=0.

[2] Measure of the shape of the probability distribution. High values of kurtosis involve distributions with sharp peaks and thick tails.

Regarding the economic performance, the obtained diesel should be sold at a minimum price of \$11-106 $gal^{-1}$ with a probability of 90%. This range is wider than the estimates between \$0.9-43 $gal^{-1}$ from previous assessments (Lundquist et al., 2010; Richardson et al., 2012; Sun et al., 2011). The variability of the results is linked to the large number of variable process and economic parameters that are considered in the current study. In contrast, most of the previous techno-economic assessments considered a single case study or a limited number of changes. Despite the high variability of the indicator, the probability of maintaining a minimum selling price below \$28 $gal^{-1}$ exceeds 50%, whereas only 25% of the situations would result in prices above \$46 $gal^{-1}$. This finding is consistent with the aforementioned range from the literature.

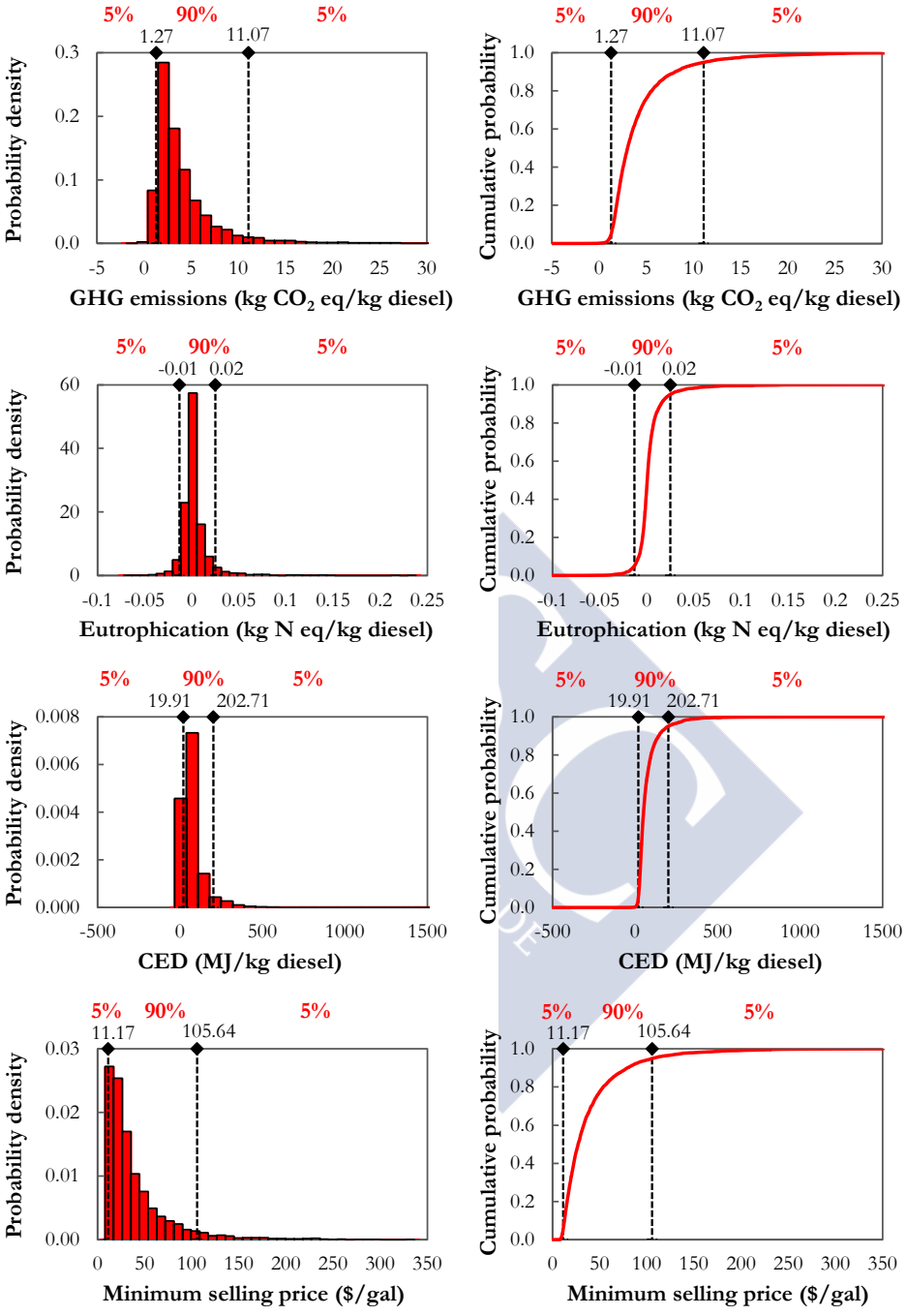


**Figure 9.6.** Probability density function and cumulative distribution function for economic and environmental indicators, including percentiles 5% and 95%.

❖ **Correlations between the economic and environmental indicators**

The scatter plots showing the variability of the results from the Monte Carlo simulation for each pair of indicators are presented in **Figure 9.7**. Pearson's and Spearman's correlation coefficients are shown in **Table 9.7**. Pearson's correlation coefficient measures the strength of the linear relationship between two variables. Spearman's coefficient is a nonparametric measure of interdependence between variables that are not necessarily linear but can be related to each other by a monotone function (Hauke and Kossowski, 2011).

According to Pearson's coefficients, GHG emissions and minimum selling price have the strongest linear relationship. This connection can be partially attributed to the GHG emissions derived from the use of electricity, which also involve significant operating costs. However, the Pearson's coefficient that reflects the environmental performance of the system in terms of CED with respect to the minimum selling price and the coefficient that links GHG emissions with CED have lower values. This suggests that CED is affected by a variable parameter that has a lower influence in GHG emissions and minimum selling price. The deviation may be linked to the co-product credits, which involve remarkable reductions of environmental impact. In particular, the credits associated with the protein fraction involve a reduction between 0.3% and 52% of the CED of the production stages, whereas the same product represents reductions below 15% for GHG emissions and generates less than 11% of annual revenues. Eutrophication has low correlation coefficients with respect to the other three indicators. The values indicate the lack of a linear relationship of eutrophication with the other measured indexes. This finding is consistent with the characteristics of eutrophication, which is associated with unit processes and substances that have lower contributions to the other categories.

Despite the different type of mathematical relationship measured by Spearman's correlation coefficient, the results show common trends compared to Pearson's coefficients. Thus, the association of GHG emissions with minimum selling price has again the highest correlation coefficient. In this case, the coefficient that describes the relationship between GHG emissions and CED is remarkably higher than Pearson's value and only 0.2% lower than the coefficient describing GHG variability with respect to the economic indicator. This finding reflects the

clear interdependence of the two parameters, despite the non-linearity of the relationship. Spearman's correlation coefficients for eutrophication also show the low connection of this parameter with the other indicators.

**Figure 9.7** also indicates the probability of combinations in each quadrant. Regardless of the pair of performance indicators, most of the simulated scenarios are included in the third quadrant. The results indicate a high probability of achieving production scenarios with GHG emissions below 4.17 kg $CO_2$ eq·kg$^{-1}$ diesel, eutrophication potential below 0.003 kg N eq·kg$^{-1}$ diesel, CED lower than 74 MJ·kg$^{-1}$ diesel and a minimum selling price below \$39 gal$^{-1}$.

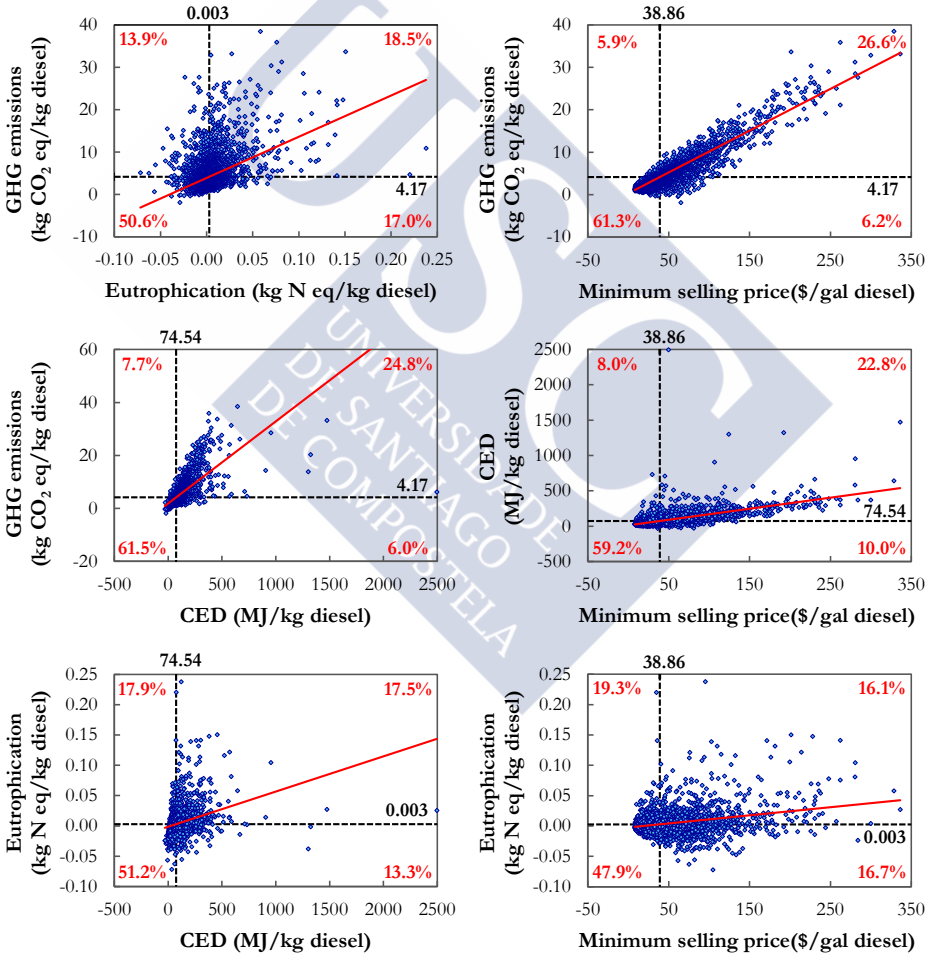


**Figure 9.7.** Correlations between the analyzed performance indicators.

**Table 9.7.** Pearson's and Spearman's correlation coefficients for the analyzed economic and environmental indicators

| | Performance indicator | GHG emissions (kg $CO_2$ eq·$kg^{-1}$ diesel) | Eutrophication (kg N eq·$kg^{-1}$ diesel) | CED (MJ·$kg^{-1}$ diesel) | Minimum selling price ($·$gal^{-1}$ diesel) |
|---|---|---|---|---|---|
| **Pearson's coefficients** | **GHG emissions (kg $CO_2$ eq·$kg^{-1}$ diesel)** | 1 | | | |
| | **Eutrophication (kg N eq·$kg^{-1}$ diesel)** | 0.408 | 1 | | |
| | **CED (MJ·$kg^{-1}$ diesel)** | 0.704 | 0.315 | 1 | |
| | **Minimum selling price ($·$gal^{-1}$ diesel)** | 0.925 | 0.297 | 0.647 | 1 |
| **Spearman's coefficients** | **GHG emissions (kg $CO_2$ eq·$kg^{-1}$ diesel)** | 1 | | | |
| | **Eutrophication (kg N eq·$kg^{-1}$ diesel)** | 0.329 | 1 | | |
| | **CED (MJ·$kg^{-1}$ diesel)** | 0.830 | 0.390 | 1 | |
| | **Minimum selling price ($·$gal^{-1}$ diesel)** | 0.831 | 0.127 | 0.666 | 1 |

❖ **Effect of individual parameter uncertainty on the economic and environmental results**

The Monte Carlo simulation allowed the evaluation of the possible scenarios and the likelihood of each economic and environmental performance. The analysis considered a wide range of variable parameters simultaneously. However, the model may be more sensitive to changes in certain parameters than to others. To analyze the different effects of process parameters, characterization factors and economic parameters, three additional simulations were conducted. Each simulation was carried out by varying one group of parameters separately. The results are summarized in **Figure 9.8**.

Process parameters are the main cause of uncertainty for all the analyzed indicators, whereas characterization factors have a moderate contribution to the variability of eutrophication potential and CED. Economic parameters are a limited source of uncertainty for the minimum selling price.

In the Monte Carlo simulation for the analysis of process parameters, GHG emissions ranged between 1.30 and 10.90 kg $CO_2$ eq·kg$^{-1}$ renewable diesel with a probability of 90%. This interval is nearly as wide as the global variability of the indicator presented in **Figure 9.6**. Characterization factors had a very limited effect and involve changes lower than 10% with respect to the median.

Eutrophication potential varies from -0.009 to 0.016 kg N eq·kg$^{-1}$ diesel in 90% of the scenarios, when considering the uncertainty of process parameters. This interval represents 67% of the global range of values presented in **Table 9.6**. Although the variation with respect to the characterization factors is more limited, they involve significant changes in the indicator, which has a 90% probability of values between -0.005 to 0.010 kg N eq·kg$^{-1}$ diesel.

CED also has a remarkable level of uncertainty associated with the variability of process parameters. Thus, 90% confidence interval includes values from 25 to 188 MJ·kg$^{-1}$ diesel, which correspond to an uncertain EROI between 0.19 and 1.40 MJ produced per MJ consumed. The results point out the need of a careful optimization of the operating conditions to obtain a favorable energy balance. The characterization factors have a lower influence in the indicator and lead to variations from 25 to 74 MJ·MJ$^{-1}$.

As in the case of GHG emissions, most of the uncertainty of the minimum selling price is due to the uncertainty of the process parameters. Thus, the diesel price may range between \$10 and \$98 $gal^{-1}$ with a probability of 90% depending on the process parameters, while this interval is limited to \$20-26 $gal^{-1}$ when the effect of economic assumptions is analyzed separately.

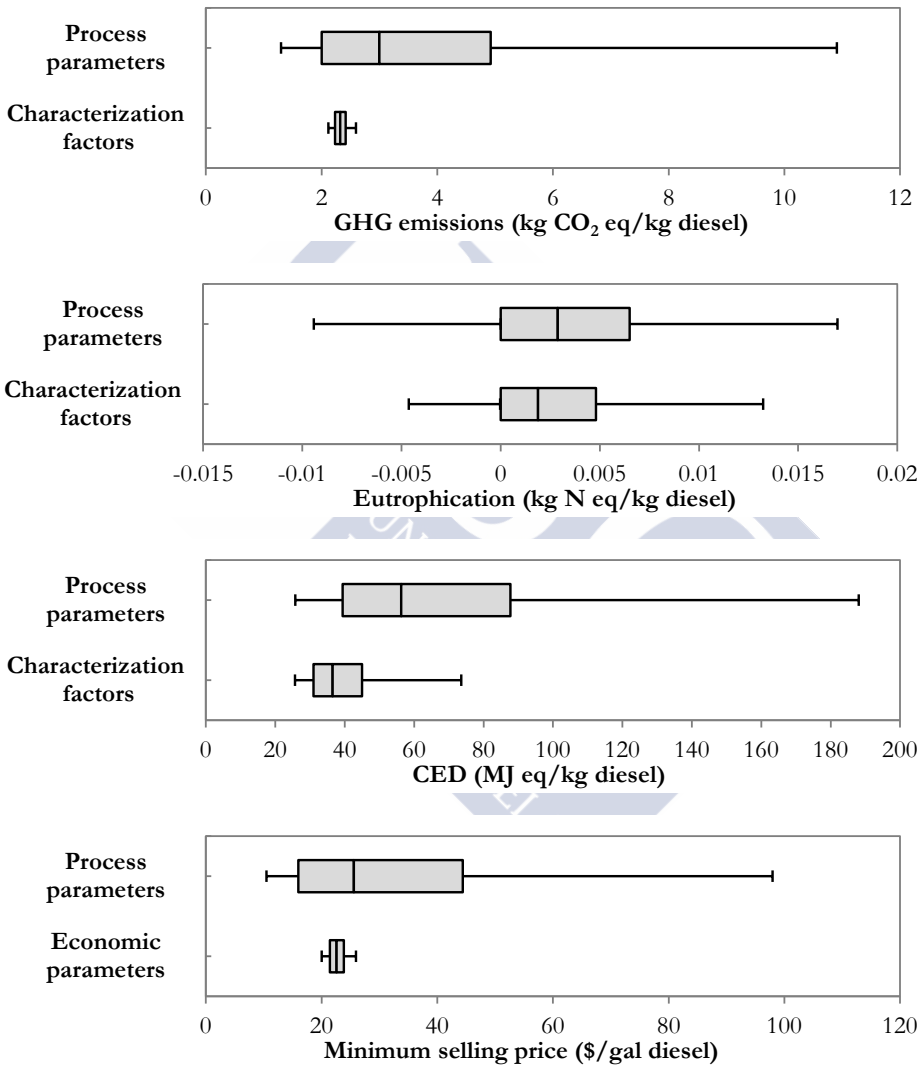


**Figure 9.8.** Variability of the performance indicators with each group of input parameters, represented according to percentiles 5%, 25%, 50%, 75% and 95%.

Since process parameters were identified as the main cause of uncertainty for all the analyzed indicators, the individual effect of each variable included in this category is presented in **Figures 9.9**, **9.10**, **9.11** and **9.12**. According to the results, biomass productivity is a key factor affecting all the environmental indicators. Thus, the uncertainty of this variable leads to the wider interval of likely values in the three categories. Productivity also have a remarkable influence in the minimum selling price, although the variability of this economic indicator is higher with respect to the lipid content. The possible values of the lipid fraction result in a wide range of prices from \$12 $gal^{-1}$ up to \$78 $gal^{-1}$, while the indicator has a 95% probability of values below \$52 $gal^{-1}$ for the complete range of productivities. The high variability of GHG emissions and minimum selling price with respect to lipid content may be one of the reasons of the stronger mathematical relationship between those indicators compared to the correlations with CED. In addition, parameters related to the protein recovery (i.e. protein content and microalgal to commercial substitution ratio) have a higher secondary contribution in the case of CED than for the other two indicators. This suggests that environmental credits associated with the protein may involve higher reductions of impact for this environmental category. The eutrophication potential is also affected by the uncertainty of protein content. Methane yield has a moderate contribution to the uncertainty of the environmental indicators, but it barely affects the economic performance. These results indicate the environmental benefits of energy recovery, which are more limited in economic terms due to the low relative contribution of operating costs to the total costs of the facility (already shown in **Figure 9.5**). Other process parameters included in the uncertainty assessment such as carbohydrate content, nutrient excess or nitrogen and phosphorus bioavailability have a very limited effect on all the performance indicators.

The main findings of this step of the analysis confirm the key role of productivity and lipid content in the global performance of microalgal systems, already highlighted in previous studies (Davis et al., 2012; Sills et al., 2013). In addition, the results show moderate benefits of co-product credits, which are especially significant in certain environmental categories such as eutrophication and energy balance.

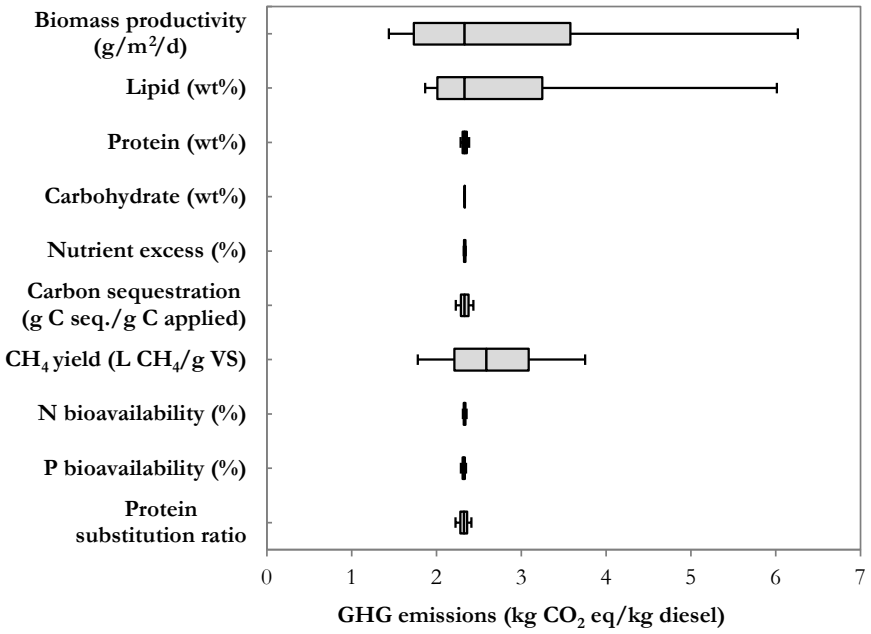


**Figure 9.9.** Individual effect of process parameters on GHG emissions.

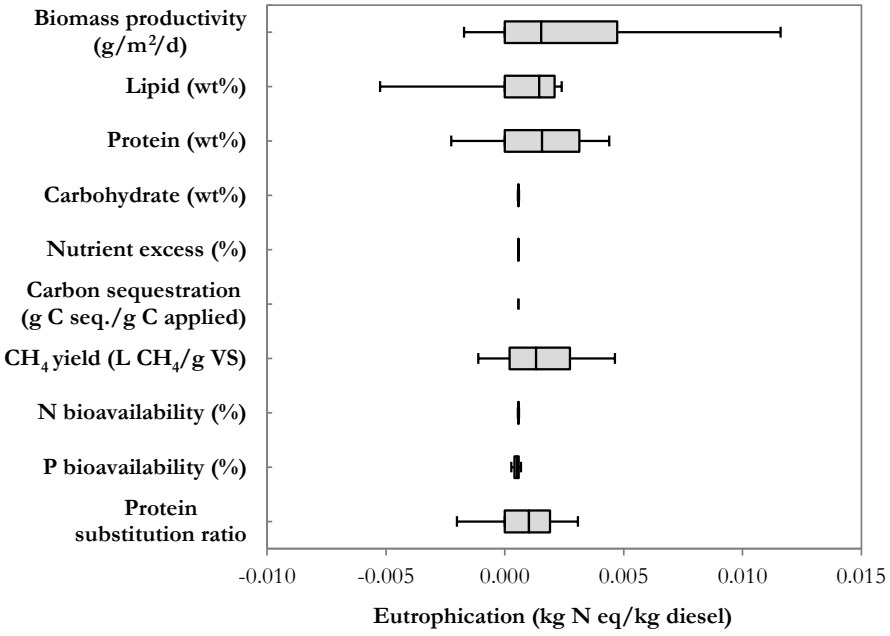


**Figure 9.10.** Individual effect of process parameters on eutrophication.

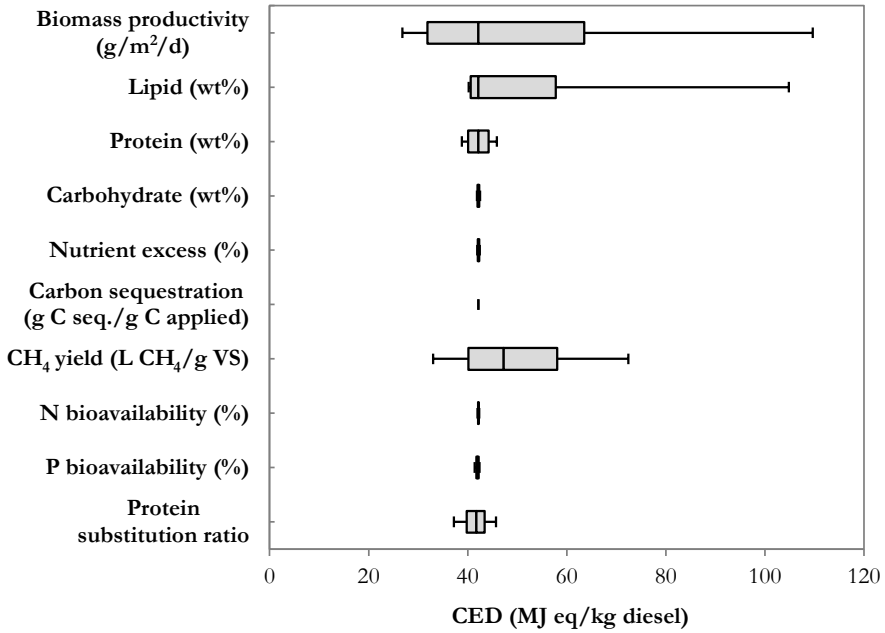


**Figure 9.11.** Individual effect of process parameters on CED.

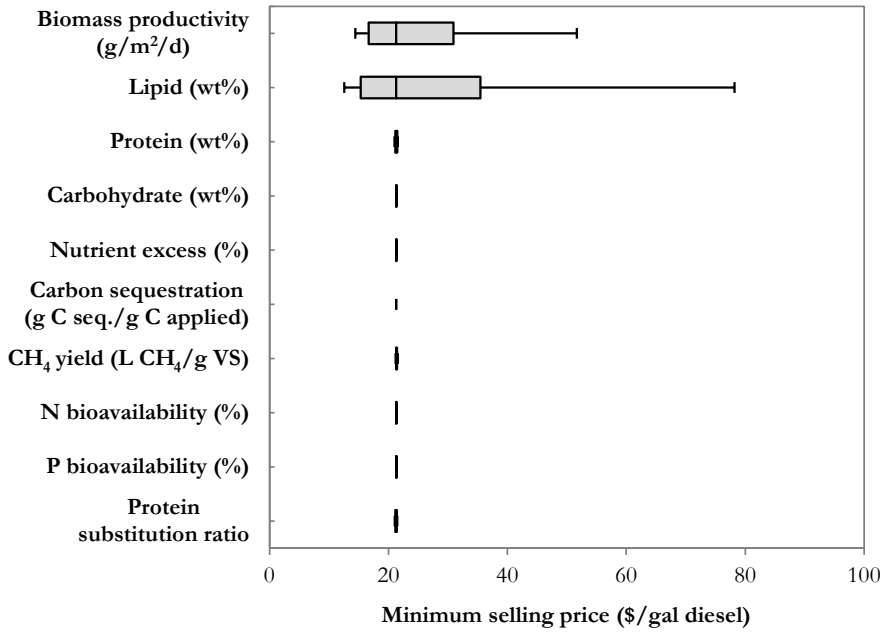


**Figure 9.12.** Individual effect of process parameters on minimum selling price.

## 9.5. Conclusions

This chapter presents one of the first integrated economic and environmental assessments for a multi-product biorefinery scheme in which the wide variability of parameters is analyzed according to a probability-based uncertainty method. The assessment was based on standardized models that are widely applied in the US and included three environmental indicators (GHG emissions, eutrophication and CED) and one economic indicator (minimum selling price).

The developed model was first validated according to a harmonized model. For this set of parameters, the determined impacts were consistent with other LCA studies, although the environmental benefits of co-products allowed significant improvements in the profile, especially in terms of eutrophication.

In the second stage, the Monte Carlo simulation was applied to evaluate a large number of alternative production scenarios. The different scenarios were randomly generated according to a set of probability distribution functions, which were estimated from both experimental data and available literature. As expected, the results from this stage had a wide range of possible values for the measured economic and environmental indicators. Despite the variability, the simulation showed a high probability of operating in conditions that have favorable environmental performance for all the evaluated categories and a minimum selling price in the same range reported in previous works.

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# SECTION IV

## CONCLUSIONS

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# Chapter 10

# General conclusions

The purpose of this thesis was to analyze the environmental aspects associated with the production of high value molecules and commodities by the diverse species of aquatic organisms. These products are included in the field of blue biotechnology, an emerging industry with an increasing global market that constitutes a strategic activity in the current European context due to the favorable location and the wide variety of aquatic ecosystems.

LCA methodology has proved its usefulness as an environmental management tool that provides strategic information for the design of novel processes. Thus, the application of LCA allowed identifying the problematic stages, as well as the improvement opportunities in the analyzed processes. It was also applied to compare the performance of alternative technologies for the production and extraction of the target compounds. Moreover, social and economic indicators were included in the assessment of representative pilot and large-scale systems in order to achieve a holistic evaluation of the potential of blue biotechnology. The main findings and general conclusions that were obtained from the research presented in Sections II and III are detailed below:

**Section II: High value added molecules from aquatic organisms**

This section focused on the production of a wide variety of high-value compounds and bioactive molecules with applications in industries such as cosmetics, functional food and pharmaceutical sectors. The evaluated products were obtained from different aquatic sources, including micro- and macroalgae, sponges, bacteria, chromists and fungi. Detailed life cycle inventories were mainly based on primary data obtained by on-site measurements for real systems at lab and pilot scale.

The evaluated systems applied diverse cultivation approaches and novel photobioreactor designs, as well as both conventional and innovative extraction routes. After identifying the main contributors to the environmental impact (hot spots) of the production processes, alternative scenarios and improved routes to enhance the performance were analyzed.

The main conclusions for the production of high value molecules from microalgae *H. pluvialis*, *P. tricornutum* and *T. suecica* were:

- The environmental impacts were mainly related with the cultivation stages. Regardless of the production scale, the preparation of the inoculum and culture medium and the growth in reactor were the main causes of the impact. These environmental burdens were related to the production of nutrient sources and electricity required in the process.
- The reactor selection was found to be one of the most significant choices for process development. The reactor design had a high influence in the electricity consumption, linked to medium pumping, aeration and especially artificial illumination. The substitution of inefficient reactors by system with better light distribution patterns resulted in improvements between 60% and 80%.
- The simulation of cultivation scenarios based on alternative nitrogen sources and the addition of nutrient recovery and recycling steps led to significant reductions of impact.
- The process upscaling had a remarkable effect on the environmental results. When comparing real systems at lab, semi-pilot and pilot scale, the larger system was found to have between one and two orders of magnitude lower than the other process.
- Technological advances and innovative alternatives such as the use of supercritical $CO_2$ extraction instead of conventional solvent extraction also showed a great potential to reduce the environmental burdens.
- The socio-economic assessment identified workers' salary and working hours, and product benefits to consumers as the main strengths of the producing companies. The evaluation highlighted some management

strategies to improve key issues of the social behavior. The economic indicators estimated a significant profitability and the potential to recover the initial investment in a relatively short period of time.

The environmental implications of the use of macroalgae *S. muticum* and *O. secundiramea* as sources of high value molecules were:

- The high electricity consumption was the main hot spot for both cultivation and extraction stages. As in the case of microalgae, most of the electricity required for cultivation was related to reactor illumination, whereas supercritical extraction and non-isothermal autohydrolysis were the most energy-intensive extraction stages.
- In multi-functional systems, the selection of the functional unit was a key decision that could affect the outcomes of the LCA study. In addition, the chosen operating conditions led to differences in biochemical composition that also have a significant influence in the environmental performance. When some fractions were present in a very low concentration, the integral valorization of the algae was not necessarily linked to a better environmental performance.
- LCA was applied as an optimization tool that led to the proposal of optimized scenarios with remarkable impact reductions (8-50% reduction compared to the baseline scenario).

The comparison of advanced techniques for the cultivation of sponges *C. crambe* and *S. spinosulus* and subsequent extraction of bioactive molecules with potential applications as pharmaceutical ingredients drew the following conclusions:

- *In situ* cultivation of marine sponges in sea-based farming structures had low environmental impacts compared to the downstream processing (which requires the use of large quantities of organic solvents) to separate the target compounds from the harvested biomass.
- The alternative process, consisting of the maintenance of sponge biomass in indoor aquaria under artificial illumination, had remarkably higher environmental burdens than *in situ* cultivation due to the electricity requirements.

- Regardless of the cultivation alternative, the production of solvents for the extraction and purification stages was the main contributor to the environmental impacts.
- The combined optimization of key aspects of the *ex situ* cultivation (e.g. solvent reuse, lighting regime, use of renewable energy sources) led to improved scenarios with comparable or lower environmental impacts than the *in situ* system.

The main findings for the production of biocompounds by other aquatic organisms were comparable to the results for microalgae, seaweeds and sponges:

- Cultivation was the main stage responsible for the environmental burdens of the production of DHA by the chromist *U. visurgensis*, as well as for the production of lipase enzymes by marine fungi *C. laurentii* and *G. pannorum*. This impact was mainly due to the high electricity requirements of both processes.
- The production of chemicals for the extraction and purification was the main hot spot of the production of coenzyme $Q_{10}$ by epiphytic bacteria isolated from macroalgae *F. spiralis* and *S. coronopifolius*.

**Section III: Microalgal biorefineries**

Two key aspects for the commercial implementation of microalgal processes were addressed in this section: the advantages and drawbacks of the most common reactor configurations and the integration of economic criteria and model uncertainty in the environmental evaluation of the processes.

The main conclusions to be considered for an appropriate selection of the reactor were:

- The environmental profile of the evaluated reactor configuration was strongly dependent on the maximum biomass concentration that could be achieved in each system. For this reason, the efficiency of open raceway systems (ORPs) in environmental terms was extremely restricted to the weather conditions, especially for locations with low temperatures and sunlight intensities.

- The profile of the flat-panel reactor showed a higher potential than the ORP due to the high concentrations achieved in the system. However, the global efficiency depended on strategic decisions during the process development, such as the choice of substitution or reuse of plastic bags after each cultivation cycle.
- Tubular reactors showed the most robust performance and lowest dependence on weather conditions. They allowed good average environmental response that could be maintained for longer periods of time.
- For the inventoried facility, transport of seawater was the main hot spot contributing to the environmental burdens. Its relative contribution was higher for systems with lower biomass concentration (i.e. ORP system).
- When excluding transport from the system boundaries (to obtain a more representative assessment of the hypothetical implementation at commercial scale), the electricity production was the key aspect related to the cultivation. Regardless of the reactor configuration, most of the requirements were associated with the temperature control system, which included heating and cooling to maintain the temperature between 20-30ºC.

The integration of economic and environmental criteria in a single analysis was considered to evaluate the sustainability of the combined production of biofuels and other microalgal co-products. Due to the high variability of the parameters to model microalgal production scenarios, an uncertainty analysis was also performed by applying the Monte Carlo method. The integrated assessment led to the following conclusions:

- For the deterministic model analyzed in the first stage, cultivation was the main cause of the environmental burdens. The environmental benefits of co-products obtained during the process allowed remarkable reductions of impact and led to a negative value in the case of eutrophication (which involves that the process could substitute alternative production routes with higher environmental burdens).

- The single-point results were comparable to the values from previous studies, although the estimated minimum selling price of the obtained diesel was slightly higher, mainly due to different assumptions in the conversion stage.
- Capital costs (related to the equipment and the establishment of the facility) were the main contributor to the final selling price of the produced diesel. Operating costs involved less than 20%, whereas land costs were below 5% of the total production costs.
- The uncertainty analysis revealed a high variability of the economic and environmental results due to the numerous variable parameters and the wide range of possible values for each input.
- Strong correlations between GHG emissions, energy demand and diesel selling price were obtained. The association of GHG emissions with the minimum selling price presented the clearest linear relationship. Eutrophication had low correlation coefficients with respect to the other indicators, which indicates that this category was mainly linked to unit processes with limited GHG emissions and energy consumption.
- Among the three categories of input parameters analyzed (i.e. process parameters, characterization factors and economic parameters), process parameters were the main source of uncertainty. In particular, biomass productivity and lipid content had the most significant effect on the selected indicators. Some parameters related to co-products, including methane yield and protein fraction, also had a relevant influence in certain environmental indicators such as eutrophication and energy demand.
- Despite the uncertainty, a high probability of operating in conditions with a favorable environmental performance and a minimum selling price in the same range of previous techno-economic assessments was obtained.

# ADDITIONAL CONTENTS

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# Appendix I

# Acronyms

| | |
|---|---|
| AD | Anaerobic digestion |
| ADP | Abiotic depletion potential |
| AP | Acidification potential |
| APD | Algae Process Description model |
| API | Active pharmaceutical ingredient |
| Ara-A | Adenine arabinoside |
| Ara-C | Cytosine arabinoside |
| CBA | Cost-Benefit Analysis |
| CED | Cumulative energy demand |
| CERA | Cumulative Energy Requirement Analysis |
| CEPCI | Chemical Engineering Plant Cost Index |
| CML | Centre of Environmental Science of Leiden University (The Netherlands) |
| CNRS | Centre National de la Recherche Scientifique (France) |
| DAF | Dissolved air flotation |
| DCM | Dichloromethane |
| DfE | Design for the Environment |
| DHA | Docosahexaenoic acid |
| DMI | Direct material inputs |
| DMSO | Dimethyl sulfoxide |
| DOE | United States Department of Energy |
| DW | Dry weight |
| EA | Environmental Auditing |
| EDTA | Ethylendiaminetetraacetic acid disodium salt |
| EEA | Environmental European Agency |
| EIA | Environmental Impact Assessment |
| EP | Eutrophication potential |
| EPA[1] | Eicosapentaenoic acid |
| EPA[2] | United States Environmental Protection Agency |
| EPE | Environmental Performance Evaluation |
| ERA | Environmental Risk Assessment |
| EROI | Energy return on investment |
| EU | European Union |
| FAME | Fatty acid methyl esters |
| FAO | Food and Agriculture Organization |
| FCI | Fixed capital investment |
| FEOW | Freshwater Ecoregions of the World |

| | | | |
|---|---|---|---|
| FEP | Freshwater aquatic ecotoxicity | LDPE | Low density polyethylene |
| FFA | Free fatty acids | LED | Light-emitting diode |
| FP6 | Sixth Framework Programme (EU research funding programme) | MACRS | Modified Accelerated Cost Recovery System |
| FP7 | Seventh Framework Programme (EU research funding programme) | MAIA | Material Intensity Analysis |
| FP7 | Seventh Framework Programme (EU research funding programme) | MCA, MCDA | Multi-Criteria Analysis (also Multi-Criteria Decision Analysis) |
| FU | Functional Unit | MESR | Fench Ministry of Higher Education and Research |
| GDP | Gross domestic product | | |
| GHG | Greenhouse gas | MET | Material, Energy and Toxic analysis |
| GREET | Greenhouse Gases, Regulated Emissions and Energy Use in Transportation | MFA | Material Flow Accounting |
| | | MIPS | Material Intensity Per Unit Service |
| GWP | Global warming potential | MRI | Midwest Research Institute (Kansas City, Missouri) |
| HDPE | High density polyethylene | NMVOC | Non-methane volatile organic compounds |
| HPLC | High performance liquid cromatography | NP | Net profit |
| IPCC | Intergovernmental Panel on Climate Change | NPV | Net present value |
| IPP | Integrated Product Policy | NR-F | Non-renewable fossil energy |
| ISO | International Organization for Standardization | NR-N | Non-renewable nuclear energy |
| KBBE | Knowledge-based Bio-Economy (FP7 call) | NR-B | Non-renewable biomass energy (from primary forests) |
| LC | Land competition | ODP | Ozone layer depletion potential |
| LCA | Life Cycle Assessment | OECD | Organization for Economic Co-operation and Development |
| LCC | Life Cycle Costing | ORP | Open pond reactor |
| LCI | Life Cycle Inventory | PBR | Photobioreactor |
| LCIA | Life Cycle Impact Assessment | PBS | Phosphate buffered saline solution |
| LCSA | Life Cycle Sustainability Analysis | PDA | Potato Dextrose Agar |

| | |
|---|---|
| PET | Polyethylene terephtalate |
| PM | Particulate matter |
| PMI | Process Mass Intensity |
| PMMA | Polymethyl methacrylate |
| POFP | Photochemical oxidants formation potential |
| PP | Polypropylene |
| PPP | Purchasing power parity |
| PUFAs | Polyunsaturated fatty acids |
| PVC | Polyvinyl chloride |
| RA | Resource assessment model |
| R-B | Renewable biomass energy |
| RD | Renewable diesel |
| RFS | Renewable Fuel Standard Program (Research program established by DOE, EPA and the United States Department of Agriculture) |
| R-HYD | Renewable hydropower |
| Rio+20 | Rio Earth Summit, 2012 |
| R-WSG | Renewable wind, solar and geothermal energy |
| SETAC | Society of Environmental Toxicology and Chemistry |
| SFA | Substance Flow Analysis |
| SLCA | Social Life Cycle Assessment |
| SME | Small and medium enterprises |
| SOMLIT | Service d'Observatio en Milieu LIToral (CNRS) |
| TAGs | Triacylglycerols (triglycerides) |
| TDC | Total direct cost |
| TEA | Techno-economic analysis model |
| TEP | Terrestrial aquatic ecotoxicity |
| TFA | Trifluoroacetic acid |
| TMR | Total Material Requirement |
| TRACI | Tool for the Reduction and Assessment of Chemical and other environmental Impacts |
| TS | Total solids |
| UNCED | United Nations Conference on Environment and Development (Rio Earth Summit, 1992) |
| UNEP | United Nations Environment Programme |
| US, USA | United States of America |
| UV | Ultraviolet |
| VS | Volatile solids |
| WCED | World Commission on Environment and Development |
| WMO | World Meteorological Organization |
| WoRMS | World Register of Marine Species |
| wt% | Weight percent |
| WWF | World Wildlife Fund |

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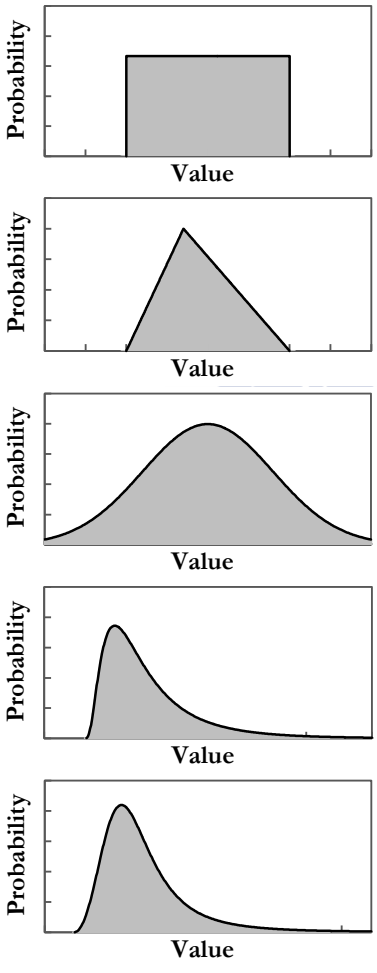
# Appendix II

# Supplementary information




**Uniform distribution**

Min = continuous lower boundary parameter

Max = continuous upper boundary parameter

**Triangular distribution**

Min = continuous boundary parameter

Most likely = continuous mode parameter

Max = continuous boundary parameter

**Normal distribution**

μ = continuous location parameter (mean)

σ = continuous scale parameter (standard deviation)

**Lognormal distribution**

μ = mean

σ = standard deviation

RiskShift = additional parameter to shift the domain of the distribution

**Loglogistic distribution**

γ = continuous location parameter

β = continuous scale parameter

α = continuous shape parameter

**Figure II.1.** Probability distribution functions and associated parameters used to model uncertainty in @RISK.

**Table II.1.** Distribution functions and parameters for process inputs

| Effect | Parameter | Type of distribution | Min | Max | Mode | Source |
|---|---|---|---|---|---|---|
| **Biomass composition** | Lipid content (wt%) | Uniform | 4.56% | 45.10% | | Experimental data for *C.sorokiniana*, *N. oculata* and *N. oleabundans* |
| | Protein content (wt%) | Uniform | 3.49% | 14.01% | | |
| | Carbohydrate content (wt%) | Uniform | 25.39% | 54.96% | | |
| **Algal yield** | Specific (aerial) productivity ($g \cdot m^{-2} \cdot d^{-1}$) | Uniform | 3.39 | 19.15 | | |
| **Fertilizer requirements** | Nutrient excess (%) | Uniform | 0 | 10% | | Sills et al. (2013) |
| **Carbon sequestration** | Carbon sequestered in AD residue (g C seq. per g C applied) | Triangular | 0 | 0.16 | 0.08 | Frank et al. (2011a) |
| **Biogas from AD** | Methane yield (L $CH_4 \cdot g^{-1}$ VS) | Triangular | 0.1 | 0.3 | 0.4 | Sills et al. (2013) |
| **Co-product substitution ratio** | Microalgae to commercial protein ratio ($g \cdot g^{-1}$) | Triangular | 0.64 | 1.85 | 1.16 | Bryant et al. (2012) |
| | Nitrogen bioavailability (%) | Triangular | 30% | 40% | 35% | Frank et al. (2011a); Mulbry et al. (2005) |
| | Phosphorous availability (%) | Triangular | 39% | 75% | 57% | |

**Table II.2.** Distribution functions and parameters for GHG characterization factors (values are expressed in kg $CO_2$ eq per FU)

| Process | FU | Type of distribution | μ | σ | Shift | γ | β | α |
|---|---|---|---|---|---|---|---|---|
| Electricity production (US mix) | 1 kWh | Loglogistic | | | | 0.626 | 0.121 | 6.119 |
| Ammonia (liquid) | 1 kg | Lognormal | 1.975 | 0.682 | 0.166 | | | |
| Diammonium phosphate, as N | 1 kg | Lognormal | 1.687 | 0.303 | 1.107 | | | |
| Concrete, normal | 1 $m^3$ | Lognormal | 235.570 | 51.979 | 52.498 | | | |
| Reinforcing steel | 1 kg | Lognormal | 0.841 | 0.156 | 0.741 | | | |
| High-density polyethylene | 1 kg | Lognormal | 0.452 | 0.142 | 1.995 | | | |
| Excavation (considering diesel burned in building machine) | 1 $m^3$ | Normal | 4.707 | 0.407 | | | | |
| Cast iron | 1 kg | Lognormal | 1.071 | 0.202 | 0.566 | | | |
| Aluminum sulfate (flocculant) | 1 kg | Lognormal | 0.506 | 0.161 | 0.136 | | | |
| Natural gas | 1 MJ | Lognormal | 0.011 | 0.004 | 0.003 | | | |
| Hexane | 1 kg | Lognormal | 0.377 | 0.099 | 0.605 | | | |
| Hydrogen | 1 kg | Normal | 1.660 | 0.077 | | | | |
| Single superphosphate, as $P_2O_5$ | 1 kg | Lognormal | 2.577 | 0.780 | 0.726 | | | |
| Ammonium nitrate, as N | 1 kg | Lognormal | 8.340 | 1.423 | 0.338 | | | |
| Soybean meal | 1 kg | Lognormal | 0.340 | 0.058 | 0.128 | | | |

**Table II.3.** Distribution functions and parameters for eutrophication characterization factors (in kg N eq per FU)

| Process | FU | Type of distribution | μ | σ | Shift | γ | β | α |
|---|---|---|---|---|---|---|---|---|
| Electricity production (US mix) | 1 kWh | Lognormal | $1.914 \cdot 10^{-3}$ | $1.927 \cdot 10^{-3}$ | $2.750 \cdot 10^{-4}$ | | | |
| Ammonia (liquid) | 1 kg | Loglogistic | | | | $4.530 \cdot 10^{-4}$ | $1.070 \cdot 10^{-3}$ | 2.517 |
| Diammonium phosphate, as N | 1 kg | Lognormal | $3.757 \cdot 10^{-3}$ | $3.617 \cdot 10^{-3}$ | $1.780 \cdot 10^{-3}$ | | | |
| Concrete, normal | 1 $m^3$ | Lognormal | 0.127 | 0.098 | 0.049 | | | |
| Reinforcing steel | 1 kg | Lognormal | $5.024 \cdot 10^{-3}$ | $3.330 \cdot 10^{-3}$ | $1.050 \cdot 10^{-3}$ | | | |
| High-density polyethylene | 1 kg | Loglogistic | | | | $4.750 \cdot 10^{-4}$ | $9.100 \cdot 10^{-4}$ | 2.152 |
| Excavation (considering diesel burned in building machine) | 1 $m^3$ | Loglogistic | | | | $2.700 \cdot 10^{-3}$ | $3.280 \cdot 10^{-3}$ | 5.111 |
| Cast iron | 1 kg | Lognormal | $4.729 \cdot 10^{-3}$ | $3.410 \cdot 10^{-3}$ | $1.650 \cdot 10^{-3}$ | | | |
| Aluminum sulfate (flocculant) | 1 kg | Loglogistic | | | | $4.350 \cdot 10^{-4}$ | $1.210 \cdot 10^{-3}$ | 2.131 |
| Natural gas | 1 MJ | Loglogistic | | | | $1.996 \cdot 10^{-6}$ | $3.890 \cdot 10^{-6}$ | 2.221 |
| Hexane | 1 kg | Loglogistic | | | | $2.600 \cdot 10^{-3}$ | $1.43010^{-3}$ | 3.021 |
| Hydrogen | 1 kg | Lognormal | $3.952 \cdot 10^{-4}$ | $2.249 \cdot 10^{-4}$ | $2.100 \cdot 10^{-4}$ | | | |
| Single superphosphate, as $P_2O_5$ | 1 kg | Loglogistic | | | | $8.610 \cdot 10^{-3}$ | 0.011 | 3.067 |
| Ammonium nitrate, as N | 1 kg | Loglogistic | | | | $3.215 \cdot 10^{-3}$ | 2.265 | $3.080 \cdot 10^{-3}$ |
| Soybean meal | 1 kg | Lognormal | $9.467 \cdot 10^{-3}$ | $1.908 \cdot 10^{-3}$ | $1.550 \cdot 10^{-3}$ | | | |

**Table II.4.** Distribution functions and parameters for CED characterization factors (in MJ per FU)

| Process | FU | Type of distribution | μ | σ | Shift | γ | β | α |
|---|---|---|---|---|---|---|---|---|
| Electricity production (US mix) | 1 kWh | Loglogistic | | | | 5.040 | 4.721 | 2.207 |
| Ammonia (liquid) | 1 kg | Lognormal | 36.634 | 15.568 | 5.904 | | | |
| Diammonium phosphate, as N | 1 kg | Lognormal | 45.513 | 10.134 | 11.67 | | | |
| Concrete, normal | 1 $m^3$ | Loglogistic | | | | 785.630 | 681.271 | 3.676 |
| Reinforcing steel | 1 kg | Loglogistic | | | | 15.990 | 6.559 | 3.406 |
| High-density polyethylene | 1 kg | Loglogistic | | | | 81.059 | 4.010 | 2.437 |
| Excavation (considering diesel burned in building machine) | 1 $m^3$ | Normal | 71.135 | 7.309 | | | | |
| Cast iron | 1 kg | Loglogistic | | | | 15.804 | 8.589 | 3.721 |
| Aluminum sulfate (flocculant) | 1 kg | Loglogistic | | | | 4.144 | 4.446 | 2.331 |
| Natural gas | 1 MJ | Normal | 1.115 | 0.107 | | | | |
| Hexane | 1 kg | Lognormal | 19.912 | 3.267 | 41.610 | | | |
| Hydrogen | 1 kg | Normal | 69.759 | 3.261 | | | | |
| Single superphosphate, as $P_2O_5$ | 1 kg | Loglogistic | | | | 20.181 | 25.752 | 2.867 |
| Ammonium nitrate, as N | 1 kg | Lognormal | 43.045 | 14.420 | | | | |
| Soybean meal | 1 kg | Lognormal | 14.163 | 2.240 | | | | |

**Table II.5.** Distribution functions and parameters for economic inputs

| Effect | Parameter | Type of distribution | Min | Max | Mode | μ | σ | Source |
|---|---|---|---|---|---|---|---|---|
| **Financial** | Desired return on investment | Triangular | 9% | 13% | 10% | | | Oxera (2011); Davis et al. (2014b) |
| | Contingency factor | Triangular | 10% | 30% | 14% | | | Aden (2002); Davis et al. (2011; 2012; 2014a; 2014b); Humbird (2011); Lundquist (2010). |
| | Labor burden | Triangular | 60% | 90% | 75% | | | |
| | Maintenance factor | Triangular | 2.0% | 3.0% | 2.5% | | | |
| | Property insurance and taxes | Triangular | 1.5% | 0.7% | 1.0% | | | Davis et al. (2011, 2014a) |
| **Direct** | Base cost of ponds ($·ha$^{-1}$) | Normal | | | | 34000 | 3400 | Davis et al. (2012) |
| | Base cost of pond liners ($·ha$^{-1}$) | Normal | | | | 50590 | 5059 | |
| | Base cost of $CO_2$ system ($·ha$^{-1}$) | Normal | | | | 9550 | 955 | |
| | Land cost ($·ha$^{-1}$) | Lognormal | | | | 9518 | 8430 | USDA (2014) |
| **Price of raw materials** | Power (cts·kWh) | Triangular | 0.0639 | 0.0701 | 0.0679 | | | Variability for the period 2007-2014, EIA (2015) |
| | Flocculant ($·kg$^{-1}$) | Triangular | 0.177 | 0.231 | 0.230 | | | Variability for the period 2007-2014, U.S. Bureau of Labor Statistics (2015) |
| | Hydrogen ($·kg$^{-1}$) | Triangular | 1.10 | 2.00 | 1.57 | | | Davis et al. (2014a) |
| **Price of** | Price of protein ($·kg$^{-1}$) | Triangular | 0.35 | 0.41 | 0.38 | | | IndexMundi (2015) |
| | Price of naphtha ($·gal$^{-1}$) | Triangular | 2.75 | 3.25 | 3.75 | | | According to minimum and maximum values evaluated from the sensitivity assessment by Davis et al. (2014) |
| | Price of N-fertilizer ($·kg$^{-1}$) | Triangular | 0.03 | 0.70 | 0.50 | | | |
| | Price of P-fertilizer ($·kg$^{-1}$) | Triangular | 0.03 | 0.70 | 0.50 | | | |

# Appendix III

# Resumo

Os ríos e océanos ocupan un 71% da codia terrestre e teñen constituído, ao longo da historia, unha fonte esencial de alimentos e produtos naturais de alto valor engadido. A diversidade de organismos que habitan nos ecosistemas acuáticos levou á obtención de máis de 24000 produtos naturais con numerosas aplicacións en sectores como a industria farmacéutica, a industria alimentaria e nutracéutica ou a produción de cosméticos.

Nos últimos anos, a biotecnoloxía mariña (tamén denominada biotecnoloxía azul) estase a converter nun sector emerxente a nivel mundial cunha facturación estimada arredor de $4800 millóns para o ano 2020. O potencial actual da biotecnoloxía azul, especialmente destacado no contexto europeo debido á súa localización xeográfica e á variedade de ecosistemas, levou ao desenvolvemento de políticas para potenciar o avance tecnolóxico do sector.

A crecente preocupación pola escaseza de recursos naturais e o deterioro dos ecosistemas do planeta fixo posible a aparición de ferramentas de xestión ambiental para medir a sustentabilidade dos procesos de produción. Con este fin, a metodoloxía de Análise de Ciclo de Vida (ACV) estendeuse e estandarizouse para a avaliación de impactos asociados aos procesos sobre diversas categorías ambientais.

A ACV está baseada nun enfoque global dos procesos que pretende avaliar os impactos ó longo de todo o ciclo de vida dos produtos analizados considerando non só o impacto causado directamente na instalación industrial senón tamén o efecto asociado coas actividades previas de extracción e procesado de materias primas así como as cargas ambientais orixinadas polo produto e polo tratamento dos residuos derivados do proceso. No caso dos produtos de orixe mariña, a

ACV emprégase con frecuencia no estudo dos aspectos ambientais dos biocombustibles obtidos a partir de microalgas. Tamén existen diversos estudos ambientais que utilizan esta metodoloxía na avaliación de procesos de produción de ingredientes activos e compostos de interese farmacéutico, aínda que a súa aplicación é más limitada e non se estende a compostos obtidos en procesos de biotecnoloxía mariña. Ademais de analizar os aspectos ambientais, a perspectiva de ciclo de vida estase a incorporar na avaliación das dimensións social e económica do desenvolvemento sustentable.

Esta tese doutoral ten como principal finalidade a análise detallado dos aspectos ambientais asociados coa produción de moléculas de alto valor engadido e outros produtos de menor valor obtidos a partir de diversos tipos de organismos mariños. Adicionalmente, propóñense un conxunto de indicadores socio-económicos para conseguir unha avaliación global da sustentabilidade dos procesos de biotecnoloxía azul.

O documento está dividido en catro seccións, segundo a estrutura representada na Figura III.1. As devanditas seccións abordan os seguintes aspectos relacionados coa biotecnoloxía azul e o aproveitamento dos recursos procedentes de ecosistemas acuáticos:

- Sección I: Introdución ó estudio, inclúe os Capítulos 1 e 2, nos que se presenta o ámbito da biotecnoloxía azul e a produción de biomoléculas e biocombustibles por parte dos organismos mariños e de augas doces, así como una descrición das principais ferramentas de xestión ambiental e análise de sustentabilidade.
- Sección II: Moléculas de alto valor engadido procedentes de organismos acuáticos, formada polos Capítulos 3 a 7, nos que se presentan os inventarios de ciclo de vida e os resultados da avaliación de impacto ambiental para diversas especies produtoras de compostos bioloxicamente activos.
- Sección III: Biorrefinerías de microalgas, na que se analizan en detalle certos aspectos clave do cultivo de microalgas para a obtención de diversos co-produtos que abarcan biocompostos de alto valor e biocombustibles. Esta sección consta dos Capítulos 8 e 9.

- Sección IV: Conclusións, presentadas no Capítulo 10, no que se resumen as principais implicacións dos resultados obtidos ao longo da tese.


**Figura III.1.** Esquema da estrutura da tese.

## Sección I: Introdución ao estudo

O Capítulo 1 presenta os aspectos relacionados co aproveitamento de recursos vivos procedentes de océanos e augas doces. En primeiro lugar, destácase o potencial dos hábitats acuáticos debido á variedade de características químicas e físicas que dan lugar a unha grande biodiversidade e descríbese a utilización destes recursos por parte do ser humano ao longo da historia. A continuación, enuméranse diversos produtos obtidos a partir de organismos acuáticos, incluíndo numerosas moléculas bioactivas e diversos biocombustibles, e amósanse as principais técnicas de cultivo empregadas na actualidade. En terceiro lugar, introdúcese o potencial económico da biotecnoloxía azul e

preséntanse as iniciativas e programas máis recentes enfocados a favorecer os avances tecnolóxicos no sector.

No Capítulo 2 descríbese a orixe e o desenvolvemento do actual concepto de sustentabilidade, xunto cos esforzos das institucións internacionais para establecer un marco conxunto orientado á mellora dos procesos actuais. Para a medida da sustentabilidade propóñense unha serie de ferramentas de xestión ambiental, entre as que destaca a ACV como unha metodoloxía estandarizada (segundo as normas ISO 14040 e 14044) e mundialmente aceptada de avaliación de impacto. Esta técnica obxectiva de análise das cargas ambientais asociadas aos procesos produtivos ao longo de todo o ciclo de vida aplícase con frecuencia na avaliación de procesos de obtención de biocombustibles a partir de microalgas e tamén ten sido utilizada para determinar os impactos de certos compostos bioactivos e de interese farmacéutico. Ademais, o seu uso está a estenderse á avaliación dos outros dous pilares da sustentabilidade: a dimensión económica e a dimensión social. Por todo isto, a metodoloxía de ACV foi seleccionada como principal ferramenta para a análise dos procesos nesta tese.

**Sección II: Moléculas de alto valor engadido a partir de organismos acuáticos**

Esta sección céntrase na análise dos procesos de obtención de moléculas bioactivas para aplicacións en industrias tales como o sector farmacéutico, alimentario ou cosmético. Os procesos avaliados inclúen diversos organismos produtores como micro- e macroalgas, esponxas, bacterias epifíticas, cromistas e fungos.

As avaliacións de impacto levadas a cabo seguen unha perspectiva "do berce á porta", segundo a cal se teñen en conta as etapas de produción das materias primas requiridas no proceso, as operacións de limpeza e esterilización de equipos, o crecemento dos organismos en diversos sistemas de cultivo e o procesado da biomasa obtida nas etapas de concentración de biomasa w extracción de compostos bioactivos. As etapas típicas incluídas dentro dos límites do sistema nos estudos ambientais realizados resúmense na Figura III.2.

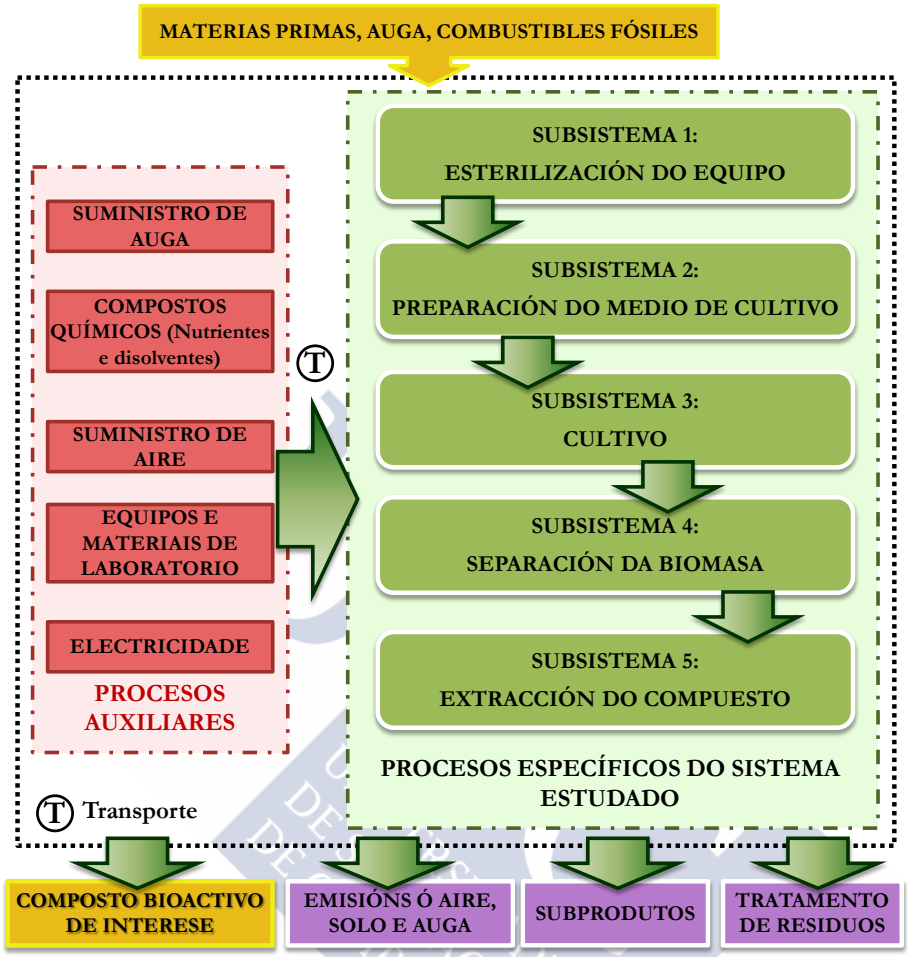



**Figura III.2.** Etapas habituais do proceso de produción de compostos biolóxicamente activos de alto valor engadido por organismos mariños.

En xeral, os inventarios de ciclo de vida presentados nesta sección están baseados en datos primarios obtidos mediante medidas directas en sistemas de produción reais que se atopan actualmente en funcionamento. A avaliación de impacto de ciclo de vida permite identificar os principais puntos problemáticos (hot spots) en termos ambientas e, en varios casos, cuantificar o potencial de mellora dunha serie de escenarios alternativos propostos para a optimización do proceso.

Nos Capítulos 3 e 4 avaliouse a produción de compostos como ácidos omega-3 ou carotenoides, extraídos de microalgas en procesos a escala laboratorio e piloto. Os resultados ambientais revelaron que as etapas de preparación de medio de cultivo e crecemento da biomasa no reactor son os puntos máis críticos desde un punto de vista ambiental, debido ao elevado consumo de electricidade e, en menor medida, ao requirimento de nutrientes. Ademais, estimáronse as posibles melloras asociadas á substitución dos nutrientes por compostos alternativos e á optimización do consumo eléctrico mediante o uso de reactores alternativos con distribucións de intensidade luminosa máis eficientes. No Capítulo 4 realizouse tamén unha avaliación socio-económica que permitiu identificar as principais fortalezas e as estratexias de mellora máis aconsellables para dúas empresas reais do sector de biotecnoloxía mariña.

O Capítulo 5 consistiu na aplicación da ACV para a análise comparativa das distintas rutas de extracción para a valorización de macroalgas invasivas recollidas do medio natural como medida de control. Tamén se analizou un proceso novidoso de cultivo de macroalgas en fotobiorreactores pechados, similares aos empregados comunmente no cultivo de microalgas. De novo, a electricidade foi identificada como o principal responsable dos impactos, tanto en procesos de cultivo como en posterior na extracción de moléculas.

No Capítulo 6 presentáronse dous sistemas avanzados de cultivo de esponxas *in situ* e *ex situ* para a produción de compostos con elevada actividade biolóxica e posibles aplicacións na industria farmacéutica. Neste caso, o consumo de disolventes orgánicos durante a etapa de extracción foi principal responsable dos impactos ambientais. En canto á análise comparativa dos dous sistemas, o escenario inicial de cultivo *ex situ* presentaba unhas cargas ambientais considerablemente máis elevadas que un sistema *in situ* cunha produción de biomasa equivalente. Non obstante, a optimización combinada de diversos aspectos do cultivo *ex situ* permitiu unha notable redución do impacto global, de modo que os escenarios de mellora propostos presentaban perfiles ambientais similares ou mesmo máis eficientes que a alternativa de cultivo *in situ*.

No Capítulo 7 completáronse os inventarios de ciclo de vida para a produción de compostos (coenzima $Q_{10}$, ácidos omega-3 e enzima lipasa), por parte doutros organismos mariños, incluíndo fungos, cromistas e bacterias epifíticas

illadas de macroalgas. Na avaliación ambiental destes procesos observáronse comportamentos similares aos resultados obtidos para outros compostos bioactivos de orixe mariña. O elevado consumo eléctrico na etapa de cultivo e o uso de disolventes orgánicos na extracción convencional dos compostos de interese foron novamente os principais responsables das cargas ambientais derivadas dos procesos.

**Sección III: Biorrefinerías de microalgas**

Nesta sección analizáronse dous aspectos fundamentais para a implantación comercial de procesos de microalgas: o efecto da configuración de reactor no perfil ambiental e a incorporación de criterios económicos e incerteza na simulación de procesos a escala comercial mediante modelos matemáticos.

Con respecto á selección de sistemas de cultivo apropiados, a análise realizada no Capítulo 8 demostrou a forte dependencia do perfil ambiental con respecto á máxima concentración de biomasa que permite alcanzar cada tipo de reactor. De acordo con esta dependencia, a eficiencia dos reactores abertos tipo "open pond" está limitada a condicións climáticas favorables. A produción destes sistemas en continuo resulta moi difícil de manter durante períodos de tempo prolongados, especialmente en lugares con baias temperaturas e intensidades solares limitadas. Os fotobiorreactores planos mostraron un grande potencial relacionado coas elevadas concentracións que se poden alcanzar nestes sistemas, pese a que o perfil global dependía en grande medida das estratexias aplicadas para o mantemento e substitución das bolsas de polietileno que compoñían o sistema. Finalmente, os reactores tubulares presentaron un comportamento máis robusto e unha menor dependencia das condicións climáticas. Os resultados suxiren a posibilidade de manter a operación nestes sistemas cun perfil ambiental relativamente eficiente durante períodos de tempo prolongados.

O Capítulo 9 presenta unha avaliación integral dos aspectos económicos e ambientais. Esta análise levouse a cabo para un proceso de cultivo de algas e posterior extracción dos compoñentes principais que incluía a obtención de diversos co-produtos nun único sistema. Debido á escaseza de datos reais a escala comercial e á variabilidade inherente dos parámetros de cultivo, a avaliación ambiental e económica dos procesos de obtención de produtos a partir de algas en base aos modelos matemáticos dispoñibles está afectada por

unha incerteza significativa. Para abordar este problema, utilizouse a técnica de simulación de Monte Carlo, baseada na xeración de escenarios aleatorios en base á definición de distribucións de probabilidade para as variables de entrada do modelo. Este método permitiu obter as funcións de distribución de probabilidade dos indicadores económicos e ambientais avaliados para determinar o rango de posibles escenarios. Tamén se obtiveron as correlacións entre estes indicadores e se analizaron por separado tres tipos de variables: variables de proceso, factores de caracterización e parámetros económicos. A avaliación permitiu identificar ao conxunto de variables de proceso como a principal causa de incerteza. Dentro desta categoría, a produtividade e o contido de lípidos foron os dous parámetros con maior contribución á incerteza dos indicadores seleccionados. Entre as variables con contribucións secundarias, o contido de proteína e o rendemento de metano foron os únicos parámetros cunha influencia moderada na variabilidade dos indicadores.

**Sección IV: Conclusións**

Os principais resultados e as implicacións prácticas das avaliacións ambientais realizadas ao longo da tese resúmense no Capítulo 10, que presenta as conclusións xerais do traballo. En conxunto, a tese demostrou a utilidade da metodoloxía de ACV como ferramenta de xestión ambiental que aporta información estratéxica para mellorar a toma de decisións no desenvolvemento de procesos innovadores. A identificación dos puntos problemáticos mediante esta metodoloxía permitiu propoñer escenarios alternativos de mellora que amosan o potencial de optimización dos procesos analizados para acadar sistemas de aproveitamento de recursos mariños sustentables no futuro.

# Appendix IV

# Resumen

Los ríos y océanos ocupan un 71% de la superficie terrestre y han constituido, a lo largo de la historia, una fuente esencial de alimentos y productos naturales de alto valor añadido. La diversidad de organismos que habitan en los ecosistemas acuáticos ha hecho posible la obtención de más de 24000 productos naturales con numerosas aplicaciones en sectores como la industria farmacéutica, la industria alimentaria y nutracéutica o la producción de cosméticos.

En los últimos años, la biotecnología marina (también denominada biotecnología azul) se ha convertido en un sector emergente a nivel mundial con una facturación estimada en torno a $4800 millones para el año 2020. El potencial actual de la biotecnología azul, especialmente destacado en el contexto europeo debido a su localización y variedad de ecosistemas, ha llevado al desarrollo de políticas para potenciar el avance tecnológico en el sector.

La creciente preocupación por la escasez de recursos naturales y el deterioro de los ecosistemas del planeta ha hecho posible la aparición de herramientas de gestión ambiental para medir la sostenibilidad de los procesos de producción. Con esta finalidad, la metodología de Análisis de Ciclo de Vida (ACV) se ha extendido y estandarizado para la evaluación de impactos asociados a los procesos sobre diversas categorías ambientales.

El ACV se basa en un enfoque holístico de los procesos que pretende evaluar los impactos a lo largo de todo el ciclo de vida de los productos analizados considerando no solo el impacto causado directamente en la instalación industrial sino también el efecto asociado con las actividades previas de extracción y procesado de materias primas así como las cargas ambientales originadas por el producto y por el tratamiento de los residuos generados en el

proceso. En el caso de los productos de origen marino, el ACV se emplea con frecuencia en el estudio de los aspectos ambientales de los biocombustibles obtenidos a partir de microalgas. También existen diversos estudios ambientales que utilizan esta metodología en la evaluación de procesos de producción de ingredientes activos y compuestos de interés farmacéutico, aunque su aplicación es más limitada y no se ha extendido a compuestos obtenidos en procesos de biotecnología marina. Además de analizar los aspectos ambientales, la perspectiva de ciclo de vida se ha empezado a incorporar recientemente en la evaluación de las dimensiones social y económica del desarrollo sostenible.

Esta tesis doctoral tiene como principal finalidad el análisis detallado de los aspectos ambientales asociados a la producción de moléculas de alto valor añadido y otros productos de menor valor obtenidos a partir de diversos tipos de organismos marinos. Adicionalmente, se proponen un conjunto de indicadores socio-económicos para conseguir una evaluación global de la sostenibilidad de los procesos de biotecnología azul.

El documento se encuentra dividido en cuatro secciones de acuerdo con la estructura representada en la Figura IV.1. Dichas secciones abordan los siguientes aspectos relacionados con la biotecnología azul y el aprovechamiento de los recursos procedentes de ecosistemas acuáticos:

- Sección I: Introducción al estudio, incluye los Capítulos 1 y 2, en los que se presenta la contextualización en el ámbito de la biotecnología azul y la producción de biomoléculas y biocombustibles por parte de los organismos marinos y de aguas dulces, así como una descripción de las principales herramientas de gestión ambiental y análisis de sostenibilidad.

- Sección II: Moléculas de alto valor añadido procedentes de organismos acuáticos, formada por los Capítulos 3 a 7, en los que se presentan los inventarios de ciclo de vida y los resultados de la evaluación de impacto ambiental para diversas especies productoras de compuestos biológicamente activos.

- Sección III: Biorrefinerías de microalgas, en la que se analizan en detalle ciertos aspectos clave del cultivo de microalgas para la obtención de

diversos co-productos que abarcan biocompuestos de alto valor y biocombustibles. Esta sección se compone de los Capítulos 8 y 9.

- Sección IV: Conclusiones, presentadas en el Capítulo 10, en el que se resumen las principales implicaciones de los resultados obtenidos a lo largo de la tesis.

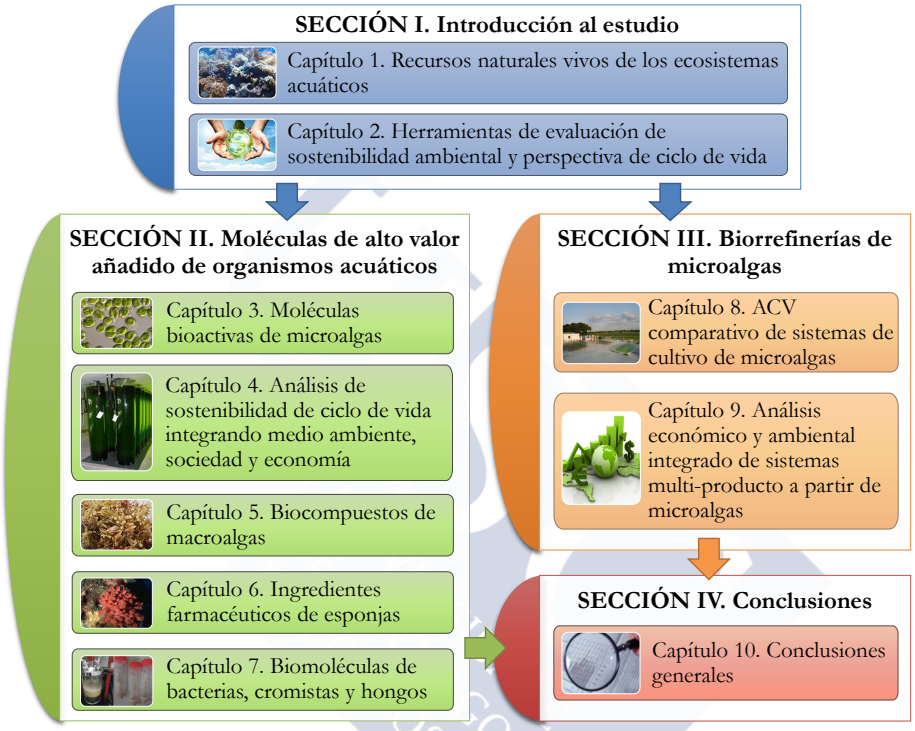



**Figura IV.1.** Esquema de la estructura de la tesis.

## Sección I: Introducción al estudio

El Capítulo 1 presenta los aspectos relacionados con el aprovechamiento de recursos vivos procedentes de océanos y aguas dulces. En primer lugar, se destaca el potencial de los hábitats acuáticos debido a la variedad de características químicas y físicas que dan lugar a una gran biodiversidad y se describe la utilización de estos recursos por parte del ser humano a lo largo de la historia. A continuación, se enumeran diversos productos obtenidos a partir de organismos acuáticos, incluyendo numerosas moléculas bioactivas y diversos biocombustibles, y se muestran las principales técnicas de cultivo empleadas en

la actualidad. En tercer lugar, se introduce el potencial económico de la biotecnología azul y se presentan las iniciativas y programas más recientes enfocados a favorecer los avances tecnológicos en el sector.

En el Capítulo 2 se describen los orígenes y el desarrollo del actual concepto de sostenibilidad, junto con los esfuerzos de las instituciones internacionales para el establecimiento de un marco conjunto orientado a la mejora de los procesos actuales. Para la medida de la sostenibilidad se proponen una serie de herramientas de gestión ambiental, entre las que destaca el ACV como una metodología estandarizada (de acuerdo con las normas ISO 14040 y 14044) y mundialmente aceptada de evaluación de impacto. Esta técnica objetiva de análisis de las cargas ambientales asociadas a los procesos productivos a lo largo de todo su ciclo de vida se aplica con frecuencia en la evaluación de procesos de obtención de biocombustibles a partir de microalgas y también ha sido utilizada para determinar los impactos de ciertos compuestos bioactivos y de interés farmacéutico. Además, su uso se ha extendido recientemente a la evaluación de los otros dos pilares de la sostenibilidad: la dimensión económica y la dimensión social. Por todo ello, la metodología de ACV ha sido seleccionada como la principal herramienta para el análisis de los procesos en esta tesis.

**Sección II: Moléculas de alto valor añadido a partir de organismos acuáticos**

Esta sección se centra en el análisis de los procesos de obtención de moléculas bioactivas para aplicaciones en industrias tales como el sector farmacéutico, alimentario o cosmético. Los procesos evaluados incluyen diversos organismos productores como micro- y macroalgas, esponjas, bacterias epifíticas, cromistas y hongos.

Las evaluaciones de impacto llevadas a cabo siguen una perspectiva "de la cuna a la puerta", según la cual se tienen en cuenta las etapas de producción de las materias primas requeridas en el proceso, las operaciones de limpieza y esterilización de equipos, el crecimiento de los organismos en diversos sistemas de cultivo y el procesado de la biomasa obtenida en las etapas de concentración de biomasa (o cosechado) y extracción de compuestos bioactivos. Las etapas típicas incluidas dentro de los límites del sistema en los estudios ambientales realizados se resumen en la Figura IV.2.

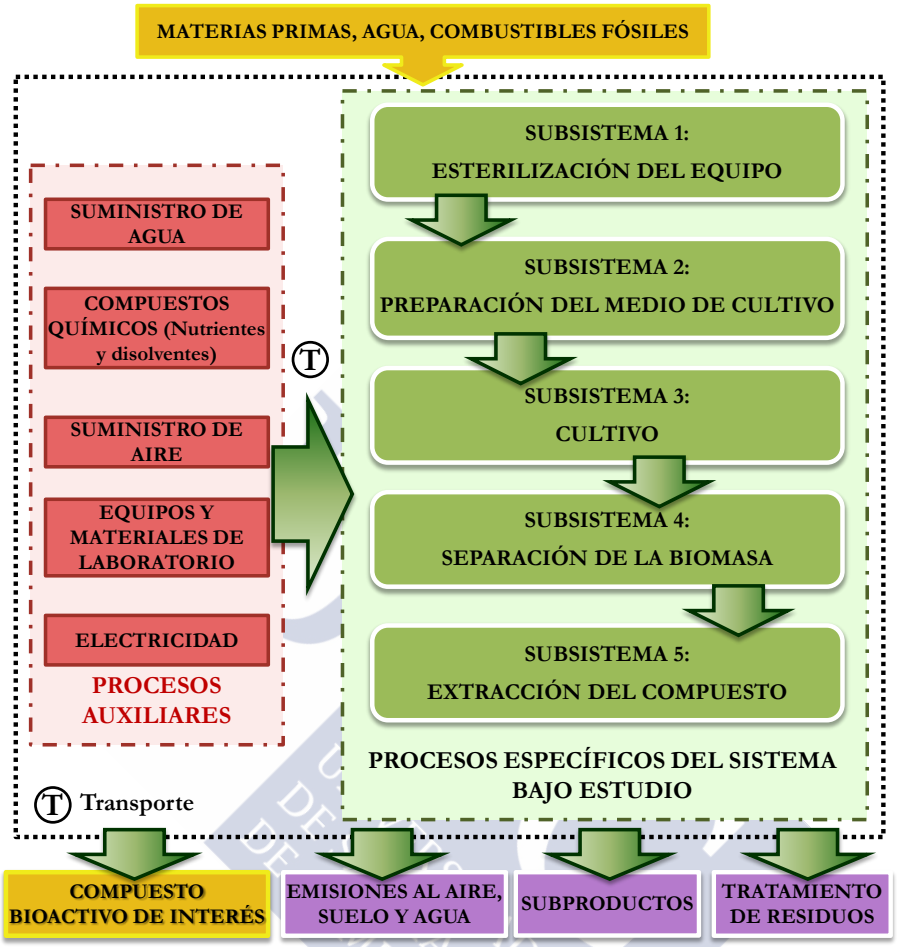



**Figura IV.2.** Etapas habituales del proceso de producción de compuestos biológicamente activos de alto valor añadido por organismos marinos.

En general, los inventarios de ciclo de vida presentados en esta sección se basan en datos primarios obtenidos mediante medidas directas en sistemas de producción reales que se encuentran actualmente en operación. La evaluación de impacto de ciclo de vida permite identificar los principales puntos problemáticos (hot spots) en términos ambientales y, en varios casos, cuantificar el potencial de mejora de una serie de escenarios alternativos propuestos para la optimización del proceso.

En los Capítulos 3 y 4 se evaluó la producción de compuestos como ácidos omega-3 o carotenoides, extraídos de microalgas en procesos a escala laboratorio y piloto. Los resultados ambientales revelaron que las etapas de preparación de medio de cultivo y crecimiento de la biomasa en el reactor son los puntos más críticos desde un punto de vista ambiental, debido al elevado consumo de electricidad y, en menor medida, al requerimiento de nutrientes. Además, se estimaron las posibles mejoras asociadas a la sustitución de los nutrientes por compuestos alternativos y a la optimización del consumo eléctrico mediante el uso de reactores alternativos con distribuciones de intensidad lumínica más eficientes. En el Capítulo 4 se realizó también una evaluación socio-económica que permitió identificar las principales fortalezas y las estrategias de mejora más aconsejables para dos empresas reales del sector de biotecnología marina.

El Capítulo 5 consistió en la aplicación del ACV para el análisis comparativo de distintas rutas de extracción para la valorización de macroalgas invasivas recogidas del medio natural como medida de control. También se analizó un proceso novedoso de cultivo de macroalgas en fotobiorreactores cerrados, similares a los empleados comúnmente en el cultivo de microalgas. De nuevo, la electricidad fue identificada como el principal responsable de los impactos, tanto en procesos de cultivo como en posterior extracción de moléculas.

En el Capítulo 6 se presentaron dos sistemas avanzados de cultivo de esponjas *in situ* y *ex situ* para la producción de compuestos con elevada actividad biológica y posibles aplicaciones en la industria farmacéutica. En este caso, el consumo de disolventes orgánicos durante la etapa de extracción fue el principal responsable de los impactos ambientales. En cuanto al análisis comparativo de los dos sistemas, el escenario inicial de cultivo *ex situ* presentaba unas cargas ambientales significativamente más elevadas que un sistema *in situ* con una producción de biomasa equivalente. No obstante, la optimización combinada de diversos aspectos del cultivo *ex situ* permitió una notable reducción del impacto global, de modo que los escenarios de mejora planteados presentaban perfiles ambientales similares o incluso más eficientes que la alternativa de cultivo *in situ*.

En el Capítulo 7 se completaron los inventarios de ciclo de vida para la producción de compuestos (coenzima $Q_{10}$, ácidos omega-3 y enzima lipasa), por parte de otros organismos marinos, incluyendo hongos, cromistas y bacterias epifíticas aisladas de macroalgas. En la evaluación ambiental de estos procesos se observaron comportamientos similares a los resultados obtenidos para otros compuestos bioactivos de origen marino. El elevado consumo eléctrico en la etapa de cultivo y el uso de disolventes orgánicos en la extracción convencional de los compuestos de interés fueron nuevamente los principales responsables de las cargas ambientales derivadas de los procesos.

**Sección III: Biorrefinerías de microalgas**

En esta sección se analizaron dos aspectos fundamentales para la implementación comercial de procesos de microalgas: el efecto de la configuración de reactor en el perfil ambiental y la incorporación de criterios económicos e incertidumbre en la simulación de procesos a escala comercial mediante modelos matemáticos.

Con respecto a la selección de sistemas de cultivo apropiados, el análisis realizado en el Capítulo 8 demostró la fuerte dependencia del perfil ambiental con respecto a la máxima concentración de biomasa que permite alcanzar cada tipo de reactor. De acuerdo con esta dependencia, la eficiencia de los reactores abiertos tipo "open pond" está limitada a condiciones climáticas favorables. La producción de estos sistemas en continuo resulta muy difícil de mantener durante periodos de tiempo prolongados, especialmente en lugares con bajas temperaturas e intensidades solares limitadas. Los fotobiorreactores planos mostraron un gran potencial relacionado con las elevadas concentraciones que se pueden alcanzar en estos sistemas, si bien el perfil global dependía en gran medida de las estrategias aplicadas para el mantenimiento y sustitución de las bolsas de polietileno que componían el sistema. Finalmente, los reactores tubulares presentaron un comportamiento más robusto y una menor dependencia de las condiciones climáticas. Los resultados sugieren la posibilidad de mantener la operación en estos sistemas con un perfil ambiental relativamente eficiente durante periodos de tiempo prolongados.

El Capítulo 9 presenta una evaluación integral de los aspectos económicos y ambientales. Este análisis se llevó a cabo para un proceso de cultivo de algas y posterior extracción de los componentes principales que incluía la obtención de diversos co-productos en un único sistema. Debido a la escasez de datos reales a escala comercial y a la variabilidad inherente de los parámetros de cultivo, la evaluación ambiental y económica de los procesos de obtención de productos a partir de algas en base a los modelos matemáticos disponibles se encuentra sujeta a una incertidumbre significativa. Para abordar dicho problema, se utilizó la técnica de simulación de Monte Carlo, basada en la generación de escenarios aleatorios en base a la definición de distribuciones de probabilidad para las variables de entrada del modelo. Este método permitió obtener las funciones de distribución de probabilidad de los indicadores económicos y ambientales evaluados para determinar el rango de posibles escenarios. También se obtuvieron las correlaciones entre dichos indicadores y se analizaron por separado tres tipos de variables: variables de proceso, factores de caracterización y parámetros económicos. La evaluación permitió identificar al conjunto de variables de proceso como la principal causa de incertidumbre. Dentro de esta categoría, la productividad y el contenido de lípidos fueron los dos parámetros con mayor contribución a la incertidumbre de los indicadores seleccionados. Entre las variables con contribuciones secundarias, el contenido de proteína y el rendimiento de metano fueron los únicos parámetros con una influencia moderada en la variabilidad de los indicadores.

**Sección IV: Conclusiones**

Los principales resultados y las implicaciones prácticas de las evaluaciones ambientales realizadas a lo largo de la tesis se resumen en el Capítulo 10, que presenta las conclusiones generales del trabajo. En conjunto, la tesis ha demostrado la utilidad de la metodología de ACV como herramienta de gestión ambiental que aporta información estratégica para mejorar la toma de decisiones en el desarrollo de procesos innovadores. La identificación de los puntos problemáticos mediante esta metodología ha permitido plantear escenarios alternativos de mejora que ponen de manifiesto el potencial de optimización de los procesos analizados para alcanzar sistemas de aprovechamiento de recursos marinos sostenibles en el futuro.

# Appendix V

# Curriculum vitae

## PERSONAL DATA


| | |
|---|---|
| **Name:** | Paula Pérez López |
| **Address:** | C/Santa Comba, nº 23-25, 7ºA |
| **City:** | Ferrol (A Coruña) |
| **Phone:** | 650216319 - 981324016 |
| **e-mail:** | paula.perez@usc.es//paula_perez_lopez@yahoo.es |
| **ID card:** | 32708748-B |
| **Date of birth:** | 09/12/1986 |
| **Nationality:** | Spanish |
| **Marital status:** | Single |


## ACADEMIC BACKGROUND

| | |
|---|---|
| **2004 - 2010** | **Chemical Engineering**<br>Specialities of Process Control and Chemical and Biochemical Processes.<br>School of Engineering, University of Santiago de Compostela |
| **2010 - 2011** | **Master of Science in Chemical and Environmental Processes Engineering**<br>School of Engineering, University of Santiago de Compostela |
| **Fall 2015 (expected)** | **PhD in Chemical and Environmental Engineering**<br>School of Engineering, University of Santiago de Compostela |

## LANGUAGES

| | |
|---|---|
| **Mother tongues:** | Spanish and Galician |
| **English:** | Preliminary English Test (PET). Cambridge University. June, 2000 |
| | First Certificate English (FCE). Cambridge University. June, 2002 |
| | Certificate in Advanced English (CAE). Cambridge University. June, 2003 |
| | English level C1.2. Centre for Modern Languages (CLM). University of Santiago de Compostela. June, 2013 |
| **French:** | Elementary. High school credit hours |
| **German:** | Level A1. Centre for Modern Languages (CLM). University of Santiago de Compostela. June, 2014 |

## RESEARCH STAYS

| | |
|---|---|
| **May 2012** | **Science Faculty (University of Vigo).** Ourense (Spain). |
| **April-August, 2013** | **Algae Production and Research Centre (AlgaePARC, Wageningen University and Research Centre).** Wageningen (The Netherlands). |
| **September-November, 2014** | **Department of Civil and Environmental Engineering (Northeastern University).** Boston (MA, United States). |
| **June-July, 2015** | **Shannon ABC (Limerick Institute of Technology).** Limerick (Ireland). |

## PARTICIPATION IN EU-FUNDED RESEARCH PROJECTS

**White biotechnology for added value products from renewable plant polymers: Design of tailor-made biocatalysts and new industrial bioprocesses (BIORENEW)**

Funding body: European Union (NMP2-CT-2006-026456)

Project Leader: María Teresa Moreira Vilar

Partners: 27 partners from 12 EU countries, including public and private organisations from Austria, Belgium, Denmark, Finland, France, Germany, Norway, Portugal, Spain, Sweden, The Netherlands and United Kingdom.

**Sustainable production of Biologically Active Molecules of Marine Based Origin (BAMMBO)**

Funding body: European Union (FP7-KBBE-2010-4-265896)

Project Leader: María Teresa Moreira Vilar

Partners: 11 public and private organizations from 6 EU countries (Belgium, France, Ireland, Italy, Portugal, Spain), jointly with the Research Institute Genetika (Russia) and the Universidade Estadual de Campinas (Brazil).

**Monitoring air quality using moss (MOSSClone)**

Funding body: European Union (FP7-ENV.2011.3.1.9-1)

Project Leader: José Ángel Fernández Escribano

Partners: 10 partners from 5 EU countries (France, Germany, Ireland, Italy, Spain).

## PARTICIPATION IN RESEARCH CONTRACTS WITH COMPANIES

**Ecologic design of wood and furniture products (DECOFIM)**

Funding body: CENFIM, Generalitat de Catalunya

Project leader: Gumersindo Feijoo Costa

**Eco-design pilot project**

Funding body: ENISA

Project leader: Gumersindo Feijoo Costa

**Compensation of carbon emissions generated in the Abertis motorway network through forestry measures**

Funding body: Abertis Foundation

Project leader: Gumersindo Feijoo Costa

## SCHOLARSHIPS AND CONTRACTS

| | |
|---|---|
| **July - September 2009** | Training period at the Group of Environmental Engineering and Bioprocesses, Department of Chemical Engineering, University of Santiago de Compostela. Programme of Environmental Management – Product and process sustainability: Life Cycle Assessment and Carbon Footprint |
| **October 2009 - March 2013** | Research contract at the University of Santiago de Compostela at the Group of Environmental Engineering and Bioprocesses at the Department of Chemical Engineering of the University of Santiago de Compostela. |
| **March 2013- January 2015** | FPU scholar at the Group of Environmental Engineering and Bioprocesses at the Department of Chemical Engineering of the University of Santiago de Compostela |

## COURSES

| | |
|---|---|
| **September 2010** | *Advances on Wastewater Treatment* (3 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **September 2010** | *Assessment of the Design and Operation of Wastewater Treatment Plants* (8 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **February 2011** | *Instrumentation Identification and Simbology. ANSI/ISA Standard* (5 h)<br>ISA – USC Section (International Society of Automation), Santiago de Compostela |
| **March 2011** | *SIS. HAZOP risk assessment* (10 h)<br>ISA – USC Section, Santiago de Compostela |
| **April 2011** | *Multivariable Predictive Control* (10 h)<br>ISA – USC Section, Santiago de Compostela |
| **May 2011** | *International Seminar in Social Life Cycle Assessment* (10 h)<br>Cemagref, SubAgro, Montpellier |
| **June 2011** | *Getting Ready to Write a Scientific Article: How to Prepare* (2 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **November 2011** | *Field instrumentation* (10 h)<br>ISA - USC Section, Santiago de Compostela |
| **March 2012** | *Eco-design as a key tool for innovation in companies* (12.5 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **April 2012** | *ATEX, facilities in explosive atmospheres* (8 h)<br>ISA – USC Section, Santiago de Compostela |
| **January 2013** | *Building on our strengths* (16 h)<br>Department of Chemical Engineering, Santiago de |

Compostela

**COURSES**

| | |
|---|---|
| **September 2013** | *Energy and global environment - A process engineering approach* (15 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **January 2014** | *Characteristics, biotechnological production and applications of bioplastics* (3 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **January 2014** | *Development of Influencing and Negotiating Skills* (12 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **January 2015** | *Stress Management Workshop* (7 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **September 2010** | *Advances on Wastewater Treatment* (3 h)<br>Department of Chemical Engineering, Santiago de Compostela |
| **September 2010** | *Assessment of the Design and Operation of Wastewater Treatment Plants* (8 h)<br>Department of Chemical Engineering, Santiago de Compostela |

## CONFERENCES

**2012**

1. **Authors:** J.M. Lema, A. Hospido, E. Álvarez, **P. Pérez**, I. Vidal
   **Title:** Integration and visualisation of basic concepts in Chemical Engineering through a case study, based on information and communication technologies
   **Participation:** Oral
   **Conference:** 1st Congress on Teaching Innovation in Chemical Engineering
   **Place:** Granada (Spain), 26-27 January, 2012

**2013**

1. **Authors:** **P. Pérez-López**, S. González-García, C. Allewaert, A. Verween, W. Vyverman, P. Murray, M.T. Moreira, G. Feijoo
   **Title:** Environmental assessment of the sustainable production of polyunsaturated fatty acids by *Phaeodactylum tricornutum*
   **Participation:** Poster
   **Conference:** 9th European Congress of Chemical Engineering - 2nd European Congress of Applied Biotechnology
   **Place:** The Hague (The Netherlands), 21-25 April, 2013

2. **Authors:** **P. Pérez-López**, S. González-García, E. McHugh, D. Walsh, P. Murray, S. Moane, G. Feijoo, M.T. Moreira
   **Title:** Sustainable pilot-scale production of carotenoid compounds from the green microalga *Haematococcus pluvialis*
   **Participation:** Oral
   **Conference:** LCA avniR Conference 2013
   **Place:** Lille (France), 4-5 November, 2013

## CONFERENCES

**2013**

3. **Authors:** **P. Pérez-López**, E. Ternon, S. González-García, G. Genta-Jouve, G. Feijoo, O.P. Thomas, M.T. Moreira
   **Title:** Environmental optimisation for the sustainable production of biologically active molecules from sponge *Crambe crambe*
   **Participation:** Oral
   **Conference:** SETAC Europe 19th LCA Case Study Symposium
   **Place:** Rome (Italy), 11-13 November, 2013
   **ISSN:** 2310-3191

**2015**

1. **Authors:** **P. Pérez-López**, E.M. Balboa, G. Feijoo, H. Domínguez, M.T. Moreira
   **Title:** LCA as a decision tool in the design of macroalgae-based biorefineries for biomass valorization
   **Participation:** Poster
   **Conference:** LCM 2015
   **Place:** Bordeaux (France), 30 August - 2 September, 2015

## PUBLICATIONS

**Published**

1. P.M. Murray, S. Moane, C. Collins, T. Beletskaya, O.P. Thomas, A.W.F. Duarte, F. S. Nobre, I.O. Owoyemi, F.C. Pagnocca, L.D. Sette, E. McHugh, E. Causse, **P. Pérez-López**, G. Feijoo, M.T. Moreira, J. Rubiolo, M. Leirós, L. M. Botana, S. Pinteus, C. Alves, A. Horta, R. Pedrosa, C. Jeffryes, S.N. Agathos, C. Allewaert, A. Verween, W. Vyverman, I. Laptev, S. Sineoky, A. Bisio, R. Manconi, F. Ledda, M. Marchi, R. Pronzato, D.J. Walsh (2013). Sustainable production of biologically active molecules of marine based origin. *New Biotechnology* 30(6):839-850

2. **P. Pérez-López**, C.M. Gasol, J. Oliver-Solà, S. Huelin, M.T. Moreira, G. Feijoo (2013). Greenhouse gas emissions from Spanish motorway transport: Key aspects and mitigation solutions. *Energy Policy* 60:705-713

3. **P. Pérez-López**, S. González-García, C. Allewaert, A. Verween, P. Murray, G. Feijoo, M.T. Moreira (2014). Environmental evaluation of eicosapentaenoic acid production by *Phaeodactylum tricornutum*. *Science of the Total Environment* 466-467:991-1002

4. **P. Pérez-López**, S. González-García, R.G. Ulloa, J. Sineiro, G. Feijoo, M.T. Moreira (2014). Life cycle assessment of the production of bioactive compounds from *Tetraselmis suecica* at pilot scale. *Journal of Cleaner Production* 64:332-344

5. **P. Pérez-López**, S. González-García, C. Jeffryes, S.N. Agathos, E. McHugh, D. Walsh, P. Murray, S. Moane, G. Feijoo, M.T. Moreira (2014). Life cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. *Journal of Cleaner Production* 64:323-331

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7. **P. Pérez-López**, E.M. Balboa, S. González-García, H. Domínguez, G. Feijoo, M.T. Moreira (2014). Comparative environmental assessment of valorisation strategies of the invasive macroalgae *Sargassum muticum*. *Bioresource Technology* 161:137-148

8. E. Sanyé-Mengual, **P. Pérez-López**, S. González-García, R. Garcia Lozano, G. Feijoo, M.T. Moreira, X. Gabarrell, J. Rieradevall (2014). Eco-designing the use phase of products in sustainable manufacturing: the importance of maintenance and communication-to-user strategies. *Journal of Industrial Ecology* 18(4):545-557

9. **P. Pérez-López**, G. Feijoo, M.T. Moreira (2014). Aplicación de la metodología de Análisis de Ciclo de Vida para la producción sostenible de ingredients actives a partir de organismos marinos. Revista Alimentaria 455:40-46 (In Spanish)

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1. M. Montazeri, L. Soh, **P. Pérez-López**, J.B. Zimmerman, M.J. Eckelman. Time-dependent variation in life cycle assessment of microalgal biorefinery co-products. *Biofuels, Bioproducts & Biorefining.*

2. **P. Pérez-López**, C. Jeffryes, S.N. Agathos, G. Feijoo, G. Rorrer, M.T. Moreira Environmental life cycle optimization of essential terpene oils produced by the macroalga Ochtodes secundiramea. *Science of the Total Environment*

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