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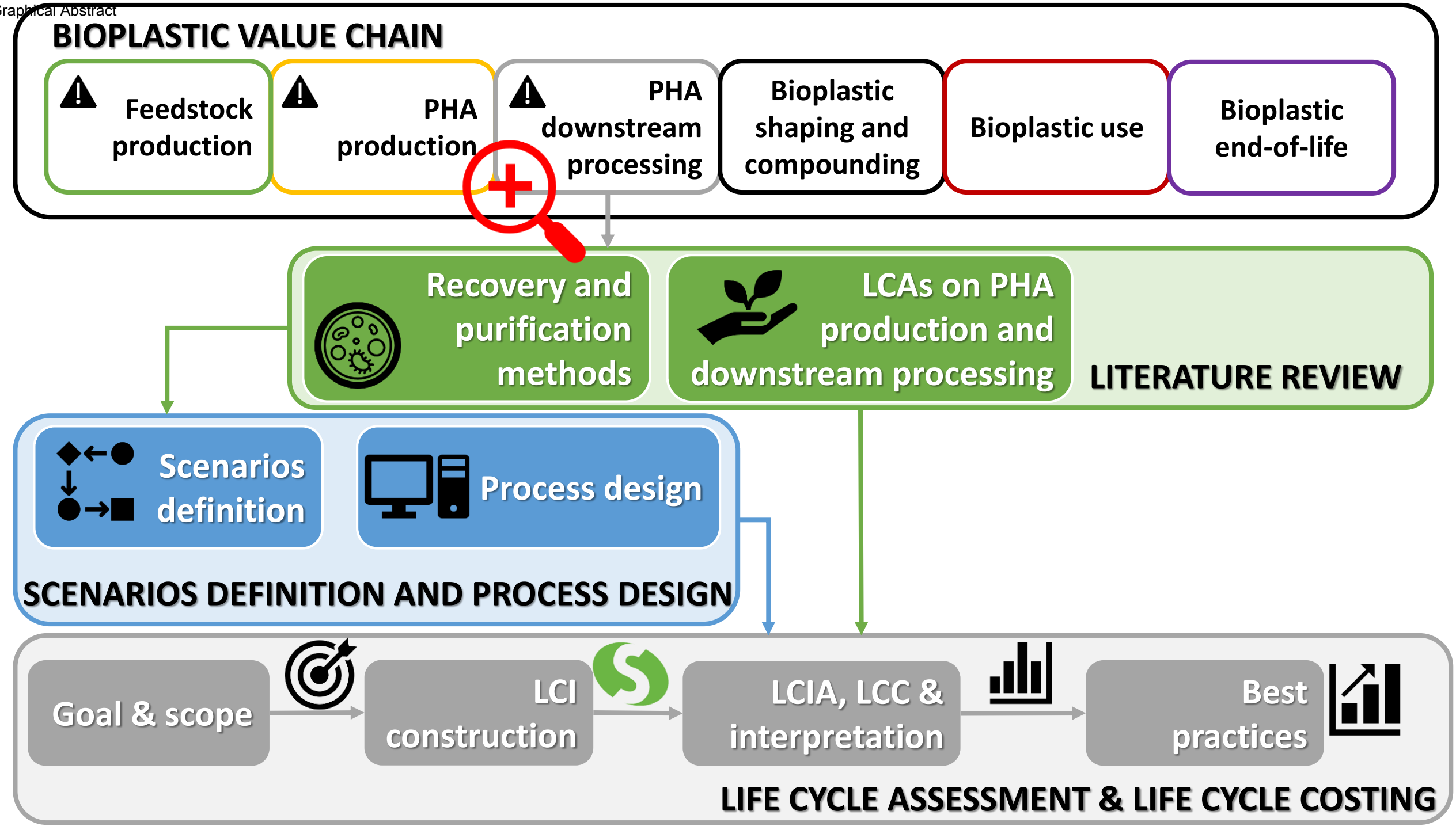
Graphical Abstract



Highlights

- PHA downstream processes and their environmental performance are reviewed
- Eight downstream processes for low- and high-grade PHA are selected and assessed
- The environmental and economic performance are estimated by LCA and LCC
- Energy and chemical intensive downstream processing steps are identified
- Opportunities for integrating PHA downstream within biorefineries are proposed

Evaluation and optimization of the environmental performance of PHA downstream processing

Mateo Saavedra del Oso, Miguel Mauricio-Iglesias* and Almudena Hospido

CRETUS Institute, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782, Santiago de Compostela, Spain

*Corresponding author.

E-mail address: miguel.mauricio@usc.es

Abstract

Biobased and biodegradable materials such as polyhydroxyalkanoates (PHA) have great potential as an alternative for conventional oil-based plastics in consumer goods and medical applications, but their total market share is still marginal due to their high production costs. Downstream processing, with high energy demand and significant requirements in oil-derived solvents and chemicals, has been identified as one bottleneck in the PHA value chain. Hence, a thorough study of the environmental performance of PHA recovery processes is essential to promote their applicability. This work provides valuable insights on PHA downstream processing environmental hotspots and how to optimize them accordingly. Eight PHA downstream alternative processes for both high-grade and low-grade purification are evaluated from a techno-economic and an environmental perspective, assessing scale-up possibilities and challenges. To reach this goal, both scenario definition and process design were supported by a systematic review of available PHA downstream methods and related life cycle assessments. Methods relying on solvent extraction require large amounts of energy for solvent recovery, and thus, their higher performance in impurity removal also entails larger costs and impacts in all categories, when compared to mechanical disruption or chemical digestion. Therefore, solvent extraction is only recommended for those cases where a higher quality is required, or solvents can be reasonably obtained from an integrated biorefinery. Chemical digestion can be optimized by adding a chemicals recovery unit, while mechanical disruption appears to be the most promising technology in terms of environmental performance. Through this technoeconomic and environmental assessment, it is proved that PHAs can be attractive materials for a sustainable bioeconomy if the process and product design incorporate life cycle assessment such as the developed in this work.

Keywords

Polyhydroxyalkanoate extraction; Biorefinery; Biobased materials; Process optimisation; Life cycle assessment

Abbreviations

AD – Annual depreciation

AP – Acidification potential

ATP – Aquatic toxicity potential

CH – Switzerland

COD – Chemical Oxygen Demand

C_{TCI} – Total capital investment

DMC – Dimethyl carbonate

EP – Eutrophication potential

FAETP – Ecotoxicity for aquatic fresh water

FD – Fossil depletion

FE – Freshwater eutrophication

FEX – Freshwater ecotoxicity

FOFP – Photo-oxidant formation potential

FU – Functional unit

GWP – Global warming potential

HT – Human toxicity

HTPE – Human toxicity potential by either inhalation or dermal exposure

HTPI – Human toxicity potential by ingestion

L – Labor costs

LCA – Life Cycle Assessment

LCC – Life Cycle Cost

LCI – Life Cycle Inventory

M – Maintenance costs

m – Materials costs

NCPM – Non-cellular PHA mass

NREU – Non-renewable energy use

ODP – Ozone depletion potential

OFP – Photochemical ozone formation

PE – Polyethylene

PET – Polyethylene terephthalate

PHA – Polyhydroxyalkanoates

PHB – Polyhydroxybutyrate

PP – Polypropylene

RER – Europe

SDS – Sodium dodecyl sulfate

TA – Terrestrial acidification

TAC – Total annual costs

TTP – Terrestrial toxicity potential

U – Utilities costs

1 Introduction

The environmental impact derived from the ubiquitous use of plastics has been linked to two of society's present main environmental concerns: climate change and the spoilage of marine environments caused by improperly disposed plastics. In this situation replacing oil-based non-biodegradable plastics with alternative bio-based plastics that have similar properties could have notable benefits. Among bioplastics, polyhydroxyalkanoates (PHAs) are biobased, biodegradable –even in marine environments by ASTM standards– and biocompatible polymers with multiple applications [1,2]. They can be produced, through bacterial fermentation, from a feedstock other than starch/glucose such as a diverse range of complex organic substrates including by-products from agriculture and food industry [3–5]. PHAs display thermoplastic properties, which depend on the choice of substrate, bacteria and fermentation conditions. Thus, PHAs are ideal substitutes for conventional oil-based plastics such as polyethylene (PE), polyethylene terephthalate (PET) or polypropylene (PP) [6].

The largest driver for replacing conventional plastics by PHA would be a better environmental performance [7]. Studies based on life cycle assessment (LCA), a technique that identifies and quantifies the potential environmental impacts associated with a product, process or service [8], have reported that conventional plastics may have lower carbon footprint [9,10] than PHA albeit biodegradable and based on renewable resources. High energy requirement was reported in overall PHA production life cycle, especially during feedstock cultivation, sterilization equipment within PHA fermentation and the PHA downstream processing [2,11]. Furthermore, PHAs production at large scale is still limited due to its high production cost –2.2 to 5 €/kg–compared with conventional oil-based plastic –less than 1 €/kg– [12]. Within PHA production, the downstream processing of PHAs has been reported as a cost and environmental hotspot for PHAs production. Specifically, it can account for up to 50% of the production costs [13], largely due to the use of great amounts of solvents and high energy requirements [11,14].

PHAs downstream processing is commonly comprised by several steps (Fig. 1), starting with the physical separation of the biomass, and probably followed by a pretreatment step to enhance the yield and purity in the extraction step. In the extraction step, PHA can be recovered by two different

ways: (1) by solubilizing the non-cellular PHA mass (NCPM) through chemical digestion or mechanical disruption or (2) by solubilizing the PHA through solvent extraction. Then, PHA is separated from the NCPM by basic operations such as precipitation, filtration or sedimentation, or more alternatives for higher-value products such as liquid-liquid extraction or air classification. Finally, in most cases and regardless of the final product requirements, PHA should be purified: re-dissolution with water or ethanol can be enough, although sometimes a bleaching step with hydrogen peroxide or sodium hypochlorite takes place; additionally, ozone treatment or activated charcoal seems to be effective too in order to obtain a purified PHA [13,15,16].

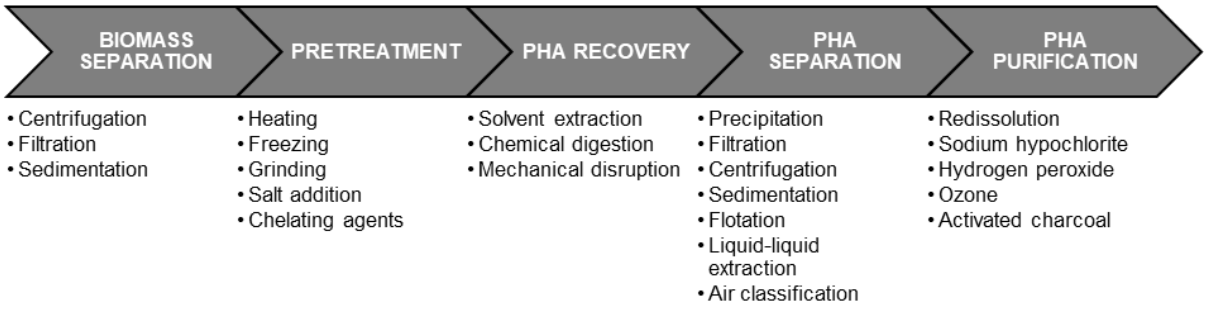


Fig. 1. Conventional steps in the PHA downstream processing and most common methods for each step.

The objective of this study is to provide valuable insights on PHA downstream processing optimization by finding economic and environmental hotspots for improve its performance. For this endeavor, a literature review of LCA on PHA downstream processing was carried out providing the basis for a techno-economic and environmental assessment of eight potential downstream approaches –both low and high grade PHA purification–. Firstly, the most promising methods for PHA downstream processing were identified and, through a design and scale-up supported by articles and patents, downstream processes were classified as corresponding to low or high grade PHA purification. Then, an in-depth revision of selected LCA of PHA production and downstream processes was carried out to evaluate how the key LCA elements –such as objective, functional unit, system boundaries, impact categories …– were handled. Finally, the economic and environmental performance of the eight selected downstream processes was performed by using

life cycle cost (LCC) and LCA, respectively, and best practices guidelines for PHA downstream processes design were proposed.

2 Methods

2.1 Literature review

In order to identify the most promising methods for PHA downstream processing, a systematic review of the literature available using Scopus web search engine for articles and Google Patents for patents was performed. To do so, the following keywords were used: 'biopolymers', 'PHA', 'polyhydroxyalkanoates', 'PHB', 'polyhydroxybutyrate', 'PHBV', 'poly(3-hydroxybutyrate-co-3-hydroxyvalerate', 'downstream processing', 'extraction', 'green solvents', 'recovery', 'chemical digestion', 'isolation', 'industrial production', 'sustainable'. Among the 200 resulting elements, twelve peer-reviewed articles and patents which collect the methods for PHA recovery and purification as well as technical specifications were screened [2,13,15,17–25], being the primary source in relation to the methods used for scenarios definition and process design, described later in Section 3.

For the second literature review focused on LCAs and LCC on PHA production and downstream processes, the following keywords were added: 'technical assessment', 'techno-economic assessment', 'life cycle costing', 'LCC', 'life cycle assessment', and 'LCA' in Scopus web search engine. Thirteen LCA peer-review articles on PHA production and downstream processing [9,10,19,26–35] which include specific data about PHA recovery and purification were screened.

2.2 Process simulation

The process simulator Aspen HYSYS –provided by Aspen Technology, Inc., US– was used to estimate the process requirements in terms of fresh solvent and energy duties in heat exchangers and solvent recovery units –mostly evaporators and distillation columns–. Fluid packages were chosen according to Carlson [36] and Elliot and Lira [37] recommendations, selecting the NTRL model for acetone-water, acetone-ethanol, ethyl acetate-heptane and isoamyl alcohol-water mixtures.

2.3 Life Cycle Assessment and Life Cycle Costing

LCA is a systematic and standardized methodology which determines the process or product's environmental impacts in the design and operational phase, and it is comprised by four steps: i) the goal and scope states the intended application, the system, its function and the related functional unit, the system boundaries, the impact categories selected and the impact assessment methodology; ii) the inventory analysis involves data collection and calculation procedures to quantify relevant inputs and outputs of a product system; iii) the impact assessment transforms the inventory results into potential environmental impacts; and iv) the interpretation phase, which involves critical review, determination of data sensitivity, and result presentation [8].

Life cycle costing (LCC), which is one pillar of the full life cycle sustainability assessment, is an economic assessment aligned with LCA in terms of system boundaries, functional unit, and methodological steps, is performed [38]. In general, costs can be divided into capital and operational costs. Regarding the former, delivered equipment costs were estimated using correlations based on individual equipment's characteristics size [39]: total capital investment was estimated applying a Lang Factor of 5.03, characteristic from a solids-fluids processing plant, and a 2019 CEPCI index of 607.5; annual depreciation (AD) was calculated –see Eq. (1)– based on the total capital investment (C_{TCI}) with an interest rate (i) of 5% and a payback time (PB) of 20 years. Concerning the latter, total annual costs (TAC) were estimated using Eq. (2): utilities (U) and materials (m) costs were based on the inventories built and literature information [39], while maintenance costs (M) were assumed to account for the 3% of the total capital investment, and the labor costs (L) were assumed to be 10% of the total annual costs.

$$\boldsymbol{AD = C_{TCI} \cdot [i(1+i)^{PB}]/[(1+i)^{PB} - 1]} \quad (1)$$

$$\boldsymbol{TAC = AD + U + m + L + M} \quad (2)$$

3 Scenarios definition and process design

To define the scenarios studied in this paper, the most promising methods of PHA downstream processing were identified. To do so, twelve peer-review articles and patents were screened by the following criteria: those which include a revision of the state-of-the-art [2,13,15,17,18], and those

which include methods with technical specifications applied at lab or pilot scale [19–25]. The processes were designed according to articles and patents guidelines. Other relevant information includes the process background (substrate or type of culture employed for PHA production), the technology readiness level (TRL) and the quality of the PHA obtained (Table 1). Also, solvents selection guides and technologies maturity level were considered when designing solvent-based processes [40,41].

The requisite quality of a recovered PHA is coupled to the intended use of the bioplastic. Most of studies employ only recovered polymer purity and molecular weight as the indicator of the polymer quality [19,20,42]. However, there are other aspects than should be considered such as the consistency of the material quality, and rather than the purity, the chemical impurities that are present –e.g. the immunostimulatory lipopolysaccharide present in most Gram-negative bacteria, which is considered a endotoxin– [43]. Indeed, loss of molecular weight during melt processing is predominantly caused by trace impurities, and thus, a higher molecular weight may not mean a higher quality. In this sense, we define here *high-grade PHA* as the one used for pharmaceutical and food grade applications. It has to comply with the following requirements: high purity, high molecular weight and chemical compatibility according to the European Commission regulation No 10/2011 related to plastic materials and articles intended to come into contact with food [44]. On the other hand, PHA obtained through processes which do not comply these requirements, especially those related to impurities –e.g. salts and lipopolysaccharide– and substrate employed –e.g. methane and wastewater– were classified as *low-grade PHA* notwithstanding stringent mechanical and structural property requirements.

Table 1 Processes definition of PHA downstream processes, accounting their quality grade, substrate, type of culture, recovery method, technology readiness level and references in which are based.

Grade	PHA	Feedstock	Culture	Microorganism	Recovery method	TRL**	Reference
High (H1)	P(4HB)	Glucose	Pure	*Escherichia coli*	Acetone extraction	9	[22,24,45]
High (H2)	P(3HB)	Food industry byproducts	Pure	*Ralstonia eutropha*[*]	HPH + SDS digestion	9	[25,46]
High (H3)	P(3HB-co-4HB)	Oleic acid, ɣ-butyrolactone	Pure	*Cupriavidus* sp.[*]	NaOH + Lysol digestion	4	[45,47]
High (H4)	P(3HB-co-3HHx)	Glucose, soybean oil	Pure	*Aeromonas, Wauteria*.	Ethyl acetate extraction	6	[21]

Grade	PHA	Feedstock	Culture	Microorganism	Recovery method	TRL**	Reference
Low (L1)	P(3HB-co-4HB)	Methane	Pure	*Methylocystis hirsuta*	Acetone extraction	4	[5,48]
Low (L2)	P(3HB)	Canning industry waste	Halophilic bacteria	*Halomonas boliviensis*	Osmotic shock + SDS digestion	4	[49–51]
Low (L3)	P(3HB)	Wastewater	Mixed culture	Not applicable.	NaClO + SDS digestion	4	[32,33]
Low (L4)	P(3HB)	Sugar molasses byproducts	Pure	*Alcaligenes eutrophus**	Fusel alcohols extraction	8	[23,52]

* *Ralstonia eutropha*, *Cupriavidus sp.* and *Alcaligenes eutrophus* are the same microorganism
****** TRL was assigned according to Humbird [53]

A brief description of the selected processes for each type, high- or low-grade, is presented below plus a summary of key model assumptions (Table 2 and Table 3), while mass balances and detailed schemes along with process specifications and design outcomes are collected in Supplementary information (sections S1.1, S2.1, S3.1 and so on; note that processes H1 and L2 flow diagrams are also included there for completeness).

3.1 Downstream processes for high-grade PHA

In **process H1** (Fig. 2), which is based on a patent applied by Tepha Inc. US [24], P(4HB) enriched biomass –obtained through fermentation of glucose by *Escherichia coli* [45]– is extracted by acetone. Before being submitted into extraction, biomass is dried and washed with ethanol to remove its toxins content. After extraction, NCPM is separated by ultrafiltration, and dissolved PHA is recovered by adding an antisolvent –ethanol in this case– until the PHA precipitates. Additionally, P(4HB) is purified through ethanol washing prior to spray drying. The pretreatment and purification step with ethanol along the extraction with acetone, which is a Class 3 solvent –i.e. those solvents with low human toxic potential according to the European Medicines Agency [54]– allows that the obtained P(4HB) powder can be employed in medical and food grade applications.

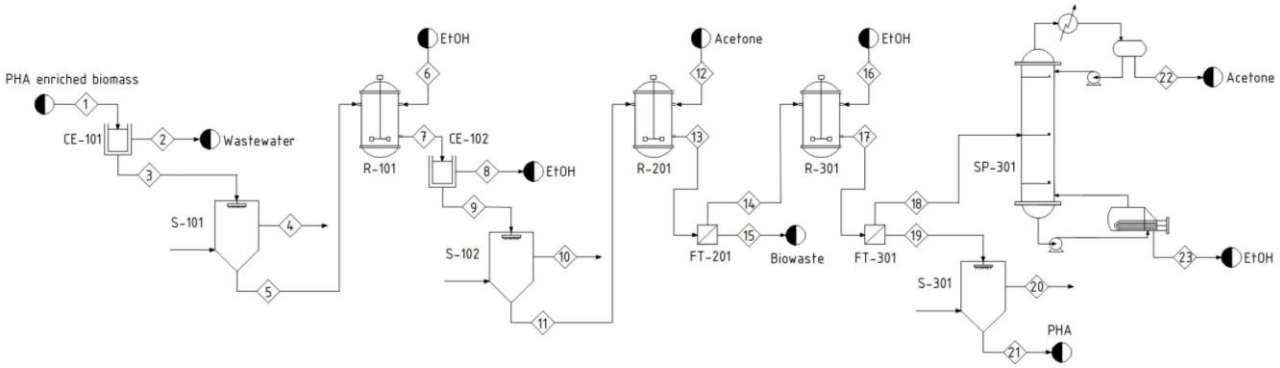


Fig. 2. H1 process flow diagram.

Process H2 is based on NCPM mechanical disruption and chemical digestion. Developed by Bio-On (Italy) as a patent [25], it is already being applied at full scale by them [46]. P(3HB) enriched biomass –produced through microbial fermentation of food industry byproducts– is sent to a high-pressure homogenization where a surfactant is added to cause simultaneously the NCPM disruption and digestion. Then P(3HB) is easily separated from dissolved biomass by a liquid-solid separation. Additionally, P(3HB) is purified by bleaching prior to spray drying.

Similarly, **process H3** is based on NCPM chemical digestion to extract the copolymer of P(3HB-co-4HB). Based on a study performed at pilot plant scale [47], PHA enriched biomass previously dewatered is sent to a NaOH and Lysol treatment, to digest NCPM. After that, a post-treatment by water washing and a spray drying is carried out.

Process H4 is based on a large-scale study [21], where the P(3HB-co-3HHx) enriched biomass is obtained by a pure culture of *Aeromonas hydrophile* that uses only glucose as a carbon source. The downstream process comprises dry biomass solvent extraction with ethyl acetate, heptane precipitation and post-treatment consisting of ethanol washing followed by a spray drying step to obtain the final PHB copolymer.

Table 2 Summary of key model assumptions for high-grade PHA downstream processes.

Process	Step	Assumptions
H1	Biomass treatment	10,000 t of P(4HB) in enriched biomass are recovered for 330 days/year 11.5 kg·m^{-3} biomass concentration with 68% P(4HB) weight content A drying step –less than 5 wt% water content – prior to 95 wt% ethanol washing in a 4:1 ratio ethanol-biomass in R-101 [b] An additional drying step to reach up to 99 wt% solids content [b]
	Extraction	A 94% P(4HB) recovery yield [c] Acetone extraction in a 20:1 acetone-biomass ratio in R-201 [b] Solvent recovery in a distillation column
	Recovery and purification	99.7% P(4HB) purity [b] P(4HB) precipitation with ethanol 95 wt% in a 1:1 ethanol-acetone ratio in R-301 [a]
H2	Biomass treatment	10,000 t of P(3HB) in enriched biomass are recovered for 330 days/year 80 kg·m^{-3} biomass concentration with 70% P(4HB) weight content [d] A 10 wt% H_2SO_4 addition obtain a pH 4.5 [d]
	Extraction	A 83% P(3HB) recovery yield [d] A high pressure homogenization step (1000 bar) at room temperature [d] A 50 wt% NaOH addition to obtain a pH 10.0 and a 4 g·L^{-1} SDS aqueous solution addition in R-201 [d] A water dilution (50% of FT-201 liquid stream is recycled) in a 1:4 stream-water ratio in R-202 [d]

	Recovery and purification	A 94% P(3HB) recovery yield and 99% purity [d] 30 wt% H_2O_2 bleaching treatment in a 1:8 stream-H_2O_2 ratio in R-301 [d] Water dilution (85% of FT-301 liquid stream is recycled) in a 1:3 stream-water ratio in R-302[d]
H3	Biomass treatment	10,000 t of P(3HB-co-4HB) in enriched biomass are recovered for 330 days/year 11.5 kg·m^{-3} biomass concentration with 70% P(4HB) weight content [ae] A dewatering step to reach up to 80 wt% solids content
	Extraction	A 90% P(3HB-co-4HB) recovery yield [e] A 0.15 M NaOH and 2 vv% Lysol addition in R-201 [e]
	Recovery and purification	93% P(3HB-co-4HB) purity [e] A water dilution in a 1:3 stream-water ratio in R-301
H4	Biomass treatment	10,000 t of P(3HB-co-3Hxx) in enriched biomass are recovered for 330 days/year 50 kg·m^{-3} biomass concentration with 50% P(3HB-co-3Hxx) weight content [f] A drying step –up to 99 wt% solids content – prior to grinding [f]
	Extraction	A 94% P(3HB-co-3Hxx) recovery yield [f] Ethyl acetate extraction in a 20:1 ethyl acetate-biomass ratio in R-201 [f] Solvent recovery in a distillation column
	Recovery and purification	95% P(3HB-co-3Hxx) purity P(3HB-co-3Hxx) precipitation with heptane in 3:1 heptane-ethyl acetate ratio in R-301 [f] A P(3HB-co-3Hxx) purification step with ethanol in a 4:1 ethanol-biomass ratio in R-302 [f]
References for assumptions: a-[45], b-[24], c-[22], d-[25], e-[47], f-[21]		

3.2 Downstream processes for low-grade PHA

Process L1 is based on a research scale and a technoeconomic study [5,48], which employs solvent extraction to isolate the copolymer P(3HB-co-4HB). PHA enriched biomass, obtained through microbial fermentation of methane by *Methylocystis hirsute*, is previously dried prior to hot acetone extraction. After NCPM separation, the stream is refrigerated and water is added to precipitate the PHB. Finally, PHB is recovered and spray dried.

Process L2 (Fig. 3), based on a patent developed by Repsol S.A. (Spain) [51], takes advantage of halophilic culture characteristics and uses an osmotic shock to disrupt NCPM [49,50]. PHA enriched biomass is partially dewatered and heated prior a recovery step where an osmotic shock combined with SDS digestion is carried out to digest NCPM. Then, PHA is separated from dissolved NCPM and spray dried after a washing step with water.

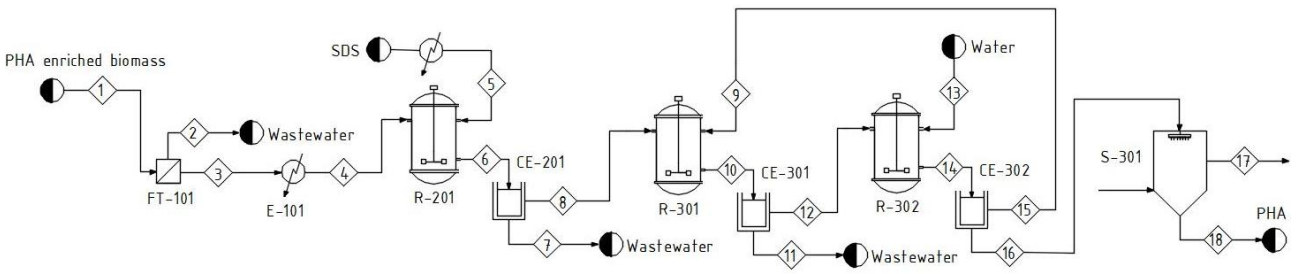


Fig. 3. L2 process flow diagram.

Chemical digestion of the NCPM is the extraction method employed in **process L3** to recover P(3HB). Based on a paper of PHA isolation from a mixed microbial culture [32,33], this process uses a chemical digestion which combines the high oxidizing potential of NaClO with a surfactant such as SDS. Then a washing step with fresh water is carried out before spray drying.

Process L4 has the peculiarity of employing a byproduct of ethanol biorefinery, such as fusel alcohols, as solvent. This process, which is based on a PHB Industrial S.A. patent [23], is comprised by a extraction step where the P(3HB) is extracted by fusel alcohols. Then, after some solid-liquid separations to separate the NCPM and concentrate the polymer, the stream is cooled to precipitate and recover the polymer prior to spray drying.

Table 3. Summary of key model assumptions for low-grade PHA downstream processes

Process	Step	Assumptions
L1	Biomass treatment	100,000 t of P(3HB-co-4HB) in enriched biomass are recovered for 330 days/year 60.7 kg·m^{-3} biomass concentration with 50% P(3HB-co-4HB) weight content A drying step –less than 5 wt% water content–
	Extraction	A 92% P(3HB-co-4HB) recovery yield [b] Hot acetone extraction –3 bar and 90°C– in a 9:1 acetone-biomass ratio in R-201 [a] Solvent recovery in a distillation column
	Recovery and purification	98% P(3HB-co-4HB) purity [a] A P(3HB-co-4HB) precipitation step comprised by cooling (E-301) and water addition (R-301) [a] A separation by microfiltration and spray drying to obtain P(3HB-co-4HB) powder
L2	Biomass treatment	1,500 t of P(3HB) in enriched biomass are recovered for 330 days/year 8 kg·m^{-3} biomass concentration with 50% P(3HB-co-4HB) weight content [c] A dewatering step –up to 60 wt% solids content – followed by a heating step (60°C) [de]
	Extraction	A 98% P(3HB) recovery yield [d] 0.1 wt% SDS chemical digestion at 60°C –less than 5 wt% solids content– [de]
	Recovery and purification	94% P(3HB) purity [d] Water washing in two step maintaining up to 5 wt% solids content –water from second washing step is recycled into first washing step– A separation by centrifugation and spray drying to obtain P(3HB) powder
L3	Biomass treatment	1,500 t of P(3HB) in enriched biomass are recovered for 330 days/year 60.8 kg·m^{-3} biomass concentration with 70% P(3HB) weight content [f] A heating step (55°C) [f]
	Extraction	A 94% P(3HB) recovery yield SDS chemical digestion in a 3:1 SDS-NCPM ratio in first recovery step [f] 15 wt% NaClO solution chemical digestion in a 8:1 NaClO-NCPM ratio in second recovery step [f] SDS recovery step comprised by: refrigeration (9 °C) of the liquid stream resulting of first recovery step, precipitation and microfiltration –80% yield– [f]

Process	Step	Assumptions
	Recovery and purification	94% P(3HB) purity Water washing in two step maintaining up to 5 wt% solids content –water from second washing step is recycled into first washing step– Separation by centrifugation and spray drying to obtain P(3HB) powder [f]
L4	Biomass treatment	100,000 t of P(3HB) in enriched biomass are recovered for 330 days/year 150 kg·m^{-3} biomass concentration with 70% P(3HB) weight content [g] A heating step –up to 95 °C– [h]
	Extraction	A 95% P(3HB) recovery yield [h] Fusel alcohols extraction at 115 °C in a 75:1 solvent-biomass ratio in R-201 – 80% of the solvent stream is introduced as a liquid stream at 105 °C while the rest of the stream is added as vapor stream at 135 °C– [h] Solvent recovery in a distillation column [h]
	Recovery and purification	98% P(3HB-co-4HB) [gh] P(3HB-co-4HB) precipitation step by cooling (E-301) [h] Separation by microfiltration and spray drying to obtain P(3HB) powder [h]
References for assumptions: a-[48], b-[55], c-[50], d-[49], e-[51], f-[32,33], g-[52], h-[23]		

4 Environmental and economic evaluation of the selected processes

4.1 State-of-the-art of LCA and LCC studies on PHA downstream processing

Even when a lack of LCAs which assess only the PHA downstream processing has been detected, useful information can be collected from the selected LCAs studies on PHA production (Table 4). In terms of motivation studies, most research in the period 2000-2010 focused on evaluating the feasibility of PHA production comparing it to oil-based plastics, while in the present decade, studies were more focused on comparing PHA production from different feedstocks, evaluating different processes configuration such as PHA production using mixed cultures, assessing more impact categories or evaluating some specific phases of bioplastic value chain such as downstream processing [19,32,34].

Table 4 Chronological summary of characteristics of reviewed LCA studies. [1]NREU: non-renewable energy use. GWP: global warming potential, OFP: photochemical ozone formation, FAETP: ecotoxicity for aquatic fresh water, AP: acidification potential, EP: eutrophication potential, FOFP: photo-oxidant formation potential, ODP: ozone depletion potential, TTP: terrestrial toxicity potential, ATP: aquatic toxicity potential, HTPI: human toxicity potential by ingestion, HTPE: human toxicity potential by either inhalation or dermal exposure. [2]PHA production costs including raw materials, fermentation and downstream processing. [3]PHA downstream processing costs.

Source	Recovery method	Solvent	Functional Unit	System boundaries	Origin of primary data	Origin of secondary data	LCIA method and impact categories[1]	LCC
[9]	Mechanical disruption	-	1 kg PHA	Cradle-to-gate	Calculations and estimates based on US Department of Energy (DOE) and United States Department of Agriculture (USDA); literature	Literature	Midpoint level NREU	-
[27]	Solvent extraction	C4-C11 alcohol	1 kg PHA	Cradle-to-gate	Monsanto's data and assumptions; literature; Air Chief, EPA 1997; Ontario Ministry of Agriculture, Food and Rural Affairs, OMAFRA	Economic Research Service (ERS) of the United States Department of Agriculture-USDA; Ecobalance's DEAM™ database	Midpoint level GWP	-
[28]	Chemical digestion	SDS + NaClO	5000 tons PHA	Cradle-to-gate	USDA and DOE; literature; own calculations; computer simulation using SuperPro Designer v4.5	Own calculations; literature; computer simulation using SuperPro Designer v4.5	Midpoint level GWP, NREU	3.53-4.77 €·kg^{-1} PHA[2]
[29]	Chemical digestion	NaClO	1 kg COD in the feed	Gate-to-gate	All the environmental figures are taken from Australian based source (local utility companies, the Australian Greenhouse Office); literature	Literature	Midpoint level GWP	4.33 €·kg^{-1} PHA[2]
[30]	Chemical digestion	NaOH + NaClO	1 kg PHA	Gate-to-gate	Literature; computer simulation (data of a simulated ethanol plant that are based on laboratory and pilot-plant results)	Data from Agricultural Resource Management Survey (ARMS); Literature; average performance of chemical industry in the U.S.	Midpoint level GWP, NREU	-

Source	Recovery method	Solvent	Functional Unit	System boundaries	Origin of primary data	Origin of secondary data	LCIA method and impact categories[1]	LCC
[31]	Mechanical disruption + Chemical digestion / Solvent extraction	SDS + NaClO / C4-C11 alcohol	1 kg PHA	Cradle-to-gate	Literature	GaBi Professional database	End-point level Ecosystem quality, human health, supply of resources	-
[33]	Chemical digestion	SDS + NaClO	1 kg PHB	Gate-to-gate	Laboratory and pilot scale data and literature data integrated with process modelling in ASPEN Plus software	EcoInvent v2.2	Midpoint level GWP, NREU	1.18 €·kg^{-1} PHA[2]
[32]	Chemical digestion (2) / Solvent extraction	SDS + NaOH / SDS + NaClO / DCM	1 kg PHB	Cradle-to-gate	Laboratory and pilot scale data and literature data integrated with process modelling in ASPEN Plus software	EcoInvent v2.2	Midpoint level GWP, NREU	1.40-1.95 €·kg^{-1} PHA[3]
[19]	Chemical digestion (3) / Solvent extraction	NaOH / NaClO / DCM / H_2SO_4	1 kg PHB	Gate-to-gate	Laboratory and pilot scale data and literature data integrated with process modelling in ASPEN Plus software	Not defined	Midpoint level GWP	1.02-6.61 €·kg^{-1} PHA[3]
[34]	Solvent extraction	DMC	1 kg PHB	Gate-to-gate (only downstream processing)	Scale-up of laboratory data; estimates; literature	Gabi Professional Database; Ecoinvent Database	Midpoint level GWP, OFP, FAETP	-
[26]	Solvent extraction	Acetone	Not stated	Cradle-to-gate (polymer rich biomass)	Literature data	GaBi 6 Professional and Ecoinvent 3.1 databases	Midpoint level GWP, AP, EP freshwater, EP marine, EP terrestrial, POFP	-
[35]	Chemical digestion	SDS + NaOCl	1 kg PHA	Gate-to-gate	Laboratory data; literature	WAR database	Midpoint level GWP, AP, ODP, TTP, ATP, HTPI, HTPE	5.77-6.12 €·kg^{-1} PHA[2]
[10]	Chemical digestion	NaOCl	1 kg PHB	Cradle-to-gate	Literature	Literature; Gabi Professional database; Ecoinvent	Midpoint level GWP, NREU, EP, AP	-

Concerning to the recovery method, chemical digestion is the most evaluated option –9 out of the 13 papers–. Indeed, chemical digestion methods have an advantage over solvent extraction, especially when the PHA content in dry cell basis is high (>80%) as it happens in these studies [28,30,31]. Mechanical disruption, which has been evaluated by Gerngross [9], can be considered as competitive as chemical digestion. Solvent extraction, which was evaluated by few authors –6 out of the 13 papers–, is a highly energy intensive process due to solvents recovery step. Thus, a heat integration within a biorefinery may solve this dependency.

Regarding the selection of the functional unit (FU), a mass-based approach is the most common option, being 1 kg or 1 ton of PHA –or specifically PHB– the preferred option –i.e. 10 of the 13 papers–. When looking at the system boundaries, 7 studies are classified as cradle-to-gate –i.e. from raw material extraction to factory gate– and the remaining 6 as gate-to-gate –i.e. from one defined point along the life cycle to a second defined point further along the life cycle–.

Looking at the inventory phase it can be concluded that non-disclosure agreements prevent the publication of primary data by industry actors [56]. Therefore, most of primary data comes from own estimations, computer simulation and previous literature. Indeed, a dense correlation among the 13 papers has been observed and an important part of primary in LCI share the same origin –two earlier studies [9,28] are used as reference for the data collection of other six references [10,26,29–31,34]–, so there is a lack of primary sources and overdependency in LCI construction. Regarding secondary data, both Ecoinvent and GaBi databases are the most employed for data collection.

Concerning the impact assessment stage, most studies followed a midpoint approach. Global warming potential (GWP) is so far the most evaluated category –11 of 13, followed by non-renewable energy use (NREU) –6 of 13–. In fact, they were the main focus in early studies (1999-2008): the goal of these studies was to compare the biopolymers carbon footprint with their respective oil-based. These days, as some solvents are considered hazardous for the ecosystem and human health, more impact categories –such as

acidification, eutrophication and human toxicity potential– are being included in the assessment [10,26,34,35].

Half of the selected LCA studies include an LCC assessment. Most of the studies which assess the overall PHA production process pointed out fermentation as responsible of major operating costs –up to 75% of total–, which range from 1.18 to 6.12 €·kg^{-1} [28,29,33,35]. Substrates represented up to 50% of fermentation costs in pure culture fermentation [28,35], while energy was the major cost in mixed culture fermentation processes [29,33]. Concerning to LCCs which asses PHA downstream processing, methods based on chemical digestion, especially those which employ NaOH and surfactants, have lower costs –which range from 1.02 to 5.23 vs 1.95 to 6.61 €·kg^{-1}– than those based on solvent extraction[18,31]. Within PHA downstream processing by NCPM digestion and PHA extraction, chemicals and heat were the major contributors to total operating costs respectively –up to 80 and 70%– [19,32].

4.2 LCA and LCC of selected processes: Goal and scope

The goal of the current LCA is the identification of the main contributors, i.e. hotspots, both from an environmental and economic perspective within the four PHA recovery processes for both groups –high- and low-grade– as well as the cross comparison within each group to identify the best alternatives. As the function to be covered by the final product is not equivalent for all the cases, two functional units were defined: 1 kg of high-grade PHA powder and 1 kg of low-grade PHA powder.

The system boundaries are common, with a gate-to-gate approach covering from the PHA enriched biomass to the purified PHA. Being a comparative assessment, both the upstream substrate and PHA production phase and afterwards bioplastic compounding and shaping, use and end-of-life are assumed to be equivalent for all considered downstream processes [57]. Indeed, not all the downstream processes are interchangeable within each group –high and low-grade PHA–, i.e. some processes are based on an halophilic culture which can be submitted to osmotic shock or a solvent which is produced in the facility. Nevertheless, identified best practices can guide the proposal and adoption of improvement actions in

different configurations, as it is commonly done in process. As almost all alternatives are still at lab or pilot scale, some well-founded assumptions were taken for extrapolation to full scale production –see Supplementary information–. Data for the processes of the background system come from the Ecoinvent v3.3 database [58].

Regarding the geographical boundaries and the time horizon, all processes are placed in the EU, including background processes such as chemicals and energy production –whenever possible–, and long-term emissions are excluded. Capital goods were excluded for the environmental assessment, and therefore only operational inputs and outputs have been collected.

The analysis of the environmental impacts followed a midpoint approach, being global warming (GWP), terrestrial acidification, freshwater eutrophication, human toxicity, freshwater ecotoxicity and fossil depletion potential the selected impact categories, according to the latest reviewed LCAs on PHA downstream processing [10,26,34,35]. Excluding GWP, where the last version of the IPCC method –for a 100-year time horizon– was used, other impacts categories were assessed using the Hierarchist ReCiPe(H) v1.13. To do so, version 8.3 of SimaPro software –PRé Sustainability, NL– was selected.

Finally, to overcome the lack of specific background data, the following assumptions were formulated:

(1) Biomass waste in processes H1, H4, L1 and L4 was assumed as composting biowaste.

(2) Wastewater stream was assumed as urban wastewater.

(3) The production process for SDS, used in processes H2, L2 and L3, was assimilated to another chemical with similar function: alkylbenzene sulfonate.

(4) The production process for Lysol, used in process H2, was approximated to its main component: *o*-cresol.

(5) The production of isoamyl alcohol from ethanol biorefinery, as is the case in process L4, was assimilated as the standard isoamyl alcohol chemical production process, which a priori is considered the worst case scenario.

(6) Solvent losses in spray drying are burnt and complete combustion is assumed so fossil CO_2 is produced.

4.3 LCA and LCC of defined processes: Results for High-grade PHA

Inputs of raw material and energy as well as emissions to air, water and solid waste or wastewater per kg of obtained PHA for the four high-grade PHA processes are summarized in Table 5. The selected background processes from Ecoinvent v3.3 are also reported for transparency.

Table 5 LCI per kg of PHA for high grade processes: H1, H2, H3 and H4.

Item	H1	H2	H3	H4	Ecoinvent v3.3
Resources					
Water (m^3)	-	0.028	0.005	-	Water, cooling, unspecified natural origin, Europe without Switzerland
Acetone (kg)	0.321	-	-	-	Acetone, liquid {RER[1]}| oxidation of butane
Materials/fuels					
Ethanol (kg)	0.678	-	-	0.223	Ethanol, without water, in 99.7% solution state, from ethylene {RER}| ethylene hydration
SDS (kg)	-	0.049	-	-	Alkylbenzene sulfonate, linear, petrochemical {RER}| production
Lysol (kg)	-	-	0.429	-	*o*-cresol {RER}| Production
Sodium hydroxide (kg)	-	0.013	0.172	-	Sodium hydroxide, without water, in 50% solution state {RER}| chlor-alkali electrolysis, membrane cell
Hydrogen peroxide (kg)	-	0.280	-	-	Hydrogen peroxide, without water, in 50% solution state {RER}| hydrogen peroxide production, product in 50% solution state
Sulfuric acid (kg)	-	0.015	-	-	Sulfuric acid {RER}| production
Ethyl acetate (kg)	-	-		0.803	Ethyl acetate {RER}| production
Heptane (kg)	-	-		0.185	Heptane {RER}| molecular sieve separation of naphtha
Electricity/heat					
Electricity (kJ)	309	1,581	128	382	Electricity, medium voltage {Europe without Switzerland} market group for
Heat duty (kJ)	62,972	3,171	3,166	110,082	Heat, in chemical industry {RER} market for
Cooling duty (kJ)	48,217	-	-	93,989	Steam, in chemical industry {RER}| steam production in chemical industry
Emissions to air					
Carbon dioxide (kg)	2.484	-	-	0.595	Carbon dioxide, fossil
Waste and emissions to treatment					
Solid waste (kg)	0.577	-	-	1.113	Biowaste {CH[2]} treatment of, composting
Wastewater (m^3)	0.136	0.032	0.171	0.041	Wastewater, average {Europe without Switzerland}| treatment of wastewater, average

[1] RER = Europe. [2] CH = Switzerland

Results from the characterization stage are displayed on Table 6 and Fig. 4, and further individual information is reported on the Supplementary Information (sections 1.2.1, 2.2.1, 3.2.1 and so on).

Table 6 Comparative of characterization results of high-grade PHA downstream processes per kg of PHA. Shading colors from red to white indicate a better environmental performance of each process compared to the others in each impact category.

Impact category	Unit	H1	H2	H3	H4
IPCC GWP 100a	kg CO_2 eq	9.259	0.806	2.394	12.956
Terrestrial acidification	kg SO_2 eq	0.023	0.003	0.009	0.044
Freshwater eutrophication	kg P eq	$4.950 \cdot 10^{-4}$	$6.770 \cdot 10^{-5}$	$2.370 \cdot 10^{-4}$	$5.600 \cdot 10^{-4}$
Human toxicity	kg 1,4-DB eq	0.172	0.036	0.068	0.331
Freshwater ecotoxicity	kg 1,4-DB eq	$2.470 \cdot 10^{-3}$	$4.400 \cdot 10^{-4}$	$2.920 \cdot 10^{-3}$	$5.890 \cdot 10^{-3}$
Fossil depletion	kg oil eq	2.675	0.290	1.223	4.503

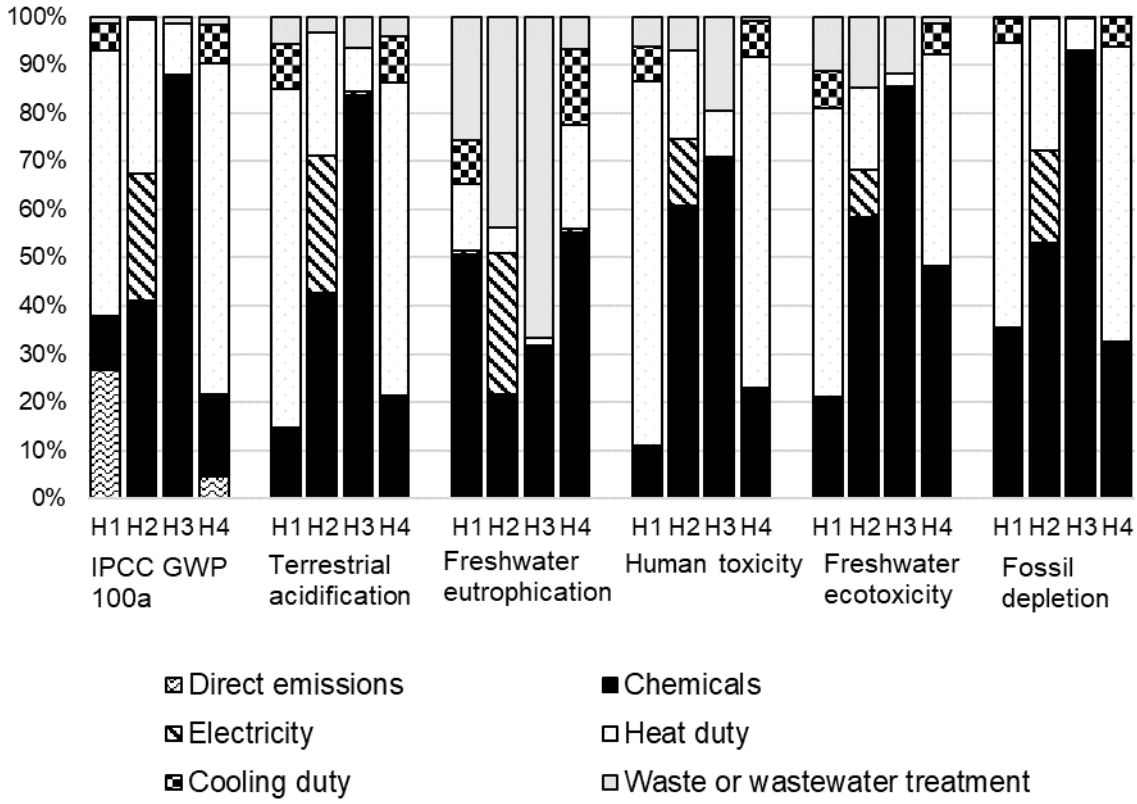


Fig. 4. Characterization of high-grade processes H1, H2, H3 and H4 respectively, and contributions of each LCI component.

Results from the life cycle costing are displayed on Fig. 5. Capital investment and operating costs calculation is collected in Supplementary material.

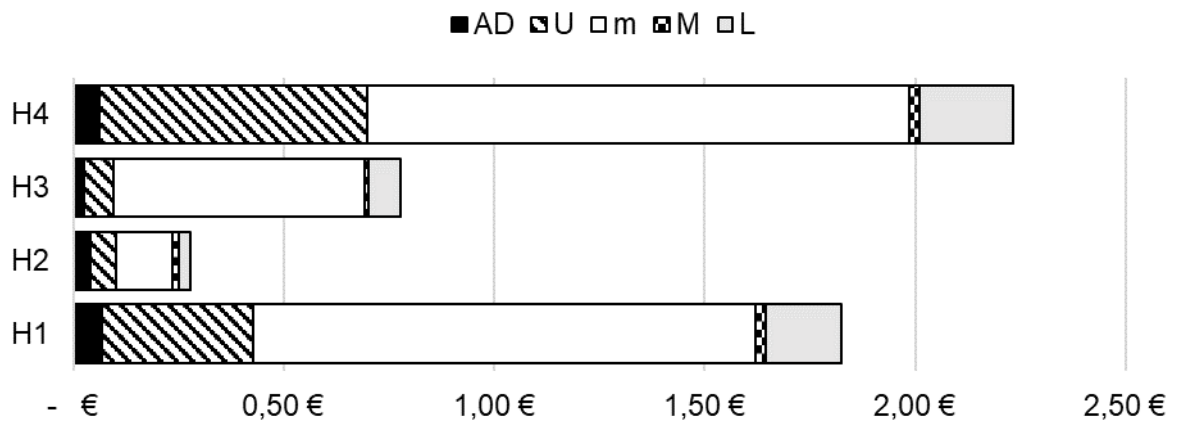


Fig. 5. Economic evaluation of high grade processes H1, H2, H3 and H4 respectively, per kg of PHA. AD, U, m, L and M are referred to annual depreciation, utilities, materials, labor and maintenance respectively.

Processes based on NCPM disruption show a better environmental and economic performance than those based on solvent extraction, while chemicals and heat production are the main contributors to environmental impacts and total operating costs in high grade processes. Going further down and looking at elements contribution (Fig. 4), most environmental impacts of both H1 and H4 processes, which are based on solvent extraction, are caused mainly by heat production, and to a lesser extent by solvent production. However, freshwater eutrophication is predominately caused by ethanol production. From an economic perspective, heat represents a 20% and a 30% of total operating costs in H1 and H4 respectively, while chemicals represent more than a 50% in both cases. Respecting H2 process, based on mechanical disruption, heat, electricity and peroxide hydrogen production have a similar weight in most impact categories. Economically, peroxide hydrogen represents almost the 50% of total operating costs. Ortocresol production is behind the major share of environmental impacts in most categories in process H3 except freshwater eutrophication, which is caused by waste stream treatment, similarly to process H2. Likewise, surfactant is responsible for 80% of the total operating costs.

4.4 LCA and LCC of defined processes: Results for low-grade PHA

Inputs of raw material and energy as well as emissions to air, water and solid waste or wastewater per kg of obtained high-grade PHA for the four processes are summarized in Table 7. The selected background processes from Ecoinvent v3.3 are also reported for transparency.

Table 7 LCI per kg of PHA for processes L1, L2, L3 and L4.

Items	L1	L2	L3	L4	Ecoinvent v3.3
Resources					
Water (m^3)	0.0002	0.314	0.039	-	Water, cooling, unspecified natural origin, Europe without Switzerland
Materials/fuels					
Acetone (kg)	0.487	-	-	-	Acetone, liquid {RER}| oxidation of butane
Isoamyl alcohol (kg)	-	-	-	0.962	3-methyl-1-butanol {RER}| hydroformylation of butane
SDS (kg)	-	0.252	0.303	-	Alkylbenzene sulfonate, linear, petrochemical {RER}| production
Sodium hypochlorite (kg)	-	-	0.414	-	Sodium hypochlorite, without water, in 15% solution state {RER}| sodium hypochlorite production, product in 15% solution state
Electricity/heat					
Electricity (kJ)	206	1,372	237	353	Electricity, medium voltage {Europe without Switzerland} market group for
Heat (kJ)	24,684	43,847	4,380	54,570	Heat, in chemical industry {RER} market for
Cooling duty (kJ)	17,470	-	1,659	48,189	Cooling energy {RER}| from natural gas, at cogeneration unit with absorption chiller 100kW
Emissions to air					
Carbon dioxide (kg)	1.109	-	-	1.578	Carbon dioxide, fossil
Waste and emissions to treatment					
Solid waste (kg)	1.172	-	-	0.710	Biowaste {CH} treatment of, composting
Wastewater (m^3)	0.006	0.316	0.065	0.009	Wastewater, average {Europe without Switzerland}| treatment of wastewater, average, capacity 1E9l/year

[1] RER = Europe. [2] CH = Switzerland

In current subsection, results of characterization of several alternative processes of PHA downstream processing for high value applications are displayed on Table 8 and Fig. 6.

Table 8 Comparative of characterization results of low-grade PHA downstream processes per kg of PHA. Shading colors from red to white indicate a better environmental performance of each process compared to the others in each impact category.

Impact category	Unit	L1	L2	L3	L4
IPCC GWP 100a	kg CO_2 eq	3.931	4.159	1.177	10.249
Terrestrial acidification	kg SO_2 eq	0.011	0.015	0.005	0.032
Freshwater eutrophication	kg P eq	$6.170 \cdot 10^{-5}$	$3.710 \cdot 10^{-4}$	$1.020 \cdot 10^{-4}$	$2.270 \cdot 10^{-4}$
Human toxicity	kg 1,4-DB eq	0.075	0.176	0.115	0.234
Freshwater ecotoxicity	kg 1,4-DB eq	$1.300 \cdot 10^{-3}$	$1.880 \cdot 10^{-3}$	$6.350 \cdot 10^{-4}$	$2.427 \cdot 10^{-3}$
Fossil depletion	kg oil eq	1.132	1.467	0.577	3.413

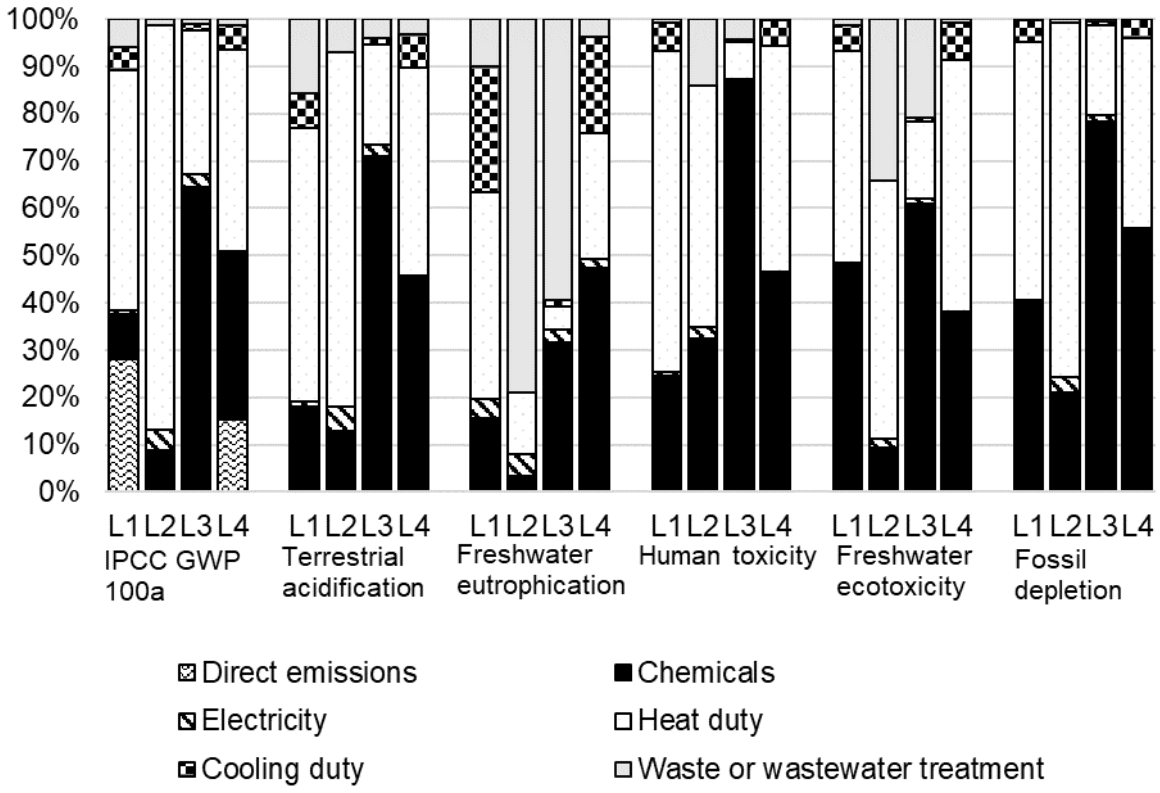


Fig. 6. Characterization of low-grade PHA processes L1, L2, L3 and L4 respectively, and contributions of each LCI component.

Results from the life cycle costing are displayed on Fig.7:

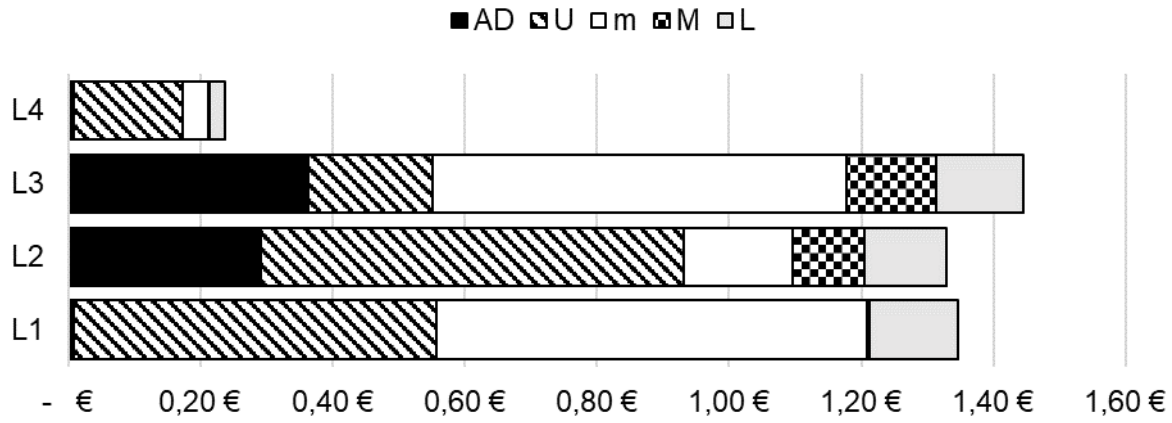


Fig. 7. Economic evaluation of low-grade processes L1, L2, L3 and L4 respectively, per kg of PHA. AD, U, m, L and M are referred to annual depreciation, utilities, materials, labor and maintenance respectively.

Process L3 and L4, based on NCPM digestion and PHA solvent extraction, show the best environmental and economic performance respectively within low-grade processes, while chemicals and heat production are the main contributors to environmental impacts and total operating costs. Regarding processes contribution to low-grade PHA processes (Fig. 6), most environmental impacts of L1, which is based on solvent extraction, are caused mainly by heat production, and to a lesser extent by acetone production. Similarly, total operating costs are caused by heat (49%) and acetone (44%). Respecting L2 process, based on osmotic shock and chemical digestion, heat production dominates almost all impact categories, except freshwater eutrophication, which is mainly caused by wastewater treatment. With regards to total operating costs, heat (36%) and annual depreciation (25%) –the plant scale determines higher AD costs– have a relevant weight. Sodium hypochlorite and SDS production are responsible by major of environmental impacts in most categories in process L3 except freshwater eutrophication, which is caused by waste stream treatment, similarly to process L2. Economically, sodium hypochlorite (36%) and annual depreciation (27%) –note that, similarly to L2, the size scale influences AD costs– represent the major costs. As regards process L4, isoamyl alcohol and heat production are the major responsible

of all environmental impacts in any category. In economic assessment, and due to fusel alcohols are a byproduct of ethanol biorefinery, heat cost represents 80% of total operating costs. Similarly, Lysol and heat production are the principal contributors to H3 environmental impacts.

4.5 LCA and LCC of defined processes: Interpretation of results

The fourth stage of the life cycle based assessments aims at deriving conclusions and assessing their robustness of conclusions. To do so, firstly a comparison among the processes assessed in this work and those from previous studies, secondly a sensitivity analysis to assess the robustness of the LCA results is carried out.

4.5.1. Comparison of results

According to results showed in Table 6 and Fig. 4, methods relying on solvent extraction (H1 and H4) require large amounts of energy for solvent recovery. The benefits of using a solvent instead of mechanical disruption or chemical digestion are outweighed in both all impact categories and costs and therefore, solvent extraction is only recommended for those cases where a higher quality is required [43]. Both economic and environmental performance of these processes can be optimized by employing more easily recoverable solvents. Mechanical disruption (H2) seems to be a priori the preferable method for downstream processing from an environmental (Fig. 8) and economic perspective –0.26 €·kg^{-1} PHA versus 0.77 €·kg^{-1} PHA–, mainly due to lower amounts of chemicals and surfactants were needed than in processes which employ only chemical digestion (H3).

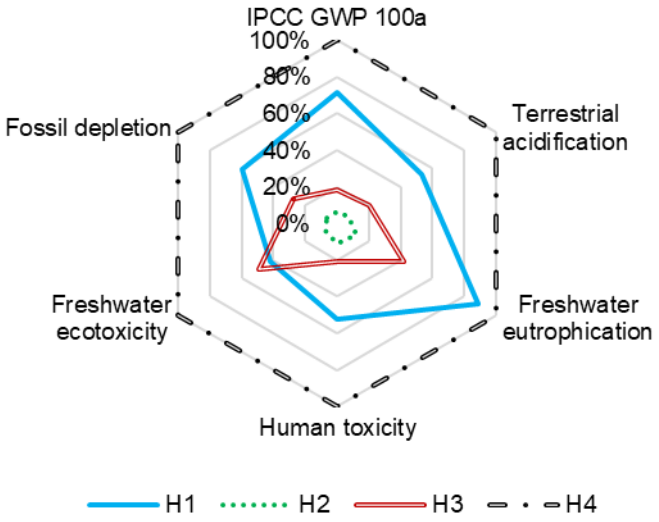


Fig. 8. Comparative of characterization of high-grade PHA processes.

With regards to results showed in Table 8 and Fig. 6, and similarly to high-grade PHA downstream processes, those based on extraction methods (L1 and L4) apparently show a worse environmental performance (Fig. 9) than those based on chemical digestion –environmental impacts are expected to be considerable lower in L4 since there was no data available to fusel alcohols production in sugar molasses industry and thus, isoamyl alcohol chemical based was employed in LCI construction–. Only in those cases (L4) where downstream processing is coupled with other own facility processes and/or products and byproducts –e.g. the utilization of residual heat or process byproducts employed as solvents–, solvent extraction methods are both environmental and economically feasible –0.24 €·kg^{-1} PHA–.The process L2 environmental impacts in categories related to freshwater seem to be exceed. Considering that a valorization of a canning wastewater is carried out in overall process, environmental impacts in freshwater ecotoxicity and freshwater eutrophication should be considerably lower when including canning wastewater treatment as avoided. Downstream processes based on SDS chemical digestion can be optimized by adding a SDS recovery unit. Indeed, it can be the key for process L3 relative low environmental impacts. Hence, the utilization of easy recoverable chemicals can lead to a better economic and environmental process performance.

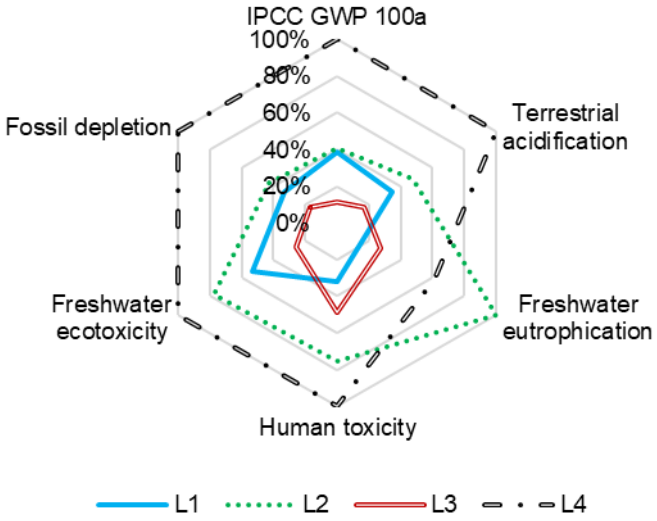


Fig. 9. Comparative of characterization of low-grade PHA processes.

Comparing current results from LCIA with those from literature [19,32,34] in GWP –other impact categories were not included as they do not have individualized data about PHA downstream processing environmental impacts–, it is verified that there is some concordance between them: a range from 0.81 to 4.16 kg CO_2 eq versus 2.6 to 6.27 kg CO_2 eq –studies from literature– for chemical digestion, and a range from 3.93 to 12.96 versus 4.94 to 28.71 kg CO_2 eq per kg of extracted PHA for solvent extraction. Likewise, from an economical perspective, there is some similarity in PHA downstream processing costs between those from literature (from 1.02 to 6.61 €·kg^{-1} PHA) and those from current study (from 0.24 to 2.23 €·kg^{-1} PHA).

4.5.2. Sensitivity analysis

Previous analysis (Sections 4.3 and 4.4) showed which factors were relevant on processes environmental impact. Based on that, the following scenarios were assessed (Fig. 10 and Fig. 11):

(1) For processes based on solvent extraction –H1, H4, L1 and L4–, the baseline scenarios, where heat was taken from European chemical industry market, are compared with two scenarios: oil (A) and natural gas (B) as heat sources.

(2) For process H2, the impact of the electricity production profile is assessed, considering that the electricity mix is not homogeneous within the geographical boundaries, so two different electricity mixes, characterized by the highest and lowest carbon footprint were chosen: the Polish electricity mix (A), dominated by coal production, and the Swedish mix (B), based on renewable energies.

(3) For processes based on chemical digestion by surfactant, base case is compared to another scenario where surfactant is or not recovered by crystallization –L2 and L3–. Additionally, in process H3 base case scenario is compared to another where Lysol, which cannot be recovered, is substituted by SDS with a recovery unit by crystallization.

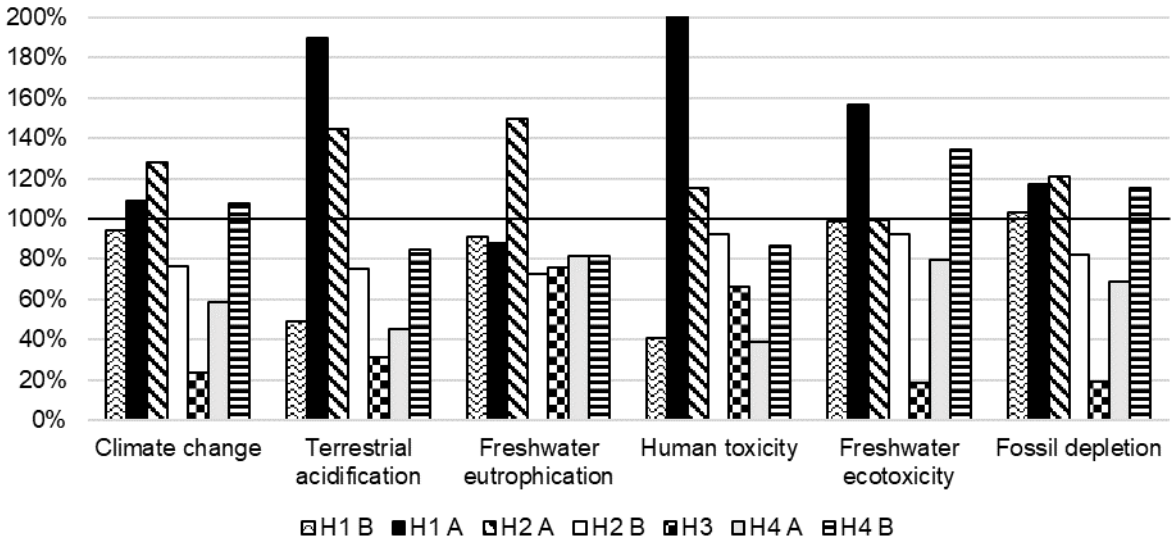


Fig. 10. Variation of LCIA on sensitivity analysis to high-grade PHA processes, being 100% each baseline process. H1 A, H4 A employ oil as source for heat production while option B employ natural gas. H2 A and H2 B employ Polish and Swedish electricity mix respectively. H3 uses SDS as surfactant and includes a crystallization unit.

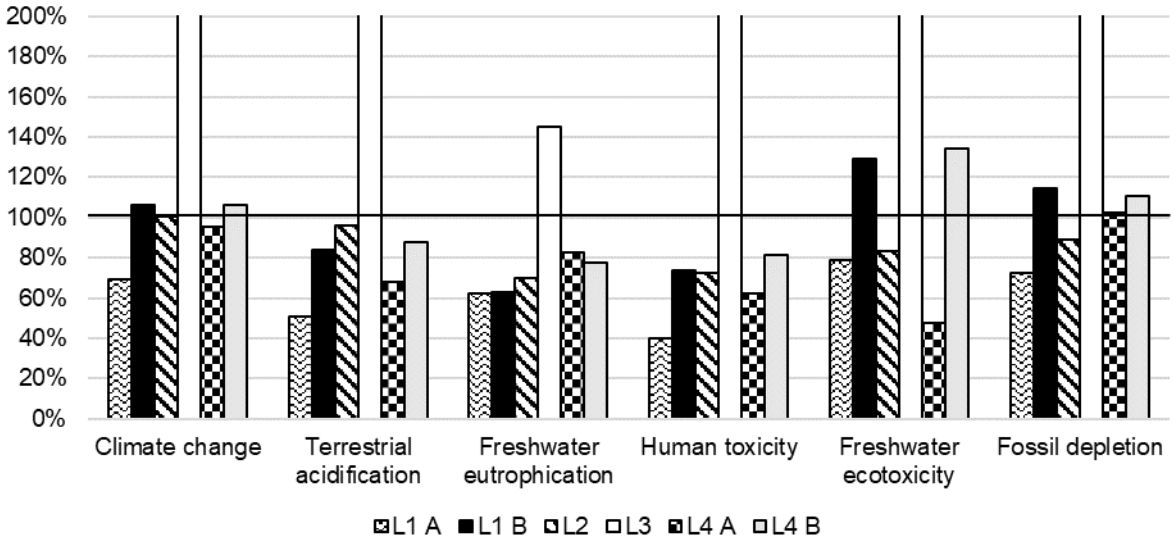


Fig. 11. Variation of LCIA on sensitivity analysis to low-grade PHA processes, being 100% each baseline process. L1 A and L4 A employ oil as source for heat production while option B employ natural gas. L2 add a crystallization unit for SDS recovery while L3 do not consider a crystallization unit.

Fig. 10 and Fig. 11 quantify how would affect these changes in environmental performance compared to base cases. First impression is that there are considerable variations in environmental performance compared to the base case. While the adoption of natural gas as heat source has negligible effect in almost all impact categories –due to heat average production in Europe has natural gas as major heat source–, the utilization of oil can aggravate environmental impacts in some categories up to 50%. The chosen electricity mix in H2 can aggravate environmental impacts in a 30% by mean considering the Polish mix, while can reduce them a 30% by mean if Swedish mix, based on renewable sources, was adopted. Changing SDS instead Lysol as surfactant and including a recrystallization unit just reaches a 4% of the total operating costs but can reduce environmental impacts by 50% due to the reduction of utilized surfactant. Introducing an SDS crystallization step in L2 has negligible effects in environmental impacts due to the small quantity of SDS employed. Contrarily, by eliminating the SDS crystallization step in L3, environmental impacts could

reach up to 125% by mean, due to a high quantity of SDS is employed in L3 process. Therefore, easily recoverable chemicals are crucial for a better environmental performance. As some processes are based on lab and pilot scale –i.e. TRL < 6–, a sensitivity analysis on the reported extraction yield was carried out showing a significant influence on the results (Table 9) both on process environmental impacts and costs (more than 10% in several indicators for L3).

Table 9. Results of sensitivity analysis to extraction yield in process environmental impacts and costs. A 5% increase and decrease of yield is considered.

	GWP		TA		FE		HT		FEX		FD		Cost	
Yield	-5%	5%	-5%	5%	-5%	5%	-5%	5%	-5%	5%	-5%	5%	-5%	5%
H3	3%	-4%	3%	-5%	1%	-5%	2%	-4%	3%	-5%	3%	-5%	3%	-5%
H4	7%	-2%	6%	-5%	6%	-5%	6%	-5%	7%	-6%	6%	-5%	5%	-5%
L1	3%	-3%	5%	-4%	4%	-4%	4%	-3%	2%	-2%	3%	-3%	3%	-3%
L2	5%	-2%	5%	-2%	5%	-2%	5%	-2%	5%	-2%	5%	-2%	4%	-2%
L3	11%	-10%	12%	-11%	8%	-7%	11%	-10%	12%	-11%	7%	-6%	15%	-14%

5. Discussion

While LCI and LCIA pointed out intensive energy use and high chemicals consumption in processes based on solvent extraction and NCPM digestion as hotspots, sensitivity analysis supports these statements and remarked the importance of reducing energy use and employing greener energy sources and introducing chemicals recovery units where it is possible. Additionally, considering the whole PHA production framework –i.e. fermentation or facility characteristics which can establish a synergy with downstream process– and technical assessment –i.e. possibility of heat integration or introducing chemical recovery unit– improvements were proposed for some processes, as summarized in Table 10. See Supplementary Information for process specifications and heat integration procedure.

Table 10. Summary of improvement actions proposed for PHA downstream processes and their effects in environmental and economic performance.

Process	Hotspots	Framework	Improvement actions	Environmental impacts reduction						Operating costs reduction
				GWP	TA	FE	HT	FEX	FD	
L2	Heat duty	Canning industry	Heat integration	83%	73%	13%	50 %	53%	73 %	32%
L4	Heat duty	Molasses biorefinery		12%	12%	11%	13 %	15%	11 %	35%

Despite being energy intensive processes, both H1 and H4 processes do not allow heat integration from residual heat sources, due to all equipment –spray dryers and distillation column– with heat duty requires low or medium pressure steam. As peroxide hydrogen, heat duty and electricity are the major contributors to H2 environmental impacts, and heat duty is consumed totally in the spray dryers as low pressure steam, the principal action to reduce environmental impact would be employing electricity from renewable source. This could reduce environmental impacts up to 20% by mean, as sensitivity analysis showed. Similarly to H2 process, Lysol and heat production are the principal contributors to H3 process environmental impacts. Thus, a heat source based on natural gas could reduce environmental impacts.

Low-grade PHA downstream processes are more prone to allow heat integration, not within the PHA production process itself, but given the most likely setups for these processes, i.e. integrated in larger facilities. Indeed, in L2 process, framed within a canning industry facility where larger amounts of residual vapor from food processing may be available [59], 4 $t \cdot h^{-1}$ of vapor at 115 °C would be enough to cover the E-101 and E-102 heat duties. Heat integration within downstream process –summarized in Supplementary information– is proposed for L4 process, where streams resulting of E-101, E-201, E-301 and E-302 can be partially integrated reducing a 25% heat duty and a 35% in operating costs.

6. Conclusions

In this work, a detailed assessment of the economic and environmental performance of PHA downstream processing has allowed identifying hotspots and provided a deeper understanding of the process, leading to the proposal of operation and design guidelines. Eight processes for both high-grade and low-grade PHA recovery were selected and designed based on articles and patents. Also, a review on LCAs of PHA downstream and production processes was realized, pointing out the key LCA-decisions in order to define the goal and scope of the economic and environmental assessment. Sensitivity analysis validates the results robustness and allowed to identify some actions that could be taken to enhance PHA downstream processes performance.

As conclusion, valuable insights are extracted from the LCIA and sensitivity analysis:

- It is verified that PHA downstream processes are highly energy intensive, especially those based on solvent extraction. Real cases of biorefineries which could provide part of the heat and solvent requirements were identified; the integration of the PHA recovery in these larger processes would reduce the environmental impacts up to 50% pointing out at the opportunities for industrial symbiosis. The utilization of so-called green solvents such as ethyl acetate, fusel alcohols, ethyl lactate or dimethyl carbonate can improve the processes environmental performance. However, a higher level of technical maturity in PHA extraction is needed to consider these alternative solvents in full scale processes.
- The use of surfactants and sodium hydroxide is a good environmental alternative compared to organic solvent extractions; still, surfactant recovery by crystallization can significantly reduce the environmental performance of the process.
- Mechanical disruption seems to be the most promising method for high-grade PHA from an environmentally point of view, even in cases where the electricity mix is carbon-intensive. Surfactant treatment can be considered as the most promising method for low-grade PHA.

This work contributes to increase the available knowledge around PHA, supporting its potential as an attractive material for a sustainable bioeconomy and framing current results from PHA downstream processing in overall PHA value chain.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] S. Philip, T. Keshavarz, I. Roy, Polyhydroxyalkanoates: Biodegradable polymers with a range of applications, Journal of Chemical Technology and Biotechnology. 82 (2007) 233–247. https://doi.org/10.1002/jctb.1667.

[2] K. Dietrich, M.J. Dumont, L.F. del Rio, V. Orsat, Producing PHAs in the bioeconomy — Towards a sustainable bioplastic, Sustainable Production and Consumption. 9 (2017) 58–70. https://doi.org/10.1016/j.spc.2016.09.001.

[3] J.M.B. Domingos, S. Puccio, G.A. Martinez, N. Amaral, M.A.M. Reis, S. Bandini, F. Fava, L. Bertin, Cheese whey integrated valorisation: Production, concentration and exploitation of carboxylic acids for the production of polyhydroxyalkanoates by a fed-batch culture, Chemical Engineering Journal. 336 (2018) 47–53. https://doi.org/10.1016/j.cej.2017.11.024.

[4] S. Campanari, F. Augelletti, S. Rossetti, F. Sciubba, M. Villano, M. Majone, Enhancing a multi-stage process for olive oil mill wastewater valorization towards polyhydroxyalkanoates and biogas production, Chemical Engineering Journal. 317 (2017) 280–289. https://doi.org/10.1016/j.cej.2017.02.094.

[5] J.C. López, E. Arnáiz, L. Merchán, R. Lebrero, R. Muñoz, Biogas-based polyhydroxyalkanoates production by Methylocystis hirsuta: A step further in anaerobic digestion biorefineries, Chemical Engineering Journal. 333 (2018) 529–536. https://doi.org/10.1016/j.cej.2017.09.185.

[6] E. Bugnicourt, P. Cinelli, A. Lazzeri, V. Alvarez, Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging, Express Polymer Letters. 8 (2014) 791–808. https://doi.org/10.3144/expresspolymlett.2014.82.

[7] PlasticsEurope, Plastics 2030: PlasticsEurope's Voluntary Commitment to increasing circularity and resource efficiency, (2018). https://www.plasticseurope.org/en/focus-areas/our-commitment (accessed March 16, 2020).

[8] International Standard Organization, ISO 14040:2006 - Environmental management — Life cycle assessment — Principles and framework, (2006). https://www.iso.org/standard/37456.html.

[9] T.U. Gerngross, Can biotechnology move us toward a sustainable society?, Nature Biotechnology. 17 (1999) 541–544. https://doi.org/10.1038/9843.

[10] I.K. Kookos, A. Koutinas, A. Vlysidis, Life cycle assessment of bioprocessing schemes for poly(3-hydroxybutyrate) production using soybean oil and sucrose as carbon sources, Resources, Conservation and Recycling. 141 (2019) 317–328. https://doi.org/10.1016/j.resconrec.2018.10.025.

[11] S. Heimersson, F. Morgan-Sagastume, G.M. Peters, A. Werker, M. Svanström, Methodological issues in life cycle assessment of mixed-culture polyhydroxyalkanoate production utilising waste as feedstock, New Biotechnology. 31 (2014) 383–393. https://doi.org/10.1016/j.nbt.2013.09.003.

[12] A. Gholami, M. Mohkam, S. Rasoul-Amini, Y. Ghasemi, Industrial production of polyhydroxyalkanoates by bacteria: Opportunities and challenges, Minerva Biotecnologica. 28 (2016) 59–74.

[13] C. Pérez-Rivero, J.P. López-Gómez, I. Roy, A sustainable approach for the downstream processing of bacterial polyhydroxyalkanoates: State-of-the-art and latest developments, Biochemical Engineering Journal. 150 (2019). https://doi.org/10.1016/j.bej.2019.107283.

[14] M. Narodoslawsky, K. Shazad, R. Kollmann, H. Schnitzer, LCA of PHA production - Identifying the ecological potential of bio-plastic, Chemical and Biochemical Engineering Quarterly. 29 (2015) 299–305. https://doi.org/10.15255/CABEQ.2014.2262.

[15] M. Koller, H. Niebelschütz, G. Braunegg, Strategies for recovery and purification of poly[(R)-3-hydroxyalkanoates] (PHA) biopolyesters from surrounding biomass, Engineering in Life Sciences. 13 (2013) 549–562. https://doi.org/10.1002/elsc.201300021.

[16] G. Mannina, D. Presti, G. Montiel-Jarillo, J. Carrera, M.E. Suárez-Ojeda, Recovery of polyhydroxyalkanoates (PHAs) from wastewater: A review, Bioresource Technology. 297 (2020). https://doi.org/10.1016/j.biortech.2019.122478.

[17] N. Jacquel, C.W. Lo, Y.H. Wei, H.S. Wu, S.S. Wang, Isolation and purification of bacterial poly(3-hydroxyalkanoates), Biochemical Engineering Journal. 39 (2008) 15–27. https://doi.org/10.1016/j.bej.2007.11.029.

[18] S. Kunasundari, K. Sudesh, Isolation and recovery of microbial polyhydroxyalkanoates, EXPRESS Polymer Letters. 5 (2011) 15. https://doi.org/10.3144/expresspolymlett.2011.60.

[19] M. López-Abelairas, M. García-Torreiro, T. Lú-Chau, J.M. Lema, A. Steinbüchel, Comparison of several methods for the separation of poly(3-hydroxybutyrate) from Cupriavidus necator H16 cultures, Biochemical Engineering Journal. 93 (2015) 250–259. https://doi.org/10.1016/j.bej.2014.10.018.

[20] G. Mannina, D. Presti, G. Montiel-Jarillo, M.E. Suárez-Ojeda, Bioplastic recovery from wastewater: A new protocol for polyhydroxyalkanoates (PHA) extraction from mixed microbial cultures, Bioresource Technology. 282 (2019) 361–369. https://doi.org/10.1016/j.biortech.2019.03.037.

[21] G.Q. Chen, G. Zhang, S.J. Park, S.Y. Lee, Industrial scale production of poly(3-hydroxybutyrate-co-3-hydroxyhexanoate), Applied Microbiology and Biotechnology. 57 (2001) 50–55. https://doi.org/10.1007/s002530100755.

[22] X. Jiang, J.A. Ramsay, B.A. Ramsay, Acetone extraction of mcl-PHA from Pseudomonas putida KT2440, Journal of Microbiological Methods. 67 (2006) 212–219. https://doi.org/10.1016/j.mimet.2006.03.015.

[23] P.E. Mantelatto, A. Minto Duzzi, N.A. Sertori Durao, C. Rocchiccioli, S. Kesserlingh, Process for recovering polyhydroxyalkanoates (“PHAs”) from cellular biomass, US 9045595 B2, 2015.

[24] D.P. Martin, K. Guo, S.F. Williams, Compositions and devices of poly-4-hydroxybutyrate, US 2014/0275325 A1, 2014. https://patentimages.storage.googleapis.com/16/ee/1a/770a64a4de5695/US20140275325A1.pdf.

[25] S. Begotti, Processes for recovering and purifying polyhydroxyalkanoates from cell cultures, US 9,683,076 B2, 2017. https://patentimages.storage.googleapis.com/6c/bb/74/62c4be858d81c0/US9683076.pdf.

[26] F. Morgan-Sagastume, S. Heimersson, G. Laera, A. Werker, M. Svanström, Techno-environmental assessment of integrating polyhydroxyalkanoate (PHA) production with services of municipal wastewater treatment, Journal of Cleaner Production. 137 (2016) 1368–1381. https://doi.org/10.1016/j.jclepro.2016.08.008.

[27] D. Kurdikar, L. Fournet, S.C. Slater, M. Paster, K.J. Gruys, T.U. Gerngross, R. Coulon, Greenhouse gas profile of a plastic material derived from a genetically

modified plant, Journal of Industrial Ecology. 4 (2000) 107–122. https://doi.org/10.1162/108819800300106410.

[28] M. Akiyama, T. Tsuge, Y. Doi, Environmental life cycle comparison of polyhydroxyalkanoates produced from renewable carbon resources by bacterial fermentation, Polymer Degradation and Stability. 80 (2003) 183–194. https://doi.org/10.1016/S0141-3910(02)00400-7.

[29] N. Gurieff, P. Lant, Comparative life cycle assessment and financial analysis of mixed culture polyhydroxyalkanoate production, Bioresource Technology. 98 (2007) 3393–3403. https://doi.org/10.1016/j.biortech.2006.10.046.

[30] J. Yu, L.X.L. Chen, The greenhouse gas emissions and fossil energy requirement of bioplastics from cradle to gate of a biomass refinery, Environmental Science and Technology. 42 (2008) 6961–6966. https://doi.org/10.1021/es7032235.

[31] Z.W. Zhong, B. Song, C.X. Huang, Environmental impacts of three polyhydroxyalkanoate (pha) manufacturing processes, Materials and Manufacturing Processes. 24 (2009) 519–523. https://doi.org/10.1080/10426910902740120.

[32] C. Fernández-Dacosta, J.A. Posada, R. Kleerebezem, M.C. Cuellar, A. Ramirez, Microbial community-based polyhydroxyalkanoates (PHAs) production from wastewater: Techno-economic analysis and ex-ante environmental assessment, Bioresource Technology. 185 (2015) 368–377. https://doi.org/10.1016/j.biortech.2015.03.025.

[33] C. Fernández Dacosta, J.A. Posada, A. Ramírez, Large Scale Production of Polyhydroxyalkanoates (PHAs) from Wastewater: A Study of TechnoEconomics, Energy Use and Greenhouse Gas Emissions, International Scholarly and Scientific Research & Innovation. 9 (2015) 6.

[34] S. Righi, F. Baioli, C. Samorì, P. Galletti, E. Tagliavini, C. Stramigioli, A. Tugnoli, P. Fantke, A life cycle assessment of poly-hydroxybutyrate extraction from microbial biomass using dimethyl carbonate, Journal of Cleaner Production. 168 (2017) 692–707. https://doi.org/10.1016/j.jclepro.2017.08.227.

[35] Y.K. Leong, P.L. Show, J.C.W. Lan, H.S. Loh, H.L. Lam, T.C. Ling, Economic and environmental analysis of PHAs production process, Clean Technologies and Environmental Policy. 19 (2017) 1941–1953. https://doi.org/10.1007/s10098-017-1377-2.

[36] E.C. Carlson, Don't gamble with physical properties for simulations, Chemical Engineering Progress. 92 (1996) 35–46. https://webpages.sdsmt.edu/~ddixon/cep_aspenmodel.pdf.

[37] J.R. Elliott, C.T. Lira, Introductory chemical engineering thermodynamics, Second Edi, Upper Saddle River, NJ: Prentice Hall PTR., 1999.

[38] M.Z. Hauschild, R.K. Rosenbaum, S.I. Olsen, Life Cycle Assessment: Theory and Practice, Springer International Publishing, 2017. https://doi.org/10.1007/978-3-319-56475-3.

[39] W.D. Seider, D.R. Lewin, J.D. Seader, S. Widagdo, R. Gani, K.M. Ng, Product and process design principles : synthesis, analysis, and evaluation, Fourth edi, 2016.

[40] R.K. Henderson, C. Jiménez-González, D.J.C. Constable, S.R. Alston, G.G.A. Inglis, G. Fisher, J. Sherwood, S.P. Binks, A.D. Curzons, Green Chemistry Expanding GSK's solvent selection guide-embedding sustainability into solvent selection starting at medicinal chemistry †, (2011). https://doi.org/10.1039/c0gc00918k.

[41] D. Prat, O. Pardigon, H.-W. Flemming, S. Letestu, ‖ Véronique, V. Ducandas, P. Isnard, E. Guntrum, T. Senac, ⊥ Stéphane, S. Ruisseau, P. Cruciani, P. Hosek, Sanofi's Solvent Selection Guide: A Step Toward More Sustainable Processes, (2013). https://doi.org/10.1021/op4002565.

[42] C. Samorì, F. Abbondanzi, P. Galletti, L. Giorgini, L. Mazzocchetti, C. Torri, E. Tagliavini, Extraction of polyhydroxyalkanoates from mixed microbial cultures: Impact on polymer quality and recovery, Bioresource Technology. 189 (2015) 195–202. https://doi.org/10.1016/j.biortech.2015.03.062.

[43] F. Valentino, F. Morgan-Sagastume, S. Campanari, M. Villano, A. Werker, M. Majone, Carbon recovery from wastewater through bioconversion into biodegradable

polymers, New Biotechnology. 37 (2017) 9–23. https://doi.org/10.1016/j.nbt.2016.05.007.

[44] European Commission, Commission Regulation (EU) No 10/2011 of 14 January 2011 on plastic materials and articles intended to come into contact with food, (2011). https://eur-lex.europa.eu/eli/reg/2011/10/2019-08-29 (accessed July 6, 2020).

[45] X.Y. Zhou, X.X. Yuan, Z.Y. Shi, D.C. Meng, W.J. Jiang, L.P. Wu, J.C. Chen, G.Q. Chen, Hyperproduction of poly(4-hydroxybutyrate) from glucose by recombinant Escherichia coli, Microbial Cell Factories. 11 (2012) 54. https://doi.org/10.1186/1475-2859-11-54.

[46] Bio-on | Turn Off Pollution, (n.d.). http://www.bio-on.it/index.php (accessed March 3, 2020).

[47] N.M.N.M. Irdahayu, K. Shantini, K.-H. Huong, S. Vigneswari, N.A. Aziz, Mohd.N.Mohd. Azizan, A.-A.A. Amirul, En route to economical eco-friendly solvent system in enhancing sustainable recovery of poly(3-hydroxybutyrate- *co* -4-hydroxybutyrate) copolymer, Engineering in Life Sciences. 17 (2017) 1050–1059. https://doi.org/10.1002/elsc.201600217.

[48] I. Levett, G. Birkett, N. Davies, A. Bell, A. Langford, B. Laycock, P. Lant, S. Pratt, Techno-economic assessment of poly-3-hydroxybutyrate (PHB) production from methane - The case for thermophilic bioprocessing, Journal of Environmental Chemical Engineering. 4 (2016) 3724–3733. https://doi.org/10.1016/j.jece.2016.07.033.

[49] D.-N. Rathi, H.G. Amir, R.M.M. Abed, A. Kosugi, T. Arai, O. Sulaiman, R. Hashim, K. Sudesh, Polyhydroxyalkanoate biosynthesis and simplified polymer recovery by a novel moderately halophilic bacterium isolated from hypersaline microbial mats, Journal of Applied Microbiology. 114 (2013) 384–395. https://doi.org/10.1111/jam.12083.

[50] D. Van-Thuoc, J. Quillaguamán, G. Mamo, B. Mattiasson, Utilization of agricultural residues for poly(3-hydroxybutyrate) production by Halomonas boliviensis LC1,

Journal of Applied Microbiology. 0 (2007) 071003000434003-??? https://doi.org/10.1111/j.1365-2672.2007.03553.x.

[51] V.F.R. Gomis Antonio Marcilla, Escalona Antonio Munoz, Procedure for the extraction of polyhydroxyalkanoates from halophilic bacteria which contain them, EP 0 622 462 A1, 2001. http://www.google.com.ar/patents/EP0622462B1?cl=en (accessed March 3, 2020).

[52] R. v. Nonato, P.E. Mantelatto, C.E.V. Rossell, Integrated production of biodegradable plastic, sugar and ethanol, Applied Microbiology and Biotechnology. 57 (2001) 1–5. https://doi.org/10.1007/s002530100732.

[53] Dave Humbird, Expanded Technology Readiness Level (TRL) Definitions for the Bioeconomy : Biofuels Digest, 2018. https://www.biofuelsdigest.com/bdigest/2018/10/01/expanded-technology-readiness-level-trl-definitions-for-the-bioeconomy/.

[54] E. Medicines Agency, ICH guideline Q3C (R6) on impurities: guideline for residual solvents Step 5, n.d. www.ema.europa.eu/contactsTelephone+31 (accessed July 7, 2020).

[55] X. Jiang, J.A. Ramsay, B.A. Ramsay, Acetone extraction of mcl-PHA from Pseudomonas putida KT2440, Journal of Microbiological Methods. 67 (2006) 212–219. https://doi.org/10.1016/j.mimet.2006.03.015.

[56] European Commission, A circular economy for plastics – Insights from research and innovation to inform policy and funding decisions, 2019. https://op.europa.eu/en/publication-detail/-/publication/33251cf9-3b0b-11e9-8d04-01aa75ed71a1/language-en/format-PDF (accessed June 10, 2020).

[57] A. Hospido, J. Davis, J. Berlin, U. Sonesson, A review of methodological issues affecting LCA of novel food products, International Journal of Life Cycle Assessment. 15 (2010) 44–52. https://doi.org/10.1007/s11367-009-0130-4.

[58] [Dataset] ecoinvent, ecoinvent 3.3, (2016). https://www.ecoinvent.org/database/ecoinvent-33/ecoinvent-33.html.

[59] M.C. Barros, A. Magán, S. Valiño, P.M. Bello, J.J. Casares, J.M. Blanco, Identification of best available techniques in the seafood industry: a case study, Journal of Cleaner Production. 17 (2009) 391–399. https://doi.org/10.1016/j.jclepro.2008.08.012.