INTERNATIONAL DOCTORAL
SCHOOL OF THE USC

Alba
Roibás Rozas

PhD Thesis

Challenges implementing circular economy principles in the management of industrial saline effluents: from technology development to holistic assessment.

Santiago de Compostela, 2022

Doctoral Programme in Chemical and Environmental Engineering

TESE DE DOUTORAMENTO

CHALLENGES IMPLEMENTING CIRCULAR ECONOMY PRINCIPLES IN THE MANAGEMENT OF INDUSTRIAL SALINE EFFLUENTS: FROM TECHNOLOGY DEVELOPMENT TO HOLISTIC ASSESSMENT

Alba Roibás Rozas

ESCOLA DE DOUTORAMENTO INTERNACIONAL DA UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

PROGRAMA DE DOUTORAMENTO EN ENXEÑARÍA QUÍMICA E AMBIENTAL

SANTIAGO DE COMPOSTELA 2022

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

D./Dna. **Alba Roibás Rozas**

Título da tese: **Challenges implementing circular economy principles in the management of industrial saline effluents: from technology development to holistic assessment.**

Presento a miña tese, seguindo o procedemento axeitado ao Regulamento, e declaro que:

1) A tese abarca os resultados da elaboración do meu traballo.
2) De ser o caso, na tese faise referencia ás colaboracións que tivo este traballo.
3) Confirmo que a tese non incorre en ningún tipo de plaxio doutros autores nin de traballos presentados por min para a obtención doutros títulos.
4) A tese é a versión definitiva presentada para a súa defensa e coincide a versión impresa coa presentada en formato electrónico

E comprométome a presentar o Compromiso Documental de Supervisión no caso de que o orixinal non estea na Escola.

En Santiago de Compostela**,** 26 de Abril de 2022**.**

Asinado: **Alba Roibás Rozas**

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

AUTORIZACIÓN DAS DIRECTORAS DE TESE

Challenges implementing circular economy principles in the management of industrial saline effluents: from technology development to holistic assessment

D./Dna. Anuska Mosquera-Corral

D./Dna. Almudena Hospido Quintana

INFORMAN:

Que a presente tese, correspóndese co traballo realizado por D/Dna. Alba Roibás Rozas, baixo a miña dirección/titorización, e autorizo a súa presentación, considerando que reúne os requisitos esixidos no Regulamento de Estudos de Doutoramento da USC, e que como director desta non incorre nas causas de abstención establecidas na Lei 40/2015.

De acordo co indicado no Regulamento de Estudos de Doutoramento, declara tamén que a presente tese de doutoramento é idónea para ser defendida en base á modalidade de Monográfica con reproducción de publicaciones, nos que a participación da doutoranda foi decisiva para a súa elaboración e as publicacións se axustan ao Plan de Investigación.

En Santiago de Compostela, 26 de Abril de 2022

Anuska Mosquera Corral **Almudena Hospido Quintana**

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

I'll tell you what freedom is to me. No fear.
Priestess Nina Simone

A mis padres, a quienes se lo debo todo.
A mi mejor amiga, que es mi madre.
A mi padre, la suerte de mi vida.

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

ABBREVIATIONS
SUMMARY ..... **I**
RESUMO ..... **IX**

1. INTRODUCTION **1**

1.1 CHALLENGES IN THE CONTEXT OF A GLOBAL CRISIS 2
1.2 ALTERNATIVE BIO-BASED PLASTICS AND BIOMATERIALS 4
1.2.1. Technological development in PHA production and mixed microbial cultures 5
1.2.2. Environmental validation of PHA 9
1.2.3. Looking for integral validation: is there enough information about PHA production costs? 11
1.3 CIRCULAR ECONOMY AT A REGIONAL LEVEL: THE FISH CANNING INDUSTRY IN GALICIA 12
1.4 THESIS ESTRUCTURE AND OBJECTIVES. 14

2. METHODOLOGY **1**

2.1. ANALITICAL METHODS 2
2.1.1. Characterization of the liquid phase 2
2.1.1.1 Chemical Oxygen Demand (COD) 2
2.1.1.2 Total Organic Carbon (TOC), Total Carbon (TC), Inorganic Carbon (IC), and Total Nitrogen (TN) 3
2.1.1.3 Volatile Fatty Acids (VFAs) 4
2.1.1.4 Ammonium 4
2.1.1.5 Proteins 5
2.1.1.6 Carbohydrates 5
2.1.1.7 Inorganic Ions 6
2.1.2. Characterization of the solid phase 6
2.1.2.1. Solids 6
2.1.2.2. Polyhydroxyalkanoates (PHA) 7
2.1.3. Other Parameters 8
2.1.3.1 pH 8
2.1.3.2. Alkalinity 8
2.1.3.3. Dissolved Oxygen and Temperature 9
2.2. ENVIRONMENTAL AND ECONOMIC EVALUATION METHODOLOGIES 9
2.2.1. Life Cycle Assessment (LCA) 9
2.2.2. Techno-economic Analysis (TEA) and Life Cycle Costing (LCC) 13

3. STRATEGIES FOR THE VALORISATION OF A PROTEIN-RICH SALINE WASTE STREAM FROM THE FISH CANNING INDUSTRY INTO PHA 15

3.1 INTRODUCTION 16
3.2 MATERIAL AND METHODS 16
3.2.1 Experimental set-up 16
3.2.1.1 CSTR for acidification 17
3.2.1.2. Enrichment SBR 17
3.2.1.3. FBR 19
3.2.2. Analytical methods 19
3.2.3. Calculations 20
3.2.3.1. CSTR mass balances 20
3.2.3.2. SBR mass balances 21
3.2.3.3. PHA content and kinetic parameters 22

3.3. RESULTS AND DISCUSSION 22
3.3.1. Protein fermentation in the acidification reactor (CSTR) 22
3.3.2 Enrichment (SBR) and accumulation (FBR) reactors 24
3.3.2.1. SBR operation using acidic-pH feeding 24
3.3.2.2. SBR operation using neutral-pH feeding 30
3.4 CONCLUSIONS 31

4. HOW CAN WE VALIDATE THE ENVIRONMENTAL PROFILE OF BIOPLASTICS? TOWARDS THE INTRODUCTION OF PHA IN THE VALUE-CHAINS 33

4.1 INTRODUCTION 34
4.2. REVIEW STRATEGY: CURRENT LITERATURE REGARDING LCA OF PHA 34
4.3 CRITICAL REVIEW OF THE CURRENT LCA STUDIES FOR PHA PRODUCTION 34
4.3.1. Review Outputs regarding methodological issues 35
4.3.1.1. Motivation of the studies, Functional Unit, and System Boundaries 35
4.3.1.2. Impact categories 37
4.3.1.3. Assessment type 37
4.3.1.4. Source of data. Is there enough information to perform accurate assessments? 38
4.3.2. Classification of the studies regarding process type 39
4.3.3. Main outputs 39
4.3.3.1. Full value-chain vision. What do we know about the use and EoL stages? 40
4.3.4. Lessons learned 40
4.4. FUTURE OUTLOOK, CHALLENGES AND KEY PLAYERS 41
4.5 CONCLUSIONS 41

5. ENVIRONMENTAL ASSESSMENT OF COMPLEX WASTEWATER VALORISATION BY PHA PRODUCTION 43

5.1 INTRODUCTION 44
5.2 GOAL AND SCOPE DEFINITION 45
5.2.1. Functional Unit definition and feedstock quantification 45
5.2.2. System boundaries and scenarios definition 47
5.2.2.1. Baseline scenario: the linear economy approach 48
5.2.2.2. Circular economy approach 49
5.2.2.3. Mass balances and stream compositions 51
5.2.3 Life Cycle Impact Assessment: impact categories and method selection 51
5.3. LIFE CYCLE INVENTORY 52
5.3.1. Inventory of the linear economy scenario 52
5.3.2. Inventory of the circular economy scenario 54
5.3.3. Background processes 56
5.4 LIFE CYCLE IMPACT ASSESSMENT 56
5.4.1. Individual environmental performance of each scenario 56
5.4.2. Comparison of scenarios 58
5.5 DISCUSSION 58
5.5.1. Results obtained versus available literature 58
5.5.2. Benefits of process integration strategies 59
5.5.3. Sensitivity analysis 60
5.5.4. Future outlook 62
5.6. CONCLUSIONS 63

6. ASSESSING A CENTRALIZED BIOREFINERY APPROACH UNDER A TECHNO-ECONOMIC AND ENVIRONMENTAL PERSPECTIVE 65

6.1. INTRODUCTION 66
6.2. MATERIALS AND METHODS 67
6.2.1. Discharged effluent quality and WWTPs design considerations 67
6.2.2. System and scenarios definition in the first stage 67
6.2.3. Description of scenarios of System A (SA): PHA production from MCW 68
6.2.4. Description of scenarios of System B (SB): PHA and TAG production from FPW 70
6.2.5. Approach in the second stage of the evaluation 72
6.2.6. Features of the processes selected for the second stage 72
6.2.6.1. Waste treatment 72
6.2.6.2. DSP for the systems extracting PHA only 72
6.2.6.3. DSP for the systems extracting PHA and TAG (one scenario selected for FPW-SB) 73
6.2.7. Description of the Life Cycle Thinking based strategies used 74
6.2.8. Systems up-scaling, process scale, and mass and energy balances 75
6.3 RESULTS AND DISCUSSION – 1ST STAGE 76
6.3.1. Results of the mass and energy balances for the WWTPs 76
6.3.2. Economic Analysis: TEA and LCC 77
6.3.2.1. System A (SA) with Mussels Cooking Wastewater (MCW) 77
6.3.2.2. System B (SB) with Fish Processing Wastewater (FPW) 79
6.3.3. Environmental Analysis 81
6.3.3.1. Environmental impacts of System A scenarios with MCW 81
6.3.3.2. Environmental impacts of System B scenarios with FPW 82
6.4 RESULTS AND DISCUSSION – 2ND STAGE 83
6.4.1 Centralized biorefinery for PHA production: economic results 84
6.4.2. Centralized biorefinery for PHA and TAG production: economic results 85
6.4.3. Environmental performance of the centralized DSP approaches 85
6.5 DISCUSSION AND COMPARISON WITH THE AVAILABLE LITERATURE 86
6.6 FUTURE OUTLOOK 88
6.7 CONCLUSIONS 89

7. MODELING THE IMPACT OF SALINITY VARIATIONS ON AQUATIC ENVIRONMENTS: INCLUDING NEGATIVE AND POSITIVE EFFECTS IN LIFE CYCLE ASSESSMENT 91

7.1 INTRODUCTION 92
7.2 MATERIALS AND METHODS 93
7.2.1. Fate Factor (FF) 93
7.2.3. Effect Factor (EF) 95
7.3. EFFECT FACTOR FOR SALINITY VARIATIONS IN AQUATIC ENVIRONMENTS 95
7.4. APPLICATION OF THE METHODOLOGY TO A CASE STUDY 97
7.4.1 Fate Factor Calculation 97
7.4.2. Effect Factor calculation 100
7.4.3. Characterization Factor calculation 101
7.5 DISCUSSION 102
7.6. FUTURE OUTLOOK 104

CHAPTER 8: CONCLUSIONS 107

8.1 INTEGRAL DISCUSSION OF THE MAIN OUTCOMES 107
8.2 TAKE-HOME MESSAGES 109

APPENDIX 1: SUPPLEMENTARY INFORMATION FOR CHAPTER 3 111
APPENDIX 2: SUPPLEMENTARY INFORMATION FOR CHAPTER 4 113
APPENDIX 3: SUPPLEMENTARY INFORMATION FOR CHAPTER 5 115
APPENDIX 4: SUPPLEMENTARY INFORMATION FOR CHAPTER 6 123
APPENDIX 5: SUPPLEMENTARY INFORMATION FOR CHAPTER 7 133
REFERENCES 147
LIST OF PUBLICATIONS 171

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

ABBREVIATIONS

ACR	Acute to Chronic Ratio
ADF	Aerobic Dynamic Feeding
AGS	Aerobic Granular Sludge
AP	Acidification Potential
BAT	Best Available Techniques
CAS	Conventional Activated Sludge
CEPCI	Chemical Engineering Plant Cost Index
CF	Characterisation Factor
COD	Chemical Oxygen Demand
CSTR	Continuously Stirred Tank Reactor
DAF	Dissolved Air Flotation
DAR	Fossil (abiotic) Depletion Potential
DO	Dissolved Oxygen
DSP	Downstream Process
EC	European Commission
EC50	Effect Concentration for 50% of the population
EF	Effect Factor
EoL	End of Life
EP-F	Freshwater Eutrophication
EP-M	Marine Eutrophication
EU	European Union
FAS	Ferrous Ammonium Sulphate
FBR	Fed-Batch Reactor
FF	Fate Factor
FU	Functional Unit
GHG	Greenhouse gas
GWP	Global Warming Potential
HC50	Hazardous Concentration for 50% of the ecosystem
HRT	Hydraulic Retention Time
HT	Human Toxicity

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

IC	Inorganic Carbon
IS	Impact Score
JRC	Joint Research Centre
LCA	Life Cycle Assessment
LCCA	Life Cycle Cost Assessment
LCI	Life Cycle Inventory
LCIA	Life Cycle Impact Assessment
LLW	Low-loaded wastewater
M-ADF	Modified-Aerobic Dynamic Feeding
MCW	Mussels Cooking Wastewater
ME	Marine Eutrophication
MMC	Mixed Microbial Cultures
NdN	Nitrification and Denitrification
NREU	Non-renewable energy use
ODP	Ozone Layer Depletion
OLR	Organic Loading Rate
PAF	Potentially Affected Fraction of Species
PBS	Polybutylene Succinate
PDF	Potentially Disappeared Fraction of Species
PE	Polyethylene
PET	Polyethylene terephthalate
PHA	Polyhydroxyalkanoates
PHB	Polyhydroxybutyrate
PHV	Polyhydroxyvalerate
PLA	Polylactic Acid
POCP	Photochemical Oxidation Potential
PP	Polypropylene
PPE	Personal Protective Equipment
PS	Polystyrene
SB	System Boundaries
SBR	Sequencing Batch Reactor
SDS	Sodium Dodecyl Sulphate

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

SPI	Sustainable Process Index
SRT	Solid Retention Time
SSD	Species Sensitivity Distribution
SUP	Single-use Plastics
TAC	Total Annual Costs
TAETP	Terrestrial Ecotoxicity
TAG	Triacylglycerides
TC	Total Carbon
TEA	Techno-Economic Analysis
TN	Total Nitrogen
TOC	Total Organic Carbon
TS	Total Solids
TSS	Total Suspended Solids
TVS	Total Volatile Solids
VFA	Volatile Fatty Acids
VSS	Volatile Suspended Solids
WRA	Waste Reduction Algorithm
WWTP	Wastewater Treatment Plant
XF	Exposure Factor

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

SUMMARY

The fish canning industry is one of the most important sectors in Galicia, generating 85% of the total sector profits in Spain and about 10% of the world's production. It is estimated that, yearly, about 300,000 t of fish and shellfish are processed in this region, reaching an economic value of 1,25 billion €. But this activity also results in a high generation of effluents that need to be treated before being discharged. These effluents are characterized by a high content of organic matter, nutrients (like nitrogen), and salt. For this reason, fish canneries need to perform high investments in wastewater treatment facilities and effluent management. This is even more important in a context in which the sustainability of the sector is highly dependent on the water quality, as the raw material of this industry comes from the ocean.

In recent years, political and governmental institutions, including the EU, are increasingly committed to an economic model that aims to maintain goods and materials in the value chains as long as possible. Here, waste is not only treated with the aim of being managed for dumping, but also for its recovery and use as a raw material. This model is known as **circular economy**, and it is clearly promoted from the European institutions by executing strategies like the Green Deal or the Circular Economy Action plan.

The implementation of circular economy in a sector like the Galician fish canning industry would have special relevance, as this traditionally conservative industry applied moderate innovation patterns that hindered its development in the past. In this context, institutions like the National Association of Fish Canning Producers (by its acronym in Spanish, ANFACO) are advocating for the transformation of the approach applied in the sector, dealing with the specific problems of the industry (like the management of saline effluents).

Problems like the climate and the plastic crises, and the COVID-19 pandemic, among others, are causing fluctuations in the petrol cost, and are raising the urgency of implementing new economic and environmental strategies that guarantee the sustainability of the global economic model and of the life system in the Planet. Therefore, the challenges currently faced are not only urgent, but also globally significant, and circular economy seems to be one of the most promising solutions to solve these issues.

In this sense, the TREASURE-TECHNOSALT[i] project aims to provide solutions to increase the circularity applied in the fish canning sector. Here, the challenges of implementing circular economy in the fish canning industry (where saline waste streams are generated) were addressed, as TREASURE-TECHNOSALT targets to unravel the effects of salinity in wastewater treatment and valorisation.

These challenges were not limited to technological development, but also to the methodological gaps linked to the environmental evaluation of effluent disposal (as saline

[i] TREASURE – TECHNOSALT: Facing the treatment/recovery of saline wastewater to assure future water availability. CTQ2017-83225-C2-1-R. National Spanish Agency of Research. https://biogroup.usc.es/treasure

streams might need different theoretical frameworks), and to the economic assessments, where the body of knowledge on resource recovery processes based on saline streams is still scarce. The unravelling of these challenges would aim to increase the readiness level of the technologies linked to application of the circular economy in the fish canning industry and could be extrapolated to other sectors.

In this context, this thesis addressed these challenges, and aimed to transform wastes coming from the fish canning industry in value-added products using mixed microbial cultures (MMC). These value-added compounds are, mainly, Polyhydroxyalkanoates (PHA) and Triacylglycerides (TAG). They are completely biodegradable, and their production involves microbiological processes using waste streams (renewable sources), so their introduction in the value chains would fit the principles of circular economy in the context of resource recovery.

PHA, a biopolymer that has the potential to replace conventional petrochemical plastics, is currently produced on an industrial scale, although such production is scarce and restricted to processes in which pure cultures of microorganisms are used (under sterile conditions). Moreover, this full-scale production is highly limited and circumscribed to some applications, like biomedical.

TAG, a biomaterial produced by some microorganisms when they are in the presence of fats and oils, can be used to produce paints, varnishes, and biodiesel, among other applications. However, pilot-scale or full-scale applications are not even under development, and the current research works for TAG production only cover lab-scale.

The industrial-scale production of these and other biomaterials has been hindered as the use of axenic cultures and pure substrates (like corn or glucose) significantly raises process costs. Moreover, it could have other side effects, like subliminal distress of food supply, as land would be used for materials generation instead of food production. The production of biopolymers from waste (which are unexpensive raw materials) and MMC (which do not need sterile conditions) have been a major advance to increase the feasibility of these processes on an industrial scale.

In the last years, a lot of work has been performed regarding this production pathway for biomaterials (using MMC and waste streams). Recently, the use of saline streams as a feedstock for these processes has gained more interest, as they present some potential advantages. For example, Downstream Process (DSP) might have operational costs lower than those without saline conditions, due to the use of osmotic shocks to break the cell wall, and the decrease of probability for potential contamination by unwanted microbial strains.

However, technical viability itself is not enough to guarantee the feasibility of introducing a new process or product in the value chains, and environmental and economic validations are necessary before a new good is stablished in the market. Life Cycle Assessment (LCA) has proven to be the tool for assessing the environmental impacts of different industrial processes. Particularly, it has been pointed out as the most accurate tool to validate resource recovery processes and emerging technologies, like those in which residual streams are valorised into biopolymers.

LCA evaluates the environmental impacts of a product or service along its life cycle, i.e., the stages defining the existence of a process or good, from raw materials extraction to product waste management. LCA is being extended to cover other vectors also defining sustainability, like societal or economic dimensions. Here, the life cycle approach can be

applied to financial aspects by Life Cycle Cost Assessment (LCCA), providing another powerful tool to perform integral evaluations of products and processes by combining different vectors of sustainability (not only environmental, but also economic). For systems based on emerging technologies, LCCA can be combined with other strategies for the economic assessment like the Techno-Economic Analysis (TEA), that specifically studies the performance of industrial processes, to provide a more robust framework to analyse a process' viability.

Therefore, the life cycle approach allows to analyse the performance of processes and products, assessing from a holistic perspective the consumption of resources and energy, the generation of waste and emissions, and expressing environmental burdens in a set of impact categories (such as climate change, eutrophication, human toxicity, etc.).

However, there are still methodological gaps to be covered in LCA, as modelling all environmental interactions is a difficult task. For example, most LCA studies ignore the effect of salinity, although it is known that modifications in the salt concentration of ecosystems can cause significant environmental damage. This is important when circular economy implementation is being assessed in a sector like the fish canning industry, as effluents are generally saline, and the effects of wastewater disposal might need tools that consider this effect as well. Therefore, the assessment of salinity effects in LCA represents one of the current methodological gaps found that needs to be addressed in the field and, for this reason, TREASURE-TECHNOSALT addressed this issue.

On the other hand, the challenges for process validation are not only linked to methodological gaps. Moreover, because there is no data on the production of PHA/TAG on an industrial scale using MMC, LCA studies assessing the production of some biomaterials are still scarce. Therefore, the environmental benefits generated by the production of these substances compared to the production of traditional materials have not yet been properly validated.

Furthermore, as circular economy-based systems are multifunctional (waste is managed, and by-products are generated), validation goes in two directions. Then, the assessments need to include not only a comparison between conventional (petrochemical) and innovative (biobased) materials. Moreover, also circular economy-based effluent management techniques (including resource recovery) need to be compared to traditional linear economy-based waste treatment systems (where valorisation is not included).

This scenario is appliable not only to LCA, but also to analogous life-cycle approach methodologies needed to obtain the integral validation of a technology, like LCCA. Here, although some efforts were made to evaluate the economic profile of PHA production, studies show wide ranges of results, and generally agree that economic validation is still not achieved. For TAG and other innovative biomaterials, the number of studies is highly limited, so it is even more difficult to analyse issues like the marketability or the current technology status.

In this sense, the lack of full and pilot-scale data is also hindering the economic validation of PHA production and, this, the maturity level increase. Therefore, new case studies and economic assessments are needed to improve the current framework for PHA and biomaterials validation. For this purpose, integral studies where several dimensions of the process validation (like technological, environmental, and economic) are properly assessed and included are needed. Otherwise, the readiness level of the technology is stuck.

The challenges faced by this thesis focus on solving some of the problems that appear in the management of fish canning industrial effluents, trying to find strategies to improve the degree of circularity applied in this sector. Because these streams have a high concentration of salt, especial attention has been paid to determine how the salinity affects these biological processes and how these features could be addressed in the field of environmental evaluation. Therefore, the issues addressed were framed in a context where circular economy needs to be implemented in different sectors and industries. Thus, a systematic approach to assess the readiness of an emerging technology based on resource recovery process was stablished.

To do so, this thesis aims at i) studying and optimising the production of high value-added storage substances (PHA) from the organic fraction of fish canning industrial effluents, using mixed (non-sterile) cultures of microorganisms, ii) assessing and identifying the current tools available to validate emerging technologies and try to fill some of the potential gaps found in the field, and iii) evaluating the technological solution through a life cycle approach, including both the environmental and the economic vectors. By doing so, the final goal is to provide a robust framework for technology readiness level increase in emerging technologies based on circular economy, with a special focus in the fish canning sector.

In **Chapter 1**, the necessity of producing biomaterials is contextualized in the current plastic and climate crises. The state of the art of PHA production is analysed[ii], and an overview of the application of circular economy and valorisation strategies applied in the fish canning industry is also provided. Special attention is paid to technical, environmental, and economic challenges linked to the plastic crisis and PHA production. Finally, the objectives and structure of this thesis are explained.

In **Chapter 2**, there is an overview of the materials and methods used. Considering the scope of this thesis, it is divided in two sections covering: i) the experimental protocols and analytical methods used to follow up the bioreactors operation, and ii) the evaluation methodologies used for the environmental and techno-economic assessment of the technology (i.e., LCA, which special focus on the Life Cycle Impact Assessment (LCIA) stage, LCCA, and TEA).

Chapter 3 assesses the production of PHA using wastewater coming from the fish canning industry. To do so, saline Mussels Cooking Wastewater (MCW) was valorised to produce PHA with MMC in a three-stage system consisting on: 1) an acidification unit, where Chemical Oxygen Demand (COD) is fermented into Volatile Fatty Acids (VFA), 2) an enrichment unit, where the VFA-rich stream is used to feed a Sequencing Batch Reactor (SBR), and 3) an accumulation unit to maximise PHA storage in a Fed-Batch Reactor (FBR).

One of the major findings was that the relatively high protein content in the wastewater hindered biopolymer production and MMC enrichment, as they are hardly fermented into VFA, and their presence in the SBR difficult the enrichment performance. Due to this relatively high protein content (1.8 – 5.7 g COD_{PROT}/L), PHA accumulating capacity was initially below 10 % in the system, so several strategies were tested during the operational time to improve its transformation.

[ii] Please note that even when TAG production was addressed by TREASURE-TECHNOSALT, its technology development is not covered by this thesis.

In the acidification unit, sodium bicarbonate ($NaHCO_3$) was added to supply alkalinity, which was also low in the wastewater, to avoid the low pH and VFA production inhibition. This action increased protein conversion into VFA from 10.3% to 69.2%, and the subsequent PHA accumulation raise from 6.9 to 14.7%.

In the enrichment unit, the strategy was to modify the Aerobic Dynamic Feeding (ADF) used to enrich the MMC by incorporating a settling stage after the feast phase. This provoked a shift in the proteins' oxidation profile, as their consumption switched from the feast to the famine phase of the cycle, where the nitrogen released was used by the MMC for growth during the starvation period. This increased the biomass concentration, so the tolerated COD by the system was also higher (from 1.6 to 4.2 g VSS/L and from 2.2 to 4.4 g COD/L, respectively).

Finally, increasing the proteins/VFA ratio for MMC acclimation to proteins was done by modifying the wastewater proportions in the SBR feeding. This modification allowed to increase PHA accumulation from 8.8 to 41.5 % in the FBR, overcoming the proposed threshold for industrial viability of 40%.

Chapter 4 is a review on the methodological choices applied to perform LCA of PHA production. As already said, the fact that a material is biodegradable or renewable, does not automatically guarantee environmental benefits linked to its production, so a validation through LCA is needed. It was found that the few LCA studies for PHA production using MMC obtained contradictory results, mainly due to the lack of actual data, but also to the absence of a consensus methodological framework.

The review covered the research works published in the last 20 years, hoping to find the methodological gaps hampering the environmental validation of the process. Consequently, it focuses on key methodological characteristics of any LCA, such as the functional unit and the allocation choices, but also on the data sources of the existing studies, to have a clear picture of the present knowledge related to the environmental profile of biomaterials like PHA.

Besides some methodological gaps found; the main output of this study is that most of the LCA performed till date on PHA production do not use primary data. Contrarily, they usually employ data from references based on studies performed 20 years ago. Therefore, LCA practitioners need to stop relying on data generated decades ago to evaluate nowadays processes, and environmental assessments based on primary information need to be urgently performed.

Chapter 5 aims at covering one of the main outputs of Chapter 4, the need of studies using primary information. Therefore, the results from a two-year laboratory-scale operation for PHA production using MCW and MMC (Chapter 3) were scaled-up to define and compare a circular economy scenario with the current linear approach (i.e., effluent generation, treatment, and discharge). Here, the multifunctionality of the PHA production system is addressed, as the replacement is not only referred to the material (petrochemical plastic vs biopolymer), but also to the waste management system.

USC
DE SANTIAGO
DE COMPOSTELA

Results show an average improvement of ca. 25% for nine out of ten impact categories evaluated if the circular economy approach is implemented, where the only category performing worse (ozone depletion) is linked to the DSP. The sludge management strategy was a key factor for the environmental validation of the process, and if composting is applied instead of anaerobic digestion, the improvement is lost in the fossil depletion impact category but maintained in the remaining 8 categories.

Moreover, when a conservative replacement yield of fossil-based plastic was tested, the circular economy approach was the preferable option in 8 out of 10 categories (in this case, also fossil depletion performed worse, as less petrochemical plastic is avoided). The significance of the DSP was also confirmed by this study, although it was not a barrier to show the feasibility of producing value-added bioproducts under a circular economy approach.

Accordingly, **Chapter 6** addresses the integration of techno-economic and environmental evaluations to perform a holistic assessment that aims to contribute to the readiness level of the technology. To do so and considering the framework of the TREASURE-TECHNOSALT project, industrial effluents (wastewater and oil) from the fish canning industry were valorised to produce PHA and TAG using MMC at bench-scale. Once validated at lab scale, those process systems were up-scaled to perform a techno-economic and environmental analysis and to compare them with the treatments currently applied in the industry. Here, especial attention was paid to the effects of process scale and (des)centralization. In this sense, one of the major findings was that centralized extraction was an attractive alternative when DSP on-site was not technically feasible due to the process scale. Under this approach, wastewater treatment plants (WWTP) would act as PHA-rich biomass suppliers where a centralized biorefinery approach would oversee the DSP of the solids generated in the factories.

For system that used wastewater as a feedstock, effluent management cost would decrease from 0.53 €/kg COD removed when wastewater is only treated, to 0.48 €/kg COD when wastewater is valorised if the extraction process is not included. This is mainly due to the replacing of activated sludge reactors, with residence times of several days for industrial wastewater, by sequential ones, which have a lower volume due to their shorter residence times of 24 hours (so lower direct and indirect costs). If several of these industrial WWTPs provided biomass for centralized biomaterials extraction, PHA production cost could be 0.95 – 1.18 €/kg PHA.

When oil is separated from the WWTP influent and used to produce biomaterials, wastewater treatment cost decreases from 0.52 to 0.30 – 0.33 €/kg COD if DSP is not included. However, the cost of biomaterials production (thus, the extraction process performance) increases with respect to the systems that used wastewater instead of oil to produce biomaterials (1.56 – 3.35 €/kg PHA, and 6.75 – 9.21 €/kg TAG) due to the low biomass production in the biological reactor fed with oil.

Finally, the environmental validation of the processes is linked to the operational approach selected and the wise use of chemicals, as well as to the use of low-cost extraction processes to produce low grade PHA.

As mentioned above, Chapter 5 aimed to solve some of the general gaps linked to PHA environmental validation. However, other research necessities were raised in Chapter 4 when particularly assessing the use of saline streams in industrial processes, as it was found that there was no methodological framework to assess the effects of salinity variations in aquatic environments. **Chapter 7** aims at covering this gap by addressing the issue of salinity variations in aquatic environments in LCA. Variations in the salt concentration (both derived directly from anthropogenic activities, like irrigation, treated wastewater discharge or dam management, but also indirectly from climate change) do impact on ecosystems, but there was no methodology to quantify or describe those impacts.

Salinity is changing in aquatic systems and, although there are studies on the effects of salinity variations on individual species, little is known about the effects on overall ecosystems. Moreover, these impacts could be more uncertain in transitional waters such as estuaries or fiords, and, particularly, in the Galician *rías* or estuaries.

Complementary to the current models used for ecotoxicity assessment, where an increase in the concentration of a pollutant generates an increase in the impacts (thus, disregarding the effects of water freshening), this thesis introduces a general framework to address the impacts of salinity variations, including emission-related positive effects. To do so, both negative and positive impacts linked to a decrease in the concentration of a chemical (in this case, sodium chloride) were modelled for the first time.

The framework developed was validated by applying it to the Galician *ría* of *Arousa*, where sharp drops in the salt concentration have caused mass mortalities of shellfish in recent decades. The characterization factors developed ranged 0 – 0.89, in units of Potential Disappeared Fraction of species (PDF)·m^3·month/kg.

Chapter 8 summaries the main knowledge contributions of this thesis, .i.e. the provision of a systematic approach that can be used to increase technology readiness level for resource recovery emerging processes based on circular economy, with special emphasis to the ones employing saline effluents and/or streams coming from the fish canning industry.

Firstly, a process for PHA production using saline waste streams and MMC was developed and validated, both technically and environmentally. Then, a state-of-the-art overview of the methodological framework available for this evaluation was provided, and part of the research work developed after that aimed to contribute to some of the nowadays general necessities found after this review, to provide general validation for PHA production systems.

Therefore, an economic assessment is provided after the environmental validation. Here, it was proven that WWTPs could work as raw materials providers for centralised biorefinery schemes. The scope of this evaluation was extended by including other research works in the frame of TREASURE-TECHNOSALT for TAG production, validating the centralized biorefinery approach for more resource recovery systems.

This thesis proved the feasibility of implementing circular economy-based approaches and processes in the Galician fish canning industry and provided a framework to assist the increase of the readiness level of emerging technologies, as explained in Chapter 1. Moreover, it proved the usefulness of life thinking strategies to holistically validate the feasibility of developing processes based on circular economy, like the resource recovery ones.

Finally, this thesis paid special attention to the particular challenges that need to be addressed when working with saline streams. Therefore, the results obtained led to the generation of a new methodology to assess the impacts of salinity variations in aquatic environments, including both negative and positive effects in emission-related impact assessment for the first time, and providing a new field of discussion in the LCA community.

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

RESUMO

A industria conserveira de peixe é un dos sectores máis importantes de Galicia, xerando o 85% dos beneficios totais do sector en España e preto do 10% da produción mundial. Estímase que, anualmente, nesta rexión se procesan preto de 300.000 t de peixe e marisco, alcanzando un valor económico de 1.250 millóns de euros. Pero esta actividade tamén dá lugar a unha alta xeración de efluentes que precisan ser tratados antes de ser vertidos. Estes efluentes caracterízanse por un alto contido en materia orgánica, nutrientes (como o nitróxeno) e sal. Por este motivo, as conserveiras de peixe necesitan realizar grandes investimentos en instalacións de tratamento de augas residuais e xestión de efluentes. Isto é aínda máis importante nun contexto no que a sustentabilidade do sector depende moito da calidade da auga, xa que a materia prima desta industria procede do océano.

Nos últimos anos, as institucións políticas e gobernamentais, incluída a UE, apostan cada vez máis por un modelo económico que pretende manter os bens e materiais nas cadeas de valor o maior tempo posible. Aquí os residuos non só son tratados co obxectivo de ser xestionados para o seu vertido, senón tamén para a súa valorización e utilización como materia prima. Este modelo coñécese como **economía circular**, e está claramente impulsado dende as institucións europeas mediante a execución de estratexias como o Green Deal ou o Plan de Acción de Economía Circular.

A implantación da economía circular nun sector como o da conserva de peixe galega tería especial relevancia, xa que os patróns de innovación moderados dificultaron no pasado o desenvolvemento dunha industria tradicionalmente conservadora. Neste contexto, institucións como a Asociación Nacional de Fabricantes de conservas de Pescados e Mariscos (ANFACO) avogan pola transformación do enfoque aplicado no sector, tratando os problemas específicos da industria (como a xestión dos efluentes salinos).

Problemas como a crise climática e a dos plásticos, así coma a pandemia de COVID-19, entre outros, están a provocar flutuacións no custo do petróleo, e xerando a urxencia de implantar novas estratexias económicas e ambientais que garantan a sustentabilidade do noso modelo económico e da nosa forma de vida no planeta. Polo tanto, os retos aos que se nos enfrontamos na actualidade non só son urxentes, senón tamén significativos a nivel mundial, e a economía circular parece ser unha das solucións máis prometedoras para resolver estes problemas.

Neste sentido, o proxecto TREASURE-TECHNOSALT[*] pretende aportar solucións para aumentar a circularidade aplicada no sector da conserva de peixe. Aquí abordáronse os retos da implantación da economía circular na industria conserveira de peixe (onde se xeran fluxos de residuos salinos), xa que TREASURE-TECHNOSALT pretende desvelar os efectos da salinidade no tratamento e valorización das augas residuais.

USC DE SANTIAGO DE COMPOSTELA

[*] TREASURE – TECHNOSALT: Facing the treatment/recovery of saline wastewater to assure future water availability. CTQ2017-83225-C2-1-R. Axencia Nacional de Investigación. https://biogroup.usc.es/treasure.

Estes desafíos non se limitaron ao desenvolvemento tecnolóxico, senón que tamén abordaron algunhas lagoas metodolóxicas vinculadas á avaliación ambiental do vertido de efluentes (xa que as correntes salinas poden necesitar marcos teóricos diferentes), e as avaliacións económicas, onde o coñecemento sobre os procesos de recuperación de recursos no contexto da economía circular e empregando efluentes salinos é aínda escaso. A resolución destes retos e problemáticas tería como obxectivo aumentar o grao de madurez das tecnoloxías vinculadas á aplicación da economía circular na industria conserveira de peixe, e os resultados obtidos poderían ser extrapolados a outros sectores.

Neste contexto, esta tese abordou estes desafíos, e polo tanto tivo como obxectivo transformar os residuos procedentes da industria conserveira de peixe en produtos de valor engadido utilizando cultivos mixtos de microorganismos (CMM). Estas substancias de valor engadido son, principalmente, Polihidroxialcanoatos (PHA) e Triacilglicéridos (TAG). Estes materiais son completamente biodegradables, e a súa produción implica procesos microbiolóxicos que utilizan fluxos de residuos, polo que a súa introdución nas cadeas de valor encaixaría cos principios da economía circular no contexto da recuperación de recursos.

O PHA é un biopolímero que ten potencial para substituír aos plásticos petroquímicos convencionais. Prodúcese na actualidade a escala industrial, aínda que esa produción é escasa e restrinxida a procesos nos que se utilizan cultivos puros de microorganismos (en condicións estériles). Ademais, esta produción a gran escala é moi limitada e circunscrita a algunhas aplicacións, como a biomédica.

O TAG é un biomaterial producido por algúns microorganismos cando están en presenza de graxas e aceites. Pode utilizarse para producir pinturas, vernices e biodiesel, entre outras aplicacións. Non obstante, as aplicacións a escala piloto ou a escala real nin sequera están en desenvolvemento, mentres que a produción actual de TAG só se realiza a escala de laboratorio.

A produción a escala industrial destes e doutros biomateriais viuse obstaculizada debido a que o uso de cultivos en condicións estériles e substratos puros (como o millo ou a glicosa) aumentaban significativamente os custos do proceso. Ademais, este enfoque de proceso podería ter outros efectos secundarios, como a influencia sobre a produción de alimentos, xa que a terra utilizaríase para a xeración de materiais en lugar de para o abastecemento dos seres humanos. A produción de biopolímeros a partir de residuos (que son materias primas sen custo) e o uso de CMM (que non precisan de condicións estériles) supuxeron un gran avance para aumentar a viabilidade destes procesos a escala industrial.

Nos últimos anos traballouse moito nesta vía de produción de biomateriais (utilizando CMM e fluxos de residuos). Recentemente, o uso de correntes salinas como materia prima para estes procesos ten adquirido máis interese, xa que presentan algunhas vantaxes potenciais. Por exemplo, o proceso de extracción augas abaixo (polas súas siglas en inglés, *Downstream Process* ou DSP) pode ter custos máis baixos debido ao uso de choques osmóticos con auga doce para romper a parede celular, e a que sería máis improbable a contaminación do cultivo mixto por cepas microbianas non desexadas.

Non obstante, a validación técnica en si non é suficiente para garantir a viabilidade de introducir un novo proceso ou produto nas cadeas de valor, xa que é necesario levar a cabo tamén validacións ambientais e económicas antes de levar a cabo a devandita introdución no mercado. Neste senso, a Análise do Ciclo de Vida (ACV) demostrou ser a ferramenta para avaliar os impactos ambientais dos diferentes procesos industriais.

Particularmente, sinalouse como a ferramenta máis precisa para validar procesos de recuperación de recursos e tecnoloxías emerxentes, como aquelas nas que os fluxos residuais son valorizados en biopolímeros.

O ACV avalía os impactos ambientais dun produto ou servizo ao longo do seu ciclo de vida. É dicir, as etapas que definen a existencia dun proceso ou ben, desde a extracción de materias primas ata a xestión de residuos. Ademais, o ACV estase a estender e ampliar para poder abarcar outros vectores que tamén definen a sustentabilidade, como as dimensións sociais ou económicas. Aquí, o enfoque do ciclo de vida pódese aplicar aos aspectos financeiros mediante a Avaliación dos Custos do Ciclo de Vida (ACCV), proporcionando outra poderosa ferramenta para realizar avaliacións integrais de produtos e procesos combinando diferentes vectores de sustentabilidade (non só ambiental, senón tamén económico). Para sistemas baseados en tecnoloxías emerxentes, o ACCV pódese combinar con outras estratexias, como a Análise Tecnoeconómica (ATE), que estuda especificamente o rendemento dos procesos industriais, para proporcionar un marco máis sólido mediante o cal analizar a viabilidade dun proceso.

Polo tanto, o enfoque do ciclo de vida permite analizar o rendemento de procesos e produtos, avaliando dende unha perspectiva holística o consumo de recursos e enerxía, a xeración de residuos e emisións, e expresando as cargas ambientais nun conxunto de categorías de impacto como o cambio climático, a eutrofización, a toxicidade humana, etc.

Non obstante, aínda quedan lagoas metodolóxicas por cubrir no ACV, xa que modelar todas as interaccións ambientais é unha tarefa difícil. Por exemplo, a maioría dos estudos de ACV ignoran o efecto da salinidade, aínda que se sabe que as modificacións na concentración salina dos ecosistemas poden causar importantes danos ambientais. Isto é importante cando se está a avaliar a implantación da economía circular nun sector como o da industria conserveira de peixe, xa que os efluentes son xeralmente salinos, e os efectos da eliminación de augas residuais poden necesitar ferramentas que teñan en conta este efecto tamén. Polo tanto, a avaliación dos efectos da salinidade en ACV representa unha das lagoas metodolóxicas actuais atopadas que cómpre abordar no campo e, por este motivo, TREASURE-TECHNOSALT acometeu esta cuestión.

Por outra banda, os retos para a validación de procesos non só están ligados a lagoas metodolóxicas. Ademais, como non hai datos sobre a produción de PHA/TAG a escala industrial mediante CMM, os estudos de ACV que avalían a produción dalgúns biomateriais aínda son escasos. Polo tanto, os beneficios ambientais xerados pola produción destas substancias fronte á produción de materiais tradicionais aínda non foron debidamente validados.

Ademais, como os sistemas baseados na economía circular son multifuncionais (xestionanse residuos e xéranse subprodutos), a validación vai en dúas direccións. Deste xeito, as avaliacións non só deben incluír unha comparación entre materiais convencionais (petroquímicos) e innovadores (de base biolóxica). Ademais, tamén hai que comparar as técnicas de xestión de efluentes baseadas na economía circular (incluída a recuperación de recursos) cos sistemas tradicionais de tratamento de residuos baseados na economía lineal (onde non se inclúe a valorización).

Este escenario é aplicable non só ao ACV, senón tamén a metodoloxías análogas de enfoque do ciclo de vida necesarias para obter a validación integral dunha tecnoloxía, coma o ACCV. Aquí, aínda que se realizaron algúns esforzos para avaliar o perfil

económico da produción de PHA, os estudos amosan unha ampla gama de resultados e, en xeral, coinciden en que aínda non se logra a validación económica. Para TAG e outros biomateriais innovadores, o número de estudos é moi limitado, polo que é aínda máis difícil analizar cuestións como a comercialización ou o estado actual da tecnoloxía.

Neste sentido, a falta de datos completos e a escala piloto tamén está a dificultar a validación económica da produción de PHA e, iso, o aumento do nivel de madurez. Polo tanto, son necesarios novos estudos de caso e novas avaliacións económicas que aporten información para mellorar o marco actual para acadar a validación integral do PHA e doutros biomateriais. Polo tanto, precísase aportar máis estudos integrais onde se analicen varias dimensións da validación do proceso (como tecnolóxica, ambiental e económica). Se non, o nivel de preparación da tecnoloxía está atascado.

Os retos aos que se enfronta esta tese céntranse en resolver algúns dos problemas que aparecen na xestión dos efluentes industriais das conservas de peixe, tratando de buscar estratexias para mellorar o grao de circularidade aplicado neste sector. En particular, debido a que estas correntes presentan unha alta concentración de sal, prestouse especial atención a como a salinidade afecta a estes procesos biolóxicos e como se podería abordar o tema no ámbito da avaliación ambiental. Polo tanto, as cuestións acometidas enmarcáronse nun contexto no que a economía circular debe implantarse en diferentes sectores e industrias. Así, estableceuse un enfoque sistemático para avaliar a preparación dunha tecnoloxía emerxente baseada no proceso de recuperación de recursos.

Para iso, esta tese pretende i) estudar e optimizar a produción de substancias de alto valor engadido (PHA) a partir da fracción orgánica dos efluentes industriais das conservas de peixe, utilizando cultivos mixtos (non estériles) de microorganismos, ii) avaliar cales son as ferramentas dispoñibles actualmente para validar tecnoloxías emerxentes coma a produción de PHA, e cubrir algunhas das posibles lagoas atopadas no campo, e iii) avaliar a solución tecnolóxica proposta a través dun enfoque de ciclo de vida, incluíndo tanto os vectores ambientais como os económicos. Ao facelo, o obxectivo final é proporcionar un marco sólido para o aumento do nivel de madurez das tecnoloxías emerxentes baseadas na economía circular, con especial atención ao sector das conservas de peixe.

No **Capítulo 1** contextualízase a necesidade de producir biomateriais na actual crise plástica e climática. Analízase o estado da arte da produción de PHA[†], e tamén se ofrece unha visión xeral da aplicación da economía circular e das estratexias de valorización aplicadas na industria conserveira de peixe. Préstase especial atención aos retos técnicos, ambientais e económicos vinculados á crise do plástico e á produción de PHA.

No **Capítulo 2**, hai unha visión xeral dos materiais e métodos empregados no traballo da tese. Tendo en conta o alcance desta tese, este capítulo divídese en dous apartados que abranguen: i) os protocolos experimentais e os métodos analíticos utilizados para o seguimento do funcionamento dos biorreactores, e ii) as metodoloxías empregadas para a avaliación ambiental e tecnoeconómica da tecnoloxía (é dicir, o ACV, centrándose especialmente na fase de Avaliación de Impacto do Ciclo de Vida (AICV), ACCV e ATE).

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

O **Capítulo 3** avalía a produción de PHA utilizando augas residuais procedentes da industria conserveira de peixe. Para iso, valorizáronse as augas residuais salinas

[†] Aínda que a produción de TAG foi abordada en TREASURE-TECHNOSALT, o desenvolvemento tecnolóxico deste proceso non foi acometido nesta tese.

procedentes da cocción de mexillóns (ou auga de cocción de mexillón, ACM) para producir PHA empregando CMM nun sistema de tres etapas. Estas etapas foron: 1) unha unidade de acidificación, onde a demanda química de osíxeno (DQO) se fermenta en ácidos graxos volátiles (AGV), 2) unha unidade de enriquecemento, onde o fluxo rico en AGV se usa para alimentar un reactor secuencial por lotes (polas súas siglas en inglés, *Sequencing Batch Reactor* ou SBR) e 3) unha unidade de acumulación para maximizar o almacenamento de PHA nun reactor alimentado por lotes (polas súas siglas en inglés, *Fed-Batch Reactor* ou FBR).

Un dos principais descubrimentos destacados neste capítulo foi que o contido relativamente alto de proteínas nas augas residuais dificultou a produción de biopolímeros e o enriquecemento de CMM, xa que case non se fermentan en AGV, e a súa presenza no SBR dificulta o rendemento do enriquecemento. Debido a este contido proteico relativamente alto (1,8 – 5,7 g COD_{PROT}/L), a capacidade de acumulación de PHA estaba inicialmente por debaixo do 10 % no sistema, polo que se probaron varias estratexias durante o tempo operativo para mellorar a súa transformación.

Na unidade de acidificación, engadiuse bicarbonato de sodio ($NaHCO_3$) para proporcionar alcalinidade, que tamén era baixa na ACM, para evitar o baixo pH e a inhibición da produción de AGV. Esta acción aumentou a conversión de proteínas en AGV do 10,3% ao 69,2% e a posterior acumulación de PHA do 6,9% ao 14,7%.

Na unidade de enriquecemento, a estratexia foi modificar a Alimentación Dinámica Aeróbica utilizada para enriquecer o MMC incorporando unha fase de sedimentación despois da fase de saciedade. Isto provocou un cambio no perfil de oxidación das proteínas, xa que o seu consumo pasou da fase de saciedade á fase de fame do ciclo, onde o nitróxeno liberado foi utilizado pola CMM para o crecemento durante o período de fame. Isto aumentou a concentración de biomasa, polo que o DQO tolerada polo sistema tamén foi maior (de 1,6 a 4,2 g VSS/L e de 2,2 a 4,4 g DQO/L).

Finalmente, variar a relación proteínas/AGV co obxectivo de aclimatar o CMM á alta concentración de proteínas mediante a modificación das proporcións de auga residual na alimentación SBR permitiu aumentar a acumulación de PHA do 8,8 ao 41,5 % no FBR, superando o limiar proposto para a viabilidade industrial do 40%.

O **Capítulo 4** é unha *review* que se centra principalmente nas eleccións metodolóxicas que foron aplicadas para realizar o ACV da produción de PHA nos estudos dos últimos anos. Como xa se dixo, o feito de que un material sexa biodegradable ou renovable non garante automaticamente que existan beneficios ambientais vinculados á súa produción, polo que é necesaria unha validación mediante ACV. De feito, descubriuse aquí que os poucos estudos de ACV para a produción de PHA utilizando CMM ata a data obtiveron resultados contraditorios, principalmente debido á falta de datos reais, pero tamén á ausencia dun marco metodolóxico de consenso.

A revisión abrangue os traballos de investigación publicados nos últimos 20 anos, coa esperanza de atopar as lagoas metodolóxicas que dificultan a validación ambiental do proceso. En consecuencia, céntrase nas características metodolóxicas clave de calquera ACV, como a unidade funcional e as opcións de asignación (en inglés, *allocation*), pero tamén nas fontes de datos dos estudos existentes, para ter unha imaxe clara do estado actual do coñecemento do perfil ambiental dos biomateriais como PHA.

Ademais dalgunhas lagoas metodolóxicas atopadas; o principal resultado deste estudo é que a maioría dos ACV realizados ata a data na produción de PHA non utilizan

datos primarios. Pola contra, adoitan empregar datos de referencias baseadas en estudos realizados hai 20 anos. Polo tanto, os profesionais do ACV deben deixar de empregar datos xerados hai décadas para avaliar procesos actuais, sendo así de manifesto a urxencia de realizar novas avaliacións ambientais baseadas en información primaria.

O **Capítulo 5** pretende cubrir un dos principais retos atopados no capítulo 4: a necesidade de realizar estudos que utilicen información primaria. Polo tanto, os resultados da operación de dous anos a escala de laboratorio para a produción de PHA usando ACM e CMM (capítulo 3) foron ampliados para definir e comparar un escenario de economía circular co enfoque lineal actual, que abrangue só a xeración, tratamento e vertido de efluentes). Aquí abórdase a multifuncionalidade do sistema de produción de PHA, xa que a substitución non só se refire ao material (plástico petroquímico vs biopolímero), senón tamén ao sistema de xestión de residuos.

Os resultados mostran unha mellora media de aproximadamente 25 % para nove de cada dez categorías de impacto avaliadas se se implanta o enfoque da economía circular, onde a única categoría con peor rendemento (esgotamento da capa de ozono) está vencellada ao proceso augas abaixo ou DSP. A estratexia de xestión de lodos foi un factor chave para a validación ambiental do proceso, e se se aplica compostaxe en lugar da dixestión anaerobia, a mellora pérdese na categoría de impacto de esgotamento de recursos fósiles, pero mantense nas 8 categorías restantes.

Ademais, cando se probou unha relación de substitución (referido á cantidade de bioplástico que substituiría ao plástico petroquímico) conservador, o enfoque de economía circular foi a opción preferible en 8 de cada 10 categorías (neste caso, o esgotamento dos fósiles tamén foi peor, xa que se evita a produción de menos plástico petroquímico). Este estudo tamén confirmou a importancia do DSP, aínda que tamén probou que este non foi unha barreira para mostrar a viabilidade dos bioprodutos de valor engadido baixo un enfoque de economía circular.

En consecuencia, o **Capítulo 6** aborda a integración de avaliacións tecnoeconómicas e ambientais para realizar unha análise holística do proceso que pretende contribuír ao nivel de madurez da tecnoloxía. Para iso, e tendo en conta o marco do proxecto TREASURE-TECHNOSALT, avaliáronse distintos efluentes industriais (augas residuais e aceite) procedentes da industria conserveira para producir PHA e TAG empregando CMM a escala laboratorio. Unha vez validados a dita escala, eses sistemas foron escalados para realizar unha análise tecnoeconómica e ambiental, e comparalos así cos tratamentos aplicados na actualidade na industria. Aquí prestouse especial atención aos efectos da escala do proceso e da (des)centralización. Neste sentido, un dos principais descubrimentos foi que a extracción centralizada era unha alternativa atractiva cando DSP *in situ* non foi tecnicamente viable debido á escala do proceso. Baixo este enfoque, as estacións depuradoras de augas residuais (EDAR) actuarían como provedores de biomasa rica en PHA, onde, baixo este enfoque, unha biorrefinería centralizaría o DSP dos sólidos xerados nas fábricas e a extracción do PHA e dos biomateriais.

Para o sistema que utilizaba as augas residuais como materia prima, o custo de xestión do efluente diminuiría de 0,53 €/kg de DQO eliminado cando as augas residuais só se tratan, a 0,48 €/kg de DQO cando estas tamén se valorizan. Isto débese fundamentalmente á substitución dos reactores de lodos activos, con tempos de residencia de varios días para a auga residual industrial, por outros secuenciais, que teñen un menor volume debido aos seus tempos de residencia máis curtos, de 24 horas (polo que tamén presentan menores custos directos e indirectos). Se varias destas EDAR industriais

proporcionasen biomasa para a extracción centralizada de biomateriais, o custo de produción de PHA podería ser de 0,95 a 1,18 €/kg de PHA.

Cando as graxas e os aceites son separados do influente que alimenta outra EDAR industrial para producir TAG e PHA, o custo de tratamento de augas residuais diminúe de 0,52 a 0,30 – 0.33 €/kg DQO, pero o custo de produción destes biomateriais aumenta con respecto aos sistemas que empregaban augas residuais sen aceite coma materia prima (1,56 – 3,35 €/kg de PHA, e 6,75 – 9,21 €/kg TAG) debido á baixa produción de biomasa no reactor biolóxico alimentado con aceite.

Finalmente, a validación ambiental dos procesos está ligada ao enfoque operativo seleccionado e ao uso moderado de produtos químicos, así como á implantación de procesos de extracción de baixo custo para producir PHA de baixo grao (usado, por exemplo, para embalaxes).

Por último, nesta liña, o capítulo 5 tivera como obxectivo resolver algunhas das lagoas xerais relacionadas coa validación ambiental PHA. Non obstante, detectáronse outras necesidades de investigación á hora de valorar particularmente o uso de correntes salinas nos procesos industriais, xa que se comprobou que non existía un marco metodolóxico para avaliar os efectos das variacións de salinidade nos medios acuáticos. O **Capítulo 7** pretende por tanto cubrir esta lagoa abordando o problema da salinidade no ACV. As variacións na concentración de sal (derivadas directamente de actividades antropoxénicas, como o rego ou a xestión de encoros, pero tamén indirectamente polo cambio climático) repercuten nos ecosistemas, pero ate o momento non había unha metodoloxía para cuantificar ou describir eses impactos.

A salinidade está cambiando nos sistemas acuáticos e, aínda que existen estudos sobre os efectos das variacións da salinidade en especies individuais, pouco se sabe sobre os impactos sobre os ecosistemas globais. Ademais, estes efectos poderían ser máis incertos en augas de transición como rías ou fiordos e, en particular, nas rías galegas.

Complementariamente aos modelos actuais utilizados para a avaliación da ecotoxicidade, onde un aumento da concentración dun contaminante xera un aumento dos impactos (polo tanto, sen ter en conta os efectos da dulcificación da auga), esta tese introduce un marco xeral para abordar os impactos das variacións da salinidade, incluíndo efectos positivos relacionados cos impactos por emisións (tradicionalmente só negativos). Para iso, modeláronse por primeira vez tanto impactos negativos como positivos vinculados á diminución da concentración dun produto químico (neste caso, cloruro de sodio), introducindo así este novo marco.

O devandito marco desenvolvido validouse aplicándoo á ría galega de Arousa, onde fortes descensos da concentración de sal provocaron mortalidades masivas de marisco nas últimas décadas. Os factores de caracterización estimados neste capítulo oscilaron entre 0 e 0,89, en unidades de fracción de especies potencialmente desaparecidas·m^3·mes/kg.

O **Capítulo 8** ofrece as principais achegas ao coñecemento desta tese. Polo tanto, co traballo aquí realizado, ofrécese un enfoque sistemático que pode ser utilizado para aumentar o nivel de madurez tecnolóxica dos procesos emerxentes de recuperación de recursos baseados na economía circular, con especial énfase nos que empregan efluentes salinos e/ou correntes procedentes da industria conserveira de peixe.

En primeiro lugar, desenvolveuse e validouse un proceso para a produción de PHA utilizando fluxos de residuos salinos e CMM, tanto a nivel técnico coma a nivel ambiental. A continuación, presentouse unha visión xeral do estado da arte do marco metodolóxico dispoñible para esta avaliación, e parte do traballo desenvolvido posteriormente tivo como obxectivo contribuír a algunhas das necesidades xerais que se atoparon tras esta revisión, coa fin de proporcionar unha validación xeral para sistemas de produción de PHA.

Polo tanto, nesta tese realizouse unha avaliación económica despois da validación ambiental. Aquí, comprobouse que as EDAR poderían funcionar como provedores de materias primas para esquemas de biorefinería centralizada. O alcance desta avaliación ampliouse incluíndo outros traballos de investigación no marco de TREASURE-TECHNOSALT para a produción de TAG, validando o enfoque de biorefinería centralizada para máis sistemas de recuperación de recursos.

Esta tese demostrou a viabilidade de implantar enfoques e procesos baseados na economía circular na industria conserveira de peixe galega, e proporcionou un marco para aumentar o nivel de madurez das tecnoloxías emerxentes. Ademais, demostrou a utilidade das estratexias de pensamento de ciclo de vida para validar holísticamente a viabilidade de desenvolver procesos baseados na economía circular, como os de recuperación de recursos.

Finalmente, esta tese prestou especial atención aos retos particulares que se deben abordar cando se traballa con correntes salinas. Polo tanto, os resultados obtidos levaron á xeración dunha nova metodoloxía para avaliar os impactos das variacións da salinidade nos medios acuáticos, incluíndo por primeira vez os efectos tanto negativos como positivos na avaliación dos impactos relacionado efectos de emisións, e proporcionando un novo campo de discusión na comunidade do ACV.

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

1. INTRODUCTION

SUMMARY

This chapter provides an overview of the motivation of the thesis and its scope. Here, the necessity of producing bio-based materials like Polyhydroxyalkanoates (PHA) in the framework of circular economy arises, contextualized in the current climatic and plastic crises. The current state-of-the-art of biomaterials production (mainly PHA, but also triacylglycerydes) is assessed, and the status of PHA environmental and techno-economic evaluations, which are the base for technology maturity increase, is assessed. Finally, an overview of the thesis structure and how that is related to technology development, is provided.

Part of the content of this chapter was published as A. Roibás-Rozas[1], M. Saavedra del Oso[1], G. Zarroli[1,2], M. Mauricio-Iglesias[1], A. Mosquera-Corral[1], S. Fiore[2], and A. Hospido[1] (2022). **How can we validate the environmental profile of bioplastics? Towards the introduction of Polyhydroxyalkanoates (PHA) in the value-chains**. Chapter 20 in: C. Teodosiu, S. Fiore, A. Hospido (Eds.), Assessing Progress Towards Sustainability, Elsevier. https://www.elsevier.com/books/assessing-progress-towards-sustainability/teodosiu/978-0-323-85851-9

[a] CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

[b] DIATI (Department of Engineering for Environment, Land and Infrastructures), Politecnico di Torino, 10129 Torino, Italy

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

1.1 CHALLENGES IN THE CONTEXT OF A GLOBAL CRISIS

The plastic and the climate change crises are two of the most relevant problems that need to be addressed by the humankind to avoid a global collapse. Although they have been treated in the past as separated issues, the truth is that they are fundamentally linked. Primarily, plastics contribute to greenhouse gas (GHG) emission in all the stages of its life cycle (from raw materials extraction to manufacturing, transport, and end of life (EoL)). Secondly, both issues are narrowly related with the oceans and the marine environment. Finally, the root cause of both crises is the same: the overconsumption of finite resources and a bad EoL management. In this sense, engagement solving plastic pollution can increase action against climate change, where integrated solutions can be summed up with strategies like the implementation of a circular economy (Ford et al., 2022).

Plastic goods have been in our lives for no longer than one century. However, in about seventy years, they have become one of the greatest concerns and environmental threats worldwide. The growth of plastics market during the twentieth century was remarkably fast, only surpassed by some construction goods, as steel and cement. Plastics' production increased from 2 Mt in 1950 to 380 Mt in 2015, yielding in about 83,000 Mt of total produced virgin plastics in that period (Geyer et al., 2017). Moreover, the plastic sector employs about 1.5 M people in the European Union (EU) and generates around 340 billion € yearly (European Commission (EC), 2018a). However, its production and use are linked to the generation of extremely high amounts of wastes. It is estimated that, of all the plastics ever produced, only about the 30% of them still remain in the value chains, so the remaining 70% of them were wasted. Of this enormous amount of waste, only 9% is recycled, 12% is incinerated, and 79% is accumulated in landfills or directly disposed in the natural environment with no treatment at all (Geyer et al., 2017).

The related impacts on ecosystems, health and economy cannot be quantified yet due to the lack of consistent frameworks and actual data (Boulay et al., 2021). However, the number of studies on this topic is growing very fast, and their results show that these effects are serious and global (Halsband and Herzke, 2019; Saling et al., 2020). In fact, plastics are slowly decomposing and leaking in the environment. First, they affect terrestrial ecosystems (de Souza Machado et al., 2018), from where they are transferred into freshwater (Wagner et al., 2014). And finally, plastics reach the oceans in the forms of macro, micro and nano plastics (Galloway et al., 2017; Geyer et al., 2017; Halsband and Herzke, 2019; Saling et al., 2020).

Modern plastics are complex mixtures of polymers, residual monomers, and chemical additives, some of them being endocrine disruptors that alter metabolic and reproductive patterns (Galloway et al., 2017). Plastic debris accumulate in the environment and are transferred to many trophic levels through food chains, thereby affecting animal and human health (Akhbarizadeh et al., 2020). Moreover, unmanaged plastic waste also affects the economy, seriously impacting fishing, aquaculture, and tourism sectors, and it implies direct costs for governments and administrations. As an example, the Netherlands spend millions of euros each year on removing litter from beaches and coastal areas (Conejo-Watt and Luisetti, 2019).

Micro and nano plastics are vehicles that transport potentially hazardous and toxic substances, like heavy metals or organic pollutants. Moreover, these carried pollutants interact sometimes with the carriers (the micro and nano plastics), boosting the harmful effects of each pollutant by itself (Liu et al., 2022; Xiang et al., 2022). Humans uptake

plastic by several exposure pathways, mainly: drinking water, the consumption of food products, and air (inhalation of dust), potentially generating pulmonary damage, oxidative stress, cellular damage, DNA damage, and inflammatory and immune reactions, among other effects (Xiang et al., 2022).

Moreover, the COVID-19 pandemic crisis resulted in an unprecedent surge of production, consumption, and disposal of single-use plastics (SUP) due to the necessity of using disposable personal protective equipment (PPE), like face masks. This not only increased the amount of plastic pollution, but also increased health risks due to mismanaged potentially infectious materials disposed in urban and natural environments (Ammendolia and Walker, 2022). In fact, contaminated PPE pollution is considered a new type of environmental hazard, linked to the increase of plastic pollution (they are a new source of micro and nano plastics), and to their status of potential carriers for harmful contaminants (Kutralam-Muniasamy et al., 2022).

Unfortunately, the introduction of plastics in the trophic chains has already been confirmed extensively. High amounts of microplastics were found in some of the most fished and consumed species (Masiá et al., 2022). About 80% of the cans examined in a study containing tuna and mackerel had at least one microplastic that is going to be consumed by humans (Akhbarizadeh et al., 2020). Consequently, it is estimated that a person intake between several tens of thousands to several millions of microplastic particles annually, where the main source of exposure might be the ingestion of water bottled in plastic. Here, factors like size, chemical composition, or hydrophobicity, would shape the type and magnitude of the effects, ranging from cytotoxicity to hormone disruption, among other effects (Kannan and Vimalkumar, 2021).

Regrettably, the presence of plastic particles in humans has already been confirmed, as they were found in all tested samples of liver and adipose tissue (ACS Newsroom, 2020) and human stool (Schwabl et al., 2019). Results were linked with strong evidences of relationship between the presence of these particles in human tissues and harmful effects on the lungs and the immune system (Zarus et al., 2021). Moreover, maternal transfer was already proved by finding pieces of plastic in human placentas (Ragusa et al., 2021).

Therefore, (micro)plastic is everywhere (Lim, 2021). In this sense, the amount of plastic accumulated in the ocean is uncertain, and recent studies are pointing out that the current measurements are in fact underestimating the magnitude of the problem (Way et al., 2022). Nevertheless, some studies estimate discharges of about 6.4 million tons of plastic debris yearly, where about 80% of this debris originates from land (remaining 20% correspond to ship or military operations). Here, the biggest contributor to this plastic release is China (Jambeck et al., 2015).

However, this is not only due to waste mismanagement in this country, but to the global plastic waste trade networks. In fact, the main sources of plastic waste are found in Europe and the United States (Wang et al., 2020), as rich states sell their waste to poorer and/or developing countries. As the importer countries use these wastes as raw materials to recycle and convert them into new products, these world trades were linked with reductions in GHG emissions (Liu et al., 2021). However, other effects, like the ones linked with marine debris and human toxicity, can only increase under this trade model that does not include reduction.

Moreover, China recently imposed an import ban on plastic waste, generating a shift in the trade directions to poorer countries and creating a new discussion about waste management in developed countries (Wang et al., 2020). As the global peak of waste is not supposed to be reached until at least 2100 due to increased population and consumption patterns, long-term solutions need to be designed. Here, developed and rich countries have a huge responsibility, specially towards some Asian and African countries, were the harmful effects of the traded plastic from rich regions are being substantial (Browning et al., 2021; Jambeck et al., 2015).

As seen, the issue of plastic pollution is not only an environmental and public health one, but also an economic, social, and political problem, and its consequences can be as catastrophic as uncertain. Therefore, when searching for solutions to the plastic/climate crisis, several vectors need to be considered, mostly in a bioeconomic framework. Here, integral solutions can be provided by the application of circular economy approaches.

1.2 ALTERNATIVE BIO-BASED PLASTICS AND BIOMATERIALS

Around 50% of the produced plastics are SUP (Galloway et al., 2017), which constitute the 49% of the marine litter in the EU (EC, 2018b). Moreover, it is estimated that about 95% of the economic value of plastic packaging materials is lost due to the single-use life cycles (EC, 2018a). Therefore, the EC recently addressed this issue by publishing a directive on the reduction of the impact of plastics on the environment (EC, 2019b), and by generating a new legal framework in the European Green Deal (EC, 2019) and the new *Circular Economy Action Plan for a cleaner and more competitive Europe* (EC, 2020); including actions to develop a framework on sourcing, labelling and use of bio-based, biodegradable and/or compostable plastics.

While biodegradable plastics can have petrochemical origin, bioplastics include a broad number of renewable, biodegradable and bio-based polymers that are expected to substitute conventional plastics, as they have similar physicochemical, thermal and mechanical properties (Mannina et al., 2020). Nowadays, the annual production of bio-based and/or biodegradable plastics is less than 1% of the global plastic production (Changwichan et al., 2018), being polyhydroxyalkanoates (PHA), polylactic acid (PLA) and polybutylene succinate (PBS) the biomaterials with more potential in the market. The three of them are produced involving microbiological processes.

For PLA and PBS, the building blocks of the polymer (the monomers, lactic, succinic acid and 1,4-butanediol, respectively) are produced by the activity of microorganisms, but monomer polymerization is not a natural process. However, for PHA, polymerization occurs naturally by the microorganisms, so the production is totally biological. Recently, PHA is gaining more attention, but its sustainability needs to be ensured before entering the value chains.

Therefore, the replacement of petrochemical materials by biomaterials could be very promising to solve the climate crisis and stop relying on oil. This is the main motivation for PHA production, but also for the generation of other bioproducts, like triacylglycerydes (TAG), which are lipids that can be used as a feedstock for biodiesel production (Pinto-ibieta et al., 2021).

TAG can be stored by microorganisms as intracellular granules and could replace petrochemical materials in a broad number of applications besides biodiesel, such as the pharmaceutical, cosmetical or food-processing industry (Argiz et al., 2020b; Tamis et al., 2015). However, not much is currently known about its production using biotechnology,

so other research works in the frame of TREASURE-TECHNOSALT[1] project aimed to produce it through the valorisation of waste streams. Although this introduction includes TAG, as its production process was one of the assessed ones in this thesis, this Chapter will focus on PHA, as it was the main biomaterial produced and evaluated in the present research work.

In summary, it is clear that, in the transition from linear to circular economy where value-added products from waste are generated to replace petrochemical materials, the strong commitment for bioplastics is one of the main targets of institutions like the EU (Di Bartolo et al., 2021).

1.2.1. Technological development in PHA production and mixed microbial cultures

PHAs are produced by some microorganisms subjected to stress conditions. If feedstock and nutrients are available transiently, some microbes respond by accumulating extra carbon that can be used in the case of feeding deprivation (Valentino et al., 2017). That extra carbon is stored inside the cells in the form of polyesters, which, after extraction, can be employed as base materials to replace petrochemical plastics. Therefore, in PHA production processes, carbon-rich feedstocks are transformed into value-added products by microorganisms, so they are an opportunity to fit the circular economy action plans by EU.

First studies on PHA production were based on pure substrates, mostly coming from dedicated crops such as sugar cane or palm oil, to feed pure cultures of microorganisms consisting in one single strain of bacteria able to store PHA. Under this circumstance, accumulating capacities are usually very high, reaching up to 90% of the cell content, with biomass concentrations that can be of 10 – 50 g/L (Marciniak and Mozejko-Ciesielska, 2021). However, the associated costs were high, reaching 45% of the total expenses due to high purity feedstocks use (Kourmentza et al., 2017). Besides, pure culture processes require sterile conditions, which implies high energy costs for reactor sterilisation (Bengtsson et al., 2017b). Finally, the use of dedicated crops involves an ethic conflict, as a global production of bioplastic based on agricultural feedstocks could alter the word's food supply chain (Sabapathy et al., 2020).

Research efforts are currently oriented to replace pure feedstocks by unexpensive substrates, such as waste streams. They are carbon-rich, and sometimes nitrogen and nutrient-rich, so they can also be used to feed the microorganisms in charge of PHA production. Moreover, waste streams are not competing at all with the food-chain supplies or land use, and they are environmentally burden-free (Yadav et al., 2020).

Accordingly, in the last decade, a wide amount of waste effluents were tested to produce PHA: cheese whey, sewage sludge, oil mill wastewater, glycerol, municipal wastewater, food processing effluents, molasses, paper mill wastewater, hardwood liquor, or Kraft mill effluents are just examples of the used streams reported in literature (Sabapathy et al., 2020). Moreover, the feasibility of using saline effluents has been also recently assessed (Argiz et al., 2020a; Pedrouso et al., 2020).

[1] TREASURE – TECHNOSALT: Facing the treatment/recovery of saline wastewater to assure future water availability. CTQ2017-83225-C2-1-R. From 2017 to 2021. National Spanish Agency of Research. https://biogroup.usc.es/treasure.

When waste streams are used for PHA production, pure cultures are often replaced by mixed microbial cultures (MMC), where a high diversity of microorganisms are present. Consequently, a selection strategy is required to favour the growth of the desired strains and disfavour the thrive of the non-storing populations of microorganisms (Kourmentza et al., 2017). In any case, On the other hand, although some processes using waste streams and MMC reached accumulations of 70 – 80%, most of the systems result in storages of 20 – 50% with biomass concentrations significantly lower than the ones of pure cultures (Mannina et al., 2020; Sabapathy et al., 2020). However, for axenic-based PHA productions, the benefits of high biopolymer production might be buffered by the costs of sterilisation, so MMC-based productions represent a big opportunity.

Therefore, although full-scale PHA production is currently dominated by pure culture systems (Sabapathy et al., 2020), which are generally crop-based (Yates and Barlow, 2013), the pilot-scale projects reported in literature and operated for PHA production are nowadays working with MMCs (Rodriguez-Perez et al., 2018), showing a switch trend in the culture type used.

As seen in Figure 1.1, this production pathway is generally performed in a three stage system (Figure 1.1) (Valentino et al., 2017):

i) The first step is a pre-treatment where the organic sources present in the feedstock (like carbohydrates or proteins) are transformed into suitable forms of carbon for PHA production through anaerobic fermentation (i.e. volatile fatty acids (VFA)), which will be naturally esterified and accumulated by bacteria (Kosseva and Rusbandi, 2018));

ii) Once the feedstock is fermented, the VFA-rich stream will be fed transiently to an open MMC that will be enriched in microorganisms with the capacity of accumulating PHA. This step is generally performed in a Sequencing Batch Reactor (SBR).

iii) The outgoing stream, rich in storing biomass, will be fed with the same VFA-rich stream where the MMC accumulating capacity will be maximized (Sabapathy et al., 2020). This step is generally performed in a Fed-Batch Reactor (FBR) and generates PHA-rich biomass.

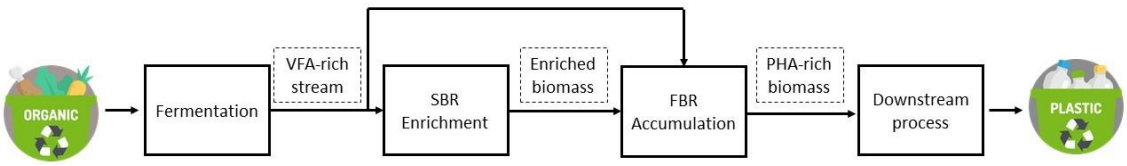


Figure 1.1. Flowchart of a typical MMC-based PHA production process

Depending on the substrate characteristics, this three-stage strategy has been also used to produce other biomaterials, like TAG (Fra-Vázquez et al., 2018; Tamis et al., 2015), achieving high accumulating capacities around 80% (Argiz et al., 2020b). However, when the feedstock employed is oily, there are other process features that need to be considered. For example, substrate concentration needs to be usually low, as oil normally presents high concentrations of Chemical Oxygen Demand (COD) per gram of substrate. This naturally leads to low biomass productions, so the productivity of the process is low although PHA accumulation is high. Nevertheless, this type of substrates also presents some advantages. As oily streams are composed by long chain fatty acids, the TAG production process can skip the acidification step by performing fatty acid

hydrolysis and MMC enrichment in the same reactor (Argiz et al., 2021, 2020b), providing new opportunities for the generation of this biomaterial

In any case, after microorganisms transformed the organic matter present in the waste stream into PHA or TAG, it is necessary to extract the stored material from the microbial cells (Figure 1.1). So, the Downstream Process (DSP) involves the activities required to recover and purify the PHA (or any accumulated biopolymer) from the biomass and make it available for its transformation into useful materials (i.e., compounding and shaping, see Figure 1.2). For PHA, studies still allocate 30 – 50% of the total production costs to DSP (Colombo et al., 2020), being, economically and environmentally, the most expensive stage of the whole MMC-based production process (Fernández-Dacosta et al., 2015). However, room for environmental and economic improvements are also present for low cost processes aiming to generate low grade PHA (Saavedra del Oso et al., 2020).

Nowadays, the existing DSPs are divided in two categories: the ones that separate biomass from PHA by dissolving the polymers, and the ones that dissolve or mechanically disrupt the cellular material to release the PHA (Jiang et al., 2018; Kosseva and Rusbandi, 2018; Yadav et al., 2020) (Figure 1.2). Generally, the process starts with the concentration of the PHA-rich biomass (Pérez-Rivero et al., 2019). Then, different treatments are used to weaken the cell membrane before it is disrupted. Although the most applied pre-treatment involves freeze-drying the biomass (Rodriguez-Perez et al., 2018), it presents technical and economic difficulties for its full-scale application, so other techniques, like thermal dehydration (Kourmentza et al., 2017) or osmotic shocks (Koller et al., 2013a; Kourmentza et al., 2017; Rathi et al., 2013) have been researched.

Once the cell is weakened or broken up, the methods directly dissolving the biopolymeric material usually employ solvents that change the permeability of the cell membrane. It provides a high-quality final product, but the use of chlorinated solvents is economically and environmentally detrimental (Fernández-Dacosta et al., 2015; Saavedra del Oso et al., 2020).

The alternative to PHA direct solving is cell digestion, which is normally chemical: acids, alkalis, or, recently, surfactants (Fernández-Dacosta et al., 2015; Mannina et al., 2019; Rathi et al., 2013). Here, the sodium dodecyl sulphate (SDS) is the most employed one (Pérez-Rivero et al., 2019). This detergent enters the lipid membrane and increases the volume of the cell envelope until it breaks down, so the PHA, is released. This detergent enters the lipid membrane and increases the volume of the cell envelope until it breaks down, so the PHA is released. This method obtains good results when combined with other treatments, like alkali addition (Fernández-Dacosta et al., 2015; Rathi et al., 2013), so at present surfactant treatment is, economically and environmentally, the most promising alternative for low-grade PHA extraction (Fernández-Dacosta et al., 2015; Saavedra del Oso et al., 2020).

In fact, the costs of solvent-based PHA extraction, normally aiming to produce high grade biopolymer to be used in applications like pharmaceutical, are estimated to range 0.25 – 2.25 €/kg PHA. On the other hand the cost of PHA obtained by cell digestion/mechanical disruption, which produces low grade biopolymer to be used in low value applications like packaging costs around 0.25 – 1.50 €/kg PHA (Saavedra del Oso et al., 2020).

Once the polymer is separated, its final uses are multiple and depend on its properties (Roohi et al., 2018). These vary with the feedstock composition and the extraction method

(Bengtsson et al., 2017b; Melendez-Rodriguez et al., 2018), as monomer distribution and chain length are two of the main issues defining these final properties (Raza et al., 2018). Although much is still to be known until the tailoring of the PHA materials is perfectly understood, it seems likely that polymers only composed by butyrate monomers (polyhydroxybutirate (PHB)) can substitute some polypropylene (PP) and polyethylene terephthalate (PET) applications (Lamberti et al., 2020; Melendez-Rodriguez et al., 2018; Roohi et al., 2018). Moreover, the presence of valerate monomers (so polyhydroxyvalerate or PHV) can also affect the final properties of the polyester, modifying the crystallinity or the melting temperature in comparison to polymers composed only by PHB (T Palmeiro-Sánchez et al., 2016).

Common PHA manufacturing techniques are moulding and injection (Bengtsson et al., 2017b; Changwichan et al., 2018) as well as electrospinning (Melendez-Rodriguez et al., 2018) (Figure 1.2). Besides, it has been stated that the combination of PHA and PLA fibres can produce materials with improved final properties (Bengtsson et al., 2017b; Lamberti et al., 2020), and composites formed by PHA, PLA and PBS reinforced with non-woven flax fibres achieve the necessary requirements to be employed in the car industry (Pantaloni et al., 2020).

Finally, poor-grade PHA (that did not achieve the market specifications) or wasted (after several use cycles) PHA films can be further valorised in feedstock recycling to produce crotonic acid and its oligomers (Figure 1.2), which are used as building blocks in the production of some chemicals, like paints or adhesives (Fernández-Dacosta et al., 2016; Lamberti et al., 2020).

In any case, after consumer use, these bio-based materials need to be properly disposed and managed at their EoL (Figure 1.2). Here, the waste management options are landfilling, incineration, anaerobic digestion, composting and recycling (Yates and Barlow, 2013). Although PHAs are biodegradable, their degradation is complex and hard to model, as it is dependent of many factors (Laycock et al., 2017).

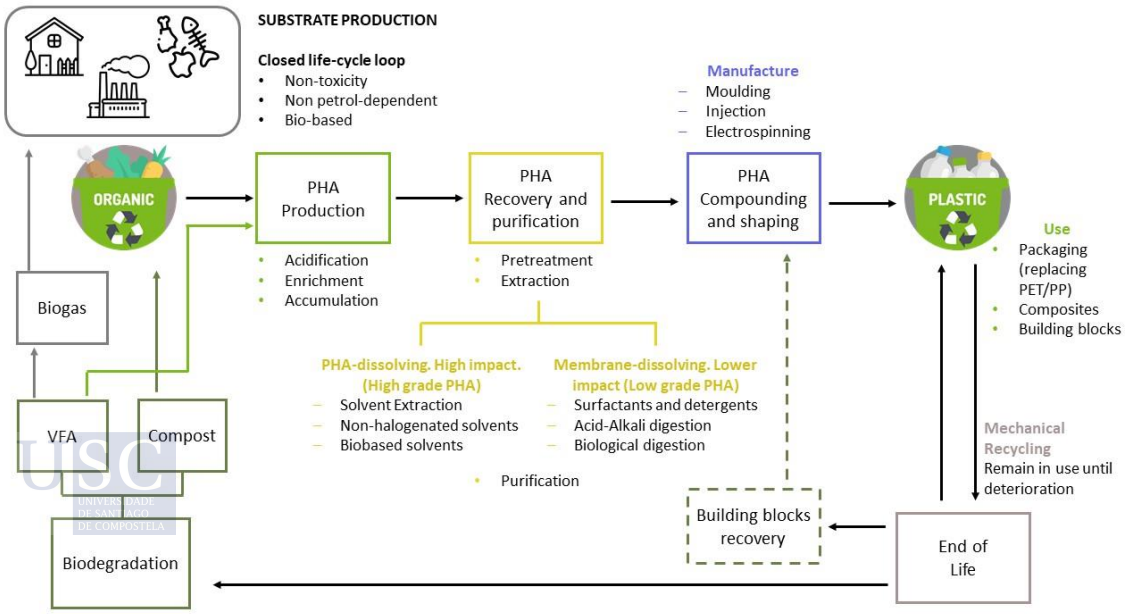


Figure 1.2. Life cycle of a biopolymer in the frame of circular economy

There is some sort of paradox regarding bioplastics at this point, as they need to be completely biodegradable, but their properties need to endure for some time, responding to products' shelf-life and life cycle uses. PHA readily biodegrades in compost, soil and marine environments, although, in absence of microorganisms and ambient conditions (mainly UV radiation), it remains stable (Lamberti et al., 2020). Moreover, PHA shows a good anaerobic biodegradability (Battista et al., 2021) and it is completely composted after 10-22 months (Arcos-Hernandez et al., 2012). However, the EN 13432 standard states a time from three to six months of biodegradation time for a material to be defined as compostable (EN, 2007), so the management of PHA as organic/compostable waste can be complex.

Despite the lack of global data, suitable EoL routes have been proposed. They start with several use cycles in which mechanical recycling is involved, as PHA is biodegradable but endurable, and its properties are maintained after several extrusion cycles (PHBV remains almost unaltered after five cycles), where the number of re-uses might be improved by combining PHA with PLA (Lamberti et al., 2020). Environmentally, the EoL has low contributions to the total impact in the lifecycle (Yates and Barlow, 2013), and recent studies confirmed that mechanical recycling is the best alternative for the EoL of SUP (Changwichan et al., 2018). Therefore, after several use-cycles, the circular economy loop can be closed employing the deteriorated PHA to produce biogas through anaerobic digestion, compost through aerobic mineralization, new PHA (as its carbon can be used as biomass feedstock), or to generate crotonic acid as a building block in the green chemistry industry by mild temperature pyrolysis (Lamberti et al., 2020).

There is still a regulatory gap between bioplastics production and its market, and specific regulations have not yet been implemented due to several factors, like the lack of full-scale demonstrations and the few data generated regarding the final properties and applications of the product. Nonetheless, policies are being proposed and the EU stablished a legal framework to begin shaping the general regulations which will rule bioplastic production, use and management for the next years (EC, 2020, 2018a, 2018b). Here, the EC engages to develop specific policies to manage the use of bio-based and biodegradable plastics and the gradual change to a circular industrial and consumption mode. According to the OECD (2013), the proposed policies must provide supportive environments which will let bioeconomy growth by employing different tools, like funding research and innovation, or supporting the early market (for example, by tax incentives). A good example of the efforts that are being performed to uniformly introduce these products in the market ensuring environmental safety and legal consistency is, por example, the EU Taxonomy, that aims to to create an EU-wide classification system for sustainable activities in order to meet the EU's climate and energy targets for 2030 and reach the objectives of the European Green Deal (EC, 2022).

1.2.2. Environmental validation of PHA

New sources for plastic production like PHA are being researched and it is mandatory to prove the environmental benefits of those alternatives compared to the non-renewable ones, to stablish criteria for their applications and safe disposal (European Commission, 2018a). In this context, Life Cycle Assessment (LCA) has already been widely used to assess the environmental performance of different processes and products, and particularly of PHA production processes (Bengtsson et al., 2017b; Fernández-Dacosta et al., 2015; Morgan-Sagastume et al., 2016).

However, recent investigations pointed out the urgent need of studies covering all process stages for waste-based PHA production, as its non-existence is drawing back the scale-up and commercialization of PHA (Mannina et al., 2020). So, due to the use of sterile operating conditions and pure substrates (like wheat, corn or glucose), the bioplastic production by pure cultures result in higher environmental impacts (like GHG emissions) than the ones of producing petrochemical materials (Bengtsson et al., 2017b; Fernández-Dacosta et al., 2015), providing then only a partial solution, or even worsening the problem.

Moreover, although the proportion of land used to produce bioplastics is marginal, the so-called first-generation bioplastics raised some controversy regarding the competitive use of these crops. In fact, even if non-food crops (like lignocellulosic biomass) is used to produce bioplastics, this could subliminally distress food production by striving for land with food crops (Yadav et al., 2020). Furthermore, the production of PHA from dedicated substrates could highly impact in categories such as eutrophication or acidification due to the use of fertilizers to harvest the raw materials (crops) (Yadav et al., 2020).

Therefore, the second-generation bioplastics use residues (like wastewater, oil, or the organic fraction of municipal waste) to generate biomaterials, accomplishing the principles of circular economy and providing potential ways to reduce the environmental and societal impacts linked to first-generation bioplastics.

Although literature on MMC-based PHA production using waste substrates is very extensive (more than 400 documents are found in Scopus when typing "polyhydroxyalkanoates" and "mixed cultures"), the truth is that the number of studies that focuses on their environmental performance is scarce. In fact, only 60 studies assessing the environmental impacts of PHA production were published in the last two decades, of which only 11 evaluate the environmental profile of MMC-based processes (Roibás-Rozas et al., 2022b). Considering that biopolymers production like PHA is considered one of the main tools to face the environmental/plastic crises, it is clear that more studies validating their environmental profile are necessary and urgent.

Furthermore, despite the mentioned efforts to uniformize the research outputs and provide standard regulations, there is still a long way to go until consensus is reached. In fact, although a general methodology has been stablished to perform LCA of bio-based materials (particularly, bioplastics) (Nessi et al., 2020), different sectors and institutions linked with the circular economy and its application already expressed disconformity with the proposed protocols (European Bioeconomy Alliance, 2021). Therefore, research efforts should be oriented to provide a structure and a framework for this consensus.

Moreover, several methodological gaps are hindering the mentioned environmental validation of PHA. Mainly, the fact that plastic leaks in the environment are hard to quantify (Plastic Leak Project, 2020), and their effects are still being discussed (Boulay et al., 2021; MarILCA, 2020), so it is also hard to include the impacts of these leaks in the environmental frameworks as well as to model the expected positive effects of biomaterials EoL management (Roibás-Rozas et al., 2022b). Therefore, it is necessary to critically improve the current frameworks and to provide new case studies to generate a robust background for evaluation.

Finally, there is a specific gap linked to the use of halophilic microorganisms and saline streams for biomaterials production, both involving the use and disposal of high

salinity effluents (Mosquera-Corral and Campos Gómez, 2021). Until now, methodologies modelling the effects of salinity variations in aquatic environments did not exist, as current LCA methodologies lacked frameworks to assess these effects, presenting a new challenge for the evaluation of MMC-based PHA production using saline streams.

The need of these models is fuelled as the use of saline streams is increasing. In fact, recently, halophilic biomass was proposed for PHA production and biotechnological processes due to some potential advantages over regular (non-halophile) biomass (Kourmentza et al., 2017; Simó-Cabrera et al., 2021). For example, contamination of the selected MMC is less likely because salinity offers an extra selective pressure for the culture. Moreover, DSP could be improved, as cell lysis for extraction could be performed by using osmotic shocks by tap water addition to the saline media, avoiding the use of chemicals (Mitra et al., 2020). Therefore, research regarding the implications of using and disposing high salinity streams is necessary.

1.2.3. Looking for integral validation: is there enough information about PHA production costs?

Nowadays, 1 kg of most petrochemical conventional plastics cost less than 1 €, while the commercial prices of pure culture PHA still range between 1.5 and 5.0 €/kg (Kumar et al., 2020). Therefore, the MMC-based route is a promising alternative to increase competitiveness of bioplastics production, and costs may range 2 – 3 €/kg PHA (Crutchik et al., 2020; Fernández-Dacosta et al., 2015).

It is clear then that there is still a great economical drawback, mainly linked to the lower overall yields of the typical three-stage process for MMC-based PHA compared to the ones of pure cultures (Valentino et al., 2017). In fact, pure culture PHA processes reach 90% accumulation inside the biomass with 1.0 – 1.3 g PHA/(L·h) productivity, while MMC-based PHA reaches up to 75% accumulation and production rates of 1.2 g PHA/(L·h) (Mannina et al., 2020).

Besides, there is an extra cost for MMC-based PHA linked to the required fermentation unit (Kumar et al., 2020), which is usually the biggest reactor (thus, linked to higher capital costs). In this sense, it is important to notice that the production of PHA from oily streams (which can potentially lead to the simultaneous production of PHA and TAG) would save the acidification stage by combining the hydrolysis and enrichment of the MMC in the same reactor (Argiz et al., 2021). However, other drawbacks, like low biomass concentration, still need to be addressed. Moreover, DSP operations needed to extract the PHA from the bacterial cells in mixed cultures can be even more expensive than those for pure cultures due to different cell wall fragility with respect to single strains (Mannina et al., 2020). In fact, it was found out that PHA granules might have a structural function for bacteria, precisely to avoid the cell wall breakdown when the microorganism is exposed to variable conditions in the media (Sedlacek et al., 2019). This can hinder cell disruption for non-genetically engineered strains and suppose a drawback for PHA production by MMC.

In general, studies for the economic validation of PHA production are still scarce, and most of the works are focused on pure cultures. Moreover, full validation of emerging technologies cannot be performed by just providing economic or environmental outputs. Integral results must include multidimensional approaches (Talan et al., 2020) which could also cover stakeholder inclusion or marketability assessment, among others

(Kehrein et al., 2020). Therefore, meaningful results of a technology evaluation in the framework of bioeconomy can only be expected if integral approaches are considered. In this context, at least economic and environmental outputs should be included, although, ideally, also societal impacts should be assessed (Pérez et al., 2020a; Talan et al., 2020).

Few studies assessed the techno-economic plus environmental feasibility of PHA production using MMC, pointing out the necessity of generating new information to validate the economic/environmental profile of these processes (Mannina et al., 2020). Nevertheless, the scarce studies that addressed the issue (Andreasi Bassi et al., 2021; Bengtsson et al., 2017b; Fernández-Dacosta et al., 2015; Gurieff and Lant, 2007; Pérez et al., 2020b; Vega et al., 2020) confirmed that the DSP is still one of the main drawbacks for the production of these biomaterials, and that MMC-based processes are preferable than systems based on pure strains. Finally, the economic evaluations of PHA production processes might be subjected to similar uncertainties than the environmental ones. As PHA value chains are still not fully stablished, and bioplastics were just recently (and limitedly) introduced in the market, it is hard to really quantify some potential benefits linked to the circular economy value creation.

Therefore, the generation of new research works and case studies will shape a general framework to start understanding how biobased materials like PHA can add value to the market chains. There are already studies proving that PHA could overcome the economic performance of some petrochemical materials when produced from different waste streams (Andreasi Bassi et al., 2021). For this reason, now that technical feasibility of PHA production has already been proved, the scientific community should orient their efforts to provide a robust socio-economic and environmental framework that could help policy makers to regulate the introduction of biomaterials in the value chains.

1.3 CIRCULAR ECONOMY AT A REGIONAL LEVEL: THE FISH CANNING INDUSTRY IN GALICIA

The canning and processing industry of seafood is one of the main economic driving forces of Galicia (Northwest of Spain), where 85% of the canned fish and seafood of the entire Spanish territory is produced. Here, it is estimated that in around 300,000 tons of fish and seafood are processed annually (mainly tuna, although the volume of sardines, mussels and cockles is also relevant), reaching an economic value of about 1.25 billion euros per year (González-López, 2018).

However, in addition to significant economic revenues, this sector generates high amounts of waste streams, as fish canning effluents are highly polluted. They usually have high organic load, and they normally contain significant concentrations of nitrogen, salt, and solids, among other components (Argiz et al., 2020b; Cristóvão et al., 2015; Fra-Vázquez et al., 2020; Pedrouso et al., 2020). Although the amount of wastewater generated is hard to estimate and depends on the process and the processed raw material, effluent generation can be in the range of 5 m^3 of wastewater per ton of cooked product (Barros et al., 2009; Bello Bugallo et al., 2012).

For this reason, nowadays, fish canning companies need to make large capital investments for the construction and operation of wastewater treatment systems to minimize their impact on the receiving media, mostly the coastal areas of the estuaries. This is especially important in a context in which the viability of canneries depends, among other factors, on the quality of the waters from which the raw material (fish and shellfish) is obtained.

The quality of the coastal and offshore waters is also highly affected by plastic pollution, since approximately 90% of the waste on the beaches and Atlantic coasts is attributed to plastic (Lacroix et al., 2020). In this sense, recent reports already confirm the negative economic impacts that the fishing, aquaculture and tourism sectors, among others, are suffering due to this environmental crisis (Conejo-Watt and Luisetti, 2019). Moreover, these effects are expected to be extensive to other dimensions of human life, like food security (Muluneh, 2021).

It is at this point where bioplastics production arises as a key player to reduce plastic waste pollution and to foster circular economy strategies on the management of canning industries effluents, which would not only be useful when it comes to improving environmental performance in the sector, but it would also have a special relevance when it comes to improving its economic viability.

The valorisation of effluents from the fish canning industry in Galicia is present in the Biogroup[2] research agenda since 2011, thanks to the national funded project PLASTICWATER[3] that aimed to the production of bioplastics employing wastewater and sludge, and evaluated the influence of salinity in the production of PHA using a synthetic influent (T. Palmeiro-Sánchez et al., 2016). Then, the feasibility of the production of biopolymers employing real feedstocks (Fra-Vázquez et al., 2020, 2018; Pedrouso et al., 2020) was explored by the FISHPOL[4] project, where PHA production employing mussels cooking wastewater (MCW) was assessed. Furthermore, the integration of nitrogen removal and PHA production was evaluated (Fra-Vázquez et al., 2019; Santorio et al., 2019).

Finally, the TREASURE-TECHNOSALT project aimed to unravel the effects of salinity in wastewater valorisation, by proving that circular economy-based and resource recovery strategies are suitable to provide feasible solutions for the fish canning industry. To do so, only raw effluents from the fish canning industry were used (avoiding the use of synthetic mixtures or tap water dilution). Besides, the lessons learnt after several years of experience were evaluated to stablish a potential pilot or full-scale production. Finally, some methodological gaps hampering the evaluation of these processes were addressed, like the methodological tools to assess the effects of salinity variations in aquatic environments in LCA. In this sense, the outputs here obtained supported, by providing a technical background, new projects framed in the same research line are aiming to include industrial stakeholders to prove the technology at pilot scale.

Finally, at present, the project ECOPOLYVER (currently ongoing) aims to continue the work carried out in TREASURE-TECHNOSALT. Therefore, the validation of the processes developed in TREASURE project is tested at pilot scale in ECOPOLYVER. Here, the aspects linked to the holistic validation of the technology (employing environmental and economic assessments), and developed in TREASURE, will be considered.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

[2] https://biogroup.usc.es/

[3] Recycling of wastewaters and sludges to produce bioplastic materials (CTQ2011-22675) from 2011 to 2014.

[4] Sustainable system for the valorisation of fish canning wastewater for biopolymers production (CTQ2014-55021-R). From 2015 to 2018. https://www.usc.es/biogroup/fishpol

1.4 THESIS ESTRUCTURE AND OBJECTIVES.

The present thesis addresses the implementation of circular economy strategies by considering some of the essential stages necessary for the introduction of a new process or product in the value chains after technical validation: i.e. techno-economic and environmental assessment (Kehrein et al., 2020; Talan et al., 2020). It focuses on PHA production, but also touching the feasibility of generating other bioproducts, such as TAG and VFA.

It was carried out in the timespan of four years with the financial support of the TREASURE-TECHNOSALT project and it aimed to develop a technology from its early stage to its potential evaluation in the full-scale market (Figure 1.3). According to the circular economy framework, the technologies developed and evaluated used waste streams from the fish canning industry to valorise them into biomaterials (mainly PHA).

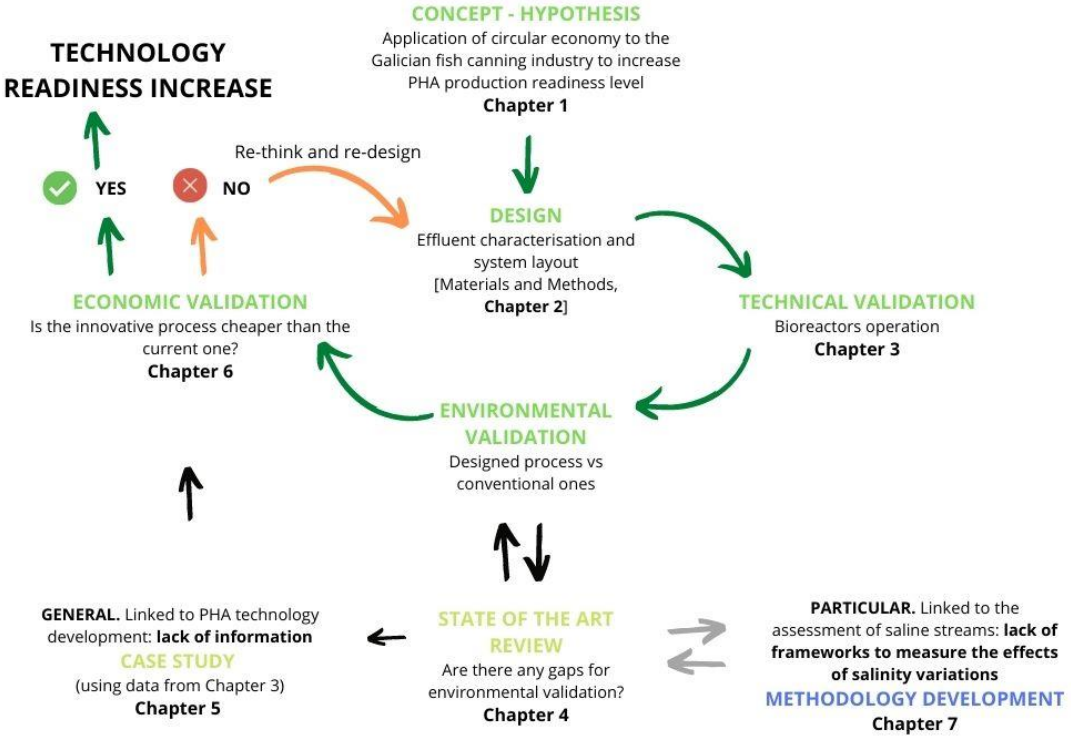


Figure 1.3. Scheme of the technology development stages followed in the course of the present work

Therefore, our hypothesis is that it is feasible and economically attractive to apply strategies based on circular economy in the Galician fish canning sector. If proved, the readiness level of the innovative processes here tested would be ready to increase. The present thesis aims to bring some light into technology development and PHA introduction in the value chains in the frame of circular economy and in the context of a global crisis. Therefore, the main objectives of this research work are:

1. Prove the technical feasibility of transforming complex waste effluents coming from the fish canning industry into value-added products, focusing on the production of PHA (**Chapter 3**).

2. Assess the current available framework to evaluate the environmental profile of biomaterials and processes based on circular economy and emerging technologies by performing a state-of-the-art review (**Chapter 4**).

3. Validate the environmental profile of the designed process and of the generated product by LCA, acknowledging the considerations extracted from the review (**Chapter 5**).

4. Prove the economic validation of the proposed systems, ensuring that the economic vector is acknowledged when technologies are evaluated in a bioeconomic framework (**Chapter 6**).

5. Fill some of the methodological gaps found when environmentally assessing bioprocesses and biomaterials, particularly focusing on the ones using saline effluents. In this case, a new methodology to assess the effects of salinity variations in aquatic environments was proposed (**Chapter 7**).

As seen, this thesis aims to contribute to the readiness level increase of emerging technologies based on circular economy by providing a systematic structure to validate the performance of the assessed technology. Therefore, the methodology shapes the structure of the thesis itself (Figure 1.3):

Chapter 1 and Chapter 2 provide an introduction and a methodological overview of this work:

- **Chapter 1**. provides the **hypothesis** to be tested in this document. Here, the necessity of generating new biomaterials in the context of the plastic and environmental crises is exposed. The **concept** to be tested (a MMC-based PHA production system fed by MCW) is presented.

- **Chapter 2** describes the methods used to perform the current research work: experimental protocols for technical validation and assessment methodologies for the environmental and economic validation. Therefore, the tool for the **design** of the MMC-based system and this methodology itself are presented.

Then, Chapters from 3 to 7 address the results obtained.

- **Chapter 3**. First, the concept presented in Chapter 1 (PHA production process under saline conditions) was tested by subjecting it to **technical feasibility validation**. Therefore, the system of bioreactors was first designed. Then, the design was put into practice by the laboratory-scale operation of about two years.

- **Chapter 4.** Here, the challenges faced to evaluate the environmental profile of MMC-based processes were assessed in a methodological **state of the art review**. Therefore, the research works addressing this issue were critically analysed hoping to stablish a robust methodological framework to perform the **environmental validation** of the studied process.

- **Chapter 5.** Once identified the existing tools to evaluate the environmental profile of the developed process, the data generated in Chapter 3 was used to perform an **environmental evaluation** of the proposed MMC-based PHA production process through a **case study**. This chapter aims to contribute to fill one of the general gaps found in Chapter 4: the lack of data for environmental verification of PHA production systems based on circular economy.

- **Chapter 6.** Once the technical feasibility and the environmental benefits of introducing these materials in the value chains are proved, it is necessary to provide

economic validation of the assessed processes as well. Therefore, techno-economic analysis (TEA) and Life Cycle Cost Assessment (LCCA) was performed.

- As Chapter 5 aimed to contribute to fill a general gap found for PHA production systems, **Chapter 7** aims to improve to the assessment of technologies using saline streams by contributing to **methodology development** in LCIA. Therefore, a new methodology to assess the effects of salinity variations in aquatic environments was stablished, addressing both negative and positive effects of emission-related impacts.

Chapter 8 sums up the main conclusions of the work.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

2. METHODOLOGY

SUMMARY

This chapter provides an overview of the materials and methods used for the research done. It is then divided in two sections, acknowledging the work carried out experimentally and in the field of process evaluation. They cover: i) the experimental protocols and analytical methods used to follow up the bioreactors operation, and ii) the evaluation methodologies used for the processes assessment to increase technology readiness level, including Life Cycle Assessment (LCA) for the environmental evaluation, with special focus on the Life Cycle Impact Assessment (LCIA) stage, and Life Cycle Cost Assessment (LCCA) and Techno-Economic Analysis (TEA) for the economic evaluation.

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

2.1. ANALITICAL METHODS

2.1.1. Characterization of the liquid phase

2.1.1.1 Chemical Oxygen Demand (COD)

The COD is defined as the amount of oxygen required to oxidise all the organic matter present in a sample (like wastewater) using a strong chemical oxidant (in this case, potassium dichromate) in acidic conditions. The catalyst silver sulphate is used to ensure the total oxidation of all organic compounds during the digestion of the samples. The global reaction of the process is:

$$Cr_2O_7^{-2} + \text{Organic Matter} + H^+ \leftrightarrow Cr_3^+ + CO_2 + H_2O$$

Hence, this measurement is performed to determine the concentration of organic matter in an analysed sample. After digestion, the remaining unreduced $K_2Cr_2O_7^{2-}$ is titrated with ferrous ammonium sulphate (FAS) to determine the amount of potassium dichromate consumed, being the amount of oxidable (reduced) matter calculated in terms of oxygen equivalents.

As the presence of Cl^- interferes with the reaction, the method described in Soto et al. (1989), that acknowledges the presence of salinity in the water samples, was applied when they had a concentration of salt that generated a ratio Cl^-/COD above 2 (g/g). This method was an adaptation of the one stablished by APHA-AWWA-WEF (2017), which was used for the remaining samples. Total COD and soluble COD (referred in the text as COD_T and COD, respectively) were measured by these two methods.

The reagents are:

- Potassium dichromate digestion solution: 10.23 g of $K_2Cr_2O_7$, 33 g of $HgSO_4$ and 167 mL of concentrated H_2SO_4 are diluted in 1 L of distilled water.
- Sulphuric acid catalytic solution: 10.7 g of Ag_2SO_4 are dissolved in 1 L of concentrated H_2SO_4. The solution can be used after 2 days of preparation.
- Ferroin indicator solution: 1.49 g of $C_{18}H_8N_2 \cdot H_2O$ (phenanthroline monohydrate) and 0.69 g of $FeSO_4 \cdot 7\ H_2O$ are dissolved in 100 mL of distilled water.
- Standard potassium dichromate solution 0.05 N: 1.23 g of $K_2Cr_2O_7$, previously dried at 105°C for 2 hours, are dissolved in 500 mL of distilled water.
- Ferrous ammonium sulphate titrant (FAS) 0.035 N: 13.72 g of $Fe(NH)_4(SO)_2 \cdot 6$ H2O and 20 mL of concentrated H_2SO_4 are dissolved in 1 L of distilled water.

Determination procedure:

A volume of 2.5 mL of each sample is placed in 10-mL Pyrex® glass tubes. Then, 1.5 mL of digestion solution and 3.5 mL of sulphuric acid reagent are added to the tube with the sample. A blank sample using distilled water is prepared in the same way to provide a reference for the zero value. The glass tubes are sealed with Teflon® and covered with Bakelite® caps to avoid the leakage of gases and liquids due to possible boiling. Then, the tubes are shaken and placed for 2 h in the thermodigester (VELP ECO16) preheated to 150 °C. After digestion, the tubes are cooled to room temperature. Then, the content of the tubes is transferred to a beaker and, after the addition of a couple of drops of ferroin indicator, the solution is titrated while stirring with standard FAS. The FAS solution is standardised as follows: 5 mL of distilled water are put into a small beaker and 3.5 mL of sulphuric acid reagent are added as well. Then, the solution is cooled to room temperature and 5 mL of standard potassium dichromate solution are added together

with several drops of ferroin indicator to enable the titration with standard FAS. The endpoint is visualised by a sharp colour change from blue green to red. The COD concentration (in mg COD/L) of the different wastewater samples is calculated with the Equation 2.1:

$$\text{COD (mg/L)} = \frac{(\text{A} - \text{B}) \cdot 8000 \cdot \text{N}_{\text{FAS}}}{\text{V}} \quad \text{Eq. 2.1}$$

Where A is the volume of FAS consumed by the blank (mL), B is the volume of FAS consumed by the sample (mL), 8,000 represents the milliequivalent weight of oxygen in 1,000 mL/L, V is the volume of sample used in the titration procedure, equal to 2.5 mL, and N_{FAS} is the normality of the FAS solution (N), calculated as in Eq. 2.2:

$$\text{N}_{\text{FAS}} = \frac{\text{V}_{\text{K}_2\text{Cr}_2\text{O}_7} \cdot \text{N}_{\text{K}_2\text{Cr}_2\text{O}_7}}{\text{V}_{\text{FAS}}} \quad \text{Eq. 2.2}$$

Where $V_{K_2Cr_2O_7}$ is the added volume of the standard $K_2Cr_2O_7$ solution, equal to 5 mL. $N_{K_2Cr_2O_7}$ is the normality of the standard $K_2Cr_2O_7$ solution, equal to 0.05 N. V_{FAS} is the volume of FAS solution consumed in the titration procedure.

2.1.1.2 Total Organic Carbon (TOC), Total Carbon (TC), Inorganic Carbon (IC), and Total Nitrogen (TN)

Organic carbon in liquid samples may include a variety of organic compounds. Measurement of Total Organic Carbon (TOC) is performed according to the method 5310 of the Standard Methods for the Examination of Water and Wastewater (APHA-AWWA-WEF, 2017). To determine the quantity of organically bound carbon in a liquid sample, the organic molecules must be broken down, so the amount of carbon is quantified. The TOC concentration is determined by a Shimadzu analyser (TOC-L CSN) as the difference between the Total Carbon (TC) and the Inorganic Carbon (IC) concentrations.

A curve comprising calibration points in the range of 0.5 to 1,000 mg C/L is used for the quantification. Potassium phthalate ($C_8H_5KO_4$) and a mixture of sodium carbonate and bicarbonate (Na_2CO_3/$NaHCO_3$, 3:4 w/w) are used as standards for TC and IC determination, respectively. The TC concentrations is determined by the amount of CO_2 produced during the combustion of the sample at 720 °C, using a platinum catalyst. The IC concentration is obtained from the CO_2 produced from the reaction at room temperature with a hydrochloric acid (HCl) 1 N reaction at room temperature. High purity air is used as carrier gas with a flow of 150 mL/min. The streams are cooled and dried at room temperature. The CO_2 produced is optically measured with a nondispersive infrared detector (NDIR), which generated a peak with an area related to the concentration of the compounds.

The Total Nitrogen (TN) is determined in the same Shimadzu analyser (TOC-L CSN). The sample is injected in the combustion tube where the oxidative pyrolysis occurs at 720 ˚C, to convert all nitrogenous compounds into nitric oxide gas (NO). The flow of gas is cooled to eliminate any possible condensation. Then, it reaches a detector where NO reacts with ozone (O_3) to obtain nitrogen dioxide in an unstable excited state (NO_2*). The NO_2* reaches its elemental state by emitting a photon in the range of 590 - 299 nm. Then, it is detected by a chemiluminescence detector (CLD). The calibration is carried out by a standard commercial solution of ammonium (Fluka) in the range of 0.5 - 777 mg N/L.

2.1.1.3 Volatile Fatty Acids (VFAs)

The VFAs are those monocarboxylic acids that contain 2 to 5 carbon atoms, from acetic to n-valeric acid. They are intermediate products in the anaerobic digestion for the production of biogas and generally necessary to produce Polyhydroxyalkanoates (PHAs) from wastewater using Mixed Cultures. As such, measuring their concentration is of key importance when monitoring the PHA production processes. VFAs were determined by gas chromatography (GC) (AGV-DB1 method).

To a volume of 1 mL of sample 10 µL of H_3PO_4 (85%) are added. The sample (1 µL) is injected to a gas chromatography (GC) (6850 Series II, Agilent Technologies). The instrument is equipped with a flame ionisation detector (FID) and an automatic injector. The determination is performed using a glass column (30 m x 0.250 mm x 0.25 µm). Nitrogen is the carrier gas at a flux of 45 mL/min. The injector and detector temperatures are maintained at 250 and 300 °C, respectively. The oven is programmed to begin at 70 °C and increase the temperature at a rate of 15 °C/min to 130 °C; increase at a rate of 3 °C/min to 150 °C; increase at a rate of 10 °C/min to 180 °C and remain there for 1 min; increase at a rate of 65 °C/min to 250 °C, and hold at 250 °C for 5 min. Air and H_2 are used as auxiliary gases with flows of 400 and 40 mL/min, respectively. The VFAs are separated in the column according to their molecular weights.

A commercial VFA mixture dissolved in deionised water is employed as standard (Supelco, USA). The obtained chromatogram gives information about the area of the obtained peaks corresponding to each analysed VFA. Each VFA is identified by the retention time of its corresponding peak. This is made with a calibration curve for each acid in the range of concentrations of 0 - 0.6 g/L, which is related with the area of each peak.

2.1.1.4 Ammonium

Ammonium concentration is determined spectrophotometrically using the method proposed by Bower and Holm-Hansen (1980). Here, indophenol blue is produced by the reaction of the ammonium ions, at pH 12.6, with salicylate and hypochlorite ions, in the presence of sodium nitroprusside as catalyst. The characteristic blue colour produced by increasing concentrations of ammonia makes the assay useful for the direct, visual estimation of ammonia in culture systems.

The reagents are the following ones:

- Reagent A: solution of 0.28 g/L of sodium nitroprusside ($C_5FeN_6Na_2O$) and 440 g/L of sodium salicylate ($C_7H_5NaO_3$).
- Reagent B: solution of 18.5 g/L of sodium hydroxide (NaOH) and 120 g/L of trisodium citrate ($Na_3C_6H_5O_7$).
- Reagent C: standard commercial solution of sodium hypochlorite (NaOCl) with 4.00 to 4.99 % active chlorine.
- Reagent D: solution prepared by mixing 7 parts of reagent B and 1 part of reagent C. Reagent D is stable for 1 hour after preparation.

Determination procedure:

Reagent A (600 µL) and reagent D (1 mL) are added to 5 mL of sample. This mixture needs to be performed under a gas extraction device due to possible leakage of hazardous vapour. Samples with reagents are shaken and stored in obscurity, to react for more than

2 hours but less than 3 (otherwise procedure is not valid). The measurements of the coloured samples are done with a spectrophotometer (Shimadzu, UV-1800) at a wavelength of 640 nm. The quantification is done with a calibration curve in the range of 0 – 0.9 mg NH_4^+-N/L using a commercial solution of NH_4Cl.

2.1.1.5 Proteins

The protein determination is done according to the Lowry's method (Lowry and Randall, 1951). This procedure is based on two reactions. First, Biuret reaction, in which the alkaline cupric tartrate reagent complexes the peptide bonds of the protein. Then, the reduction of the Folin and Ciocalteu's phenol reagent, which yields a purple colour, indicates the presence of proteins.

Note that the reagents must be stored at 4 °C. Solution A and B must be prepared the same day of the analysis. Therefore, the reagents are:

- Copper sulphate solution (1%): 1 g of $CuSO_4$ is dissolved in 100 mL of distilled water.
- Sodium tartrate solution (2%): 2 g of $C_4H_4O_6Na_2$ are dissolved in 100 mL of distilled water.
- NaOH 1 N solution.
- Solution A: 2 g of Na_2CO_3 and 1 mL of $CuSO_4$ solution and sodium tartrate solution are dissolved in 100 mL of distilled water.
- Solution B (Folin and Ciocalteu's phenol reagent): 1 mL of commercial Folin and Ciocalteu's phenol reagent are dissolved in 10 mL of distilled water.

Determination procedure:

A sample volume of 0.5 mL is rapidly mixed with 0.5 mL of NaOH 1 N solution and 5 mL of solution A and left for 10 minutes at room temperature. Then, 0.5 mL of solution B are added and mixed, and after 30 minutes the absorbance is ready to be measured. A blank with distilled water and the same reagents as the samples is also measured as reference for zero value. The measurements of the coloured samples are done with a spectrophotometer (Shimadzu, UV-1800) at a wavelength of 700 nm. The quantification of protein is done with a calibration curve using bovine serum albumin (BSA) as standard (Sigma), in a range of 0 - 0.6 g BSA/L.

2.1.1.6 Carbohydrates

The carbohydrate concentration is measured with the Dubois et al. (1956) method and expressed in equivalent glucose. Carbohydrates react with the anthrone reagent under acidic conditions to yield blue-green colour.

The reagents are:

- Anthrone solution (0.2%): 0.2 g of anthrone are dissolved in 100 mL of sulphuric acid (98%). This solution is not stable, and it is necessary to prepare it the same day of the analysis.

USC
DE SANTIAGO
DE COMPOSTELA

Determination procedure

A volume of 2 mL of samples (diluted if necessary) is placed in 10-mL Pyrex® glass tubes, and 4 mL of anthrone solution are slowly added. A blank sample using distilled water is prepared in the same way, acting as reference for zero value. Then, the tubes are shaken and placed for 10 minutes in the block digester (VELP ECO16) preheated to 100

°C. To stop the reaction, the tubes are rapidly removed from the bath and cooled immediately with ice. Then, the measurements of the coloured samples are done with a spectrophotometer (Shimadzu, UV-1800) at a wavelength of 625 nm. The quantification of carbohydrates is done with a calibration curve using glucose (Sigma), in a range of 0 to 1 g glucose/L.

2.1.1.7 Inorganic Ions

Several inorganic ions were measured in the wastewater samples by chromatography. The anions are nitrite (NO_2^-), nitrate (NO_3^-), chloride (Cl^-), bromide (Br^-), phosphate (PO_4^{3-}), sulphate (SO_4^{2-}), and thiosulphate ($S_2O_3^{2-}$). The cations are sodium (Na^+), ammonium (NH_4^+), potassium (K^+), magnesium (Mg_2^+), and calcium (Ca_2^+). They are determined using an 861 Advanced Compact ion chromatography system equipped with a CO_2 suppressor (MCS 853, Metrohm) and an 838 Advanced Sample Processor (Metrohm, Switzerland). Table 2.1 shows the calibration ranges for the different inorganic ion concentrations.

In this chromatographic method, the sample goes through a column where cations and anions are separated according to their retention time in the resin column (Methrohm, Switzerland). Anions are determined with a Metrosep A column (250 x 4.0 mm) and a carbonate-bicarbonate mobile phase (3.2 mM Na_2CO_3 and 1.0 mM $NaHCO_3$) at a flow rate of 0.7 mL/min.

Table 2.1. Ranges of detection for the chromatographic method of ion detecting (in mg/L)

Anion	Lower Limit	Upper Limit	Cation	Lower Limit	Upper Limit
Cl^-	1.0	100.0	Li^+	0.05	5.0
NO_2^-	0.05	5.0	Na^+	1.5	150.0
NO_3^-	0.5	50.0	NH_4^+	0.1	10.0
Br^-	0.2	20.0	K^+	0.5	50.0
PO_4^-	0.5	50.0	Mg_2^+	0.5	50.0
SO_4^-	1.5	150.0	Ca_2^+	0.5	50.0
$S2O_3^-$	1.5	150.0			

Cations were determined with a Metrosep C3 resin column (250 x 4.0 mm) and nitric acid 3.5 mM is used as mobile phase. Then, the sample passes through a conductimetric detector after the separation, where the obtained signal corresponding to each retention time is registered. Therefore, each ion has a characteristic retention time. The resulting chromatograms identify each measured ion by its retention time position together with the amount of ion, which is related to the area. Then, the obtained areas are compared with the ones obtained in the standard calibration.

2.1.2. Characterization of the solid phase

2.1.2.1. Solids

USC DE SANTIAGO DE COMPOSTELA

The measurement of Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS) are performed according to standard methods (APHA-AWWA-WEF, 2017).

For the TSS, a certain known volume of the sample is taken with a pipette. Then, the sample is poured through a fibre glass filter (Whatman, GF/C, 4.7 cm of diameter, 1.2 µm of pore size) that was previously tared. To do so, the filter is placed in an oven (Memmert) at 105 °C for half an hour, so the humidity is removed, and the filter has a constant weight. After achieving room temperature in a desiccator with silica, the filter can be weighed.

The filter containing the retained solids is then dried for at least 2 hours at 105 °C, until reaching a constant weight. After that, it is again located inside a desiccator till it achieves room temperature. Finally, it is weighed, so the increase in the weight of the filter represents the TSS. TSS concentration is calculated as in Eq. 2.4:

$$\text{TSS (g/L)} = \frac{(W_1 - W_0)}{V} \qquad \text{Eq. 2.4}$$

Where W_0 is the weight of the dried filter (g), W_1 is the dry weight of the filter containing the solids (g), and V is the sample volume (L).

Finally, for VSS determination, the filter previously dried is burnt inside a muffle furnace at 550 °C for half an hour. Then, it is located inside the desiccator to reach room temperature for another half an hour. Then, it is weighed, and the weight lost during ignition corresponds to the VSS content. VSS concentration is calculated as in Eq. 2.5, where W_2 is the dried weight of the burnt filter:

$$\text{TSS (g/L)} = \frac{(W_1 - W_2)}{V} \qquad \text{Eq. 2.5}$$

2.1.2.2. Polyhydroxyalkanoates (PHA)

The biopolymer content in the samples was determined according to Smolders et al. (1994) for the quantification of the monomer propyl esters present in lyophilized samples. Therefore, unfiltered biomass samples were centrifuged, and then the supernatant removed. After that, the remaining pellet was frozen and freeze-dried in a tube sealed with Parafilm. The sample tube was weighed three times (empty, full of sample and after freeze-drying) to calculate the total solids in the pellet (TS). The salt content of the samples was acknowledged to calculate the PHA content in the cells as g PHA/g VSS, as a total solids to total volatile solids ratio (TS:TVS) is stablished according to the TSS/VSS previously measured of the sample.

The reagents are:

- A digestion solution, where 1 L of a mixture in a ratio of 4:1 is prepared with 800 mL of 1-propanol (99.9 vol%) and 200 mL of hydrochloric acid (37 - 38 vol%). It is stored in the fridge and preserved from light.
- Commercial 1,2-dichloromethane (HPLC quality).
- Commercial standard of PHA (The ratio of Hydroxybutyric (HB) to Hydroxyvalerate (HV) is 88:12 wt.%).
- Benzoic acid is used as internal standard in a concentration of 20 g/L. Here, 1 g of benzoic acid is dissolved in 50 mL of 1-propanol. This solution is preserved from light and stored in the fridge for a maximum of 12 months.
- Commercial sodium sulphate anhydrous (99 wt.%).

Determination procedure:

Approximately 10 mg of dried samples are weighted in a high-precision scale. Then, they are located into 10 mL glass tubes and 50 µL of the benzoic acid standard solution is added in each tube. Then, 1.5 mL of the digestion solution and 1.5 mL of 1,2-dicloromethane are added under a gas extraction system to prevent the inhalation of hazardous vapours. It is important to make sure that the glass tubes are completely sealed to prevent gas leaks during the following steps of the determination procedure. Therefore, the tubes are placed in an oven at 100 °C (Memmert) for 4 hours. During this time, the

hydrochloric acid breaks the cell walls, releasing the microbial material and the PHA. The strong acid hydrolyses the PHA, that will be later esterified by the propanol. Finally, the non-polar esterified compounds are dissolved in the organic phase solvent (dichloromethane), which will be analysed in the gas chromatography unit.

After cooling the samples to room temperature, 3 mL of distilled water are added to the digested samples, and all the tubes are energetically shaken in a vortex (IKA MS1, Germany). Samples are then centrifuged at 2500 rpm for 5 minutes to separate the two liquid phases: organic and aqueous. The PHA is then present in the organic fraction, while the water contains the remaining cell material, like proteins. A sample of 1.5 mL is taken from the organic phase with a glass pipette. Then, the sample is immediately filtered and dried by making the liquid go through a pipette tip of 1 mL filled with glass wool (which acts as a filter) and sodium sulphate anhydrous (which acts as dryer agent). Finally, the filtered and dried samples are quickly located into GC vials, which are sealed with a septum.

Samples are analysed by a gas chromatographer (6850 Series II, Agilent Technologies) equipped with a flame ionisation detector (FID) with a mixture of H_2/air at 40:400 mL/min; an automatic injector, that injects 1 µL of sample at 250 °C. The used software is Chemstation®. The chromatographic column employed is an HP-INNOWAX 30 m x 0.25 mm x 0.25 µm (Agilent, USA) with Helium as the carrier gas, being supplied at a constant velocity of 1 mL/min. The oven is operated at a temperature ramp from 60 °C to 230 °C with a velocity increase of 10 °C/min. The column can measure the monomers present in the polymer, and the identified ones are HB and HV.

Therefore, a calibration curve is done by using the PHA standard (Sigma) that contains HB and HV, so the retention times of the monomers in the column can be detected and identified. Then, the areas of the peaks represent mass of HB and HV in the samples. Therefore, when compared the areas of the samples with the values of the calibration curve, the concentration of the monomers is obtained. The calibration curve is done by plotting the weight of the standard against its corresponding ratio value. This ratio is obtained by dividing the area corresponding to the peak of PHA (HB or HV) by the area of the peak of the benzoic acid used as internal standard.

2.1.3. Other Parameters

2.1.3.1 pH

The pH was measured with an electrode (52-03, Crison Instruments, USA) equipped with an automatic compensatory temperature device (21-910-01, Crison Instruments, USA) and connected to a digital pH-meter. The uncertainty of the instrument was 0.01 pH units. The electrode was calibrated at room temperature with two standard buffer solutions of pH 7.02 and 4.00.

2.1.3.2. Alkalinity

The alkalinity of a sample is linked with its capacity of neutralizing acids, so it is intimately related with wastewater pH. Therefore, this parameter is important for effluents that go through an acidification process, as it might limit the system capacity for VFA generation.

Although alkalinity is linked to the presence of salts and weak acids, at near neutral pH, the most common buffer substances are carbonate, bicarbonate and hydroxides. However, other substances (like borates, phosphates, silicates and/or other bases) might

contribute. Generally, alkalinity is measured as mg $CaCO_3$/L, where total alkalinity (TA) and partial alkalinity (PA) are acknowledged due pH equilibrium. TA is determined at pH 4.3, and it is the sum of the alkalinity due to bicarbonate together with that corresponding to the VFAs. PA is measured at pH 5.75, and it acknowledges the alkalinity due to the presence of bicarbonate.

This parameter was measured according to standard methods (APHA-AWWA-WEF, 2017). In this case, the reagent used is an H_2SO_4 solution with a concentration 0.05 – 0.5 N that varies with the expected amount of alkalinity.

The determination procedure consists in a titration of a sample volume (normally 25 mL) at room temperature with the standard acid solution reagent, to reach the desired pH (5.75 for PA, and 4.3 for TA). The alkalinity value (mg $CaCO_3$/L) is calculated according to Eq. 2.6:

$$\text{Alkalinity (mg/L)} = \frac{A \cdot N \cdot 50000}{V} \qquad \text{Eq. 2.6}$$

Being: A the volume of sulfuric acid used to decrease the pH to 5.75 for PA, or to 4.3 for TA (mL), N the concentration of sulfuric acid, in equivalents/L, and V the sample volume (mL). The number 50,000 is a conversion factor that allows to express alkalinity in units of calcium carbonate, and it represents its normality.

2.1.3.3. Dissolved Oxygen and Temperature

Dissolved oxygen (DO) concentration is measured using a digital multimeter device (HQ40D, Hach Lange, USA) equipped with a luminesce-based DO probe (Intellical LDO, Hach Lange, USA). The multimeter and the probe are digitally calibrated by the distributor of the equipment. The mentioned probes are also equipped with a thermopar that supplies a measurement of the temperature.

2.2. ENVIRONMENTAL AND ECONOMIC EVALUATION METHODOLOGIES

2.2.1. Life Cycle Assessment (LCA)

An LCA is a systematic tool that enables the analysis of environmental loads of a product throughout its entire life cycle and the potential impacts of these loads on the environment, and it was here performed according to ISO 14040 and 14044 standards (ISO, 2006). Moreover, the methodological background recently published by the European Joint Research Centre, where the specific features of biobased polymers LCA were stablished, was used (Nessi et al., 2020). The methodology to perform a LCA consists of the following four stages (figure 2.1) according to the aforementioned standards:

1) **Goal and scope definition**, where the reason of the study and the goal of the research are stated, so the assessment needs to be tailored to give answer to that question(s). Consequently, several methodological issues need to be addressed before moving on to the next steps:

- Definition of the functional unit (FU) to provide a quantitative description of the product[1] for which the assessment is performed and to stablish the reference flow for the study. The FU represents the quantified performance of a product system for use as a reference unit in the LCA. The reference flow is the amount of product or energy needed to fulfil the FU.
- Definition of the system boundaries (SB) so the activities and processes included in the evaluation are clearly identified. Stablishing the geographical and temporal boundaries is also necessary.
- Selecting the impacts that need to be addressed (linked with the impact categories).
- Addressing other issues, like the perspective of the study (consequential vs attributional), or the necessity of a critical review.

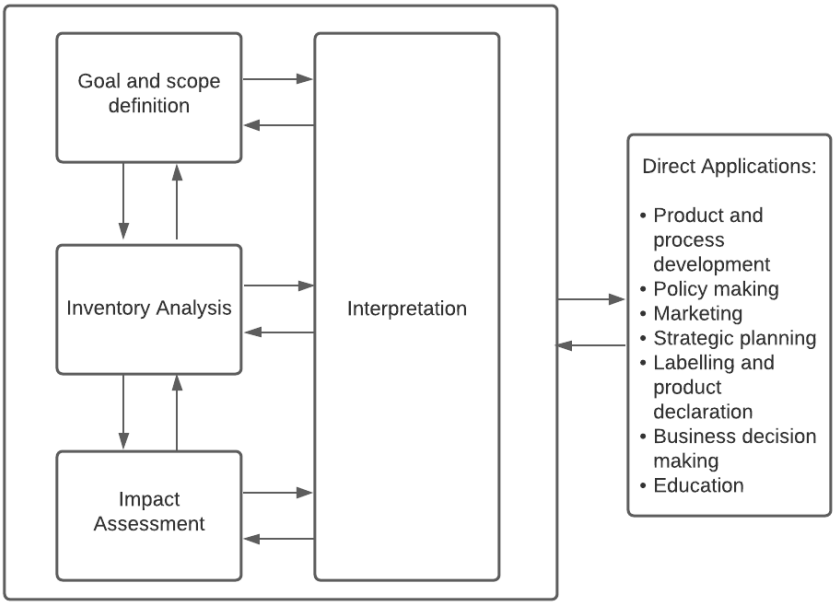


Figure 2.1. Steps for the life cycle assessment methodology

2) **Inventory Analysis**, which includes the collection of the physical flows of the processes studied: inputs of resources and materials, and outputs of emissions, wastes, and valuable products generated, to be able to bluid the Life Cycle Inventory (LCI), which is a list of the elementary flows that cross the defined system boundaries (Rosenbaum et al., 2018). This second stage is clearly the most time-consuming stage and normally the most complicated one, due to the data availability issues that might arise.

In the present thesis, primary data (so information generated *ex profeso* for the evaluation) were generated in the laboratory during the operation of the bioreactors while secondary data (coming from external sources as literature or databases) were taken from the **Ecoinvent Database** 3.8 and complemented with literature sources when required.

3) **Life Cycle Impact Assessment** (LCIA), in which the LCI is translated into impacts in the environment or in human health by applying models developed in

[1] In the context of LCA, a product is defined as the collection of materially and energetically connected unit processes that performs one or more defined function. However, it is not only referred to products, but also to services.

environmental science. According to the ISO 14040, LCIA has both mandatory and voluntary steps, being the former the three first ones of the following:

- Selection of the impact categories linked to the assessment parameters chosen in the scope definition. For each of them, a representative indicator is chosen together with an environmental model to quantify these impacts.
- Assignation of the elementary flows to the impact categories according to their impact contribution to the chosen indicator(s).
- In the characterisation step, according to the chosen environmental categories, the corresponding models will be applied. Therefore, with these models, elementary flows are transformed into impacts expressed in common metric units for each category.
- Normalisation of the characterised scores for the different impact categories to obtain their relative magnitudes.
- Grouping or weighing to provide comparison across the impact categories and rank them.

When applying LCA, the methodology employed in this thesis was Hierarchist **ReCiPe** at Midpoint, as it is a widely used consensus methodology (Huijbregts et al., 2017).

Within LCA, environmental models are on continuous development and evolution to be able to cover all the substances included in the LCI into impacts in the more accurate way within the LCIA stage. Therefore, these models are the core of LCA.

Impacts in LCA are usually divided in two types: extraction-related, and emission-related. The former is linked to resource depletion, like oil, water, or mineral extraction (for example, the environmental category fossil resource depletion). The latter are related to discharge effects, and how they affect air, soil, or water (for example, the global warming category, linked to CO_2 emissions).

For both, the methodologies in LCIA address the main cause-effect chains that determine environmental effects. These cause-effect chains are the emission/extraction of the pollutant/resource, its environmental fate, the degree of exposure for ecosystems and/or humans, the effects linked to the exposure, and the damage provoked by these effects. In general, the equation to compute impacts linked to chemical releases is Eq. 2.7:

$$IS = CF \cdot M \qquad \text{Eq. 2.7}$$

where IS is the impact score, CF is the characterization factor, and M is the mass of substance (here salts) emitted.

The CF is calculated considering the mentioned cause-effect chains linking the emission M to the environmental consequences through the modelling of fate, exposure, and effect factors (FF, XF and EF, respectively), according to Eq. 2.8 (Rosenbaum et al., 2018).

$$CF = FF \cdot XF \cdot EF \qquad \text{Eq. 2.8}$$

CFs addressing impacts on ecosystem quality at the endpoint level have units of potentially disappeared fraction of species (PDF)·m^3·time/kg, acknowledging damage in the cause-effect chains (midpoint impacts are measured as potentially affected fraction of species, PAF). As M in Eq. 2.7 is in kilograms, IS has units of PDF·m^3·time (Rosenbaum et al., 2018). FF in Eq. 2.8 is expressed in units of time (it represents the mass of a

chemical in the environment resulting after an emission flow, so units are kg/(kg/day), yielding in days). XF is dimensionless, and EF is expressed as PDF·m^3/kg. The XF represents the availability of the released chemical in a system (the fraction of the pollutant that is dissolved in the water), which can be considered as 1 for salt as it is fully dissolved.

The FF is linked to the physical behaviour and/or distribution of the substance in the environment (although it also represents the predicted mass residence of a substance in a receiving compartment). Therefore, the FF is calculated by applying mass balances to the studied compartment in which degradation and transfer processes are described.

The EF quantifies the fraction of living species that are going to potentially disappear in the aquatic ecosystem by the release of a certain chemical or a pollutant. Therefore, the EF is generally modelled using ecotoxic methodologies, such as USEtox. Under this framework, EF is calculated according to Eq. 2.9:

$$EF = {}^{0.5}/_{HC50} \qquad \text{Eq. 2.9}$$

Eq. 2.9 represents the slope of the concentration-response curve. As EF are usually calculated to assess the effects over 50% of the population (HC50), the formula is as in Eq. 2.9. However, for other ranges of effects, the formula would change accordingly (for example, for an EF based on 20% of the effects, EF = 0.2/HC20). Moreover, the units of the EF arise from the factor calculation itself. As the EF is the slope of the cause/effect (concentration variation/potentially disappeared species), the units of the factor are PAF·m^3/kg.

Therefore, the units of HC50 are kg/m^3, as it represents the concentration of chemical that generates an effect over the 50% of the species in the ecosystem Here, EC50 represents the concentration of chemical that generates effects for 50% of the population of each species studied. The tool employed to transform the effects over individual species on HH50 and EF is the species-sensitivity distribution (SSD) curve. It represents the sensitivity of an entire ecosystem to a substance, and, to shape it, several stages are needed (Fantke et al., 2018):

- Identify the endemic or representative species of the studied ecosystem.
- Gather data of chronic effects for the identified species. Data cannot be extrapolated, so, if SSD curve aims to calculate HC50, chronic effect need to measure effects for 50% of the population of the species studied. Moreover, if acute data is found, a chronic-to-acute ratio (ACR) can be applied (generally the chosen value is 2).
- Calculate the geometric mean of the EC50s obtained. That mean is the HC50.

4) **Interpretation**, where the obtained results need to be understood to answer the question asked in the goal and scope definition step, and where normalisation and weighting are involved. Here, all the decisions taken in the goal and scope definition stage need to be considered when interpreting the results. In the interpretation, substance and contribution analyses can be used to identify the main contributors for the impacts of the studied systems. Besides, sensitivity and uncertainty analysis should be performed as a part of the interpretation process to better provide conclusions and to test the robustness of the results (Rosenbaum et al., 2018).

In the context of LCA, uncertainty is referred to the probability distribution of a given variable (generally, probability density function), and it can be referred to methodological

choices (like functional unit definition) or to epistemological uncertainty (like assumptions made due to lack of relevant knowledge). A sensitivity analysis generally aims to identify the key processes and elementary flows contributing the most to the system's impacts, as it describes the extent to which the variation of an input parameter leads to variation of the model result.

For the implementation of both LCI and LCIA stages, **SimaPro v9.2** (Pré Sustainability, 2021) was used.

2.2.2. Techno-economic Analysis (TEA) and Life Cycle Costing (LCC)

The combination of LCA and economic assessments like TEA has been identified as a key issue to provide integral validation in technology development (Wunderlich et al., 2021). On the other hand, even though both LCCA and TEA have been practiced for several decades, there is a lack of commonly accepted framework to define these methodologies. Although LCCA relies on a more robust range of definitions, sometimes literature differs on how both LCCA and TEA are described, so the steps and particular approaches applied need to be clarified and reported when any of these methods are practiced, to ensure proper clarification of the limits of the study (Giacomella, 2020).

Environmental life cycle costing (LCC) summarizes all costs associated with the life cycle of a product (e.g., supplier, producer, user or consumer, and those involved at the end of life); these costs must relate to real money flows. Externalities that are expected to be internalized in the decision-relevant future comprise real money flows as well, and they must also be included. A complementary life cycle assessment (LCA), with equivalent system boundaries and functional units, is also required. Environmental LCC is performed on a basis analogous to that of LCA, with both being steady-state in nature (SETAC, 2008). Conventional life cycle costing is based on a purely economic evaluation, considering various stages in the life cycle. Environmental LCC uses system boundaries and functional units equivalent to those of LCA and is based on the same product system model, addressing the complete life cycle (SETAC, 2008).

TEA connects research and development, engineering, and business by linking process parameters to financial metrics, and it can help businesses better understand the factors that affect the profitability of their technology development projects (Burck, 2018). It is generally defined as a cost-benefit comparison resulting from the consideration of both technological and economic factors of a process, although this description might vary in the different sources. In general, it is a framework to analyse the economic performance of an industrial process or a service/product, and it includes the entire project's development (Zimmermann et al., 2020). It provides information about the relationship between technological and economic aspects, enabling to understand the potential hotspots of a projected process. The TEA has a multidisciplinary nature and is based on process modelling and engineering design. Here, process simulation might have an important role (Giacomella, 2020).

Therefore, LCCA is linked to LCA, but also to TEA, as it not only includes purchase and implementation costs, but also the associated costs, in a way that is inclined to receipt socio-environmental extensions (Giacomella, 2020). In this sense, in LCCA, special attention is paid to operational costs, discount, maintenance, etc., while TEA is more focused on direct expenditures. Consequently, the economic studies here performed combine TEA and LCCA in their approach. The economic analysis of the processes was performed in this thesis considering total capital investment plus the costs of the

operational expenditures (therefore, LCCA approach), where the technological features in an early development stage (like operational conditions or equipment selection) were directly linked to final costs (thus, TEA approach).

The estimation of the total capital investment (C_T) was performed using typical cost indexes and size-dependent equipment purchase costs (Coulson and Richardson, 1999; Seider et al., 2017). Then, Prices were updated using the Chemical Engineering Plant Cost Index (CEPCI), and a Lang factor of 5 was used to estimate C_T.

The size of the equipment and the utilities needs were calculated by solving mass balances based on laboratory-scale operation. Then, the Total Annual Costs (TAC, €/year) are estimated by Eq. 2.10.

$$TAC = AD + U + M + L + m \qquad \text{Eq. 2.10}$$

Where:

- Utilities (U) and materials costs (M) are calculated by energy and mass balances, while prices of utilities and raw materials are obtained from literature (Seider et al., 2017).
- The labour costs (L) are estimated as 3% of C_T, and maintenance (m) is approximated as in Eq. 2.11.

$$m = (AD + U + M + L) \cdot 0.1 \qquad \text{Eq. 2.11}$$

- AD is the Annual Depreciation, and it is calculated according to Eq.2.12, where the interest (i) is 5% and the project time (PT) is 20 years. Costs were obtained in $ and are expressed in € according to the present currency exchange rates.

$$AD = C_T \cdot \frac{i \cdot (1+i)^{PT}}{(1+i)^{PT} - 1} \qquad \text{Eq. 2.12}$$

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

3. STRATEGIES FOR THE VALORISATION OF A PROTEIN-RICH SALINE WASTE STREAM FROM THE FISH CANNING INDUSTRY INTO PHA

SUMMARY

Complex saline Mussels Cooking Wastewater (MCW) was valorised to produce polyhydroxyalkanoates (PHA) with Mixed Microbial Cultures (MMC) in a typical three stage system with acidification, enrichment, and accumulation units. Due to the high organic matter content in the form of proteins (protein Chemical Oxygen Demand or COD_{PROT}), which ranged 1.8 – 5.7 g COD_{PROT}/L, PHA accumulating capacity was below 10 %. Therefore, several strategies were tested with the goal of improving this storing rate. In the acidification unit, $Na(HCO_3)$ was added to manage pH and alkalinity, increasing protein conversion into Volatile Fatty Acids (VFA) from 10.3 % to 69.2 % and subsequent PHA accumulation from 6.9 to 14.7 %. Then, the proteins/VFA ratio was varied by modifying the MCW in the feeding, so MMC could gradually acclimate to the high protein concentration of the feedstock. This allowed to increase PHA accumulation to 41.5%. Finally, in the enrichment unit, the incorporation of a settling stage after the feast phase provoked a shift in the proteins' oxidation from the feast to the famine phase, where the nitrogen released during the starvation period due to protein uptake is used by the MMC for growth. This increased the biomass concentration from 1.6 to 4.2 g VSS/L, and the tolerated COD from 2.2 to 4.38 g COD/L, yielding in increased productivities.

The content of this chapter was published as A. Roibás-Rozas, Á.V. del Río, A. Hospido, A. Mosquera-Corral (2021), **Strategies for the valorisation of a protein-rich saline waste stream into polyhydroxyalkanoates (PHA)**, Bioresource Technology. https://doi.org/10.1016/j.biortech.2021.124964.

All authors' affiliation is CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

3.1 INTRODUCTION

PHA are renewable, biodegradable and bio-based polymers produced by bacteria which are expected to gradually replace conventional petrochemical plastics (Kourmentza et al., 2017). In the last decade, the use of MMC and waste streams for PHA production has been explored to reduce overall costs and environmental impacts (Roibás-Rozas et al., 2020; Yadav et al., 2020). However, the use of waste streams for PHA production is challenging due to the complexity of the substrate, containing substances that can be inhibitory for the microbial activity (Korkakaki et al., 2016a; Palmeiro-Sánchez et al., 2019).

The Spanish north-western region of Galicia is responsible for the 90 % of the production of mussels in the country, which is also the main mollusc producer of Europe (FAO, 2020). This activity generates more than 100 M€ of incomes and more than 10,000 jobs in the region (Rey-Méndez et al., 2016), and it is highly dependent on the water quality in the estuaries where seafood is harvested. Because of this, a proper treatment of the effluents generated in the fish canning and fish-processing industries, normally located at shore areas, is essential for the environmental and economic preservation of the region. Nevertheless, the treatment of these effluents is challenging due to the presence of potentially inhibitory compounds, such as sodium chloride or ammonia. More precisely, Mussels Cooking Wastewater (MCW) contains high sodium chloride concentrations (between 17.9-19.0 g NaCl/L), as well as sulphate and nitrogen (organic and in the form of ammonia) in ranges that can be inhibitory for conventional processes, like the anaerobic digestion (Palmeiro-Sánchez et al., 2013).

In fact, several attempts were made to valorise MCW in Galicia through biogas production. They were unsuccessful possibly due to the complexity of the waste stream (Barros et al., 2009; Omil et al., 1995), so the great concern regarding MCW treatment is reflected in local legislation (Consellería de Medio Rural e Mar, 2015). Because of this, and its large biodegradable organic matter concentration, the valorisation of MCW by PHA production was defined as a possible alternative to treat these effluents. The COD of MCW, ranging between 9.8-26.5 g COD/L, is about 95% soluble and mainly composed by proteins and carbohydrates. Carbohydrates are rapidly consumed in fermentation processes, but proteins are consumed slowly. Furthermore, their presence can provoke problems in the subsequent aerobic PHA production unit, so several strategies, as uncoupling the carbon and nitrogen feeding or introducing a settling stage in the process have been proposed (Argiz et al., 2020a; Oliveira et al., 2017).

The aim of this chapter is to evaluate how the presence of proteins affects the MMC-based PHA production process and to assess the feasibility of using protein-rich and saline wastewater as a substrate. To do so, several long-term strategies were tested in a three-stage system for PHA production, using a MMC fed with MCW: i) alkalinity increase in the acidification unit, ii) a settling stage in the PHA enrichment reactor, and iii) increasing the MMC capacity to consume proteins by modifying the proteins/ VFA ratio. Then, these strategies were assessed and compared to choose the optimal operational conditions.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

3.2 MATERIAL AND METHODS

3.2.1 Experimental set-up

A three-step system was utilized to produce PHA (see Appendix 1) as described in the following sections: a CSTR for acidification of the MCW; SBR for enrichment of the MMC; and a FBR for the accumulation of PHA. The experimental stages tested in the

continuous operation are summarized in Figure 3.1 and named as: A1-A6 for the acidification CSTR, E1-E5 for the enrichment SBR.

3.2.1.1 CSTR for acidification

A CSTR was fed with MCW coming from a facility in Pontevedra, NW of Spain. Although the main compounds of the MCW did not change throughout the operational time, their concentrations fluctuated highly (see Appendix 1), so the Organic Loading Rate (OLR) for CSTR stages ranged between 1.01 and 1.76 g COD/(L·d). It had a volume of 5 L and it was operated as described in Fra-Vázquez et al. (2020). This study started in the operational day 400, where VFA were produced steadily at a pH of 4.6 ± 0.6 with about 43 % of the effluent COD in the form of VFA.

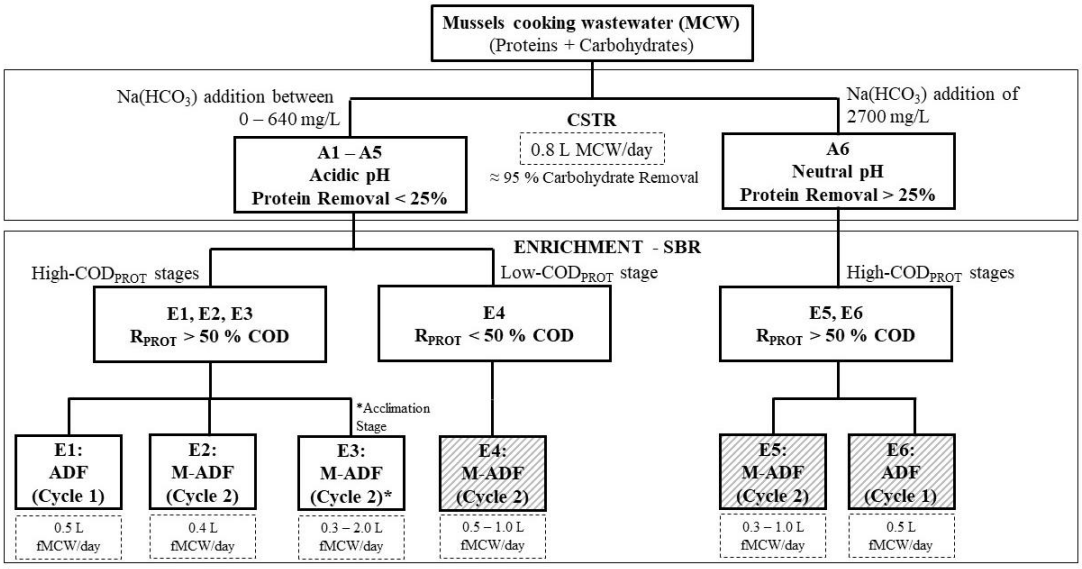


Figure 3.1: Operational periods in the three-stage PHA production system. Continuous lines represent the operational stages and dashed lines represent the mass balances for each one of them. The boxes filled with diagonal grey lines for the enrichment stages represent the periods where the volume of the reactor was reduced from 2 L to 1 L. The effluent of the CSTR is referred in the figure as fMCW (fermented MCW) to facilitate the comprehension of the scheme.

The Solid Retention Time (SRT) was fixed equal to the Hydraulic Retention Time (HRT), with a value of 6.25 days. The operational conditions by (Fra-Vázquez et al., 2020) were first maintained (stage A1), but then, different amounts of sodium bicarbonate were added (20 - 2700 mg $NaHCO_3$/L of MCW in stages A2 to A6, see Table 3.1) to increase the pH and improve protein solubility in the CSTR. The effluent produced in the CSTR was stored at 4 ºC until it was used in the following units. This feature was linked to the operation scale, and it was used for a better load control in the next units, so operational stages for the CSTR and Sequencing Batch Reactor were not synchronized. In fact, this provoked a change in the operational volume of the subsequent SBR (see Figure 3.1, and Table 3.1).

3.2.1.2. Enrichment SBR

The inoculum of the SBR was enriched in PHA accumulating biomass (accumulation capacity of 25 %), as it came from a similar bench-scale reactor that was fed with the same MCW for a year, but this feeding was diluted with tap water to decrease the salt concentration to approximately of 5 g NaCl/L (Pedrouso et al. (2020)).

For the present work, salinity was gradually increased until achieving the salinity of the MCW (17-18 g NaCl/L) in 100 days by replacing the tap water by the effluent of the same SBR as dilution agent, which means that the SBR effluent was recirculated to the influent. Then, two different operational strategies (Cycles 1 and 2) were evaluated (see Appendix 1): conventional and modified Aerobic Dynamic Feeding (ADF and M-ADF, respectively).

Table 3.1: Operational Stages throughout the operation of the acidification (CSTR) unit.

Stage	$Na(HCO_3)$ (mg/L of feeding)	Days	OLR (g COD/(L·d))	Stage in SBR[(1)]
A1	0	0-263	1.01 ± 0.21	E1-E2
A2	20	264-332	1.27 ± 0.13	E2-E3
A3	80	333-395	1.44 ± 0.23	E4
A4	320	396-466	1.76 ± 0.21	E4
A5	640	467-502	1.75 ± 0.21	E5
A6	2700	503-621	1.48 ± 0.42	E5-E6

[(1)] CSTR effluent was stored until used, so the operational time is not always coincident. This column shows the link between the effluents obtained in each acidification CSTR operational stage (employed as SBR feeding) and the SBR operational stage.

When the conventional ADF strategy (Cycle 1) was applied, air was continuously supplied while, with the M-ADF one (Cycle 2), a settling and discharge intermediate phases were included. In the case of Cycle 1, the exchanged volume was 50 % so the SRT and HRT of operation were equal to 1 day. For Cycle 2, no oxygen was supplied during the settling and supernatant discharge phases, and aeration was provided throughout the rest of the cycle phases.

After settling, 25 % of the total reactor volume (V_R) was withdrawn (supernatant discharge), so during the rest of Cycle 2, the reactor operated with 75 % of the initial volume. As the initial feeding corresponded to 50 % of V_R and the final discharge was of 25 % of V_R, the SRT and HRT were of 2 and 1 days, respectively.

The feeding of the SBR consisted of centrifuged CSTR effluent, where the dilution agent was the settled (without solids) effluent of the same SBR. The recirculation ratio (RR, i.e., volume of effluent from the CSTR / volume of effluent from the SBR used as feeding) was established according to the composition of the effluent produced in the CSTR and the organic load required to feed the SBR (Table 3.2).

The operation of the SBR was divided in six stages (from E1 to E6) depending on the percentage of COD present as proteins (R_{PROT}) at the beginning of the cycles and on the cycle type (ADF or M-ADF) (Figure 3.1). Allylthiourea was added to the feeding of the SBR at 10 mg/L to avoid nitrification, antifoaming (0.5 mL/L of feeding) to prevent spume problems and NaOH or HCl to maintain the pH value in the feeding approximately neutral in stages from E3 to E6, but pH inside the reactor was not controlled.

For all the stages, the reactor operated at 30 ºC by using a thermostatic bath (Techne Inc., USA), and air was supplied (6 L/min) through a ceramic air diffuser located at the bottom of the reactor. As the CSTR effluent was not enough to achieve the desired loads from stages E4 to E6, the SBR operation volume was reduced from 2 L (used from stages E1 to E3) to 1 L, maintaining all the operational conditions.

Table 3.2: Operational Stages throughout the operation of the enrichment (SBR) unit.

Stage	Days	Cycle	$COD_{VFA,0}$ (g COD_{VFA}/L)	R_{VFA}[(1)], % (g COD_{VFA}/g COD)	SRT (days)	RR[(2)]
E1	0-205	ADF	0.50 ± 0.26	34.98 ± 16.79	1	1:4
E2	206-263	M-ADF	0.71 ± 0.11	32.02 ± 7.32	2	1:5
E3	264-335	M-ADF	0.72 ± 0.32	33.19 ± 14.22	2	1:6 to 1:1
E4	336-425	M-ADF	2.69 ± 0.75	61.36 ± 9.97	2	1:1 or 1:0
E5	425-498	M-ADF	1.75 ± 0.48	51.28 ± 8.84	2	1:1 to 1:3
E6	499-536	ADF	0.94 ± 0.37	36.98 ± 4.73	1	1:4

(1) As the COD at the beginning of enrichment cycles was mainly VFA and proteins (COD_{CARB} was negligible), R_{PROT} can be estimated as 100 – R_{VFA}.
(2) RR: Recirculation ratio to the SBR (CSTR effluent volume: SBR recirculated effluent volume).

3.2.1.3. FBR

An FBR was employed to evaluate the maximal PHA accumulation capacity of the enriched MMC. As inoculum, it was used biomass from the SBR, and as feeding, centrifuged effluent of the CSTR (containing VFA and proteins), which was added in pulses. Analysis of the liquid phase (data no shown) showed that VFA were consumed first and proteins after, being possible to use the dissolved oxygen (DO) concentration value as indicator. For this reason, each pulse of feeding in the FBR was supplied when the DO concentration increased to intermediate values (approximately 4.0 - 4.5 mg O_2/L), which marked that VFA was depleted, and protein oxidation started.

To make assays comparable, the amount of VFA supplied in every pulse was set as 15 $Cmmol_{VFA}/(L_{REACTOR}\cdot pulse)$, except on day 388, when pulses of 70 $Cmmol_{VFA}/(L_{REACTOR}\cdot pulse)$ by increasing the volume of CSTR effluent in each pulse. Therefore, in the accumulation assays, the FBR volume was variable according with the CSTR effluent acidification degree and the number of pulses. Each experiment lasted a maximum time of 12 hours, which was equal to the enrichment cycle length. The reactor temperature was maintained at 30 °C by using a thermostatic bath (Techne Inc., USA), and air was supplied (6 L/min) through a ceramic air diffuser located at the bottom of the reactor.

3.2.2. Analytical methods

Analytical methods are described in detail in Chapter 2 of this thesis. Conductivity and pH were measured with glass electrodes (Hach-Lange 50-60 and Crisson GLP22 respectively), while DO concentration and temperature were determined in the SBR and FBR with a probe (model HQ40d, Hach-Lange, USA).

Alkalinity, TSS, VSS and total COD (COD_T) concentrations were analysed in bulk samples, and the dissolved matter was characterised after filtering with a cellulose-ester filter of 0.45 μm pore size (Advantec, Japan), according to the Standard Methods (APHA-AWWA-WEF, 2017).

Measuring included the following parameters: soluble COD (noted as COD in the text) carbohydrates, proteins, Total Organic Carbon (TOC), Total Nitrogen (TN), VFA, ammonium and other ions (Na^+, Cl^-, SO_4^{2-}). COD was generally quantified according to APHA-AWWA-WEF (2017), and based on the methodology described by Soto et al.

(1989) when samples had concentrations below 5 g COD/L. Carbohydrates were measured as indicated in Dubois et al. (1956) and expressed as COD considering that 1 g of glucose (used as standard) corresponds to 1.07 g of COD. Proteins were analysed by the Lowry method (Lowry and Randall, 1951) and expressed as COD considering that bovine serum albumin (used as standard) contains 1.32 g of COD and 0.15 g of N per gram of protein.

TOC and TN concentrations were measured by catalytic combustion in the TOC-L CNS analyser with the TNM-1 module (Shimadzu, Japan). VFA concentrations were determined in a gas chromatograph (Hewlett Packard 5890A, USA) equipped with a flame ionization detector (FID) and an automatic injector (Hewlett Packard 7673A, USA) and with ChemStation Rev. A. 10. 02 (1757) Agilent Technologies software. Ammonium was quantified by the Bower/Holm Hansen method (Bower and Holm-Hansen, 1980) and ions (Na^+, Cl^-, SO_4^{2-}) by ion chromatography (861 Advanced Compact IC, Metrohm, Switzerland).

PHA content was determined in biomass samples. To do so, unfiltered biomass samples were centrifuged, and the supernatant removed. Then, the remaining pellet was frozen and freeze-dried. The sample tube was weighed three times (empty, full of sample and after freeze-drying) to calculate the total solids in the pellet (TS). The PHA content (as g PHA/g VSS) was measured following the method described by Smolders et al. (1994) for the quantification of the monomer propyl esters present in a lyophilized sample. A commercial PHA standard (Sigma-Aldrich, USA) containing 88 % of hydroxybutyrate (HB) and 12 % of hydroxyvalerate (HV) and benzoic acid as internal standard were used. The propyl esters were analysed by means of gas chromatography in a HP innovax column equipped with an FID (Agilent, USA). Biogas composition (N_2, CH_4, CO_2, H_2S) was analyzed in a gas chromatograph (Hewlett Packard, USA) equipped with a packed column and a thermal conductivity detector (TCD) using helium as carrier gas and the percentage of each compound was calculated by analyzing 1 mL of biogas injected with a syringe.

3.2.3. Calculations

3.2.3.1. CSTR mass balances

For the CSTR, the main parameters calculated were the acidification degree (Ac., Eq. 3.1), protein and carbohydrates removal percentages (REM_{CARB} and REM_{PROT}), Eq. 3.2) and VFA production ($COD_{VFAprod}$, Eq. 3.3).

In Eq. 3.1, COD_{VFA} is the concentration of VFA in the effluent (g COD/L). Subindex i represents whether the equation refers to carbohydrates or proteins. "INF" and "EFF" subindexes represent influent and effluent streams, respectively.

$$\text{Ac.}(\%) = \frac{\text{COD}_{\text{VFA}}}{\text{COD}} \cdot 100 \qquad \text{Eq. 3.1}$$

$$\text{REM}_{\text{i}}(\%) = \frac{\text{COD}_{\text{i,INF}} - \text{COD}_{\text{i,EFF}}}{\text{COD}_{\text{i,INF}}} \cdot 100 \qquad \text{Eq. 3.2}$$

$$\text{COD}_{\text{VFAprod}} = \frac{\text{COD}_{\text{VFA,EFF}} - \text{COD}_{\text{VFA,INF}}}{\text{COD}_{\text{INF}}} \qquad \text{Eq. 3.3}$$

3.2.3.2. SBR mass balances

For the SBR, the COD concentration at the beginning of an enrichment cycle (COD_0, in g COD/L) was calculated according to Eq. 3.4 because it has a 50 % of exchange volume ratio. Accordingly, the COD concentration of proteins or VFA at the beginning of the cycle ($COD_{i,0}$, g COD_i/L) was calculated as indicated in Eq. 3.5. Analogously, this mass balance can be applied to calculate the concentration of the nitrogen forms in the cycle (substituting COD by TN or NH_4^+-N, in mg N/L). Here, "INF" refers to influent (feeding stream) and "EFF" to the effluent of the previous cycle, which coincides in concentration with the remaining volume inside the reactor. The proportion of COD_{PROT} over the total COD at the beginning of the SBR cycles (R_{PROT}, % (g COD_{PROT}/g COD)) is calculated as stated in Eq. 3.6, where the COD is mainly in the form of proteins and VFA (COD_{CARB} was negligible).

$$COD_0 = \frac{COD_{INF} + COD_{EFF}}{2} \quad \text{Eq. 3.4}$$

$$COD_{i,0} = \frac{COD_{i,INF} + COD_{i,EFF}}{2} \quad \text{Eq. 3.5}$$

$$R_{PROT} = \frac{COD_{PROT,0}}{COD_0} \cdot 100 \quad \text{Eq. 3.6}$$

A mass balance was performed to calculate protein consumption during the cycle. For conventional ADF, protein removal is only due to oxidation. However, for Cycle 2 (M-ADF), protein removal also occurs due to the withdrawal of the supernatant. Therefore, the protein removal rate due to oxidation ($REM_{PROT,OX}$, %) and to supernatant removal ($REM_{PROT,SUP}$, %) are calculated according to Eq. 3.7 and Eq. 3.8, respectively, where V_{SUP} is the volume of the supernatant withdrawal and V_R is the reactor volume at the beginning of the cycle after feeding addition.

$$REM_{PROT.OX}/_{100} = \\ = \frac{COD_{PROT,0} \cdot V_R - COD_{PROT,SUP} \cdot V_{SUP} - COD_{PROT,EFF} \cdot (V_R - V_{SUP})}{COD_{PROT,0} \cdot V_R} \quad \text{Eq. 3.7}$$

$$REM_{PROT,SUP} = \frac{COD_{PROT,SUP} \cdot V_{SUP}}{COD_{PROT,0} \cdot V_R} \cdot 100 \quad \text{Eq. 3.8}$$

For Cycle 1, V_{SUP} is zero, so $REM_{PROT,OX}$ is ($COD_{PROT,0} - COD_{PROT,EFF}$)/$COD_{PROT,0}$. These mass balances can be applied for nitrogen forms by substituting COD_{PROT} by TN or NH_4^+-N. Finally, the protein variation during the cycle phases due to oxidation ($\Delta PROT_{OX}$ in g PROT/L) is calculated as indicated in Eq. 3.9:

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

$$\Delta PROT_{OX} = \Delta PROT_F + \Delta PROT_{FAM} \quad \text{Eq. 3.9}$$

Where $\Delta PROT_F$ and $\Delta PROT_{FAM}$ (both in g PROT/L) are the variation in the concentration of proteins during the feast and the famine phases, respectively, and they are calculated as indicated in Eq. 3.10 and 3.11:

$$\Delta PROT_F = PROT_0 - PROT_{MID} \quad \text{Eq. 3.10}$$

$$\Delta PROT_{FAM} = PROT_{EFF} - PROT_{MID} \quad \text{Eq. 3.11}$$

Being $PROT_{MID}$ (g PROT/L) the protein concentration in the *middle* of the cycle (the point where feast finishes and famine begins). Accordingly, the percentage of COD_{PROT} oxidated during feast or famine ($REM_{PROT,F}$ or $REM_{PROT,FAM}$, in %) can be calculated by dividing $\Delta PROT_F$ or $\Delta PROT_{FAM}$ by $\Delta PROT_{OX}$ [10].

3.2.3.3. PHA content and kinetic parameters

For the SBR and the FBR, PHA content in the cells is calculated according to Eq. 3.12. Specific consumption and production rates (q_{VFA}, q_{PROT} and q_{PHA}, in mg/(g X·h)) are estimated from the maximum slopes of the curves obtained from the corresponding experimental data, divided by the average active biomass (X, calculated as the subtraction of the mass of PHA to the mass of VSS measured).

The active biomass composition was considered $CH_{1.8}O_{0.5}N_{0.2}$ for all the calculations. The biomass yield (Y_X) was estimated dividing the biomass production rate (g COD_X/h) by the VFA consumption rate (g COD_{VFA}/h). Finally, the ratio *r* (g VFA/g proteins) is calculated by dividing q_{VFA}/q_{PROT}.

$$\text{PHA (wt.\%)} = \frac{{}^{\text{g TS}}/_{\text{L}} \cdot {}^{\text{g PHA in sample}}/_{\text{g TS}}}{{}^{\text{g VSS}}/_{\text{L}}} \cdot 100 \quad \text{Eq. 3.12}$$

3.3. RESULTS AND DISCUSSION

3.3.1. Protein fermentation in the acidification reactor (CSTR)

The CSTR operated for 621 days in 6 operational stages according to the $Na(HCO_3)$ added in the feeding, which was increased from 0 to 2.7 g/L to improve protein solubility and promote their better degradation. Changes in the applied load (1 - 1.76 g COD/(L·d)), are due to the variability of the MCW (Table 3.1). From stages A1 to A5, pH in the CSTR was always below 5.0 due to VFA formation and low alkalinity, because the added amount of $Na(HCO_3)$ was still not enough to clearly increase the pH value. For stage A6, pH was around neutrality (pH = 6.95 ± 0.57) due to the highest added alkalinity dosage (2,7 g $Na(HCO_3)$/L), so A6 corresponds to a neutral-pH stage. Carbohydrates were almost fully consumed (over 90%) for every stage, but no proteins (Figure 3.2). For acidic-pH stages, protein removal ranged 10.30 – 21.94%, while, for neutral-pH stage A6 it increased to 42.45 ± 19.56%. The higher protein removal promoted an increase in the $COD_{VFAprod}$ and acidification from 0.21 – 0.28 g COD_{VFA}/g COD and 45.7 – 60.6% in A1-A5 to 0.43 ± 0.12 g COD_{VFA}/g COD and 71.6 ± 13.1 % in A6, respectively. Therefore, the increase in $COD_{VFAprod}$ and acidification percentages for the neutral-pH stage can be attributed to the increase in the protein consumption associated to the modified pH-environmental conditions. Successful acidification of the MCW was achieved and no methane was detected during the whole operational time. For stages A1-A5 (acidic-pH), the inhibition of methanogenic activity can be attributed to the low pH of operation (below 5) caused by VFA accumulation (Fra-Vázquez et al., 2020), but this cannot be the reason for the inhibition for the neutral-pH stage (stage A6). Although high

[10] Note that $REM_{PROT,F}$ and $REM_{PROT,FAM}$ (%) can be expressed as g/g or g COD_{PROT}/g COD_{PROT}, to obtain the same result.

concentrations of salt, and in special sodium ion, can inhibit methanogenic activity, methane can appear in long-term operations despite high salinity (Palmeiro-Sánchez et al., 2013).

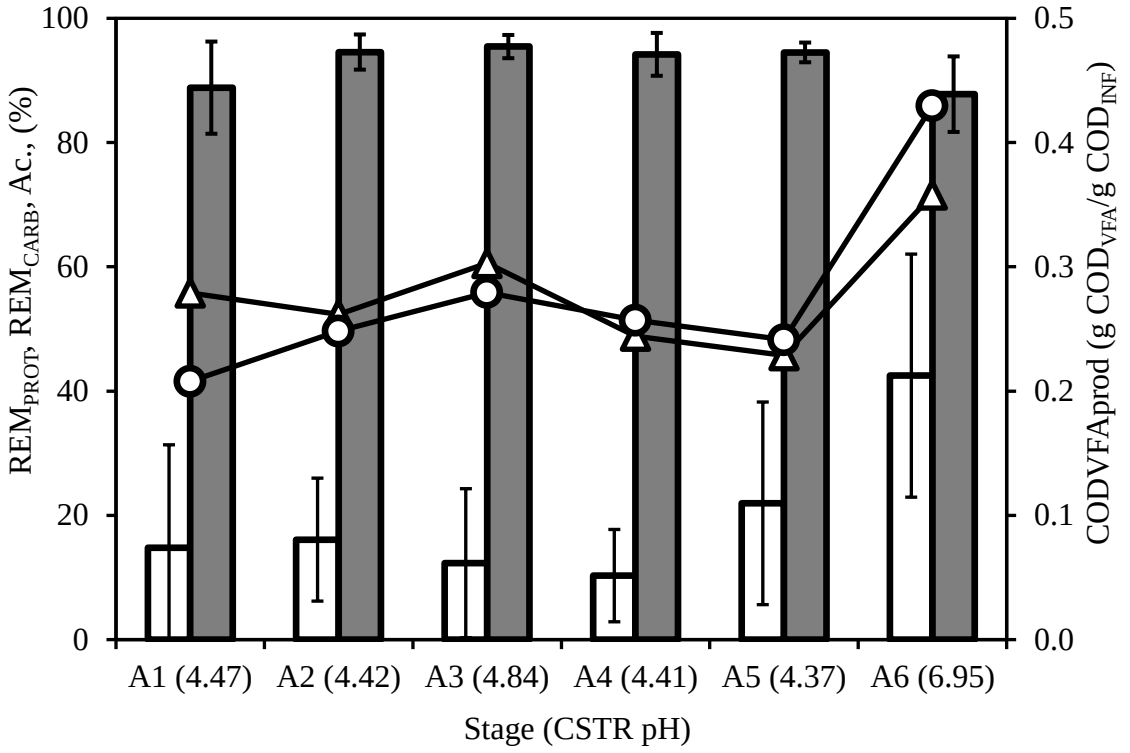


Figure 3.2: Protein Removal (REM_{PROT}, □), carbohydrates removal (REM_{CARB}, ■), and Acidification Degree (Ac, Δ), expressed as percentages, and VFA production ($COD_{VFAprod}$ ○, g COD_{VFA}/g COD_{INF}) in the CSTR.

However, when sulphate is present in the wastewater and the COD/SO_4^{-2} ratio is below 10 g/g, methanogens are inhibited due to their competition with sulphate reducing bacteria. In this case, the complex matrix of MCW makes this waste stream ideal for VFA production, as it has high concentrations of sodium due to salinity, and COD/SO_4^{-2} ratio below 10 g/g, which inhibits methanogens. Therefore, the compounds present in the MCW caused the absence of methanogenic activity during all the CSTR operation.

The transformation of proteins into VFA in anaerobic mixed culture fermentations has generated increasing interest in the last years (Bevilacqua et al., 2020; Jin et al., 2016; Liu et al., 2015; Regueira et al., 2020a, 2020b; Yang et al., 2015), as they are degraded slower than carbohydrates. Hydrolysates of proteins and carbohydrates are peptides/amino acids and glucose, respectively. As the presence of glucose represses protease formation, the uptake of glucose/carbohydrates happens first (Yang et al., 2015). Moreover, pH is one of the most relevant factors regarding VFA production from proteins (Regueira et al., 2020a).

When pH is low and VFA are formed, undissociated acids cross the membrane, releasing protons inside the cell and inhibiting the generation of new acids. Furthermore, as the concentration of protons in the solution increases, the interaction of hydrogen bonds becomes stronger. Thus, the solubility of proteins decreases, as they aggregate and even coagulate at low pH values (Liu et al., 2015). This can explain the results of the CSTR operation, where carbohydrates are always consumed, and protein uptake only happens at the neutral pH.

3.3.2 Enrichment (SBR) and accumulation (FBR) reactors

The SBR was operated during 540 days, and the experimental time was divided in six stages (Figure 3.1), selected according to: a) CSTR operational conditions (generating an acidic-pH or neutral-pH feeding), b) proportion of COD_{PROT} in SBR cycles (if R_{PROT} is higher than 50%, it is a high-COD_{PROT} stage; if R_{PROT} is below 50%, it is a low-COD_{PROT} stage and, consequently, a high-COD_{VFA} stage), and c) enrichment strategy (ADF or M-ADF).

A recirculation was performed in five of the stages (Table 3.2) with the aim of reaching stable salt concentrations avoiding the use of tap water for dilution and load control. The maximum concentrations of 28.3 ± 0.7 g NaCl/L in the SBR-FBR corresponded to the use of saline wastewater (19 g NaCl/L), the addition of alkalinity as $Na(HCO_3)$, and NaOH for feeding pH control, so the raise was gradual (data not shown) to let the biomass adapt to the highly saline conditions.

3.3.2.1. SBR operation using acidic-pH feeding

Proteins were present in the SBR feeding, although their proportion in the COD (R_{PROT}) varied (Figure 3.3). To unravel the effect of proteins on the enrichment process, two operational strategies were tested by applying the two previously defined cycle distributions (ADF and M-ADF) to two levels of protein (high and low R_{PROT}).

- **High R_{PROT} and conventional ADF**

The enrichment of PHA-accumulating organisms was initiated applying an ADF regime (stage E1). Along with the 100% consumption of VFA (data not shown), approximately 34.7% of the proteins were removed in each cycle during the feast period (Table 3.3, Figure 3.4). Therefore, the MMC consumed proteins simultaneously to VFA. The presence of non-VFA COD in the enrichment process (like proteins) can be harmful for the selection, as carbon is used by non-storing populations to grow and proliferate in the system (Argiz et al., 2020).

This issue was explored by Korkakaki et al. (2016b) for a system with methanol and VFA, where they proposed the parameter *r* (q_{VFA}/q_{MeOH}) as a control for the enrichment success. They found that *r* needs to be equal to or higher than 4.7 g HAc/g MeOH (or 5 Cmol HAc/Cmol MeOH) for MMC selection to be effective. Here, *r* (q_{VFA}/q_{PROT}) had values of 0.81-2.60 g VFA/g protein (Table 3.3), indicating poor MMC selection, which in certain periods consumed proteins even faster than VFA. Hence, maximal PHA-accumulation capacities of the MMC from E1 in the FBR were always below 10% (Table 3.3).

- **High R_{PROT} and M-ADF**

Later, the cycle distribution of the SBR was modified to M-ADF (Stage E2), so a settling phase was introduced to improve MMC selection. After withdrawal, the removed volume (which was 25% of V_R) was not substituted with any other stream due to the inexistence of a current with the same composition of the acidified wastewater but without proteins, thus only 75% of the initial volume remained inside the reactor during the rest of the cycle.

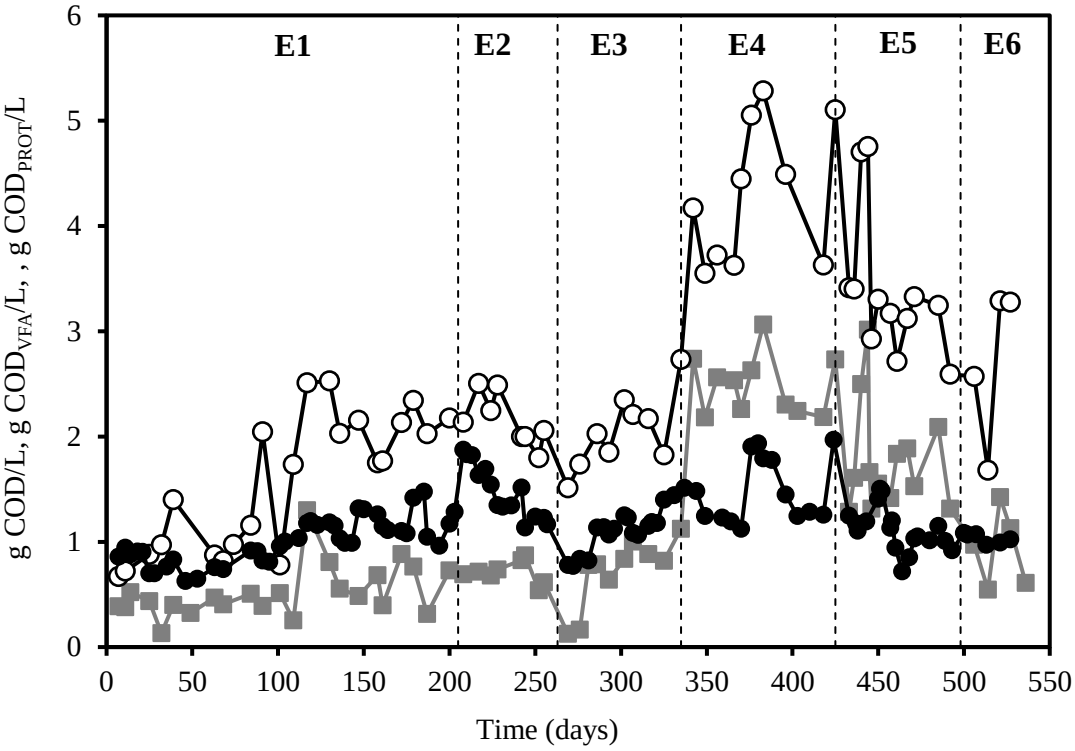


Figure 3.3. COD concentration at the beginning of SBR cycles: COD_0 (○), $COD_{VFA,0}$ (■) and $COD_{PROT,0}$ (●), in g COD/L.

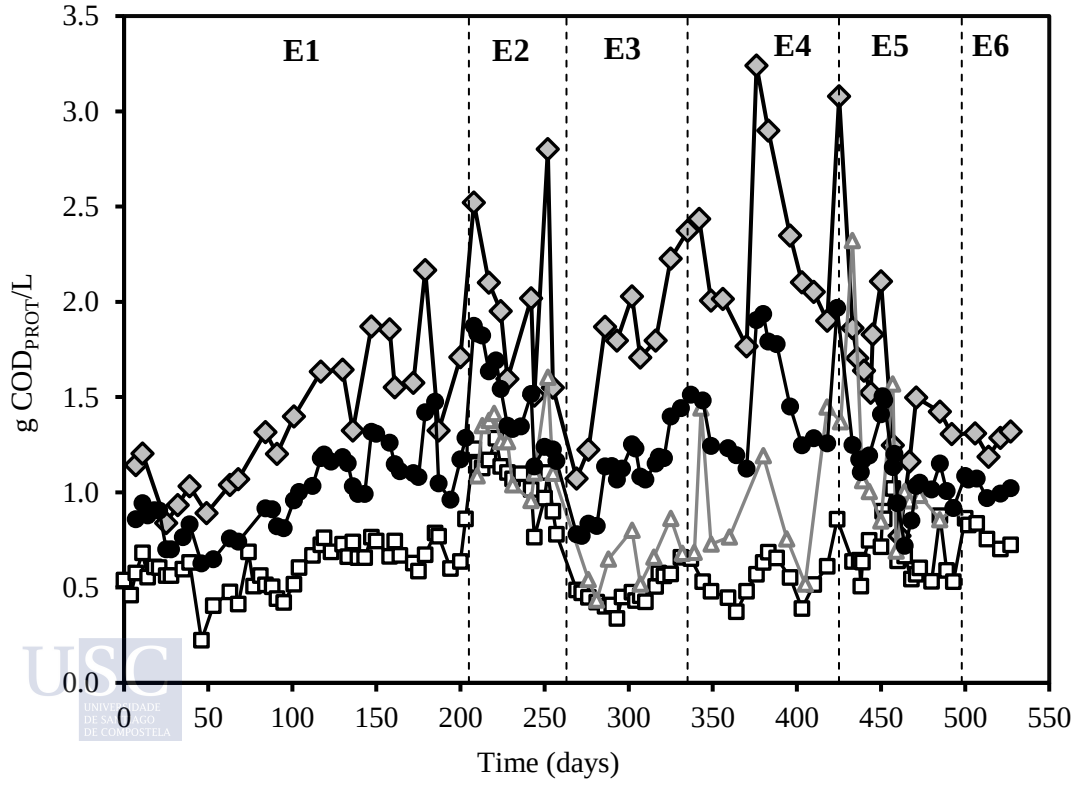


Figure 3.4. Protein concentration in SBR: in the influent ($COD_{PROT,INF}$ ◆), at the beginning of the cycle ($COD_{PROT,0}$, ●), in the supernatant ($COD_{PROT,SUP}$, △), and in the effluent ($COD_{PROT,EFF}$ □), expressed as g COD_{PROT}/L.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Except for the beginning of E1, where the applied load was still being adapted, the SBR operated in E1 and E2 at similar COD_0 and $COD_{VFA,0}$ (Table 3.2, Figure 3.3). For similar loads, *r* value generally increased from values below 3 g/g in E1, to values over 3 g/g in E2 (Table 3.3), indicating the MMC selection amelioration. Although the *r* value previously mentioned of 4.7 g HAc/g MeOH corresponded to a simple substrate (methanol), more complex substrates (as protein) could yield in a lower minimum *r* threshold.

Furthermore, a decrease in the protein oxidation by the MMC was observed with the change in the cycle type. Protein removal was about 35% in E1, while it was around 47% in E2. However, for E2, 20% of the removal happened due to supernatant withdrawal, so oxidation by the MMC was only 27%. Moreover, for E1, removal happened basically during feast, while, for E2, proteins were consumed mostly during famine phase. Even so, the accumulation capacity of the MMC did not significantly increase in E2 (Table 3.3).

It was observed that, when a low R_{PROT} (%) (this is, a high R_{VFA}) was applied to the SBR prior to the accumulation experiments in FBR (day 244 of operation), the MMC storing response improved. The accumulating capacity of the MMC in the FBR is highly dependent on the R_{VFA} (%) applied to the SBR: an R_{VFA} increase stimulate the storing response of MMCs (de Oliveira et al., 2019; Lorini et al., 2020). In fact, the accumulation capacity of the MMC in the FBR seemed to be boosted only by the R_{VFA} (g COD_{VFA}/g COD_0) of the SBR (Figure 3.5). Apparently, this behaviour is independent of the selection strategy applied (AFD, M-ADF).

On the other hand, the research studies carried out in the last years aim to incorporate the PHA production process in the wastewater treatment systems to transform treatment lines into valorisation trains. Therefore, the production of PHA must be simultaneous to the removal of pollutants. In this sense, the enrichment of the MMC can be simultaneous to processes involved in the biological nitrogen removal, like the partial nitritation (Fra-Vázquez et al., 2019), the nitritation/denitritation (Basset et al., 2016; Frison et al., 2015) or the denitrification (Santorio et al., 2019).

Nevertheless, for carbonaceous compounds not useful for PHA production (like proteins), the preferred strategy consists of removing them from the system through a settling stage after the end of the feast phase which will also increase the concentration of storing populations (Argiz et al., 2020a; Korkakaki et al., 2016b; Mulders et al., 2020). Moreover, when dealing with proteinaceous wastewater, it is also common to remove them through other means prior to the enrichment process (Colombo et al., 2019).

Here, in stage E2, the MMC partially lost its capacity of consuming proteins. Hence, COD_{PROT} is present in the reactor outflows (supernatant and effluent streams, Figure 3.4), meaning that, from a full-scale approach, further treatment would be needed before discharge. Uncoupling the carbon and nitrogen supply in the enrichment reactor has been successfully applied with protein-rich streams (Oliveira et al., 2017), but the supply of nitrogen is performed through the addition of chemicals, so the industrial application of this strategy can be hindered due to economic and environmental issues. Therefore, new strategies were tested to assess if PHA production and protein removal can occur in the same SBR cycle.

Table 3.3: Main results obtained by the monitoring of operational cycles in the enrichment (SBR) and accumulation (FBR) reactors.

Stage	E1 (ADF)				E2 (M-ADF)			E3 (M-ADF)		E4 (M-ADF)		E5 (M-ADF)	E6 (ADF)
Protein removal in SBR: total, due to oxidation in feast or famine, and due to supernatant withdrawal													
Average Total Protein Removal[(1)]	34.7				47.4			46.3		71.8		52.9	23.5
Oxidation (%) [Feast , Famine] (%)	34.7 [98.2 , 1.8]				27.0 [25.7 , 74.3]			31.9 [35.7 , 64.3]		53.6 [37.1 , 62.9]		28.2 [0.0 , 100.0]	23.5 [100.0 , 0.0]
Withdrawal (%)	-				20.4			14.4		18.1		24.8	-
SBR cycles													
Biomass (g VSS/L)	1.3 ± 0.7				2.5 ± 0.5			1.6 ± 0.4		4.2 ± 1.8		2.7 ± 1.1	1.4 ± 0.2
Cycle monitoring (day)	11	88	123	187	228	244	255	302	318	349		473	513
PHA (%)	12.4	11.5	3.5	3.6	5.4	8.2	6.7	14.6	11.0	18.8		14.0	5.1
q_{PHA} (mg PHA/(g X·h))	18.5	24.7	20.5	49.8	27.5	49.9	37.3	-	-	21.68		8.9	14.0
r (q_{VFA}/q_{PROT}), g/g	1.5	2.6	0.81	1.3	6.0	6.0	3.2	-	-	3.7		36.2	1.5
FBR accumulation assays													
Day	97		139		236	250	257			334	388[(2)]		508
PHA (%)	**6.9**		**7.8**		**7.3**	**17.8**	**8.8**			**41.5**	**28.6**		**14.7**
q_{PHA} (mg PHA/(g X·h))	13.2		10.0		13.9	6.3	17.2			68.1	36.9		24.5
Y_X (g COD_X/g COD_{VFA})	14.2		49.2		49.5	33.3	31.2			1.4	1.8		9.2
Conditions of the SBR cycle previous to the FBR-accumulation assay													
COD_0 (g COD/L)	2.0		2.5		2.5	2.0	2.1			3.6	5.3		2.6
R_{PROT} (%)	80.9		68.1		70.4	53.4	70.1			30.1	42.0		62.2

[(1)] The average protein removal is calculated using the protein concentration measured twice weekly in the SBR streams during each operational stage. The protein oxidation taking place in the feast or famine phases is calculated using the data obtained in the cycles monitored monthly throughout the operational time. Standard deviations of the elimination percentages are not provided because the wastewater composition was highly variable, so the propagation of uncertainty due to this variability generated extremely high and non-representative dispersions.

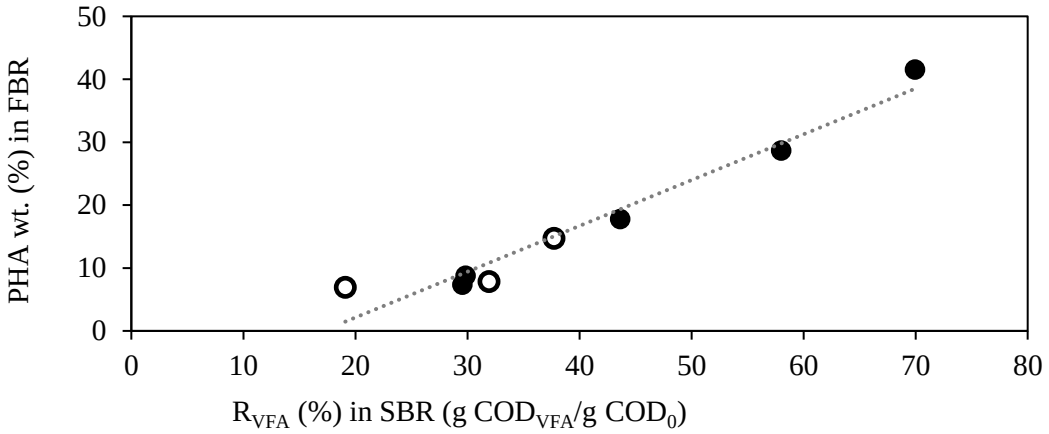


Figure 3.5: Relationship between R_{VFA} (%) in the SBR and the stored PHA (%) in the FBR, for experiments without settling (Cycle 1, ADF) (○) and with settling phase (Cycle 2, M-ADF) (●). As COD_{CARB} is negligible in the SBR-FBR system, COD is composed mainly by VFA and proteins, so R_{VFA} can be estimated as 100 – R_{PROT}.

To do so, a new approach was performed in E3, which consisted of varying the proteins/VFA ratio (thus, modifying R_{PROT} and R_{VFA}). Here, the COD_0 was maintained in the same range as in E2, but the $COD_{VFA,0}$ was varied as follows: at the beginning of E3 (days 265 to 280), the feeding of the SBR consisted of raw MCW (non-acidified) and recirculated SBR effluent. Therefore, $COD_{VFA,0}$ was negligible, while the protein content was maintained at the same levels as in prior stages (Figure 3.3), so the ratio proteins/VFA increased. Then, raw MCW was gradually substituted by the fermented MCW coming from the CSTR (containing VFA and proteins), decreasing again the ratio proteins/VFA. For E3, PHA accumulation increased in the SBR (below 10 % in E2, above 10 % in E3) along with protein oxidation (from 27 % in E2 to about 32 % in E3). Moreover, proteins were still consumed mainly in famine (about 64.3 %, Table 3.3).

- **Low R_{PROT} and M-ADF**

In stage E4 the CSTR effluent was directly fed to the SBR with no dilution, so R_{PROT} was lower than 50% for the first time throughout the experimental period. The protein elimination in the SBR in this stage was about 72%, with an 18% due to supernatant removal. Removal by oxidation (54 %) occurred in a 36% in the feast and 64% in the famine phases, so the protein consumption distribution in E4 were like that in E3 and E2 (mainly during famine). The PHA-storing capacities of the biomass in this stage were also the highest, exceeding the 40% in the FBR and being of approximately 20 % in the SBR, with an *r* value of of 3.7 g/g.

Therefore, proteins were consumed simultaneously to MMC enrichment in the SBR. The reached accumulation percentage (41.5% of PHA in the FBR) exceeded the threshold value stablished for economic feasibility (Bengtsson et al., 2017b), and it is similar to other results obtained in systems working with high proteins and/or salinity concentration. Hao et al. (2017) reported 42.3% of PHA accumulation when they used fermented sludge with high protein content, and Wen et al. (2018) obtained 42.6% of PHA when they employed fermented leachate at 15 g NaCl/L and 1.3 g COD/(L·d) in the SBR.

Besides, the biomass concentration was maximized, averaging 4.2 ± 1.8 g VSS/L in the SBR effluent, with the highest q_{PHA} and the lowest Y_X for all the stages tested (Table 3.3). When proteins were assimilated in stage E4, the total nitrogen consumption in SBR

cycles (measured as the sum of organic and inorganic nitrogen forms, see Appendix 1) is higher than the apparent ammonium nitrogen consumption during the cycles. This indicates that the nitrogen released by the proteins is employed by the MMC for growth. So, as more nitrogen is available, the concentration of biomass increased to reach its highest value in E4 (Table 3.3), and higher organic loads are tolerated.

To the best of our knowledge, few works have explored the effect of proteins in enrichment reactors. Jia et al. (2013) fed an SBR for the enrichment of an MMC with simulated excess sludge fermentation liquid, so a mixture of synthetic VFAs was initially used. Then, bovine serum albumin was added to the SBR feeding to test its effect on the enrichment process. Although the addition of proteins worsened the enrichment performance, the results showed that its consumption during the cycle facilitated the cell growth.

Moreover, a clear deterioration of the accumulation dynamic was found when R_{PROT} (g COD_{PROT}/g COD) was higher than 30% in SBR feeding. Here, with the applied loads in stage E4, the R_{VFA} was 61.36 ± 9.97%, and the best results were achieved in day 334, when R_{VFA} was as high as 69.9% (so R_{PROT} was about 30%). Furthermore, the correlation in Figure 3.5 shows that a R_{VFA} of about 70% (therefore, R_{PROT} of 30%) is necessary to produce 40% of accumulation. In fact, when Hao et al. (2017) used the guidance provided by Jia et al. (2013) to treat the excess sludge fermented liquid (containing proteins and carbohydrates), they achieved good results despite the presence of non-VFA organic matter.

Finally, Tu et al. (2020) harvested the biomass of a MMC in an enrichment reactor just after the feast phase. Then, the famine period was carried out in batch assays where different concentrations of non-VFA COD were added. In their study, a worsening in the enrichment performance was detected, as internal PHA at the end of the cycle was higher than for cycles without addition of non-VFA COD. However, a lag phase in the profile of PHA consumption of the stored carbon was detected in the MMC while non-VFA COD was consumed. This suggested that the microorganisms capable of storing PHA can also choose the substrate to be consumed (internal or external) when both are available.

Therefore, although high concentrations of proteins are harmful for the accumulation process (above 30% of the COD), their presence can stimulate the system dynamics, as proteins are non-VFA COD, but they are also nitrogen and carbon sources. Moreover, protein consumption releases nitrogen, that will be used by the two present populations (storing and non-storing) for growth in the famine phase. Consequently, proteins can enhance the process performance if a dynamic balance is stablished in the reactor medium and if proper acclimation and selection strategies are applied as demonstrated here.

Consequently, the imposed enrichment strategy (ADF or M-ADF) is not directly responsible of an increase in the MMC accumulating capacity. However, the imposition of a proper selection dynamic, with a second selective pressure (the settling stage) is necessary to increase the applied load that will rise the storing response. Moreover, the presence of proteins can be beneficial for the system if R_{PROT} is about 30%, as the released nitrogen due to protein uptake can be used by biomass for growth. As more biomass is available, the tolerated loads will be higher and PHA production can be increased.

Finally, because of the optimized behaviour in the system, two different accumulation approaches were tested as indicated in Section 3.2.1.3. The best results were obtained when 15 Cmmol/(L·pulse) were applied (day 334) (Table 3.3).

3.3.2.2. SBR operation using neutral-pH feeding

- **High R_{PROT} and M-ADF**

The proteins present in the SBR feeding in stage E5 were the ones not transformed into VFA in the CSTR in A6 (operated at 6.25 days of residence time and neutral pH), so proteins were less available for the MMC. Because of this, when the applied loads of E4 were tested in E5 (COD_0 was 4 – 5 g COD/L, Figure 3.3), the feast phase lasted 6-8 hours (feast lasted about 2.0 – 2.5 hours for stages E1 – E3 and 4.0 – 4.5 hours for E4, data not shown), and the biomass concentration in the reactor decreased to 2.7 ± 1.1 g VSS/L (Table 3.3).

Due to this instability, accumulation cycles could not be performed and, to attain steady state conditions, the applied load was diminished to reach a COD_0 of 2 – 3 g COD/L, where R_{PROT} increased due to recirculation. When no proteins are available, the selection of the MMC is supposed to be improved, as the concentration of non-VFA forms of COD decreases. However, as less nitrogen is available, less growth is promoted and the biomass concentration at the end of the cycle decreases, so organic load needs to be reduced to maintain the accumulation dynamic.

In stage E5, the value of *r* increased to its highest level basically because proteins were slightly consumed. However, PHA storing decreased to 14.03% in the SBR since the applied load could not be raised due to excessively length of the feast periods (6-8 hours) and the occurrence of the biomass loss. Moreover, q_{PHA} for the enrichment cycles in the stage E5 presented the lowest values of any tested stage of 8.9 mg PHA/(g X·h) and protein oxidation decreased to 28.2%. Nevertheless, it still happened during the famine phase (like in the other stages with M-ADF).

- **High R_{PROT} and ADF**

In stage E6, the settling phase was removed from the cycle and the ADF strategy was imposed as in the stage E1. Here, although protein removal due to oxidation decreased to 23.5%, the accumulation in the SBR and FBR worsened to values of 5.09% and 14.66%, respectively. Moreover, protein consumption occurred during the feast phase for the two stages where conventional ADF was tested (E1 and E6, Table 3.3).

Generally, when proteins are consumed during the feast phase of the cycle, the enrichment process is unsuccessful (Korkakaki et al., 2016b; Oliveira et al., 2017). Although their presence is normally considered undesirable, protein consumption during the famine stage can promote biomass growth without negatively affecting the enrichment performance when its presence is lower than 30 % of the COD (Jia et al., 2013). In the present study, although the enrichment strategy did not directly affect the accumulating capacity for the same applied loads, it had clear and significant impact on the protein consumption profile.

For the stages with ADF strategy (only relying on the Feast/Famine regime), proteins were totally or mainly consumed during the feast phase of the cycle, but, when the M-ADF strategy was imposed, they were consumed mostly during the famine phase. Therefore, M-ADF improved the culture selection, while protein consumption during famine was linked to an increase in the concentration of accumulating microorganisms. Finally, as storing populations proliferated and biomass concentration was higher, the tolerated loads and the PHA accumulation raised.

Summing up, the enrichment strategy did not directly affect the storing capacity (it is only boosted by the load increase) but it does indirectly, as a load raise is only possible if a proper enrichment strategy (M-ADF) is applied. Therefore, suitable selection of the culture (where non-VFA forms are present) is only possible if two selective pressures are imposed.

Again, the maximum COD_0 tolerated by the system was 2 – 3 g COD/L, so R_{PROT} increased due to dilution. Moreover, a change in the enrichment strategy reversed the SBR behaviour, which presented similar *r* values and biomass concentration than in stage E1, while protein oxidation occurred again during famine (as in E1, Table 3.3). Therefore, for the same operational strategy but lower protein availability, the system performance was not improved in E5 although protein consumption was lower with respect to stage E1.

3.4 CONCLUSIONS

Protein conversion into VFA in the acidification unit was enhanced from 10.3% to 69.2% with the addition of $Na(HCO_3)$ and neutral pH. In the enrichment unit, proteins can be beneficial (around/below 30% of COD) if the enrichment strategy includes a settling stage to promote their consumption in the famine, because the nitrogen released is used for biomass growth. Therefore, as biomass concentration increases, the load tolerated and the PHA produced by the system are higher. Finally, varying the proteins/VFA ratio to acclimate the MMC to proteins allowed increasing the PHA accumulation from 8.8 to 41.5%.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

4. HOW CAN WE VALIDATE THE ENVIRONMENTAL PROFILE OF BIOPLASTICS? TOWARDS THE INTRODUCTION OF PHA IN THE VALUE-CHAINS.

SUMMARY

Petrochemical plastics are threatening the environment, human health, and economy. Biodegradable plastics produced from renewable sources like polyhydroxyalkanoates (PHA) are a promising alternative to solve this crisis. However, the fact that a material is biodegradable, or renewable, does not automatically make it environmentally friendly, so a validation (normally though life cycle assessment (LCA)) is needed. Thus, the present review studied the research works published in the last 20 years about this issue. It focuses on the methodological features defining the outputs of the LCA, like the functional unit and allocation choices, but also on the data sources of the existing studies, as these issues need to be addressed by the LCA community to properly evaluate the environmental profile of biomaterials like PHA. As found in this review, the few LCA studies for PHA production obtained contradictory results, mainly due to the lack of actual data, but also to the absence of a consensus methodological framework. As one of the main results of this review, we found that many current references still use inventories generated twenty years ago. This is necessarily going to generate conflicting results and is not going to provide information in concordance to nowadays processes, so LCA practitioners need to create new inventories reflecting nowadays PHA production processes if accurate results are wanted.

Part of the content of this chapter was published as A. Roibás-Rozas[1], M. Saavedra del Oso[1], G. Zarroli[1,2], M. Mauricio-Iglesias[1], A. Mosquera-Corral[1], S. Fiore[2], and A. Hospido[1] (2022). **How can we validate the environmental profile of bioplastics? Towards the introduction of Polyhydroxyalkanoates (PHA) in the value-chains**. Chapter 20 in: C. Teodosiu, S. Fiore, A. Hospido (Eds.), Assessing Progress Towards Sustainability, Elsevier. https://www.elsevier.com/books/assessing-progress-towards-sustainability/teodosiu/978-0-323-85851-9

[a] CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

[b] DIATI (Department of Engineering for Environment, Land and Infrastructures), Politecnico di Torino, 10129 Torino, Italy

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

4.1 INTRODUCTION

Petrochemical plastics are threatening the environment, human health, and economy. Biodegradable plastics produced from renewable sources like PHA are a promising alternative to solve this crisis. However, the fact that a material is biodegradable, or renewable, does not automatically mean a better environmental profile, so a validation (normally though LCA) is needed.

The few LCA studies for PHA production obtained contradictory results, mainly due to the lack of actual data, but also to the absence of a consensus methodological framework. The present review studied the research works published in the last 20 years about this issue. It focuses on the methodological features defining the outputs of the LCA, like the functional unit and allocation choices, but also in the data sources of the existing studies, as these issues need to be addressed by the LCA community to properly evaluate the environmental profile of biomaterials like PHA.

4.2. REVIEW STRATEGY: CURRENT LITERATURE REGARDING LCA OF PHA

This review was performed using the Scopus search engine, where the following key words and their acronyms were selected: Life Cycle Assessment (LCA), Polyhydroxyalkanoates (PHA), bioplastics, sustainable process index (SPI), waste reduction algorithm (WRA), techno-economic analysis (TEA) and sustainability. The results of the review will be discussed according to: i) LCA methodological issues, ii) process issues, and iii) the studies' outputs. Regarding LCA methodological issues, a standardized protocol to perform LCA studies of PHA and bioplastics has been recently proposed by the EC and the Joint Research Centre (JRC) of the EC to conduct comparative LCA studies for bioplastics and fossil-based polymers (Nessi et al., 2020), which is based on several widely recognised guidance documents, such as ISO or EN standards. The main principles of this guidelines framed the revision and discussion presented later. Finally, besides the discussion of the main outputs, the referred process issues classification is linked to relevant PHA production features, explicitly:

- Substrate employed: ad hoc produced material vs. waste stream. Substrate selection has economic, social, ethical, and environmental implications, but it also partially defines the PHA production process (wastes are generally preferred for MMC-based systems). Moreover, the relevant environmental categories for each system type are linked to the substrate employed (in crop-based systems, land use or eutrophication are of special importance). Therefore, reviewed articles will be classified as waste streams or dedicated crops.

- Culture type: mixed culture vs. pure culture. Although there is not explicit correlation between culture type and feedstock selection, these two issues are often linked. Besides, the culture type defines the process configuration and type, so the presented studies will be also categorised regarding the microbiological features of the process, as mixed and pure cultures.

USC DE SANTIAGO DE COMPOSTELA

4.3 CRITICAL REVIEW OF THE CURRENT LCA STUDIES FOR PHA PRODUCTION

A selection of 60 papers where the environmental profile of PHA production was assessed resulted from the literature search. Most of these papers will be discussed in the following sections. Thus, the references can be found in the literature section of this

thesis. However, the research works not explicitly addressed but included among the 60 documents are included in Appendix 2. The results for LCA methodological issues and process issues are quantitatively summarised in Table 4.1, and further discussed in the consequent sections of this Chapter.

The first document addressing LCA of PHA was published back in 1998 and, since then, two studies were published per year on average, with an important growth in the last two years (i.e., 15 documents in 2019/2020). About 70% of the documents were research papers, and around 20% were reviews where LCA of biopolymers was the main issue, or where the PHA state of the art was assessed and LCA was included. The rest of the documents were 4 books or book chapters (Martinez-Hernandez et al., 2018; Gupta et al., 2019; Medina-Martos et al., 2019; Plackett, 2011), 1 report (Bengtsson et al., 2017b), and 2 methodological documents: one assessing carbon displacement of bioproducts in comparison to conventional materials (Lynd and Wang, 2003), and the JCR-EC report (Nessi et al., 2020). Nevertheless, Heimersson et al. (2014) addressed methodological issues in their review, which also included a case study.

Table 4.1 Main quantitative results of the critical review. Results are expressed as the total number of studies (NS) and, therefore, some of values include more than one of the considered features (for example, some studies compared MMC and pure culture performance, thus, they are accounted for within both culture types).

Classification of the studies attending to LCA methodological issues									
Document type	NS	Assessment type	NS	Functional Unit	NS	System Boundaries	NS	Data Source[1]	NS
Paper	40	LCA	30	Polymer mass	25	Cradle-to-gate	27	Secondary	16
Review	13	LCA + Economy	9	Feedstock	7	Cradle-to-grave	7	Primary*	26
Book/ chapter	4	SPI	4	Consumer product	6	Only Extraction	3	*Primary source type	
Methodology	2	Social + Environmental + Economic	3	Other	3	Gate-to-gate	3	Personal Communication	6
Report	1	TM-LCA	2	Not clear	2	Only EoL	1	Simulation or Mass Balance	9
TOTAL	60	Other	2			Cradle-to-cradle	1	Lab or pilot reactors	14

Classification of the studies attending to process issues			
Substrate type	NS	Culture type	NS
Pure (dedicated)	18	Pure	25
Waste	28	MMC	11
Synthetic/Not clear	3	Not specified	4

[1] The data is expressed as studies only based on previous literature works (secondary sources) and studies based on data generated explicitly for the assessment (primary sources).

4.3.1. Review Outputs regarding methodological issues

4.3.1.1. Motivation of the studies, Functional Unit, and System Boundaries

In the first years when LCA-PHA was researched, studies aimed to compare the environmental profile of PHA with the profiles of polystyrene (PS), polyethylene (PE) or PP. Nonetheless, in the last years, studies aim to investigate the most optimal routes to treat industrial streams, so the obtained results provide information about the routes with lower energy requirements or GHG emissions.

On the other hand, the question of the SB is linked to the FU definition. The most common SB were the cradle-to-gate type[1] (about 65% of the studies), which means that the EoL of the product is not considered. Thus, only the production process is included in the study, as very few data is yet available about bioplastics EoL. However, as seen previously, JCR-EC method recommends cradle-to-grave[2] SB for LCA of PHA, as, when EoL of the product is not included, biodegradability is disregarded, and the obtained results are just partial. It is also truth that fair comparison cannot be established between PHA and petrochemical plastics with the current LCA methodology, as the actual effects of plastic pollution are not yet modelled.

Thus, biodegradability and energy recovery from bioplastics are disregarded in the study if the LCA has Cradle-to-gate SB, and the devastating effects of marine and terrestrial plastic pollution are dismissed if Cradle-to-grave approach is applied. This means that, possibly, most of the studies performed to date suffer from bias in favour of petrochemical plastics, so results might not show the actual net benefits of PHA. Moreover, the scientific community must orient their efforts to gather data about the EoL of PHA and the effects of plastic leaks in the environment, as addressed in the work by Changwichan et al. (2018) or in projects like MarILCA (2020).

About 75% of the studies considered a FU linked to the final product (normally, mass of polymer or mass of final product, as plastic bags, or boxes). As stablished by Heimersson et al. (2014), Cradle-to-gate SB are enough if the studied systems have the same gate (a certain mass of PHA), and the aim of the study is to assess different bioplastic production routes. However, for assessments with a FU considering the mass of polymer, and where the aim of the evaluation is to compare the environmental performance of bio-based and petrochemical materials, the issue of the SB becomes relevant and hard to solve.

Moreover, PHA properties are different than the ones of petrochemical plastics, and they depend on the extraction protocol, the monomer proportion distribution and so on. Therefore, the assumption generally made that 1 kg of PHA will directly replace 1 kg of PE, PP or PET is not very accurate and can lead to wrong results. Unfortunately, most of the current studies reviewed follow this approach, and this might be one of the reasons why the obtained results are highly variable and sometimes conflicting.

This question needs to be considered and properly addressed, at least until more information about PHA use and EoL and plastic pollution is provided. Meanwhile, the lack of data can be managed by applying conservative replacement ratios of PHA to other polymers (Vega et al., 2019) and/or by performing sensitivity analysis (Roibás-Rozas et al., 2020).

On the other hand, a FU referred to the feedstock appears to be the most suitable option to compare different treatment/valorisation systems to produce the same polymer. This means assessments where the aim is not to compare the environmental profile of biopolymers to petrochemical polymers, but to study the performance of different process schemes. Although Gurieff and Lant (2007) considered for the first time the influent as a

[1] Cradle-to-gate refers to an LCA that considers the life cycle of a product or process, from raw material acquisition only to the factory gate. Thus, it excludes the use and disposal phases.

[2] Cradle-to-grave refers to an LCA that considers the whole life cycle of a product or process, from raw material acquisition through production, use, end of life, and final disposal (ISO, 2006).

FU more than a decade ago, the standard approach was the opposite for almost each study reported until Morgan-Sagastume et al. (2016) (except for assessments only considering the DSP).

This is linked to the process approach in the studied system, which normally was a pure culture fed by a pure substrate, so the aim was to compare the performance of the produced PHA to a conventional material. Consequently, when more process approaches appeared (not only MMCs, but also new production or extraction methods) and wastes started to be the main considered option for PHA production, the trend switched, and the chosen FU was referred to the feedstock, as the aim was not only to produce PHA but to treat a waste. In fact, 5 out of 10 studies reported in the last two years considered the influent as a FU, in opposition to the approach followed in the past decades, also indicating that the goal of these novel process approaches is not only to produce a polymer, but to establish processes that fit circularity by valorising waste streams.

4.3.1.2. Impact categories

The most assessed impact category is Global Warming Potential (GWP), as it is expected that bioproducts can mitigate the effect of climate change. Non-renewable Energy Use (NREU) or Fossil Resource Depletion are also commonly evaluated as bioplastics would replace petrochemical materials. Acidification and Eutrophication Potential were also commonly studied, and just a few papers reviewed an important number of the categories included in the ReCiPe impact methodology (Fernández-Braña et al., 2019; Harding et al., 2007; Roibás-Rozas et al., 2020; Vega et al., 2020; Vogli et al., 2020).

Again, the lack of data regarding EoL affects to the selection of impact categories, as it is not completely clear how the effects linked to polymer production, use and disposal are transferred on the environmental compartments. On the other hand, the JRC-EC method states that the whole 16 categories addressed in the Product Environmental Footprint Guidelines (Manfredi et al., 2012), so each of them must be considered from now on. In any case, it has been stated that, when the LCA is performed for polymers processed from crops, categories as Land Use, Acidification and Eutrophication must be mandatorily considered (Heimersson et al., 2014).

4.3.1.3. Assessment type

More than 60% of the assessments used pure LCA (meaning according to ISO 14040 and 14044), where the most employed software was SimaPro (Gupta et al., 2019). Around 20% combined environmental assessment (i.e. LCA) and cost assessment by adding any economic evaluation (Changwichan et al., 2018; Gurieff and Lant, 2007; Leong et al., 2017, 2016; Martinez-Hernandez et al., 2018) or implementing TEA (Fernández-Dacosta et al., 2015; Medina-Martos et al., 2019; Saavedra del Oso et al., 2020; Vega et al., 2020).

Almost 5% of the reviewed papers used Territorial Metabolism-LCA (TM-LCA), (Vega et al., 2020, 2019)which considers regional aspects of the environmental assessments, and almost 10% used SPI (Koller et al., 2013b; Kourmentza et al., 2017; Rathi et al., 2013), which is an index that normalizes impacts according to the availability of planet surface (it calculates the necessary area for industrial processes).

Recently, studies started including other sustainability aspects by linking environmental impacts with social and economic effects (Chen et al., 2020; Pérez et al., 2020a; Talan et al., 2020). In fact, the most recent findings have pointed out that the

assessment of bioproducts cannot be linked only to environmental aspects, as suggested by the triple-bottom line of sustainability, which includes environment, economy, and society.

It makes sense that, if one wants to evaluate the feasibility of bioeconomy implementation, the concept of sustainability needs to be linked not only to the environmental issues, but also to economic aspects, which are necessarily related to society.

Finally, the remaining studies combined LCA with green design principles by scoring products according to proposed Ecodesign metrics (Tabone et al., 2010) or with WRA algorithm, which links waste minimization with a decrease in ecotoxic impacts (Leong et al., 2016).

4.3.1.4. Source of data. Is there enough information to perform accurate assessments?

Yates and Barlow (2013) published the first literature review of PHA sustainability assessments (also including PLA and starch-based polymers). They reported that the studies published until that date showed unclear data sources, as some LCAs were performed using confidential information or personal communications (Hermann et al., 2007; Kim and Dale, 2005; Pietrini et al., 2007) and the reader cannot know the features of the studied system. Moreover, assessments performed in the last decade are relying on data provided by studies carried out twenty years ago. To see some examples, see Figure 4.1, where the studies reported on Table 4.1 based on secondary data (dashed lines) were tracked to find the source of information (continuous lines)

LCA practitioners need to stop relying in data generated twenty years ago to provide results referring today's processes. New inventories need to be generated adapted to the novel strategies for PHA production (MMCs, waste streams, or strategies to improve PHA yield).

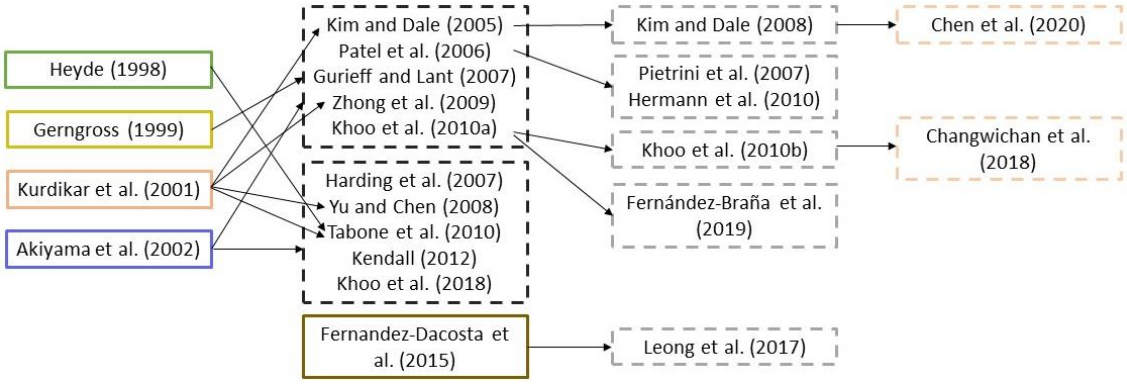


Figure 4.1 Relationship among some of the published LCA-PHA studies and their sources. References framed in continuous lines represent studies presenting original inventories (using primary and/or secondary data), and dashed lines represent the studies derived from these inventories.

Otherwise, LCA of PHA is halted and results are going to repeat the same outputs generated in the past, reproducing the same biases. Fortunately, some of the most recent studies are based on the inventories generated explicitly for the assessments, and based on up-scaled laboratory experiences (Nitkiewicz et al., 2020; Pérez et al., 2020b; Righi et

al., 2016; Roibás-Rozas et al., 2020; Vogli et al., 2020) or pilot-scale experiences (Bengtsson et al., 2017b; Morgan-Sagastume et al., 2016; Vega et al., 2020, 2019).

4.3.2. Classification of the studies regarding process type

Among the research works published until 2010, only few considered the use of any waste feedstock. However, in the last decade the trend switched, and most of the studies focused on the valorisation of waste streams, where only two studies addressed PHA production from dedicated crops (Changwichan et al., 2018; Kookos et al., 2019) using secondary data from literature (Akiyama et al. (2003), and Khoo and Tan (2010), respectively, see Figure 3) for the production of the substrate.

Regarding culture type, only Gurieff and Lant (2007) and Fernández-Dacosta et al. (2015) considered MMC-based processes until 2015. Nevertheless, and considering the recent trends in PHA production pathways, most of the papers published in the last years evaluated processed based on MMC and/or waste streams.

Moreover, it has been pointed out that data sources need to be clear and transparent (Yates and Barlow, 2013), as sometimes data sources are unclear and it is hard to understand features as important as the culture type or the feedstock employed. This becomes an important issue specially for old references and works relying on them (see Figure 3), as some of these studies were based on personal communications or confidential information provided by the company.

4.3.3. Main outputs

As mentioned, the results obtained from PHA-LCA studies were sometimes contradictory or conflicting among them, and the reasons might be linked to biases related to the lack of information for the last stages of polymers life cycle (biobased and petrochemical) and allocation choices (Heimersson et al., 2014; Kookos et al., 2019). However, studies agreed at indicating that the environmental performance of the process can be improved by introducing waste streams in the PHA production process (Chanprateep, 2010; Kim and Dale, 2008, 2005; Koller et al., 2013b; Shahzad et al., 2013), and Kendall (2012) proved that, as expected, using them is more favourable than employing dedicated crops.

Moreover, the energy source was also important, as green energy usage can switch the environmental profile of the bioproduct (Khoo et al., 2010; Kurdikar et al., 2000; Zhong et al., 2009), suggesting that impacts might be regionally dependent of the energy mix employed (Shahzad et al., 2013). Finally, the most recent outputs highlight specially that the success of the process can only be complete if several aspects of sustainability (economic, environmental and social) are considered (Chen et al., 2020; Koller et al., 2017; Kookos et al., 2019; Pérez et al., 2020b; Talan et al., 2020; Yadav et al., 2020). Results point out that this success will be reached if an integral biorefinery approach is applied (Medina-Martos et al., 2019; Nitkiewicz et al., 2020; Yadav et al., 2020), where all streams are valorised (for example, the generated sludge is converted into biogas by anaerobic digestion). In fact, the latest outputs proved that economic and environmental viability of PHA production facilities can be reached if part of the waste carbon used for biopolymer generation is derived for on-site for energy generation, like the case of anaerobic digestion (AD) (Dietrich et al., 2017; Martinez-Hernandez et al., 2018; Kookos et al., 2019; Vega et al., 2019, 2020; Pérez et al., 2020; Vogli et al., 2020)

4.3.3.1. Full value-chain vision. What do we know about the use and EoL stages?

As mentioned, little is known about the last stages of PHA life cycle. On the one hand, it has been considered that impacts derived from the use stage are the same as for petrochemical plastics, so they can be disregarded for comparative LCA with conventional materials (Hermann et al., 2007). On the other hand, it is especially urgent to generate information about the EoL as just 9 out of the 40 papers revised considered it and only 4 papers compared different management routes for the (bio)plastic materials once used.

Heyde (1998) concluded that waste bioplastic management might determine the global sustainability of the process, while Khoo and Tan (2010) highlighted that this stage usually has a low impact in relation to the full PHA life cycle. Both research works considered landfilling, composting and incineration, where landfilling showed the worst results and the success of the remaining options depended on factors like energy origin. Hermann et al. (2011) compared AD, incineration, and home/industrial composting to find that AD had generally the best performances and industrial composting the worst. Finally, Changwichan et al. (2018) performed a comparative study which included landfilling, composting, incineration and mechanical recycling, where recycling clearly showed the best results. These outputs indicate that new studies including modern approaches and recent findings are needed, as composting (the most typically considered EoL route) might not be necessarily the optimal alternative.

4.3.4. Lessons learned

PHAs are a promising alternative to solve the serious challenges we are currently facing regarding plastic pollution and climate change. However, PHA full-scale production is hindered by the economic feasibility and the lack of consistency concerning its environmental validation, which, as most studies indicated, are linked to i) the use of waste streams as feedstock, ii) employing clean or renewable energy, and iii) applying an integral biorefinery vision, where each stream is valorised (energy production on-site is encouraged). In this sense, the main aspects that need to be urgently solved for this validation are:

a) **The lack of data**. The uncertainties regarding plastic leaks in the environment for petrochemical plastics and EoL pathways for biopolymers are generating a bias in favour of conventional materials, as cradle to gate SB exclude the benefits generated from valorising wasted bioplastics and cradle to grave SB disregard the serious effects of uncontrolled plastic leaks in the environment. These gaps generate conflicting or contradictory results depending, among other factors, on the allocation choices and the hypothesis formulated when trying to fill those data requirements up.
b) **Sources of information**. Although the number of studies performed based on new inventories is increasing, there is still a big number of assessments carried out relying on old information, as seen in Section 4.1.4 (about 60 % of the research works used only secondary data sources from previous studies). This provokes results that are stuck in old technologies and process approaches, as novel strategies for PHA production are not considered, where the reproduction of biases is aggravated.
c) **Process scale**. There is still no full-scale market for PHA, so studies are based on up-scaled laboratory data or pilot-scale systems. This is another issue that can

increase the uncertainties of the assessments, as, when the environmental performance of a conventional petrochemical plastic is compared to the one of PHA, the comparison is performed between a process widely implemented at full-scale and a process with just a few pilot-scale working facilities. Although several assessment approaches have been proposed in order to fill this gap from pilot to full-scale in emerging technologies (Bergerson et al., 2019; Hung et al., 2018; Piccinno et al., 2016), to the best of our knowledge, none of them has been applied yet to PHA.

In any case, the success of the process is not only linked to the environmental validation, and future studies must mandatorily include economic and/or social aspects in the assessment.

4.4. FUTURE OUTLOOK, CHALLENGES AND KEY PLAYERS

The problem of plastic pollution is serious and real, and the necessity of looking for a suitable solution as soon as possible is a fact. However, MMC-based full-scale production of PHA from waste streams is not a reality yet due to some economic and environmental bottlenecks. Basically, the research community need to provide information about the effects of plastic leaks in the environment and about the last stages of PHA lifecycle (manufacture, use and EoL). Then, a robust framework can be established to determine the main features of the PHA value chain and, finally, that information can be employed to develop policies and regulations that fit the principles of circular economy.

Although this information is not available yet, the scientific community and agents such as the Life Cycle Initiative are gathering efforts to generate this framework, and several groups and projects deserve especial attention in this scenario. The objective of the Plastic Leak Project (2020) is to conceive a methodology to identify and inventory plastic leakage in the environment, focusing in important sectors as transport, textiles, plastic products and packaging or plastic pellets. The mentioned project MarILCA (2020) aims to determine the effects of marine litter, so they are gathering information not only regarding inventories, but also impacts, as they are trying to develop a model to describe how the release of plastic affect the different environmental compartments, and how these plastics are transferred among them.

The introduction of biodegradable plastics in the value chains is a necessity, but it is still not a reality due to several drawbacks that need to be urgently addressed. As economic issues are the main barrier in this pathway, new process configurations and strategies need to be explored, so less energy-intense routes can be proposed. However, in a bioeconomy framework, financial viability and environmental validation are two sides of the same coin. In this sense, aspects as energy efficiency optimisation will not only yield in monetary savings, but also in environmental credits.

4.5 CONCLUSIONS

The environmental validation of PHA was studied for the first time in 1998, and, since then, approximately two research works were published yearly. Although the first studies performed assessed systems based on pure cultures, the current trend is to evaluate MMC-based processes, as they are supposed to be less expensive. Moreover, current evaluations generally study systems fed with waste feedstocks, as the use of dedicated crops might not fit the circular economy principles.

The main issue found is linked to the FU and the allocation employed, as excluding EoL of the system boundaries disregards the benefits derived from PHA biodegradability and including it dismisses the effects of plastic leaks in the environment and plastic pollution. Therefore, new information regarding these topics needs to be urgently generated. In this sense, it is also necessary to provide data regarding pilot and full-scale processes, as current information is based on secondary data, simulations, or up-scaled lab results. Moreover, LCA practitioners need to be especially careful when using secondary data, as we found that many current references still use inventories generated twenty years ago. This is necessarily going to generate conflicting results and is not going to provide information in concordance to nowadays processes, so the creation of inventories for new process approaches is encouraged.

Finally, LCA of PHA need to consider each stage of the life cycle to complete the value chain, and cradle-to-grave approach is encouraged. Furthermore, it is recommended that assessments do not include only environmental aspects, but also other issues linked to bioeconomy and sustainability, as social or economic aspects, to get a full overview of the evaluation that addresses the integral approach linked to circularity.

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

5. ENVIRONMENTAL ASSESSMENT OF COMPLEX WASTEWATER VALORISATION BY PHA PRODUCTION

SUMMARY

Environmental evaluations assessing bioplastics production at full-scale are still scarce. As pointed out in Chapter 4, one of the main problems for the validation of PHA production processes (mostly the MMC-based ones) is that most of the current available studies are based on secondary data, sometimes generated twenty years ago, so primary data are lacking. So, this chapter aims to contribute to partially fill that gap by providing a new study based on primary information.

Experimental results from Chapter 3 were then used to evaluate the environmental feasibility of PHA production employing high load wastewater. To do so, results from the lab system were scaled-up to define and compare a circular economy scenario (i.e., valorisation of the wastewater by PHA production) with the current linear approach (i.e. effluent treatment and discharge). Results show an average improvement of ca. 25% for nine out of ten impact categories evaluated if the circular economy approach is implemented. Sensitivity analysis showed the significance of the sludge management strategy, the replacement yield of fossil-based plastic to biopolymer, and the downstream process. Finally, this work proposes new process integration strategies to reduce the environmental burdens of PHA production and increase the body of knowledge on MMC-based processes.

The contents of this chapter were published as Roibás-Rozas, A., Mosquera-Corral, A., & Hospido, A. (2020). **Environmental assessment of complex wastewater valorisation by polyhydroxyalkanoates production**. *Science of the Total Environment, 744*. https://doi.org/10.1016/j.scitotenv.2020.140893

All authors' affiliation is CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

5.1 INTRODUCTION

Fish canning industries play a significant role in the Spanish market, where the North-Western region of Galicia is the main mollusc producer of Europe (APROMAR, 2019). The production of mussels constitutes about the 80% of the total Spanish fish canning production, generating around 250 kt of mussels per year. This industry is an essential driving force for the region, since it is responsible for more than 100 M€ of annual income and for more than 10,000 jobs, representing between the 15% and 40% of the total active population living in the areas in which mussels are harvested and processed (Rey-Méndez et al., 2016).

However, the fish canning industry activity generates high amounts of complex wastewater containing salts, organic matter, nutrients, and solids, among other compounds. Moreover, the wastewater coming from the boiling of mussels (MCW) is a highly polluted effluent which treatment is challenging. In fact, the environmental concern regarding the treatment and disposal of MCW is high, and several measures (economic incentives, research projects, environmental policies…) were applied to solve the issue related to MCW, as reflected in specific legislation (Consellería de Medio Rural e Mar, 2015). These effluents constitute a threat for the industry, which is obviously dependent on the water quality of the areas where seafood is harvested. These areas, called *rías* or estuaries, are unique spaces with a high biological diversity, optimal for the farming and growth of seafood. Therefore, it is important to treat properly those industrial effluents to guarantee the environmental stability of the estuaries and the economic sustainability of this region.

Although the presence of salts, sulphate and other compounds which are characteristic of fish canning effluents can hinder or inhibit biological activity, biological treatments (like activated sludge or anaerobic digestion) were possible in the past when specific operational strategies were applied (Palmeiro-Sánchez et al., 2013). Considering this, it is of interest to explore alternative routes for the biological treatment of these high-salinity wastewaters, and for their valorisation under the circular economy perspective.

PHA are polyesters produced as intracellular storage materials by a wide range of microorganisms. The characteristic that makes these biopolymers an interesting alternative to conventional plastic is that they combine high functionality with biodegradability and non-toxicity. Due to the microbial origin of PHA, 40 – 48% of the total production costs are related to the employed feedstock (Rodriguez-Perez et al., 2018), while the remaining costs correspond to the energy employed to maintain sterile conditions for the microorganisms culture, and the DSP for PHA extraction (Fernández-Dacosta et al., 2015). The use of waste streams, such as industrial wastewater, and non-sterile MMC would not only reduce the cost of the final product, but also improve the environmental performance of the process (Yadav et al., 2020). Furthermore, the research for new materials that can substitute conventional plastics is essential for accomplishing the ambitious objectives targeted by the European Commission (European Commission, 2018a). Moreover, the production of bioplastics employing wastewater is of great interest, especially in coastal areas, because of the link between plastic pollution and the destruction of the marine ecosystems, which directly impact not only the environment, but also the local economy (Conejo-Watt and Luisetti, 2019)[1].

[1] http://www.cleanatlantic.eu/

In this scenario, TREASURE-TECHNOSALT project[2] aims to unravel the effects of salinity over biological processes in wastewater treatment and valorisation, and biopolymer production is being technologically assessed under high salinity conditions. However, the aspects that need to be considered for biopolymer commercialization are not only related to technical issues but also to environmental concerns. Conventional plastics, which constitute a serious problem for ecosystems and human health worldwide, are dependent on oil and petrol. Therefore, new sources for plastic production are being researched and it is necessary to prove the environmental benefits of those novel alternatives compared to the non-renewable ones and to stablish criteria for their applications and safe disposal (European Commission, 2018a). In this context, LCA seems to be the appropriate tool, since it has already been widely used to assess the environmental performance of different processes and products, and particularly of PHA production processes (Bengtsson et al., 2017b; Fernández-Dacosta et al., 2015; Morgan-Sagastume et al., 2016). However, recent investigations pointed out the urgent need for studies in which all process stages are covered, stablishing PHA biosynthesis and extraction from waste as feedstock. This lack of research works in which the whole process is holistically studied is drawing back the scale up and commercialization of PHA (Mannina et al., 2020).

The objective of this study is then to evaluate the environmental performance of a PHA production process from high salinity wastewater using MMC and to compare it with the traditional linear economy approach (i.e., wastewater treatment and discharge). All process stages, from waste generation to product extraction and effluent discharge will be evaluated. Therefore, the new data provided will contribute to increase the information available on full-scale MMC-based PHA production systems. To do so, lab activities carried out within the TREASURE-TECHNOSALT project will be used for the scaling-up of the process and complemented with literature sources when needed, and the environmental impacts of PHA production will be quantified. Here, an MMC-based PHA production process employing saline wastewater will be environmentally assessed for the first time.

5.2 GOAL AND SCOPE DEFINITION

The goal of this study is to evaluate the use of high salinity wastewater as a feedstock for PHA production. To do so, a PHA production system at lab-scale was operated using MCW coming from a local industry where molluscs were boiled.

However, the production of PHA from wastes using MMC is still under development, with only a few pilot-scale plants operating worldwide (Kourmentza et al., 2017). In this scenario, the environmental evaluation of the process is challenging, since LCA results using lab-scale data do not necessarily represent the impacts associated to a full-scale process, often resulting in an overestimation of the outcomes (Moni et al., 2020). To minimise that, the present LCA is based on an up-scaling of the system from the results obtained at laboratory-scale (18 months of stable operation), where different operational conditions were tested and optimized (Roibás-Rozas et al., 2021).

5.2.1. Functional Unit definition and feedstock quantification

The FU is usually defined in terms of the system output, and this has been the case for PHA production system, in which the FU defined is normally related to the mass of

[2] http://www.usc.es/biogroup/treasure

biopolymer generated, being 1 kg or 1 t of PHA (PHB) the preferred option (Zarroli, 2020). Having said that, literature states that the minimum amount of biopolymer needed to make a MMC-PHA production process economically feasible is around 10^3 t PHA/year (Bengtsson et al., 2017b). With the chosen substrate, MCW, and system productivity (see below), the amount of wastewater needed to guarantee the economic feasibility of the process would be approximately 1,500 m^3/day, which is a high and unrealistic flow considering the processing capacity of the region. In fact, when dealing with waste management systems, the FU might be defined in terms of the system input, that is the waste to be managed. This has been the approach taken by recent studies on PHA production when the feedstock employed was wastewater: an influent volumetric-based FU, such as the treatment of a certain amount of wastewater flow rate (Morgan-Sagastume et al., 2016). This approach might be questionable when different wastewater streams are compared, since it disregards their loads and characteristics, but not in the case where two scenarios, i.e., linear and circular economy based, are compared for the same type of wastewater as occurs in the present study. As a result, this second approach, the system input FU, is the one selected here and its quantification is described next.

As already stated, Galicia produces annually about 250 kt of mussels, of which about a 40% is consumed fresh and the 60% remaining is processed (i.e., boiled and canned). Furthermore, almost 70% of the mussel rafts are located in the area of *Arousa* (Barros et al., 2009). Currently, there are ten facilities allowed to boil mussels in Galicia, nine of them in *Arousa* (Consello Regulador do Mexillón de Galicia, 2020). Consequently, it can be assumed that the mussel processing in Galicia takes place in this estuary. Among those nine, the three biggest ones are responsible for the processing of about 45 kt of mussels/year each (Mejillones Nidal, 2018), so this study focuses on these three plants (Figure 5.1). As the distance among boiling facilities is shorter than 10 km, it is justified to evaluate a centralized wastewater valorisation and treatment plant for a full-scale PHA production scenario.

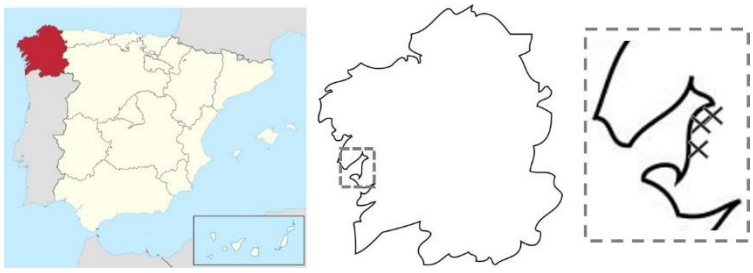


Figure 5.1. Location of the facilities where 70% of the mussels are produced and processed in Galicia (dashed-line square is Arousa area and locations of the three facilities are marked with an X)

The processing of mussels usually generates two kinds of effluents that are treated separately in the processing facility before they are discharged (Barros et al., 2009): a low-loaded wastewater stream (LLW), mainly resulting from washing operations, and a high-loaded one, from the boiling of the molluscs.

The LLW only needs basic primary treatment, such as screening, before safe discharge. It is always produced, and it is out of the scope of this study. The high-loaded one, the MCW, is our target effluent due to its high carbon content and to its treatment complexity. This stream has a highly variable composition, which average values are reported in Table 5.1.

Table 5.1 Characterization of MCW (VFA is Volatile Fatty Acids, COD is Chemical Oxygen Demand, TOC is Total Organic Carbon, TSS is Total Suspended Solids and VSS is Volatile Suspended Solids)

Parameter	Units	Range
pH	---	7.5 ± 0.7
Conductivity	mS/cm	37.9 ± 4.7
Alkalinity	mg $CaCO_3$/L	225.5 ± 74.2
Total COD	g/L	16.6 ± 5.6
Soluble COD	g/L	15.3 ± 4.4
VFA	g COD_{VFA}/L	0.025 ± 0.045
Carbohydrates	g COD_{Carb}/L	12.8 ± 4.2
Proteins	g COD_{prot}/L	3.2 ± 1.2
TOC	g C/L	6.10 ± 3.52
Inorganic Carbon	mg C/L	40.6 ± 32.5
Ammonium	mg NH_4^+-N/L	134.0 ± 75.2
Total Nitrogen	mg N/L	987.2 ± 110.0
Sodium Chloride	g NaCl/L	18.52 ± 3.94
Sulfate	g SO_4^{2-}/L	2.44 ± 0.70
TSS	g/L	3.0 ± 1.6
VSS	g/L	1.8 ± 0.8

Regarding wastewater production, a processing facility of high capacity generates about 5 m^3/t of mussels processed, of which a 6% is considered of high load (Bello Bugallo et al., 2012). As 100 - 105 kt of mussels are assumed to be processed by the three facilities per year, the centralized full-scale plant for the valorisation and treatment of MCW would have an input flow of 85 m^3/day, considering 365 working days per year (Secretaría xeral de Calidade de Avaliación Ambiental, 2013a)[3]. This is a value significantly lower than the threshold level reported above for economic feasibility. Nevertheless, and considering the expected evolution of this novel technologies, this flow is chosen as the FU for this study, as it is the available flow of the researched feedstock in the target area. This centralized scenario will be compared with the current one, in which MCW is treated in the industrial wastewater treatment plant (WWTP) of each processing facility before being released. When the fish canning facility is located in an industrial area, the effluents are usually disposed to the sanitation network with low requirements regarding discharge limits (Dirección Xeral de Calidade e Avaliación Ambiental, 2008a). One of the problems linked to mussels processing industry is that facilities are usually located in coastal areas, away from industrial zones. Therefore, after wastewater treatment, the effluent is usually disposed directly into the estuaries through a sewage pipe, with variable discharge limits.

5.2.2. System boundaries and scenarios definition

The system starts with the MCW generation, so the upstream processes, i.e., the harvesting and canning of the molluscs, are left out the system boundaries. Besides, the treatment and discharge of LLW is also left out as it is a common process for all the scenarios under evaluation. The system boundaries of the present study include all the processes involved in wastewater treatment and valorisation: water line from the influent pumping to the final discharge of the effluent in the receiving water body, sludge

[3] The feedstock quantification is based on an annual production so the number of working days will neither change the annual wastewater generated or the total PHA production.

management and disposal, and bioplastic production (extraction of the PHA from the biomass).

Sludge is produced from the MCW treatment and valorisation processes and, according to the Spanish legislation, biowaste has to be treated through anaerobic digestion or composting, encouraging administrations to promote the use of the resulting organic fertilizers in agriculture (Jefatura del Estado, 2011). A total of 19 facilities in Galicia are authorised for sludge valorisation, and only three of them work with anaerobic digestion, while the rest of them are mainly compost producers (Xunta de Galicia, 2020). Therefore, transport to the nearest of these facilities and sludge composting is considered for the conventional scenario, while anaerobic digestion at the centralized facility for the valorisation scenario is considered as the most feasible choice. However, due to the lack of information concerning the sludge characteristics, the final application of the compost and the digestate are excluded from the evaluation.

For all the stages, only the environmental impacts associated with operation have been considered in the study, while construction and decommissioning of the required infrastructures have been excluded due to lack of data.

5.2.2.1. Baseline scenario: the linear economy approach

High strength fish canning wastewater (like MCW) is usually treated in two stages: primary and secondary. In primary treatments, solids and oily matter are removed from the wastewater, while, in the secondary one, biological systems are usually employed to remove dissolved organic matter and nutrients. The choice of the best available technique (BAT) ensures the minimum environmental impact without compromising the economic performance of an installation. BAT selection, which is based on technical feasibility, environmental benefits, and economic profitability, establishes the following treatment sequence for fish canning effluent: first, a primary treatment (equalization, screening, sedimentation, pH adjustment, flocculation, flotation, or microfiltration) and then, a secondary treatment (aerobic or anaerobic). Therefore, the up-scaling of the linear economy scenario will be based on the treatments recommended in the BAT for fish processing (Tomczak-Wandzel et al., 2015), as it is commonly the base for WWTP design in MCW treatment (Mejillones Ría de Arosa, 2016).

For primary treatment, the use of Dissolved Air flotation systems (DAF) is widely spread due to their high efficiency and simplicity, and they are used to treat MCW (Barros et al., 2009). When coagulants are employed, its removal efficiency increases to 80 – 95%, since the DAF unit will be able to separate not only solids, but also soluble organic compounds that can precipitate and be removed (Tomczak-Wandzel et al., 2015). In the fish canning industry, it is recommended to operate at a pH approximately of 5 to decrease protein solubility. As MCW have high protein concentration, the use of this unit is justified (Figure 5.2), and its aim is to remove not only solids, but also organic nitrogen associated to proteins.

Concerning secondary treatment, biological processes are the preferred option (activated sludge, anaerobic digestion, granular treatments, etc.). Anaerobic digestion has been employed, but the experience has only been fully successful in facilities with low salinity wastewater (Secretaría xeral de Calidade de Avaliación Ambiental, 2013b), as the presence of sodium, ammonia and sulphate can hinder anaerobic treatments. For MCW, these ions are normally present in inhibitory concentrations, and methane production is very difficult. Despite this fact, several full-scale experiences were carried

out in the past in Galician industries trying to valorise MCW anaerobically, but with unsuccessful results where the low amounts of poor quality biogas produced were permanently burned in a torch (Barros et al., 2009). Therefore, most of the industrial plants for fish canning processing employ activated sludge processes for the wastewater secondary treatment, as it is the conventional treatment defined here. Each canning facility is supposed to have a small WWTP for the treatment of their effluents (Figure 5.2); so, assuming equal production capacities, the capacity of each WWTP for the linear economy scenario is supposed to be 1/3 of the total MCW generation, i.e., 28.3 m^3/day. Sludge is thickened and stored in an industrial container. Once the container is full, it is transported to the closest industrial composting facility.

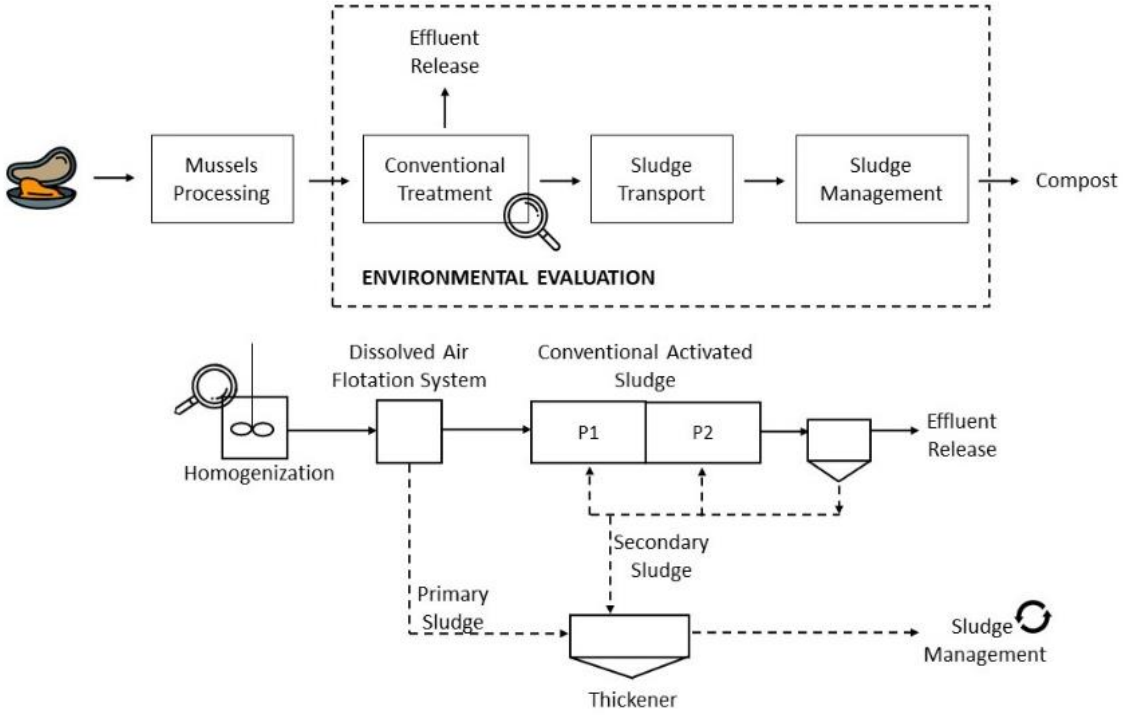


Figure 5.2. Linear economy scenario. Dashed-line square represents the system boundaries. Conventional treatment process flowchart (bottom part): P1 and P2 represent the first and second aerobic ponds, respectively. Dashed lines represent sludge streams.

5.2.2.2. Circular economy approach

The valorisation scenario was designed from the knowledge acquired in previous research projects (see Appendix 3) and the experimental results after two years operating a bench-scale three-stage system for PHA production employing MCW (Figure 5.3). For more information about the lab-scale three-stage system for PHA production, see Appendix 3 and the description provided in Chapter 3 and in Roibás-Rozas et al. (2021a). First, in the acidification stage, COD present in the MCW is transformed into VFA.

The acidification reactor and its start-up is described in Fra-Vázquez et al.(2020). After biomass separation by centrifugation, VFA are fed to two reactors: a SBR and a FBR. The SBR is an enrichment reactor for the MMC selection, while the FBR is employed to maximize PHA content in the MMC. Several operational strategies were tested throughout the experimental time (Roibás-Rozas et al., 2021). Optimal conditions were found when a mild alkalinity addition was performed by the supplementation of 80 mg $Na(HCO_3)$/L to the MCW fed to the acidogenic reactor. The enrichment reactor

operated with M-ADF, in which Feast-Famine regime was combined with a settling stage as explained Chapter 3.

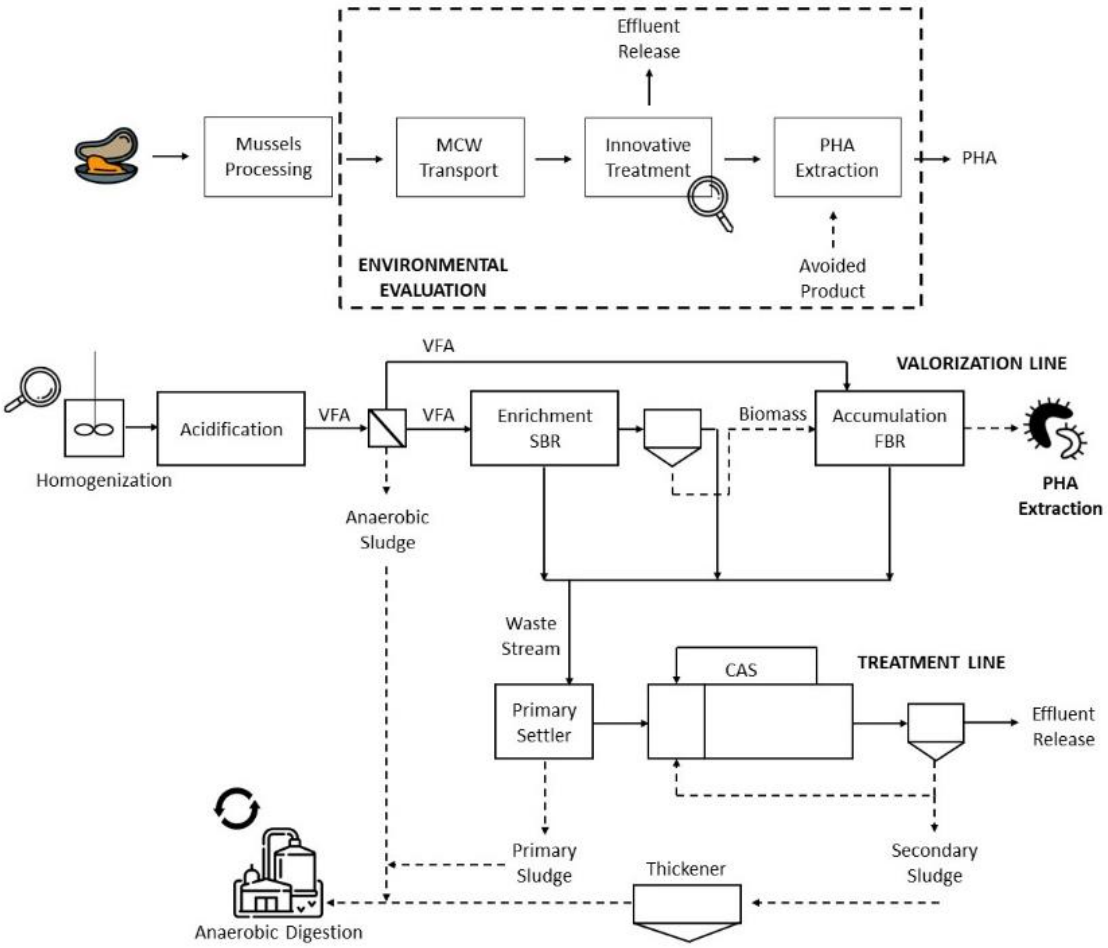


Figure 5.3. Circular economy scenario. MCW is Mussels Cooking Wastewater. Dashed-line square represents the system boundaries. Innovative process flowchart (bottom part): dashed lines represent high solids content streams. CAS is Conventional Activated Sludge, FBR is Fed-Batch Reactor, SBR is Sequencing Batch Reactor and VFA means Volatile Fatty Acids.

Initially, biological nitrification was inhibited in the SBR by allylthiourea addition (20 mg/L of feeding). When its addition was stopped, this biomass was able to produce nitrite during the famine period, with a nitritation rate of 30 mg NH_4^+-N consumed per g Volatile Suspended Solids and day (NH_4^+-N/(g VSS·d)). Denitritation occurred during the anoxic periods of feast, where all nitrite was consumed with no significant worsening of the enrichment performance (Fra-Vázquez et al., 2019).

In addition to the desired stream, the PHA-rich one, other streams are produced. They are unified in a single stream to be treated together and safely released to the environment. Therefore, a primary settler is required to remove solids present in this waste stream and to work as a homogenization unit to change from sequential operation to a continuous mode. Then, a biological treatment will be employed to remove nitrogen and organic matter in a conventional activated sludge system (CAS), in which aerobic nitrification and anoxic denitrification are performed in a step-fed type system with four anoxic/aerobic sections. Sludge generated in every stage of the process is treated anaerobically in a sludge digester.

The experiences carried out at lab-scale comprise only the valorisation steps, in which an effluent is generated for treatment and disposal, and PHA-rich biomass is produced for extraction. Therefore, experimental data developed in the frame of TREASURE-TECHNOSALT were employed for the up-scaling of the biological valorisation line. For the DSP, data from USABLE PACKAGING project[4] were used. Environmental and techno-economic analysis showed that purification of PHA employing alkaline treatment with SDS is a promising choice for PHA extraction from MMC (Saavedra del Oso et al., 2020), so data from the provided inventories are used in the present study. For the selected treatment (SDS and sodium hypochlorite), 99.9% purity was stablished according to Fernández-Dacosta et al. (2015) and an ideal recovery yield was assumed.

Concerning the avoided product, PHA properties showed similar specifications to PET, PE, PS and PP (Koller et al., 2013b). PP is similar to PHB polymers, although when other monomers are included in the chain, like hydroxyvalerate (HV), plastic properties change (Palmeiro-Sánchez et al., 2016). Biopolymer composition in TREASURE-TECHNOSALT project was approximately 25:75 HV:HB. Recent studies show that this type of biopolymer could substitute PET for packaging applications (Melendez-Rodriguez et al., 2018) so PET is the conventional plastic selected as avoided product from the PHA production. Note that neither the use nor the end-of-life stages for both conventional and bio-based plastics are included in the present study.

Finally, the design of the anaerobic reactor for the sludge valorisation was performed according to the results obtained in the frame of PLASTICWATER[5] project, where the anaerobic digestion of sludge under brackish conditions and high ammonia and sulphate concentrations was successfully assessed (Palmeiro-Sánchez et al., 2013).

5.2.2.3. Mass balances and stream compositions

Table 5.2 summarizes the composition of the main streams of every scenario and compares the achieved effluent with the discharge limits. As there is little information available regarding these limits in mussels boiling facilities, they were established according to the specifications imposed on a fish canning company discharging its effluent directly to the *Arousa* estuary or *ría de Arousa* (Dirección Xeral de Calidade e Avaliación Ambiental, 2008b), which is the area where the majority of the mussels boiling facilities are located (Fig 5.1). More information about the design of every process unit and the mass balances performed is supplied in Appendix 3.

5.2.3 Life Cycle Impact Assessment: impact categories and method selection

The choice of the relevant impact categories and impact assessment methodology is essential to provide a consistent evaluation of the environmental performance of the scenarios under study. As this research has a circular economy approach, several review papers and articles were examined to identify the most common impact categories assessed in the different areas covered by this study: WWTP operation and management, plastic production/waste and PHA production. Here, the lack of studies regarding MMC-based PHA production is noticeable, and most research works focus on pure culture processes.

[4] https://www.bbi-europe.eu/projects/usable-packaging

[5] Recycling of wastewater and sludge to produce bioplastic materials (PLASTICWATER). Supported by Spanish Government (CTQ2011-22675)

Table 5.2 Flows and compositions of the most relevant streams in every scenario.

	Influent MCW	Conventional Treatment[1]		Innovative Treatment		Discharge Limits
		DAF Effluent	Final Effluent	CAS[2] Influent	Final Effluent	
Flow (m^3/d)	85.0	26.5	20.4	74.3	67.7	-
COD (mg/L)	11,700	6,660	0.20	1,460	0.30	350.0
TN (mg N/L)	860.1	564.6	23.5	422.8	18.0	57.5
NH_4^+-N (mg N/L)	225.4	225.4	15.0	187.5	14.0	25.0
NO_2^--N (mg N/L)	0.0	0.0	0.0	6.7	0.0	-
NO_3^--N (mg N/L)	0.0	0.0	8.5	0.0	14.0	-

[1]For conventional treatment. the system is not centralized. Data shown here refer to the characteristics of each one of the three WWTP.
[2]Stream directly to biological reactors for nitrification/denitrification after primary settler.

Table 5.3 shows the result of this review. All of them will be covered in this study, paying special attention to the four first ones. For eutrophication, both freshwater and marine will be assessed (EP-F and EP-M, respectively). Besides, and considering the particularities of this study, Marine Ecotoxicity (ME) will also be considered.

The Hierarchist ReCiPe 2016 Midpoint method (v1.13) has been selected to evaluate all the selected impact categories except GWP, which was assessed by the last update of the IPCC method (v 1.03 100a). SimaPro v8.3 was used for the computational implementation of all the inventories and impact categories calculations.

5.3. LIFE CYCLE INVENTORY

Mass balances were employed to estimate the composition and flows of every stream and complemented with literature data when needed. Conventional treatment units were designed following wastewater treatment and chemical engineering handbooks (Coulson and Richardson, 1999; Metcalf & Eddy, 2014). Theoretical power equations (described in Appendix 3) were used to determine the energy requirements of every stirrer, blower, centrifuge, and pump. Chemicals` dosage was estimated using laboratory results and literature data. Results of all the calculations are summarised in Table 5.4, and detailed information is given in the following sections.

5.3.1. Inventory of the linear economy scenario

As already stated, three facilities were considered for boiling mussels. Therefore, the design was individually performed considering a third of the total flux. Then, the linear economy scenario was built up putting together the three facilities.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

- **Design of the process units:**

The DAF unit removes 85% of solids and, with the use of coagulants at low pH, about 40% of the COD. A volume of 1 L HCl per m^3 of wastewater is assumed to be added to lower pH to 5 (and equimolar amounts of NaOH are supplied after DAF to raise pH to neutrality). The removed COD is 80% of coagulated proteins, eliminating approximately a 35% of total nitrogen, and approximately 30% of carbohydrates. After DAF unit, COD/N ratio in the wastewater is high (approximately 12), so two aerobic

ponds are employed to ensure the removal of organic matter, while nitrogen is supposed to be consumed mainly due to cell growth. Mass balances were performed to determine Solids Retention Time and Hydraulic Retention Time (SRT and HRT, respectively) and the number of ponds needed to achieve discharge limits. Short SRT (5 days) guarantees avoiding nitrification in the first pond, so ammonia and COD consumption is calculated by mass balances according to the SRT. A second pond with extended aeration (SRT = 40 days) is needed to achieve COD levels according to legal requirements considering the available ammonia. Due to the long SRT in the second pond, some ammonia oxidation can take place, and it was estimated from mass balances (see Table 5.2).

Table 5.3 Categories assessed in LCA studies of WWTP operation, plastic production/waste and PHA production. and its incidence in literature. Light colour indicates relevant category and in dark colour indicates highly relevant categories.

Impact Category	Field of Interest		
	WWTP operation	Plastic/Plastic Waste	PHA with MMC
Global Warming Potential (GWP)			
Acidification Potential (AP)			
Eutrophication Potential (EP)			
Photochemical Oxidation Potential (POCP)			
Fossil (abiotic) Depletion Potential (DAR)			
Ozone Layer Depletion (ODP)			
Terrestrial Ecotoxicity (TAETP)			
Human Toxicity (HT)			
Reference	(Meneses et al.. 2015; Zang et al.. 2015)	(Antelava et al.. 2019; Koch and Mihalyi. 2018)	(Heimersson et al.. 2014; Zarroli. 2020)

- **Direct emissions to air:**

Biogenic CO_2 emissions related to COD consumption were calculated based on the emission factor reported by Campos et al. (2016): 0.08 kg CO_2/kg COD consumed. As COD/N ratio after DAF is about 12, nitrogen is consumed for biomass assimilation with no nitrification. According to the latest IPCC update for greenhouse inventories (IPCC, 2019), N_2O emissions are generally expected for aerobic systems. Depending on the stream characteristics and treatment system employed, they can vary from negligible to significant, with an emission factor range of 0.00016-0.045 kg N_2O-N/kg N in influent. In the linear economy scenario, as the main consumption of ammonia is related to cell growth, a low emission factor of 0.012 kg N_2O-N/kg N is chosen.

- **Sludge production and management:**

Sludge generated in DAF units usually has 4% of solids concentration (Metcalf & Eddy, 2014). With 85% solids removal efficiency, sludge generation is 1.8 m^3/day. Secondary sludge production was calculated according to SRT and decanters performance, producing 7.7 m^3/day of sludge with 7.43 g VSS/L. Sludge is thickened in a gravity thickener to reach a concentration of 45 g Total Suspended Solids/L (g TSS/L) (Metcalf & Eddy, 2014) and then transported to external facilities for further management. Chemically clarified supernatant is mixed with LLW, and as its contribution is less than 1.5% of its flow (6.33 versus 430 m^3/day), the influence of this stream on the LLW treatment can be disregarded. After that, sludge is composted together with other waste streams (41% average of sewage sludge as feedstock (TEN, 2020) was considered).

Table 2.4 Reference flows and Life Cycle Inventory for the linear and circular economy scenarios.

Reference flows (m^3/day)		
Stream	**Innovative**	**Conventional[1]**
Influent	85.00	28.33
Effluent	67.66	22.23
PHA-rich stream[2]	8.42	-
PHA (kg/day)	64.30	-
Downstream wastewater	3.02	5.64
Primary and centrifuge sludge	2.32	1.80
CAS sludge	6.82	4.30
Thickened Sludge	4.75	2.88
Life Cycle Inventory (units/FU)[3]		
Inputs		
Chemicals (kg)		
Triethylene glycol (L)	15.91	-
Ferrous Oxide	6.47	-
Sodium Bicarbonate	6.80	-
Sodium Hydroxide	80.0	42.00
Sodium Dodecyl Sulphate (DSP)	1.29	-
Sodium Hypochlorite (DSP)	221.8	-
Hydrochloric Acid	-	100.30
Electricity Consumption (kWh)	855[4]	1250
DSP Energy (kWh)[4]	459.5	-
Transport (tkm)	510.50	3.1[6]
Outputs		
Sludge Production (m^3)	4.75	8.63
Liquid Effluents (m^3)	67.93	66.68
COD (kg)	0.03	0.03
Nitrogen (kg)	2.8	1.9
NO_3 (kg)	1.5	0.0
NH_4^+ (kg)	1.3	1.9
Gaseous Emissions (kg)		
Biogenic CO_2	50.05	39.10
Non-Biogenic CO_2	1.8	-
N_2O	1.62	0.91

[1] Results for individual facility
[2] Containing 18.40 g Volatile Suspended Solids with 41.50% PHA
[3] Overall results for the three facilities together
[4] Electricity consumption not considering DSP
[5] Energy consumption includes electricity and cooling energy
[6] Transport of sludge is not accounted because it is included in the Ecoinvent composting process (only transport of chemicals to the plant in included)

5.3.2. Inventory of the circular economy scenario

The centralised scenario that treats the total MCW flow was up scaled from the data obtained in the lab. Theoretical equations were used to calculate power needs, the operating time of the equipment, etc (see Appendix 3).

- **Design of the process units:**

In the valorisation line, the up scaling was performed considering the obtained results. The effluent of the acidification reactor, which operated at SRT = HRT of 6.5 days, had 0.6 g COD_{VFA}/g COD. The SBR consumed 1.21 g COD_{VFA}/g biomass to reach 4 g VSS/L, and FBR needed 2.98 g COD_{VFA}/g biomass to achieve 41.5% accumulation capacity. Overall, process efficiency was of 0.2 kg COD_{PHA}/kg COD_{MCW}. In the treatment line, to ensure nitrifiers growth, SRT in the step-fed CAS was set at 15 days and HRT in the anoxic volume was 58.83 m^3. Antifoaming agent (0.5 mL triethylene glycol/L feeding) was supplied in the SBR and NaOH was used for pH adjustment in the SBR and FBR (0.75 and 1 g NaOH/L, respectively), based on laboratory-scale dosages.

- **Direct emissions to air:**

Direct emissions of the acidogenic unit were estimated from lab measurements and literature data. Waste mixtures containing carbohydrates and proteins in acidogenic fermenters generated about 279 mL biogas/g VSS, with 47% of CO_2 (Alibardi and Cossu, 2016). No methane was detected in several years of operation, and 2.4% of the reactor headspace was H_2S because of the high sulphate concentrations in MCW. For this reason, ferrous oxide pellets, which are by-products of metallurgic industry, are used to remove H_2S with a ratio of 0.6 g H_2S/g pellet (Allegue and Hinge, 2014). When pellets cannot be used anymore, they are employed as raw material for roads and bricks. Fossil CO_2 is produced due to alkalinity addition, and CO_2 emissions were calculated for operational temperature and pH. For the aerobic reactors of the valorisation line, biogenic CO_2 generation due to COD consumption is 0.08 kg CO_2/kg COD (Campos et al., 2016) and due to PHA generation is 30% (C-mol PHA/C-mol CO_2) (Jiang et al., 2011).

Regarding N_2O emissions, as nitrification and denitrification take place in the circular economy scenario, more N_2O is supposed to be generated. According to the latest IPCC update for greenhouse gas emissions (IPCC, 2019), the average value for the emission factor is 0.016 kg N_2O-N/kg N in influent for aerobic systems (as emissions for anaerobic units are not expected). This factor will be considered for the circular economy approach, assuming that nitrogen removal takes place not only in the anoxic tank of CAS but also during SBR feast.

- **Sludge production and management:**

Sludge is produced in the centrifuge after the acidification unit, in which most of the solids are removed and 1 m^3/day of sludge with 25% TSS is generated (Elías, 2012). Primary settler after the valorisation line removes 55.75% of solids and 33.96% of COD, according to theoretical equations (Metcalf & Eddy, 2014), and produces 1.3 m^3/day of sludge with 5% TSS. CAS unit generates sludge according to SRT and decanter performance, producing 4.0 m^3/day with 6.90 kg VSS/m^3, which is thickened in a gravity thickener to reach a final concentration of 45 g TSS/L (Metcalf & Eddy, 2014). The clarified stream from the thickener is diluted and treated together with the LLW[6], and the concentrated stream is treated together with the other high-solid streams in an anaerobic digester.

Regarding biogas production, sludge valorisation under high concentrations of sodium, sulphate and ammonia is feasible after proper biomass acclimation and process control. Moreover, sludge digestion generated 280 L of biogas/kg VSS (with 58% of methane) when the digester feeding composition contained 6.7 g Na^+/L and 1.5 g SO_4^{2-} per liter (Palmeiro-Sánchez et al., 2013). As these conditions are like the ones that could be found in this digester, the obtained results will be employed for the design of the digestion unit. Finally, biogas valorisation is performed considering electric and heat efficiencies of 35% and 45%, respectively (Metcalf & Eddy, 2014): i.e. 133.9 kWh/day of heat energy and 104.2 kWh/day of electricity.

The former was used to maintain the temperature of the digester at 38 ºC and the latter was sent to the grid and considered then as avoided product. An amount of 1.5% of the produced biogas is leaked, while the presence of N_2, H_2S and NH_3 in the biogas is

[6] As the supernatant only represent less than 0.5% of the LLW flow (5.64 m^3 versus 1290 m^3), this addition is considered negligible and stays out the system boundaries.

estimated in 1%, 0.05% and 0.01%, respectively (Rodriguez-Verde et al., 2014). Emissions linked to biogas combustion are calculated from literature data (Paolini et al., 2018), and ferrous oxide pellets are employed for sulphide removal before valorisation.

- **Downstream Process:**

DSP design is indicated in Fernández-Dacosta et al., 2015 (surfactant-hypochlorite method). Wastewater generated in DSP is diluted with LLW for its safe disposal (contribution of less than 0.5% in volume) and therefore left out the system boundaries[7].

- Energy integration:

Following a circular economy approach, energy integration is also applied. The temperature of MCW leaving the boilers is 125 ºC, and the bioreactors in the valorisation line operate at 38 ºC and 30 ºC, for the acidogenic reactor and the SBR-FBR respectively. Currently, this heat is dissipated naturally in the facilities, so an estimation of the available heat was done (1,000 kWh/day). It can cover the heat requirements of the biological reactors (estimated to be around 500 kWh/day) as well as the heat demand of the DSP (about 100 kWh/day). Therefore, the heat contained in MCW after the boilers is enough to cover the heat used in the process units.

5.3.3. Background processes

Data for the processes of the background system (production of electricity, chemicals, transport, plastic materials, steam and composting process) come from the Ecoinvent v3.3 database (Weidema et al., 2013). The Spanish electricity mix has been adapted with the most recent update reported, referred to 2018 (Red Eléctrica Española, 2019). No environmental burdens are allocated to the antifoaming agent (triethylen glycol) as it is a by-product of ethylene oxidation, neither to the ferrous oxide pellets as they are considered waste from the metallurgic industry. Therefore, only the transport of these products is considered. In the case of ferrous oxide, as it is employed as a component in the production of roads and asphalt, transport to the recycling facility (asphalt producer) is also included. As the SDS used at the DSP is not available in the database, its background production process has been assimilated to the one of alkylbenzene sulfonate.

5.4 LIFE CYCLE IMPACT ASSESSMENT

5.4.1. Individual environmental performance of each scenario

The contribution of the different elements to the set of impact categories for each scenario is presented in Figure 5.4.

For the linear economy scenario (Figure 5.4a), electricity has an important contribution for all the categories (between 12 – 68%) except for EP-M. Besides, chemicals consumption (which also includes the transport from the suppliers) also has a relevant impact (13 – 87%, except for EP-M), mainly due to hydrochloric acid production. This process affects HT, TAETP and ME, and direct emissions of chlorine impact on GWP. Composting (sludge management) process has significant effects on GWP, AP and POCP due to the expected emissions of CH_4, NH_3 and N_2O.

[7] Same criteria than the one applied for the supernatant of thickener, with a DSP wastewater flow of 3.02 m^3/day

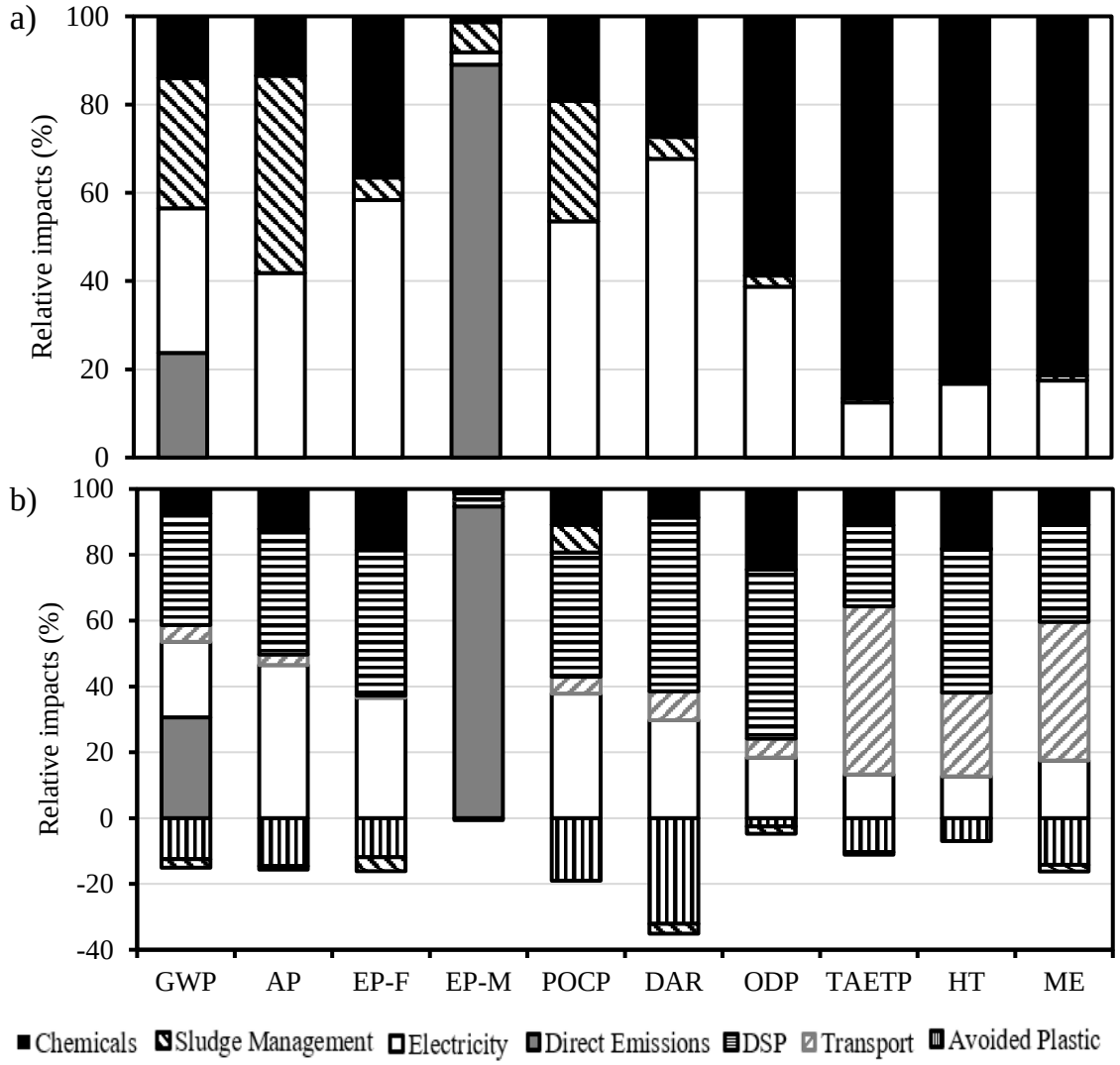


Figure 5.4. Environmental performance of **a)** Linear Economy Scenario and **b)** Circular Economy Scenario (percentage of the total impacts). Direct emissions include effluent release and air emissions. Chemicals include sodium hydroxide, hydrochloric acid and polyacrylamide for the linear economy scenario, and sodium hydroxide and sodium bicarbonate for the innovative scenario. Sludge management refers to composting in the linear economy situation and to anaerobic digestion for the circular economy one.

Behind the direct emissions label, direct emissions to air estimated in each scenario and the release to the ocean of the final effluents are included. Regarding the former, direct emissions to air also contribute to the GWP category in both scenarios, totally dominated by the N_2O release. Concerning the latter, the release of an effluent containing nitrogen (in the forms of ammonia and nitrate) is clearly impacting EP-M category for both scenarios.

In the linear economy scenario, no nitrification is expected, but small amounts of nitrate can be produced in the second pond (SRT = 40 d and low COD concentration). Total nitrogen released is 23.5 mg N_T/L, which is mainly ammonia nitrogen (15.0 mg NH_4^+-N/L and 8.47 mg NO_3^--N/L). For the circular economy scenario, nitrification and denitrification are needed as further treatment to guarantee the discharge limits accomplishment, and the total nitrogen in the effluent is 18.0 mg N_T/L, distributed between ammonia (4.0 mg NH_4^+-N/L) and nitrate (14.0 mg NO_3^--N/L). Nevertheless, the implementation of innovative process approaches, like nitrogen removal by the anammox process or phosphorous recovery units, could not only mitigate negative effects in EP-M

category, but also decrease the overall process impacts due to energy optimization, better effluent quality and substitution of chemical fertilizers (Morgan-Sagastume et al., 2016).

When looking at the circular economy scenario (Figure 5.4b), the environmental burdens are more evenly distributed among the different elements, and electricity, chemicals use, and DSP are the main contributors for most categories. The centralized characteristics of this plant, which imply the transport of the MCW by lorry to the valorisation plant (i.e., labelled transport at Figure 5.4b), have a non-negligible contribution in TAETP, HT and ME due to non-exhaust gasses emissions linked to the brake wear treatment, and containing several harmful particulate matter (like copper or antimony).

The environmental benefits related to the circular economy approach are dominated by the avoided production of plastic (i.e., PET). The most affected category is fossil depletion (DAR) (32%), as the use of crude oil and natural gas is prevented. It also has non-minor benefits for many other impact categories, like POCP, AP or ME, mainly due to the avoidance of xylene formation. The energy recovered for electricity production and sent to grid also has a net credit (net negative values for sludge management at Figure 5.4b), although with small values due to the low load entering the digester (as most carbon in MCW is employed for PHA valorisation) and the resulting low biogas production per m^3 of MCW.

5.4.2. Comparison of scenarios

Figure 5.5 shows the comparative environmental analysis of the evaluated scenarios (absolute values per category are reported in Appendix 3). The current linear economy approach shows the worst performance for all the impact categories evaluated, except for ozone depletion potential (ODP), reporting an average improvement of 24.8% when a strategy of valorisation of the wastewater as the circular economy scenario described in the present research work is applied.

The DSP is behind the worse performance of the circular economy scenario for ODP due to the chemical intensity of that stage (in particular, the hypochlorite production is the main contributor for this category). Additionally, the use of sodium hydroxide for pH control in this scenario has also negative effects on ODP. Possible improvements and approaches for this issue will be discussed in the followed sections.

5.5 DISCUSSION

5.5.1. Results obtained versus available literature

To the best of the author´s knowledge, only few papers have evaluated the environmental performance of MMC-based PHA production (Bengtsson et al., 2017b; Fernández-Dacosta et al., 2015; Gurieff and Lant, 2007; Morgan-Sagastume et al., 2016; Vega et al., 2019), showing the scarcity of original data for the environmental validation of bio-based products and biorefineries. Moreover, conflicting conclusions have been reported (Hottle et al., 2013; Yates and Barlow, 2013), showing better environmental performances for GWP when PHA is compared to PE (Gurieff and Lant, 2007), but generally worse when compared to PET (Fernández-Dacosta et al., 2015).

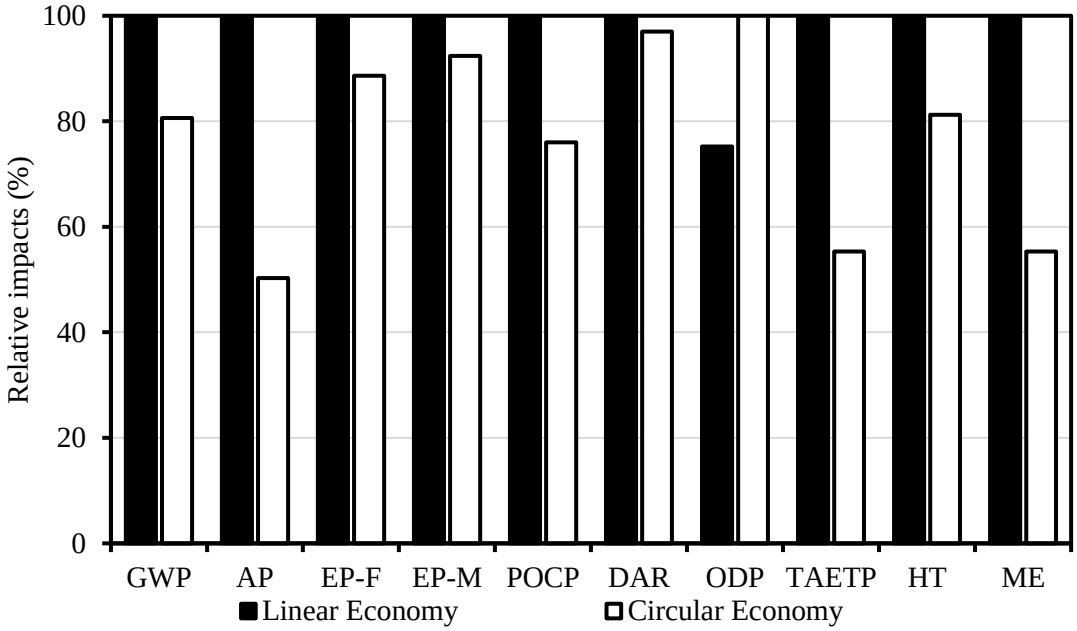


Figure 5.5. Comparison of the linear and circular economy approaches: percentage of the relative impacts of every scenario for the studied categories.

Even when the impact assessment method and the categories selected are different in each reference, some comparisons can be done between the results reported here and values available in the literature. For example, Fernández-Dacosta et al. (2015) estimated a production of 2.06 kg CO_2-eq/kg biopolymer, while 3.51 kg CO_2-eq/kg biopolymer was found for the present study, being in the current literature range for MMC-based PHA. Although no absolute values were reported, Morgan-Sagastume et al. (2016) found impact reductions for GWP, AP, EP and POCP considering a volumetric unit of feedstock as FU. Their reduction values are higher than the ones found in the present study, although in their case DSP was out of the system boundaries.

Results suggest that the environmental and economic performance of PHA production can be highly enhanced if it is integrated in existing plants (WWTP, biodiesel plants, hydrogen production factories) adapting to seasonal and operational variations (Rodriguez-Perez et al., 2018), or combining the production of several by-products, as biogas or compost (Morgan-Sagastume et al., 2016; Vega et al., 2019). The present research work has assessed the potential environmental benefits of PHA production from boiling mussel wastewater combing real data from 2-year lab-scale operation and literature, responding to the urgent need of performing new studies that can help to understand the implications of the integration of PHA production processes in WWTP.

5.5.2. Benefits of process integration strategies

Energy consumption and DSP were identified in the past as bottlenecks for environmental and economic validation of PHA production (Fernández-Dacosta et al., 2015; Gurieff and Lant, 2007; López-Abelairas et al., 2015). Moreover, Fernández-Dacosta et al. (2015) pointed out the non-negligible impacts related to heating (through steam) in the fermenter unit to maintain adequate temperature for biological reactions, and identified DSP as the main contributor to GWP category and energy consumption (60% of GWP impacts and 72% of non-renewable energy use).

In this respect, process integration was applied in this study through the use of MCW excess energy in the DSP and for reactors heating, and through anaerobic digestion of the

waste sludge. Therefore, no heat was supplied to maintain process temperature neither in the bioreactors nor in the DSP, saving approximately 500 kWh/day and 100 kWh/day, respectively.

Regarding DSP, energy consumption includes electricity and cooling energy (as heating energy is avoided, Table 5.4). It accounts for 54% of the total energy consumed in the plant, while process integration saved about 15% of the DSP needs, showing that an integral approach could improve process feasibility (environmentally, but also economically). There are only a few pilot-scale plants operating worldwide for PHA production from waste stocks (Mannina et al., 2020), and studies up-scaling PHA production with waste feedstocks are still scarce. Therefore, actual integration proposals are not yet specific and well defined, so potential environmental benefits arising from process integration and energy optimization are being disregarded from the assessment. This result shows a whole new path for the validation and for the technical, economic, and environmental optimization of bio-based products, although case-by-case studies might be needed.

Concerning anaerobic digestion, although heat provided by the exhaust gases is enough to maintain the digester temperature, the effect of anaerobic digestion in the overall process performance is low, as most of the carbon present in MCW is employed for PHA production. However, the implementation of anaerobic digestion avoids the release of harming emissions linked to composting and generates electricity, enhancing the overall environmental performance.

Moving to another topic, the results obtained also confirm the environmental benefits of the combined production of PHA and biogas as reported by other authors, such as Vega et al. (2019), who assessed the environmental performance of centralized biorefineries to produce PHA and biogas using a mixture of cow manure and grape marc.

This is linked to the idea of second generation biorefineries, in which several resource recovery methods are combined and different feedstocks can be treated, that is coming along as the future of bio-based products (Dietrich et al., 2017). Although some potential improvement strategies were not included in the present study due to uncertainties, they are mentioned here. First, the use of waste non-PHA biomass coming from the DSP was excluded from the environmental evaluation, although it contains carbon that could be used to improve biogas production. Second, the anaerobic digester could be eventually transformed into a secondary VFA provider, improving system productivity, and enhancing the plant capacity. Finally, the co-digestion of sludge and other wastes coming from local industries could stimulate biogas production and increase the economic and environmental benefits of the anaerobic digestion unit.

Finally, a centralized strategy was more beneficial than the current non-centralized approach, although the transport stage had relevant impacts that need to be considered before the centralized strategy is implemented (Figure 5.4b). Otherwise, the environmental benefit from the biorefinery process could be offset by the impacts of transportation (Vega et al., 2019).

5.5.3. Sensitivity analysis

Figure 5.5 reported a better environmental performance for the circular economy scenario, as its relative impacts are lower. In order to assess the robustness of those results, two decisions are evaluated here: the alternative defined for sludge management and the final PHA:PET equivalence (including extraction efficiency and replacement

ratio). Regarding the former, two new situations were modelled: anaerobic digestion without energy recovery and composting. The circular economy scenario is still a favourable alternative, even when no energy is recovered in the digester, compared to the current scenario (Figure 5.6) for eight of the ten categories, being only DAR the impact category that changes balance when no electricity is recovered (i.e., circular worse than linear based scenario), as the digester energy needs (basically stirring) are no longer covered by the biogas production. Direct emissions from the composting process and the removal of the benefits associated to energy recovery identified this alternative as the worse one, being the circular based scenario no longer the better option for DAR.

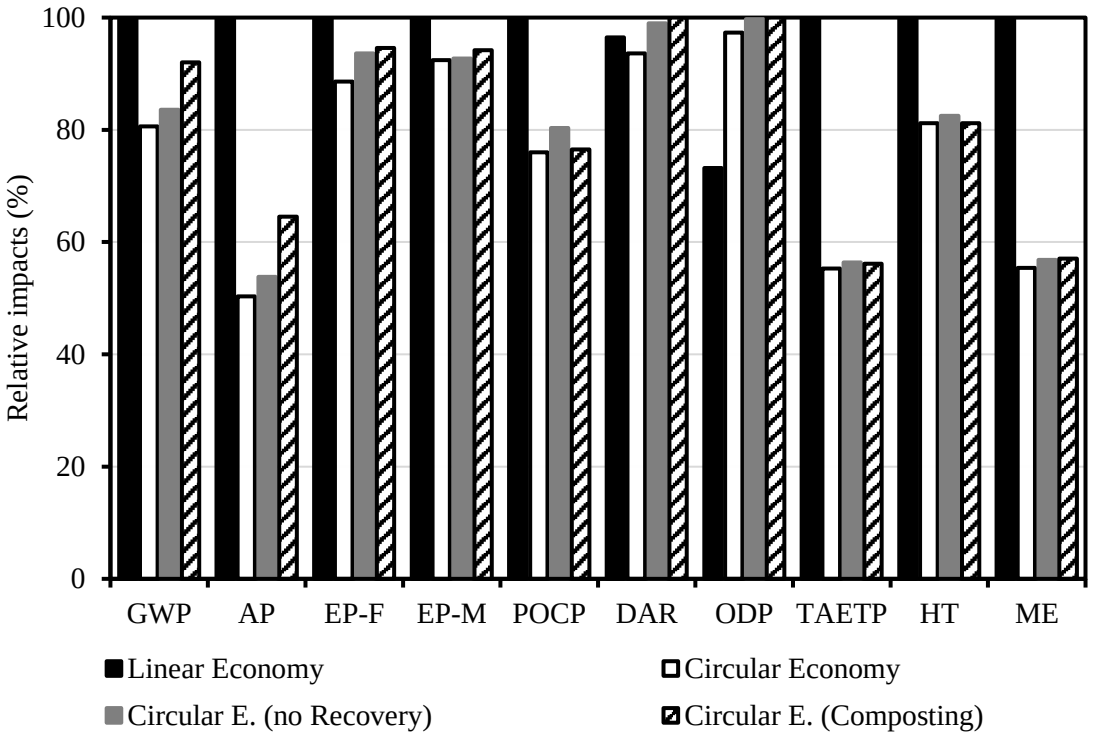


Figure 5.6. Sensitivity analysis: percentage of the relative impacts of the Linear Economy scenario with circular economy scenario in which excess sludge is treated through anaerobic digestion (Circular Economy), anaerobic digestion with no electricity production (Circular E. (no Recovery)) and composting (Circular E. (Composting)).

Concerning the latter, a replacement ratio (RR) PHA:PET of 1:1 was considered as no better approximation was available. That ratio is dependent of the yield strength and the density of the materials, and a recent study suggested that its value is of 0.93:1, i.e. 1 kg of PHA would replace 0.93 kg of conventional PET in the plastic market (Vega et al., 2019). Besides, ideal DSP efficiency was considered, that is to say, all the PHA entering the DSP process is effectively extracted during the process. However, this efficiency is hard to estimate and is dependent of several factors, such as the specific MMC used or the PHA content of the resultant stream (Mannina et al., 2019).

Averaging the results reported by (Fernández-Dacosta et al., 2015) for PHA recovery yields using diverse DSP strategies, a value of 77.17% is obtained. Combining this value with the RR of 0.93, a replacement yield PHA:PET of 0.72:1 (i.e. 0.72 kg of PET production is avoided by each kg of PHA extracted from the Circular Economy based Scenario) was applied and results are displayed in Figure 5.7.

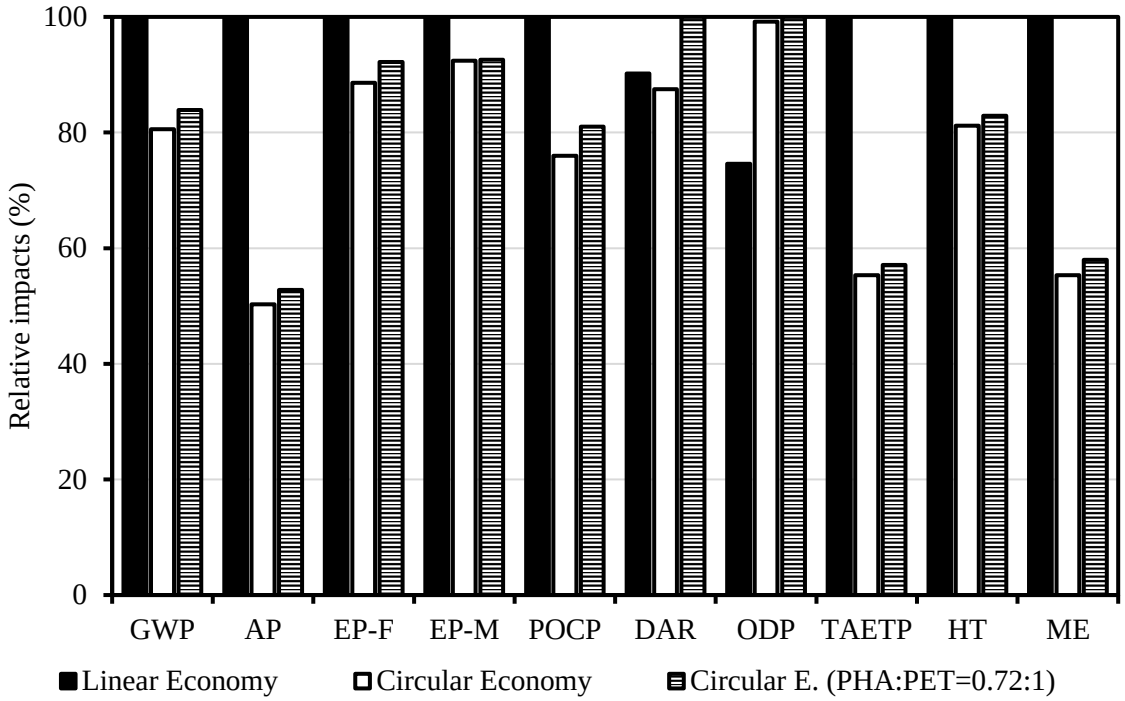


Figure 5.7. Sensitivity analysis: percentage of the relative impacts of the Linear Economy scenario with the Circular Economy one and a circular economy scenario with a replacement yield PHA:PET of 0.72:1 (Circular E. (PHA:PET=0.72:1)).

The circular economy approach is still preferable to the non-centralized linear economy current scenario, as average improvements of 25% were found for all categories except DAR and ODP. Not surprisingly, the worsening in fossil depletion category is linked to the lower amount of avoided product (PET), being this lower the environmental benefits of the circular economy approach are no longer present.

On the other hand, DSP is responsible for about 50% of the impacts in the categories that do not show any improvement compared to the circular economy scenario (DAR and ODP). The significance of the DSP is not new (Gurieff and Lant, 2007; López-Abelairas et al., 2015). Due to this fact, new research works are stepping forward towards new eco-friendly and cost-effective extraction techniques, like the ones based on switchable anionic surfactants (Mannina et al., 2019). The implementation of these new techniques could improve even more the overall environmental response of the process and decrease the negative effects reported for ODP category. Moreover, the DSP was included in the present study as new results covering the whole PHA production process are demanded by the scientific community. However, the answer to solve the DSP question may be linked to the creation of regional networks in which WWTPs are high PHA-content biomass suppliers where the biopolymer is extracted in centralized facilities (Morgan-Sagastume et al., 2016).

5.5.4. Future outlook

PHA production from MCW is still under development and optimization and, at present, the overall process conversion is relatively low (0.2 g COD_{PHA}/g COD fed). Increasing this process yield would increase environmental and economic benefits, and strategies such as the combination of several substrates for PHA production (wastewater from other industries, VFA coming from sludge management…) are potential ways to improve this efficiency (Rodriguez-Perez et al., 2018).

The present research work focuses on one single type of wastewater, the MCW. However, as stated in section 1, Galicia is responsible for about 80% of the Spanish fish canning production. Once the production of MMC-based PHA under high salinity is validated from a technical and environmental point of view, the possibilities are huge. One single fish canning facility can produce about 3,300 m^3 of wastewater per day (Dirección Xeral de Calidade e Avaliación Ambiental, 2008a), so assuming 6% of high strength effluent each facility could produce about 50 m^3/day of a PHA-rich stream (containing 380 kg PHA) on-site.

Supposing 300 working days per year, and knowing that 7 of the top ten fish canning companies (in production and incomes) are located in Galicia (Eleconomista.es, 2018), the annual regional potential is of 911 t PHA/year, which is near the minimum value reported by Bengtsson et al. (2017) for economic feasibility. Four of these fish canning plants are separated less than 25 km, although the longest distance among facilities is of 100 km. Therefore, DSP can be centralised for an optimized environmental and economic performance of bio-based products, opening an unexplored scenario where technical, economic and environmental issues need to be studied.

Finally, one challenge that is being faced by plastics and bioplastics industry is related to end of life options (Geyer et al., 2017). Due to the lack of information, this study has excluded them. Recent research works point out that mechanical recycling is preferable to composting, incineration or landfilling for bio-based materials (Changwichan et al., 2018). However, the effects of plastic litter in the water bodies, especially in the ocean, are still unknown, and the actual effects of plastic pollution worldwide are uncertain. Initiatives such as Plastic Leak Project[8] or MarILCA[9] will allow for better metrics to account for plastic leakage and to integrate potential environmental impacts of marine litter, especially plastic, in LCA results.

5.6. CONCLUSIONS

The renewable origin of bio-based plastics does not convert them automatically in an environmentally sustainable products and, even when is clear that they are promising materials, the environmental assessments comparing conventional plastics and bioplastics do not always show explicit benefits.

This study presents a detailed analysis of the environmental performance of PHA production from industrial wastewater, an area in which the LCA studies are still scarce. The current linear economy approach for mussel cooking wastewater management (generation – treatment – discharge) was compared with a circular economy approach, where PHA is produced, and wastewater is safely treated and discharged. A better environmental performance was reported for nine out of the ten impact categories under evaluation, which reflects the potential benefits of this novel approach. Process optimization through innovative systems (like anammox reactors for nitrogen removal) come along as preferred choices to improve overall performance (effluent quality and energy optimization).

The potential of bioplastics production processes is huge, although case-by-case studies are needed, evaluating the local industries and waste streams availability, as well as PHA and biogas yield for every feedstock. This study showed that the feasibility of

[8] https://quantis-intl.com/strategy/collaborative-initiatives/plastic-leak-project/
[9] http://marilca.org/

producing value-added bioproducts under a circular economy approach is linked to the integral vision of the process, where all streams are valorised, and energy use is optimized. Finally, the potential of Galician region for bioplastics production was estimated near 1 kt PHA/year, near the threshold value for economic feasibility. Accordingly, centralized extraction facilities employing novel extraction protocols could be the key for the environmental validation and the economic viability of these products, stepping towards high-scale PHA production from mixed microbial culture.

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

6. ASSESSING A CENTRALIZED BIOREFINERY APPROACH UNDER A TECHNO-ECONOMIC AND ENVIRONMENTAL PERSPECTIVE

SUMMARY

As the last step of the technology readiness increase for the processes developed, economic performance was included in the assessment. Industrial effluents (Mussels Cooking Wastewater (MCW) and oily Fish Processing Wastewater (FPW)) were valorised by polyhydroxyalkanoates (PHA) and triacylglycerides (TAG) production using mixed microbial cultures (MMC). First, the strategies carried out at lab-scale (three years of operation) were up-scaled and compared, at environmental and economic level, to the systems currently used at full-scale by the industries in two stages. In this first stage only the wastewater treatment processes were compared (downstream process (DSP) for biomaterial recovery is not included) to choose the best strategy for the valorisation of each effluent. Then, the scenarios with the best performance are chosen for the second stage of the study. Here, a centralized biorefinery scheme is proposed where ten, twenty, and fifty of these small facilities are clustered to work as raw material (PHA and TAG-rich biomass) providers for a centralized DSP factory. This scenario is compared with the current one, where factories only treat, but do not valorise waste effluents.

For the first stage (i.e., excluding DSP), the cost of MCW treatment could potentially decrease from 0.53 €/kg COD (treatment only) to 0.48 €/kg COD (when PHA-rich biomass is produced). For FPW, cost would decrease from 0.52 (treatment only) to 0.30 €/kg COD (with TAG/PHA production). Environmental impacts would decrease about 30% for FPW in some relevant categories as climate change, while for MCW the environmental validation would be linked to an optimization in the chemical's usage and in residual streams management, like treating waste solids through anaerobic digestion.

Then, under the centralized biomaterial extraction approach (the second stage of the studio), PHA production cost could be 0.95 – 1.18 €/kg PHA for MCW, which is a competitive market value. For FPW, the cost of biomaterials production increases (1.56 – 3.35 €/kg PHA and 6.75 – 9.21 €/kg TAG) due to the lower biomass concentration achieved here. Concerning to the environmental performance, the replacement of petrochemical materials by the bioproducts results in environmental credits for MCW under a centralized biorefinery approach, while the validation is less clear for FPW, where the generation of biomaterials is again lower.

This chapter proves that it is economically and environmentally feasible to manage the treatment of industrial effluents from the food industry under a circular economy approach, where centralized biorefinery schemes show results that yield in competitive product costs and decreased environmental impacts for PHA production systems based on wastewater valorisation.

This chapter was written as a result of a research stay performed in the department of biotechnology at **TU Delft (The Netherlands) under the supervision of Prof. John Posada. This research stay was financially supported by CRETUS grants for research stays 2021**, which are funded by Collaboration agreement with the Ministry of Culture, Education and University for the development of strategic R&D actions in 2021.

6.1. INTRODUCTION

Once process development and preliminary environmental evaluation have been carried out, it is necessary to assess the economic performance of the developed technology. Therefore, data from Chapter 3 on PHA production (Roibás-Rozas et al., 2021), and from other research activities carried out in the framework of TREASURE-TECHNOSALT project on PHA plus TAG production (Argiz et al., 2020b), will be used with the aim of validating the economic plus environmental performance of these circular economy-based processes to increase technology readiness level.

Studies assessing the production of biomaterials normally identified the environmental and economic burdens of the process in the DSP and the cost of raw materials (Kumar et al., 2020). By introducing MMC-based processes that use waste streams, the burden of the raw materials acquisition is addressed. However, DSP still limits the feasibility of up-scaling these systems (Bengtsson et al., 2017a; Fernández-Dacosta et al., 2015). Most of the studies performed till date (Roibás-Rozas et al., 2022b) assessed the same type of process scheme, where raw materials, coming from pure or waste sources, are transformed into PHA-rich biomass in a big-scale biorefinery[1]. Of them, only some included a DSP on-site (Fernández-Dacosta et al., 2015; Roibás-Rozas et al., 2020) while others stopped at the biomass production stage (Morgan-Sagastume et al., 2016).

However, DSP on-site is only feasible for factories of high capacity. Therefore studies including on-site DSP normally work with PHA productions of around 10^3 ton per year (Bengtsson et al., 2017a; Crutchik et al., 2020; Fernández-Dacosta et al., 2015; Gurieff and Lant, 2007), which needs large wastewater flow generations that are not representative of many food processing plants, yielding in costs of 1.5 – 2.5 €/kg PHA. This is, in fact, the case of Galician fish canneries, where many small factories are responsible for the main share of the production (González-López, 2018). Therefore, to increase the feasibility of implementing circular economy strategies in facilities with small or moderate production capacities, a different approach is needed.

The present chapter aims to assess the techno-economic and environmental performance of a biorefinery scheme to produce biomaterials from fish processing wastewater that includes a centralized set-up for biomaterials extraction. Here, data generated in the framework of TREASURE-TECHNOSALT project to produce biomaterials are used to design and up-scale several scenarios where the organic matter present in waste streams (Mussels Cooking wastewater or MCW, and Fish Processing Wastewater or FPW) is valorised into PHA and TAG.

Then, based on techno-economic and environmental criteria, three circular economy-based configurations will be selected to be further analysed: one for PHA production from MCW, one for PHA production from FPW, and one for TAG and PHA production from FPW using MMC. Moreover, a scenario where biomaterials are not produced, but innovative technologies developed in the framework of the present project are used, is assessed as well. Here, the selected scenarios will be assessed to test the economic and environmental feasibility of a centralized biorefinery scheme where extraction of PHA

[1] It is worth mentioning that, to the best of our knowledge, studies have not been carried out till date for processes producing TAG with MMC.

and/or TAG is not performed on-site but in a centralized plant. Therefore, the analysis is divided in two stages:

Firstly, data from laboratory scale experiments are used to upscale different scenarios for PHA and TAG-rich biomass production, with two goals: i) to compare, at an environmental and techno-economic level, the current systems used by the industry to treat the complex wastewater with the innovative ones, and ii) to select the best configuration (i.e., best environmental and economic performance) to be used for the valorisation perspective at the next stage. Note that only wastewater processing (for treatment or for valorisation) is considered in the first stage of the study, so DSP is not included.

Secondly, the selected valorisation configurations are used to assess the environmental and economic profile of a wastewater valorisation strategy where WWTPs act as raw materials providers for centralized biorefineries where DSP is carried out, as suggested in the previous chapter and elsewhere (Morgan-Sagastume et al., 2016). Therefore, industrial WWTPs produce PHA/TAG-rich biomass simultaneously to the wastewater treatment, and this biomass is treated in a centralized for biomaterials extraction. To do so and taking into account the characteristics of the sector in our region, ten, twenty, and fifty facilities for food processing have been defined as the three centralization scales to be evaluated for the centralized biorefinery scheme.

Therefore, on the one hand, the environmental and economic cost of switching conventional by innovative treatments for wastewater will be assessed. On the other hand, the cost of biomaterials production under the centralized scheme will be estimated, to evaluate if these materials achieve competitive market prices.

6.2. MATERIALS AND METHODS

6.2.1. Discharged effluent quality and WWTPs design considerations

The discharge limits of the effluents generated from all the evaluated treatment and valorisation alternatives are the ones stablished according to the local legislation (Boletín Oficial de la Provincia de A Coruña, 2016, see Appendix 4). Besides, chemicals are used in some solid-liquid separations, so when the use of chemicals is indicated, the dosage of coagulant is 50 mg $FeCl_3$/kg TSS for decanters, and 3 mg polyelectrolyte/kg TSS for centrifuges, to generate sludge cakes with TSS concentrations of 10% and 25%, respectively (Elías, 2012; Metcalf & Eddy, 2014).

6.2.2. System and scenarios definition in the first stage

Two long-term experiments (Chapter 3 and Argiz et al., 2020b) were carried out at lab scale with two goals: to develop more efficient treatment systems for complex industrial effluents, and to valorise the organic carbon in the waste streams into value-added materials (PHA and TAG). The effluents from two industrial fish processing facilities located in Galicia (NW of Spain) were used. As they had different origin and composition, two typologies of residual streams were stablished: non-oily effluent (MCW) and oily effluent (FPW). The strategies applied for their management were also different, as well as the bioproduct(s) obtained.

As a result, two systems were here defined and evaluated according to the feedstock used (Figure 6.1):

- System A (SA): mussel cooking wastewater (MCW), characterized by relatively high concentration of salt, nitrogen, and COD, which contained no oil (see the characterization of MCW in Table 5.1 in Chapter 5).
- System B (SB): fish processing wastewater (FPW), that came from a facility where mainly tuna is cooked and contained relatively high fats and oil concentrations and moderate concentrations of salt, nitrogen, and COD (see the characterization of FPW in Appendix 4)

Then, several scenarios were defined for both SA and SB according to the process approach and configuration (Figure 6.1). Here, the process approach refers to the treatment strategy:

- Linear economy-based scenarios (i.e., conventional): **SA0 and SB0**, for MCW and FPW, respectively.
- Circular economy-based scenarios (i.e., innovative): **SA1 to SA4**, where MCW is valorised into PHA, and **SB1 to SB5**, where the oil fraction of FPW is separated from wastewater and then valorised into PHA and TAG, while the remaining wastewater is treated using innovative treatments such as aerobic granular sludge (AGS). Finally, in scenario **SB6** the oil fraction is not biologically valorised, but sent to biodiesel production, while the wastewater is treated using AGS (thus, SB6 performs an innovative treatment, but it is not based on circular economy).

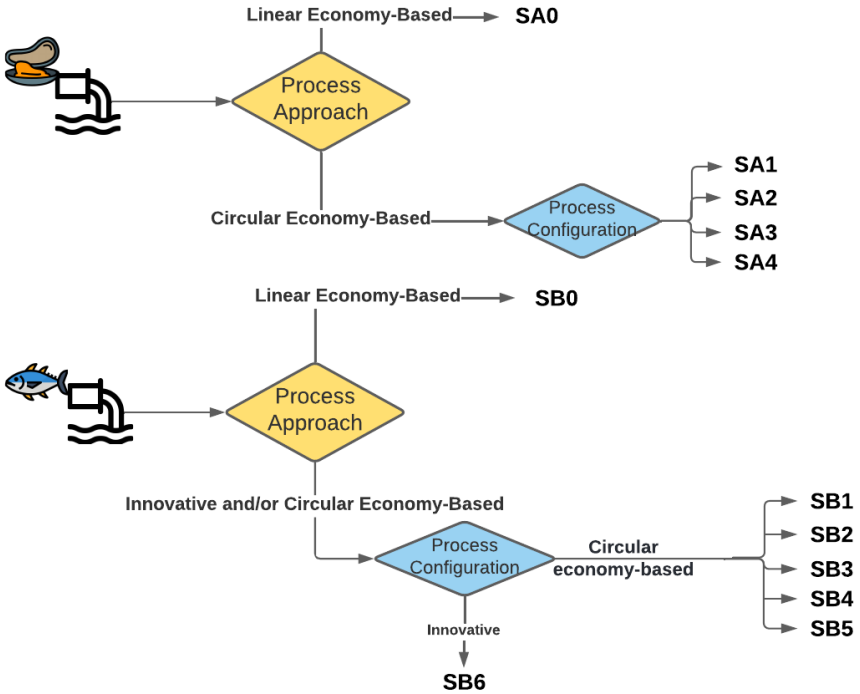


Figure 6.1. Studied scenarios at the 1st stage. The mussel represents the Mussels Cooking Wastewater for System A (MCW for SA), and the tuna fish represents the Fish Processing Wastewater for system B (FPW for SB).

6.2.3. Description of scenarios of System A (SA): PHA production from MCW

- **Linear economy-based scenario (SA0)**

SA0 (Fig. 6.2) represents the current treatment strategy applied by the industry. Its design is based on available information (Barros et al., 2009; Bello Bugallo et al., 2012) and it is similar to baseline scenario of Chapter 5 (Roibás-Rozas et al., 2020).

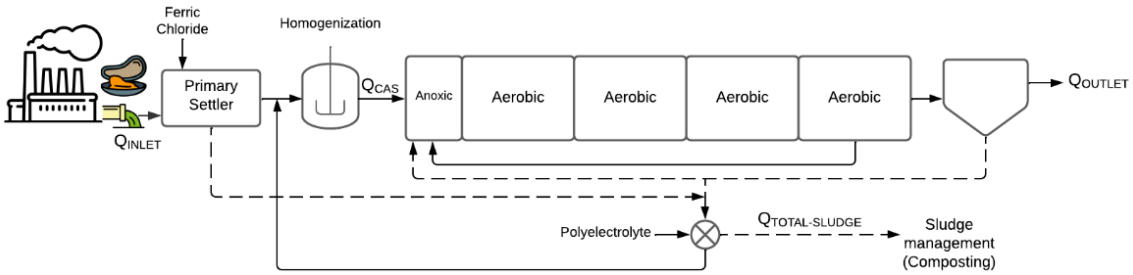


Figure 6.2. Layout of the WWTP for MCW and main streams involved. Dashed lines represent streams with high solids concentration and continuous lines represent liquid streams.

Firstly, the primary settler removes solids, enhanced by the $FeCl_3$ supply. Then, there is a homogenization tank prior to a CAS unit, which has one anoxic chamber and four aerobic ponds and a total volume of ca. 1,200 m^3. The primary and secondary sludge are dehydrated in a centrifuge, and the resultant supernatant is returned to the homogenization tank. Dehydrated sludge is here composted, the most common sludge management alternative for food industrial sludge in the region (Roibás-Rozas et al., 2020). Fig. 6.2 represents the WWTP setup and labels the key stream flows that are considered in the study.

- **Circular economy-based scenarios**

The valorisation section of the scenarios SA1 to SA4 for MCW (Figure 6.3) was based on the lab-scale results described in Chapter 3, which used a typical three-stage process (wastewater acidification, MMC enrichment and biopolymer accumulation) for PHA production. It was up-scaled after the results of that chapter (Roibás-Rozas et al., 2021) where four out of the six operational strategies tested there resulted in stable experimental periods (so **SA1**, **SA2**, **SA3**, and **SA4**).

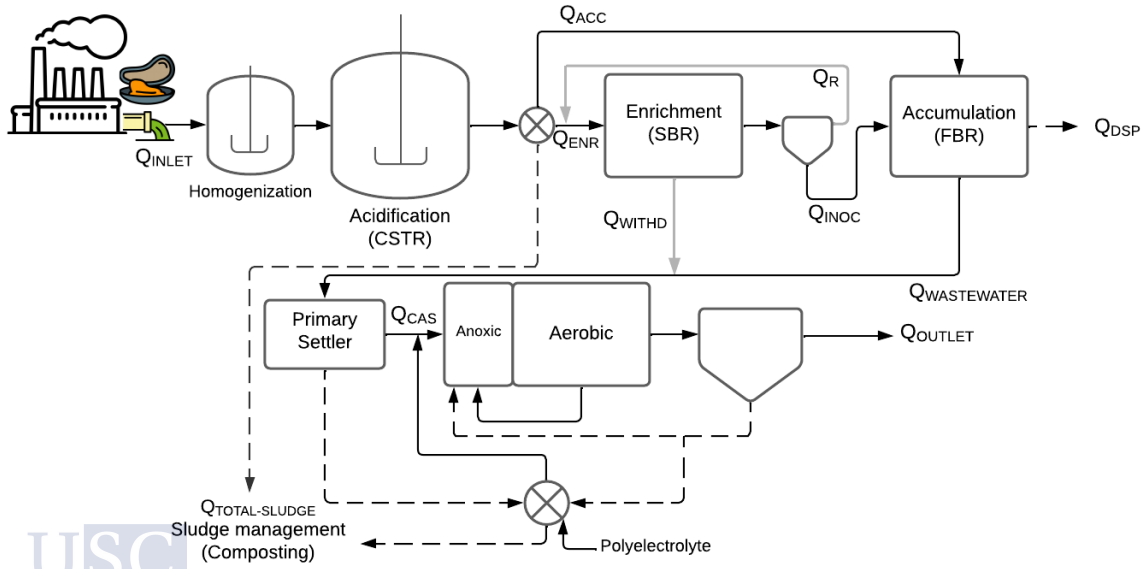


Figure 6.3. Layout of the circular economy based WWTPs for MCW and main streams involved. The grey lines represent streams that may exist or not depending on the operational scenario (see Appendix 4 for details of the scenarios based on the operational stages defined in Chapter 3). Dashed lines represent streams with high solids concentration and continuous lines represent liquid streams. The names of the stream flows are used to perform the mass balances explained in supplementary materials.

The scenarios were here classified regarding the strategy used to enrich the MMC in the SBR (Table 6.1), and the pH and alkalinity conditions in the acidification-CSTR (HRT

= 6.25 days), where the remaining waste streams are treated in a CAS. CAS and CSTR sludge are thickened in a centrifuge and treated externally by composting. Here, as the operational stages are defined according to the enrichment conditions for the obtainment of the MMC (the pH and the enrichment strategy), some streams are variable for the different stages (grey lines in Fig. 6.3). Process configurations, mass balances performed to design the reactors' volume, streams' flow, and information of each scenario are described in detail in Appendix 4.

6.2.4. Description of scenarios of System B (SB): PHA and TAG production from FPW

- **Linear economy-based scenario**

SB0 (Fig. 6.4) represents the current treatment of the oily effluent, according to the public records of the local government (Secretaría xeral de Calidade de Avaliación Ambiental, 2013a). The treatment starts at the DAF unit, that removes about 95% of the fat and 80% of the solids in the FPW. On the one hand, the remaining wastewater is treated in a 5,000 m^3 CAS biological reactor divided in eight chambers (two anoxic and six aerobic) with internal recirculation. On the other hand, the DAF sludge is settled in 1 m^3 tanks to remove the oil that naturally floats. Then, that oil is sent to an external facility where it is transformed into biodiesel. Then, the remaining non-oil DAF sludge and the centrifuged CAS waste solids are sent to another external facility that centralizes sludge treatment by anaerobic digestion.

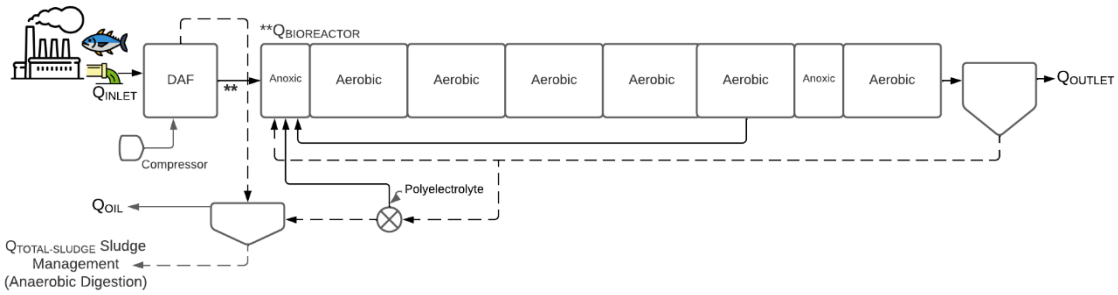


Figure 6.4. Layout of the linear economy based WWTPs for FPW and main streams involved. Dashed lines represent streams with high solids concentration and continuous lines represent liquid streams. The names of the stream flows are used to perform the mass balances explained in supplementary materials.

- **Circular economy-based scenarios**

The oily fraction of the FPW is separated in a DAF unit as in the linear scenario (Fig. 6.5). Then, the fat-free wastewater is treated in a sequencing batch reactor based on AGS (fully described in Appendix 4), and the oily fraction is valorised into PHA and TAG using an MMC, as it was recently proven by Argiz et al. (2020b) that mixed cultures feed with oil can store both compounds. Solids washed out from the AGS are decanted in a settler without additional coagulants, and then both AGS washout and DAF waste solids are concentrated in a decanter using chemicals, while supernatant is treated by the AGS. Then, the thickened solids are treated externally by anaerobic digestion.

Fig. 6.5 represents the setup of the circular economy based WWTPs that valorise FPW and labels the key stream flows required to perform the mass balances. More details about mass balances and process configuration can be found in Appendix 4.

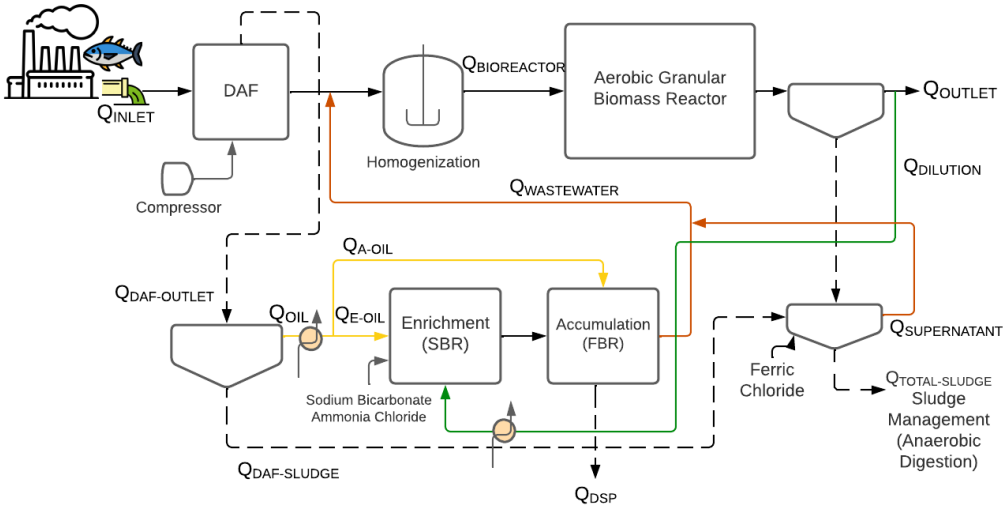


Figure 6.5. Layout of the circular economy based WWTP for FPW and main streams involved. Dashed lines represent streams with high solids concentration and continuous lines represent liquid streams. To facilitate the comprehension of the figure, waste streams that are returned to the main process line (the AGS feeding) are marked in red. The dilution water (recirculated from the outlet) is marked in green, and oil streams are highlighted in yellow.

Table 6.1. Summary of all the scenarios analysed in the first stage of the evaluation, including circular and linear economy-based scenarios for both systems, and the main differences in the valorisation sections and the enrichment strategies.

Scenario	Approach	Wastewater type	Enrichment type	Modification in the ADF and stage variation
SA0	Linear	MCW	Does not apply	-
SA1	Circular	MCW	ADF	No Low pH
SA2	Circular	MCW	M-ADF[1]	Settling stage Low pH
SA3	Circular	MCW	M-ADF	Settling stage Low pH and high load
SA4	Circular	MCW	ADF	No High pH
SB0	Linear	FPW	Does not apply	-
SB1	Circular	FPW	ADF	No
SB2	Circular	FPW	M-ADF	Uncoupling C/N feeding Dilution water addition along with the nitrogen
SB3	Circular	FPW	M-ADF	Uncoupling C/N feeding Dilution water addition along with the carbon
SB4	Circular	FPW	M-ADF	Uncoupling C/N feeding Low pH
SB5	Circular	FPW	M-ADF	Uncoupling C/N feeding Neutral pH
SB6	Innovative	FPW	Does not apply	-

[1] M-ADF: Modified Aerobic Dynamic Feeding

As mentioned above, FPW was separated in two fractions, an oily fraction that was valorised, and remaining wastewater that is treated, so the scenarios **SB1, SB2, SB3, SB4,**

and SB5 are defined according to the process configuration in the valorisation section (Table 6.1) as described by Argiz et al. (2020b). Here, the hydrolysis of the fats into long chain fatty acids plus the enrichment of the MMC are carried out simultaneously in the enrichment SBR, and the hydrolysis plus the biopolymer accumulation are carried out simultaneously in the accumulation FBR, avoiding the need for the acidification step.

Finally, when no PHA/TAG are produced (**SB6**), no biological valorisation or extraction section are required. Here, oil is managed externally by biodiesel production as in the conventional scenario SB0, but wastewater is treated by AGS instead of CAS, so a comparison between the innovative AGS process and the conventional CAS-based process can be performed.

6.2.5. Approach in the second stage of the evaluation

As a result of the defined scenarios assessment, the scenarios that showed a better techno-economic and environmental performance in the first stage of the studio are selected for the second stage. The selected scenarios will be analysed under a centralized biorefinery approach at three different scales (ten, twenty, and fifty times bigger). These scenarios are: PHA production using each of the influents (so one for PHA production using MCW, and one for PHA production using FPW), and PHA plus TAG production using FPW. Moreover, as FPW is divided into two fractions (oil to be valorised, and wastewater to be treated), a scenario where biomaterials are not produced, but innovative technologies are used, is also assessed (scenario SB6).

Under this centralized approach, the cost of wastewater treatment (while producing PHA or TAG-rich biomass) is the result of the economic analysis performed in the first stage of the studio. Consequently, the cost of PHA production is attributed only to the DSP. Therefore, in the second stage of the assessment, the cost of PHA/TAG extraction in a centralized facility where DSP is carried out will be evaluated, considering that the biomass provided is the one generated in the facilities selected in the first part of the assessment of this study. Here, the performance of a centralized DSP that extracts PHA/TAG from ten, twenty, and fifty factories that produce biomass is analysed.

Finally, and regarding SB6 (where oil fraction is not biologically valorised, but remaining waste effluents are treated under an innovative approach using AGS reactors), its environmental performance is also studied, but its economic performance is not, as neither PHA nor TAG are produced here (the cost of wastewater treatment for SB6 is the result of the first stage of the assessment).

6.2.6. Features of the processes selected for the second stage

6.2.6.1. Waste treatment

For the processes in the first stage of the evaluation, the waste solids generated in the WWTPs were treated by composting for SA scenarios, and by anaerobic digestion for SB, as it corresponds to the current reality in these factories. Here, and for the sake of cross-comparison, it is assumed that waste sludge is always treated by anaerobic digestion. Besides, it is assumed that the wastewater generated in the extraction process is treated at the DSP biorefinery by a standard primary treatment.

6.2.6.2. DSP for the systems extracting PHA only

Data obtained from the TEA of different DSPs (Saavedra del Oso et al. (2020)) were used to choose the optimal extraction route: sodium dodecyl sulphate (SDS) at low

temperature (Figure 6.6), which is used to extract PHA for low grade applications[2]. It is based on the protocols described by Fernández-Dacosta et al. (2015) and Begotti (2017).

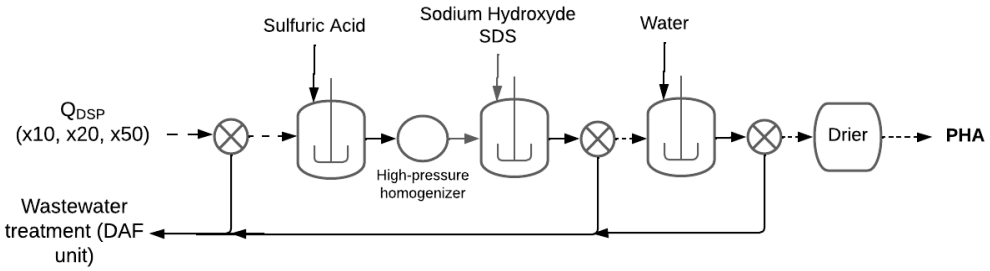


Figure 6.6 Flowchart of the Downstream Process (DSP) for PHA extraction only (no TAG), applied for one MCW scenario, and a FPW scenario at three scales (10, 20, and 50 facilities supplying PHA-rich biomass to the biorefinery).

The process consists of a microfiltration membrane to concentrate the solids in the PHA-rich stream. Then, the solid-rich stream is acidified by sulfuric acid in a reactor operated at a HRT of 0.5 hours. After that, a high-pressure homogenizer is used at low temperature (25 °C) prior to a reactor with alkaline pH, where NaOH and Sodium Dodecyl Sulphate (SDS) are added with an HRT of 1 hour to dissolve the non-polymeric cell material (NPCM). Another microfiltration membrane is used to remove the solved NPCM and concentrate the PHA solid stream. Finally, the obtained powder is washed with water in a reactor with an HRT of 0.5 hours to remove impurities, and a membrane and a spray drier are used to obtain the purified PHA. Wastewater generated in the DSP is treated in the centralized biorefinery by primary treatment (a DAF unit, Figure 6.6).

6.2.6.3. DSP for the systems extracting PHA and TAG (one scenario selected for FPW-SB)

When two bioproducts are generated (PHA and TAG), PHA is extracted as in SA, but at higher temperature (80ºC), so the resulting stream meets the conditions required for TAG extraction according to Park et al. (2021), where distillation columns are needed (Figure 6.7).

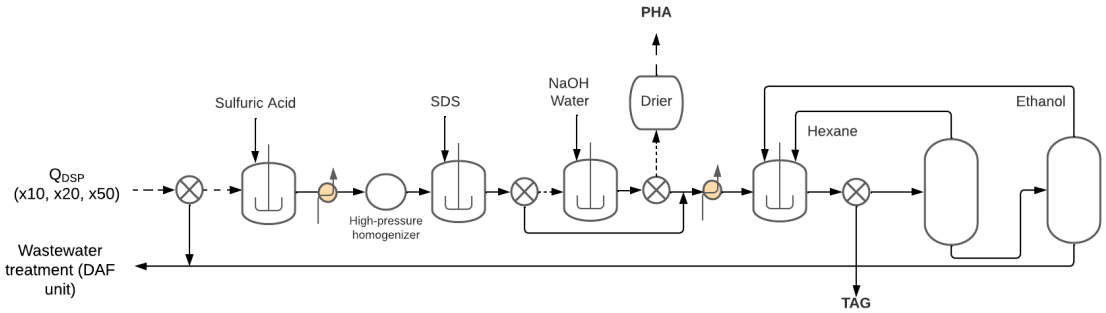


Figure 6.7 Flowchart of the Downstream Process (DSP) for PHA extraction only (no TAG), applied for one MCW scenario, and a FPW scenario at three scales (10, 20, and 50 facilities supplying PHA-rich biomass to the biorefinery).

[2] Low grade applications refer to those that will not require high products specifications, like packaging materials; while high grade applications would refer to those that require high product specifications, like biomedical materials.

First, there is a microfiltration membrane to concentrate the solids in the PHA/TAG-rich stream. Then, the solid-rich stream is acidified by sulfuric acid in a reactor with an HRT of 0.5 hours. After that, a high-pressure homogenizer is used at 80 °C prior to the reactor where SDS is added (HRT = 1 hour) to dissolve the NPCM.

Another microfiltration membrane is used to remove the solved NPCM prior to the reactor with alkaline pH (HRT = 0.5 hours). Finally, the obtained powder is concentrated with a membrane and a spray drier to obtain the purified PHA. The water streams separated in the membrane systems after the addition of sulfuric acid, SDS, and sodium hydroxide contains the storage lipids released by the biomass. Therefore, these streams are treated together with a mixture of hexane:ethanol (7:3) in a stirred reactor, so the lipidic compounds (TAG) are solved in the hexane.

6.2.7. Description of the Life Cycle Thinking based strategies used

Full description of the methodologies used in the economic and environmental analyses is performed in Chapter 2.

The goal of the study is to evaluate several resource recovery/treatment scenarios fed by two different feedstocks (MCW vs waste oil fraction of FPW) and under different experimental conditions to identify the environmental and economic profile of the processes and to choose the best strategy.

Regarding the functional unit, most of the research works found on literature consider the mass of product (i.e., kg of PHA), but this disregards the multifunctional nature of resource recovery processes, as the goal is not only to produce a biomaterial, but to treat a waste (see Chapter 4 and Roibás-Rozas et al. (2022)). Besides, using that product-based FU, it is not possible to compare a resource recovery system with a conventional treatment system (linear economy-based). Therefore, an influent-based FU is chosen.

Since the goal is to compare PHA production systems using different feedstocks, the FU cannot be volumetric (i.e., m^3 of wastewater) as the load of the waste effluents, and not only their volumes, are responsible of the environmental and economic burdens of the process. Therefore, an influent-based FU should consider the available organic matter (COD), which is the element to be valorised, and therefore the FU chosen here is 1 kg of COD in the influent. Thus, the FU is 1 kg of COD of MCW for System A, and 1 kg of COD of FPW for System B.

For the system boundaries, the first stage of the evaluation includes only the wastewater treatment to comply with discharge limits. That includes circular economy based WWTPs (where PHA/TAG-rich biomass is produced), but not the extraction. Therefore, the DSP burdens are not included, so the benefits of the avoided product are also not accounted. At the second stage, the approach is cradle-to-gate, so PHA/TAG production and the corresponding DSP are included, but the end of life is excluded.

As indicated in Chapter 2, the economic analysis (LCC and TEA) was carried out considering total capital investment plus materials and energy consumption costs (so direct costs and operational expenditures). The equations used to estimate the purchase cost of the equipment according to its size are detailed in Appendix 4. The cost of chemicals and heat was estimated from Chemical Engineering handbooks (Coulson and Richardson, 1999; Seider et al., 2017), and adapted to the prices of 2021. The cost of waste sludge management was considered 200 €/kg TS (Arif et al., 2020), and the cost of electricity was estimated as 0.12 €/kWh (GlobalPetrolPrices (2022), cost for 2021). The

cost of treating oil by transesterification for biodiesel production is 500 €/m^3 oil (Illukpitiya and de Kof, 2014).

As indicated in Chapter 2, LCA is performed according to ISO 14040 and 14044. Primary data was used from the laboratory up-scaled information and background data was employed, when necessary, from general libraries like Ecoinvent v3.4. For the second stage of the study, the avoided product in the LCA is High Density Polyethylene (HDPE) (see Chapter 5) for PHA, and diesel for TAG, as it is assumed that TAG will be used for biodiesel production, that would replace petrochemical diesel in the market.

Note that, for the first stage of the study, the LCA and LCC share the same allocation choices. However, for the second stage of the study (when DSP is included), the avoided product is accounted in the LCA (HDPE and diesel), but a more conservative approach was applied for the LCC, where there is not avoided product. This methodological choice was performed because, in order to include the avoided product for the LCC in the second stage of the study, it was necessary to allocate PHA benefits to the WWTPs, meaning that the the canneries would be the final users of the PHA, accounting also for the biomaterial benefits. This makes sense methodologically at an environmental level, but economically, it might not necessarily represent the reality of the studied system, where the possibility of a centralized biorefinery is explored.

6.2.8. Systems up-scaling, process scale, and mass and energy balances

For both systems, it was considered 330 workdays per year. Process units design (CSTR, SBR, FBR, CAS units, AGS, DAF systems, pumps, stirrers, blowers, compressors and centrifuges) and energy balances were performed according chemical engineering handbooks (Coulson and Richardson, 1999; Metcalf & Eddy, 2014; Perry, 1997; Seider et al., 2017). The design equations used are the same ones used in Chapter 5 (see Appendix 3). For the evaluation in the second stage of the study when DSP is included, the extraction sections of SA1-SA4 and SB1-SB5 were modelled using Aspen HYSYS as indicated in Saavedra del Oso et al. (2020). All evaluated scenarios were designed using Excel spreadsheets. Details about the energy consumption and selection of the equipment can be found in Appendix 4, but also in Appendix 3, as the calculations were done analogously to Chapter 5 for some processes and equipment.

For System A (SA), the process scale was chosen according to the results of Chapter 5 (Roibás-Rozas et al., 2020), and it was assumed that the daily amount of MCW is 100 m^3/day in a single facility (i.e. small-scale). MCW was sampled periodically for two years on-site in a mussels processing facility, so mass balances are accurate according to MCW composition. Mass balances were performed using laboratory-scale data published in Roibás-Rozas et al. (2021) for the valorisation section of SA1-SA4, and according to the calculations previously indicated in Chapter 5 (Appendix 3) for the treatment section (the CAS reactor).

For System B (SB), the process scale is the one that currently operated in the facility described in SB0, producing 700 m^3/day of FPW (also considered small scale, as big scale is considered here from 1,000 m^3/day). All the information (wastewater flow, system capacity, etc.) is available through the Galician government public records (Secretaría xeral de Calidade de Avaliación Ambiental, 2013a). Wastewater was periodically sampled and analysed for one year, so mass balances are accurate according to the actual conditions in the facility. Mass balances were performed using laboratory-scale data published in Argiz et al. (2020b) for the valorisation section of SB1-SB5, and laboratory

results in the framework of TREASURE-TECHNOSALT project for the treatment section of SB1-SB6 (the AGS reactor).

6.3 RESULTS AND DISCUSSION – 1ST STAGE

6.3.1. Results of the mass and energy balances for the WWTPs

In the first stage of the evaluation, the WWTPs that treat food processing wastewater are upscaled and designed to perform an evaluation of the wastewater treatment systems and to choose the ones with the best performance.

First, for SA, due to the highly variable concentration of COD and nitrogen of MCW, the acidification efficiency and the VFA composition and concentration of the CSTR effluent also differed widely according to the operational conditions. Mass balances were performed accordingly, so the volumes and stream flows of the different process units also varied on each process configuration (Table 6.2).

Table 6.2. Flows and concentrations of the main streams of SA, reactor volumes and energy use. The streams mentioned in the table are the ones identified in Figures 6.2 and 6.3. See Appendix 4 for more information about the balances. Note that the PHA referred in the table is contained in the biomass (DSP would be needed to extract it).

	SA1	SA2	SA3	SA4	SA0
	Stream flows (m^3/day)[1]				
Q_{INLET}	100	100	100	100	100
Q_{ENR}	42.5	28.3	27.2	53.4	-
Q_{ACC}	55.9	70.3	71.1	43.4	-
Q_{INOC}	42.5	12.6	13.6	54.4	-
Q_{WITH}	-	15.7	13.6	-	-
Q_{RE}	110.2	3.1	-	111.4	-
Q_{CAS}	86.9	90.7	87.6	87.9	127.2
Q_{DSP}	14.8	12.4	12.7	14.7	-
$Q_{TOTAL-SLUDGE}$	1.8	1.7	2.0	2.5	3.1
Q_{OUTLET}	83.4	85.9	85.3	82.8	96.9
PHA (kg/day)	26.0	45.3	176.9	34.2	-
	Reactor Volumes (m^3)				
V_{ENR}	152.7	31.4	27.2	165.8	-
V_{ACC}	49.2	41.5	42.3	48.9	-
V_{CAS}	92.4	199.7	115.8	147.0	1276.0
	Energy (kWh/day)				
Electricity Total	271.6	294.9	268.0	296.3	584.9
Pumps	4.1	4.6	3.4	4.3	0.4
Stirrers	178.3	201.2	177.5	192.5	35.0
Aeration	72.3	71.0	71.0	80.9	534.7
Centrifuges	16.8	18.0	16.2	18.6	14.9

[1] Q_{INLET} is the daily inflow, Q_{ENR} and Q_{ACC} are the feedings of the SBR and FBR, respectively. Q_{INOC} is the enriched biomass to the FBR from the SBR (in stages SA1, SA2, and SA4, it is the concentrated stream out of the settler after the SBR, in stage SA3 it is directly the SBR effluent). Q_{WITHD} is the withdrawal from the SBR for stages SA2 and SA3, and Q_R is the biomass-free SBR effluent used for feeding dilution in stages SA1, SA2, and SA4. Q_{DSP} contains the PHA-rich biomass to be extracted, Q_{CAS} is the wastewater flow to the conventional activated sludge (CAS) unit, and $Q_{TOTAL-SLUDGE}$ is the stream with thickened solids wasted from the system. Q_{OUTLET} is the treated wastewater effluent to be discharged to the environment.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

For SB, the composition of FPW is less variable, and the obtained waste oil did not change throughout time. Moreover, it is considered that the flow of 700 m^3 FPW/day that is fed daily to the DAF is also constant throughout time, so the flow fed to the AGS did not vary between scenarios except for SB3, due to small differences in the valorisation system that influenced the remaining system balances, and for SB6, due to the absence of

the biological valorisation section in the WWTP. The main results of the mass balances are shown in Table 6.3, where the streams represented are the ones in Figures 6.4 and 6.5.

6.3.2. Economic Analysis: TEA and LCC

6.3.2.1. System A (SA) with Mussels Cooking Wastewater (MCW)

For the conventional system SA0, the main direct cost (about 80%) is due to the CAS reactors, as well as the indirect cost, that is linked to the aeration in the reactors (Table 6.4). For the circular economy-based system, the reactors stand for around 40% the direct costs, where the pumps and centrifuge oversee about 50% of the remaining expenses.

Table 6.3. Flows of the most important streams of each scenario for SB, reactor volumes, biopolymer production and energy use. See Appendix 4 for more information about the balances. Note that the PHA/TAG referred in the table is contained in the biomass (DSP would be needed to extract it).

	SB1	**SB2**	**SB3**	**SB4**	**SB5**	**SB0**
	Stream flows (m^3/day)[1]					
Q_{INLET}	700	700	700	700	700	700
Q_{OIL}	0.53	0.53	0.53	0.53	0.53	0.53
$Q_{E\text{-}OIL}$	0.20	0.20	0.24	0.20	0.20	-
$Q_{A\text{-}OIL}$	0.32	0.32	0.29	0.32	0.32	-
$Q_{DILUTION}$	202.0	202.0	238.6	202.0	202.0	-
$Q_{BIOREACTOR}$	867.1	867.1	896.8	867.1	867.1	742.2
$Q_{TOTAL\text{-}SLUDGE}$	3.1	3.1	3.2	3.1	3.1	4.3
Q_{DSP}	40.4	40.4	47.8	40.4	40.4	-
Q_{OUTLET}	656.5	656.5	649,1	656.5	656.5	694.6
PHA (kg/day)	2.3	55.6	68.8	11.4	43.7	-
TAG (kg/day)	11.9	20.2	3.8	57.0	7.3	-
	Reactor Volumes (m^3)					
V_E	202.2	202.2	238.9	202.2	202.2	-
V_A	101.1	101.1	119.5	101.1	101.1	-
$V_{BIOREACTOR}$	361.3	361.3	373.7	361.3	361.3	5000
	Electricity (kWh/day)					
Electricity Total	593.0	593.0	632.4	593.0	593.0	2561.2
Pumps	6.6	6.6	8.2	6.6	6.6	3.2
Stirrers	34.7	34.7	35.9	34.7	34.7	300.0
Aeration	324.3	324.3	360.9	324.3	324.3	2019.6
Centrifuge	-	-	-	-	-	11.0
DAF	227.4	227.4	227.4	227.4	227.4	227.4

[1] Q_{INLET} is the daily effluent flow. Q_{OIL}, $Q_{E\text{-}OIL}$, $Q_{A\text{-}OIL}$, $Q_{DILUTION}$, are, respectively: the total flow of oil, the oil fed to the SBR and to the FBR, and the flow of wastewater effluent bypassed to the SBR as growth media for the MMC. $Q_{BIOREACTOR}$ is the flow to the AGS reactor (SB1-SB5) or to the CAS unit (SB0). $Q_{TOTAL\text{-}SLUDGE}$ is the stream with thickened solids to be externally managed.

In this sense, the cost of the reactors (40% of the direct costs) is mainly due to the CSTR for circular economy-based scenarios, as it has a high volume (HRT = 6.25 days) compared to the lower volume of the SBR and FBR (HRT = 1 day). Accordingly, the stirrers of the acidogenic reactor consume more power than the aeration of the aerobic reactors for these innovative approaches.

Therefore, reducing the residence time of the acidification unit (thus, its volume) and reducing the number of streams involved in the process (for example, the recirculation for load control, thus, reducing pumping needs, as in stage SA3) are key issues for economic feasibility, as it would decrease direct costs but also the utilities expenditure.

However, although reducing the CSTR size would also yield in a decrease in the utilities cost, the main indirect expenses are due to sludge management (Table 6.4). To

overcome this, an alternative could be the sludge transformation again into VFA in the CSTR, although this could also increase the reactor residence time, so further studies are needed to validate the expected improvement of the economic profile of the process.

Table 6.4 Equipment purchase cost of the WWTPs equipment according to their sizes, and main indirect costs for the scenarios with MCW. All direct costs are in € and indirect ones are in €/year.

	SA1	SA2	SA3	SA4	SA0
Equipment Purchase Cost (€)	**269,739**	**269,739**	**243,864**	**281,799**	**254,202**
All reactors	109,940	106,875	101,340	115,651	193,011
CSTR	72,819	72,819	72,819	72,819	-
Pumping	90,620	92,771	73,941	96,426	22,609
Stirrers	19,335	20,732	19,222	20,539	5,952
Blowers	2,561	2,561	2,561	2,561	13,130
Centrifuge	46,800	46,800	46,800	46,800	19,500
Main Indirect Costs (€/year)	**41,132**	**39,193**	**43,024**	**53,497**	**59,550**
Electricity	10,754	11,677	10,613	11,734	23,164
Sludge management	30,377	27,516	32,410	41,763	36,386

Accordingly, results show a potential decrease in the management costs of wastewater if circular economy approaches are applied. Here, the economic feasibility of this approach is linked to several factors, like a wise use of chemicals and a decrease in the residence time of the acidification reactor. For the present case, HRT is 6.25 days for SA1 – SA4, so there is room for improvement.

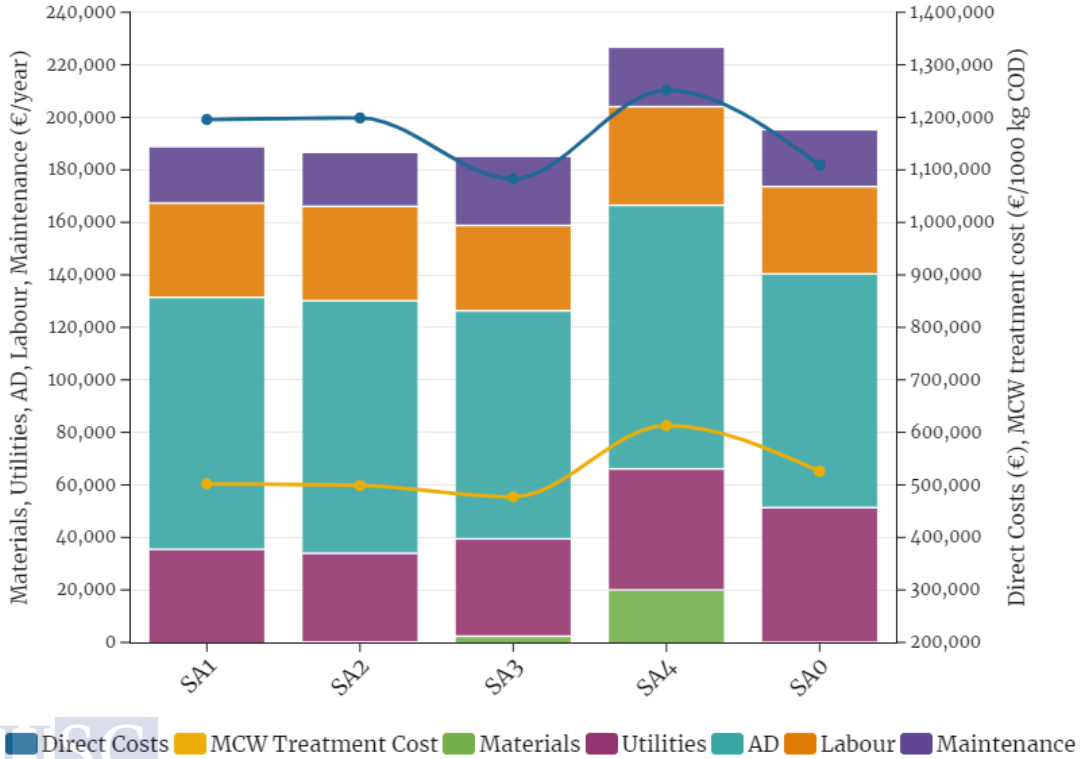


Figure 6.8. Costs of the scenarios of system SA (MCW = Mussels Cooking Wastewater, AD = Annual Depreciation. SA0 represents the current linear economy-based system and SA1 – SA4 are the circular economy-based ones.

Regarding utilities and indirect costs, the pumping costs of the circular systems are not negligible, as due to the sequential regime of the SBR and FBR, pumps need to transport high volumes in short periods of time. Finally, an efficient sludge management can make a difference, due to the important indirect cost of composting, as currently waste

sludge for these facilities is treated suing this technique. As the conventional system SA0 produces more waste sludge, sludge management increases utilities costs. Note however that, although SA0 produces more sludge, the one with higher waste management costs is SA4 because, the produced sludge has a higher solids concentration due to the use of chemicals in the process.

Therefore, the cost of treating MCW under a linear economy approach (so SA0) is 0.53 €/kg COD, while treating it using circular economy approaches yield in 0.48 – 0.61 €/kg COD (Figure 6.8). Note that, for these results, the benefits of PHA production are not accounted yet (DSP is not included). Therefore, the potential economic success of the circular economy-based process is not relying on the PHA value, but in the improved system configuration proposed.

Excluding scenario SA4, where the use of chemicals for pH control in the acidification CSTR turns into high operational costs, the cost of treating wastewater under circular economy approaches that have a wise use of chemicals (SA1, SA2, and SA3) yields in 0.49 ± 0.01 €/kg COD. The scenario with the lowest cost is SA3, with 0.48 €/kg COD.

6.3.2.2. System B (SB) with Fish Processing Wastewater (FPW)

For SB, only a fraction of the wastewater (the oil) is biologically valorised into PHA/TAG for scenarios SB1-SB5. Therefore, the non-oily wastewater is only treated, and not valorised, using an innovative treatment (AGS) for SB1-SB5, but also for SB6. For SB6 and SB0, where oil is not biologically valorised, it is transformed into biodiesel.

Here, the reactors used under an innovative approach have a volume smaller than the one used under the conventional approach (see Table 6.3) due to the lower residence times linked to the operation of the innovative AGS reactors compared to the CAS ones for this industrial wastewater treatment (AGS has a residence time of 10 hours, while the CAS has a residence time of 7.1 days). However, scenarios SB1-SB5 have more process units and pumps. In this sense, there is no CSTR unit for SB, as the oil is hydrolysed in the SBR and FBR. However, there is an AGS that also requires of high amounts of aeration. Although it is considered that the AGS would have higher oxygen needs per volume of reactor than the CAS one, the CAS reactor has a volume of 5,000 m^3, while the AGS has a volume of around 360 m^3. Consequently, operating the AGS is still cheaper than operating the CAS, despite the higher oxygen needs per volume of reactor in the AGS than in the CAS.

On the other hand, SB0 and SB6 have operational costs higher than the circular economy-based scenarios (Table 6.5). In the case of SB6, indirect costs are approximately the same as for circular scenarios SB1-SB5, since the cost of waste management (including oil treatment) is very high, but electricity needs are lower. Nevertheless, for the conventional scenario SB0, not only the costs of oil and waste sludge treatment are high, but also the energy demand is very high due to the aeration needs of the biological reactor.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Circular economy-based scenarios are economically more attractive than the current ones (Figure 6.9). However, valorising oil on-site is still more expensive than treating it externally (normally, for biodiesel production as done by SB6), as the cost of the biological valorisation of the oil into PHA/TAG is around 1,100 €/m^3, while the current

cost of oil management is around 500 €/m^3 (Illukpitiya and de Kof, 2014) (thus, approximately 0.48 €/kg COD_{OIL} for biological valorisation versus 0.22 €/kg COD_{OIL} for biodiesel).

Table 6.5 Equipment purchase cost of the WWTPs equipment according to their sizes, and main indirect costs for the scenarios with FPW. All direct costs are in € and indirect ones are in €/year.

	SB1	SB2	SB3	SB4	SB5	SB6	SB0
Equipment Purchase Cost (€)[1]	**321,728**	**321,726**	**340,225**	**321,772**	**321,707**	**149,940**	**458,549**
Reactors,	80,137	80,137	87,227	80,137	80,137	33,114	309,910
of which AGS	22,995	22,995	23,568	22,995	22,995	22,995	
Pumping	72,403	72,403	73,301	72,403	72,403	33,448	21,593
Stirrers	4,185	4,185	4,185	4,185	4,185	4,185	10,736
Blowers	7,172	7,172	7,172	7,172	7,172	3,825	40,852
Compressor	75,367	75,367	75,367	75,367	75,367	75,367	75,367
Heat Exchanger	82,462	82,462	92,967	82,462	82,462	-	-
Main Indirect Costs (€/year)	**96,261**	**107,374**	**121,616**	**107,373**	**107,366**	**122,048**	**259,009**
Electricity	23,483	23,483	25,042	23,483	23,475	18,113	101,423
Heat	55,566	66,679	78,773	66,679	66,679	-	-
Sludge management[2]	17,212	17,212	17,800	17,212	17,212	103,935	157,586

[1] The compressor is used in the DAF, and the heat exchangers are used to ensure that the temperature of the biological valorisation system maintains a temperature of 22.5 °C to ensure that oil's emulsion.
[2] For SB1-SB5, the cost of sludge management includes the price of managing waste solids, but not oil (oil is valorised in the SBR and FBR system). For SB6 and SB0, it also includes the price of oil treatment by biodiesel production.

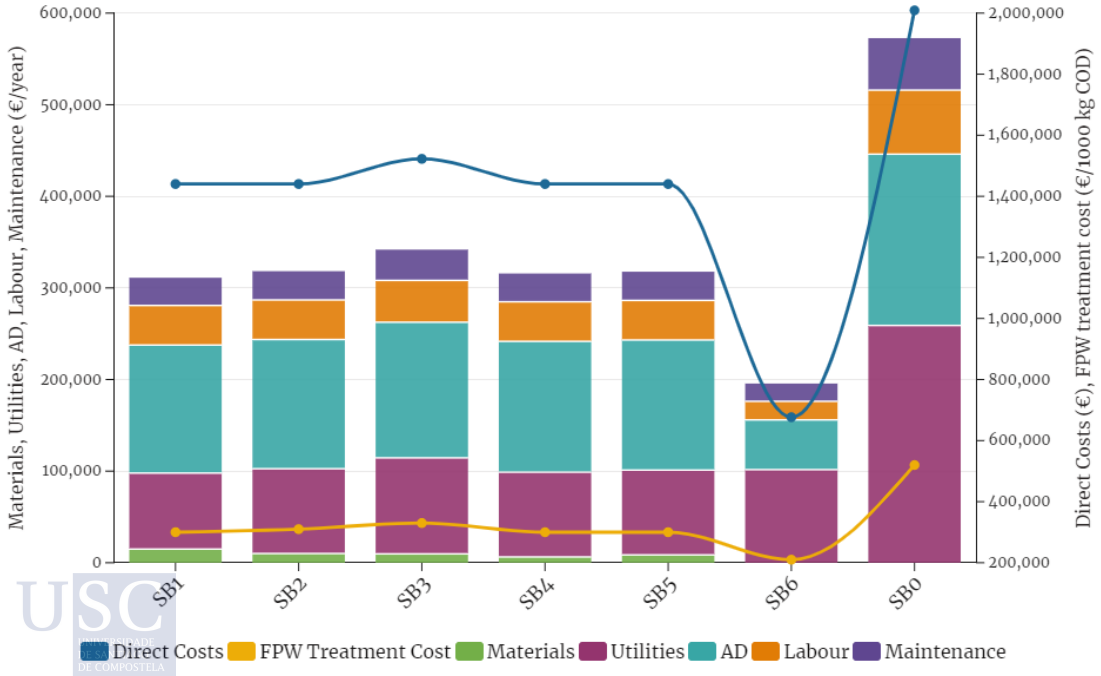


Figure 6.9. Costs of the scenarios of system SB (FPW = Fish Processing Wastewater, AD = Annual Depreciation). SB0 represents the current linear economy-based system and SB1 – SB5 are the circular economy-based ones, while SB6 is the system where oil is not biologically valorised (only the current CAS reactor is replaced by an AGS reactor).

Treating FPW under a linear economy approach costs 0.52 €/kg COD (SB0), while treating it using circular economy approaches yield in 0.30 – 0.33 €/kg COD for SB1 – SB5 (Figure 6.9). Finally, treating wastewater under an innovative approach using AGS to treat the wastewater (but not biologically valorising it into PHA/TAG) yields in 0.21 €/kg COD (SB6). Here, other criteria, such as environmental or societal, could be considered to assist the decision making for oil valorisation into PHA/TAG. In this sense, elements like the heat and electricity requirements of the biological valorisation of the oil might hinder not only the economic feasibility (see Table 6.5) but also presumably the environmental one.

The results presented then prove that replacing CAS units by AGS ones can clearly improve the economic performance of these industrial WWTPs, while they also indicate that further research and improvement might be needed before the biological oil valorisation into PHA/TAG is implemented at full-scale.

6.3.3. Environmental Analysis

6.3.3.1. Environmental impacts of System A scenarios with MCW

The environmental validation of the systems based on circular economy strongly depends on the process configuration (Figure 6.10). In fact, the option with highest impacts is SA4, due to the use of chemicals for pH control in the acidification CSTR.

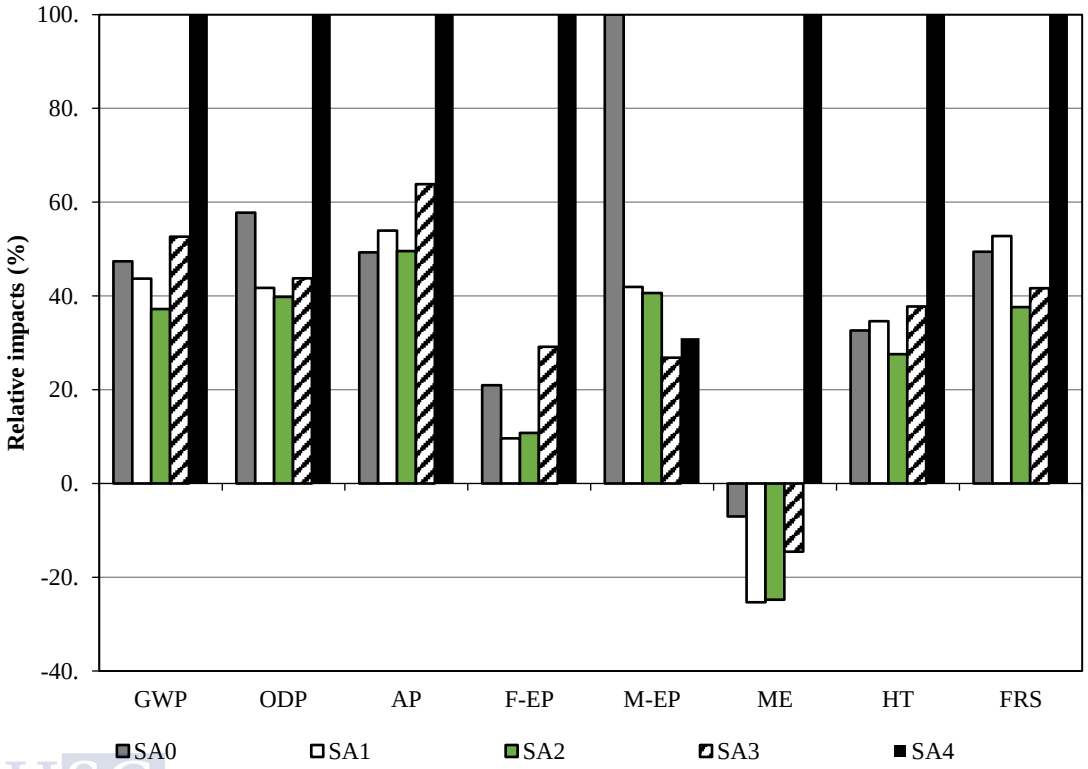


Figure 6.10. Impacts of the assessed processes for SA at small scale (DSP and PHA are not accounted). GWP = Global Warming Potential, ODP = Ozone Depletion Potential, AP = Acidification Potential, F-EP and M-EP = Freshwater and marine eutrophication potential, respectively, ME = Marine Ecotoxicity, HT = Human Carcinogenic Toxicity, FRS = Fossil Resource Scarcity.

The most profitable scenario economically (SA3) has higher impacts than the current strategy (process SA0) for some categories (about 5% for global warming (GWP), 15% for acidification (AP), 10% for freshwater eutrophication (F-EP), and 5% for human toxicity (HT)), also due to the chemical's consumption. Nevertheless, the system was

upscaled using laboratory-scale dosages of chemicals where different ranges for chemical dosage were tested, but a proper dosage optimization was not performed. In fact, it was observed that the key feature enhancing process performance was linked to the enrichment strategy, and not to the pH or the alkalinity in the CSTR (Chapter 3), so it would be expected that, at full scale, the use of chemicals for pH control would be optimized and reduced.

Moreover, sludge management by composting (the technique currently used to treat waste sludge in these factory types) generate noticeable impacts in climate change, ozone depletion and acidification, due to the process emissions (mainly ammonia, sulphur dioxide and nitrogen oxides, as well as some fossil carbon dioxide and methane).

In fact, other waste management strategies (like the centralized anaerobic digestion of SB) could potentially validate the environmental profile of the process when compared to conventional techniques, as composting generates some impacts, and anaerobic digestion usually generates environmental credits. Finally, scenarios SA1 and SA2 perform better environmentally at this 1st stage of the analysis, but they had low PHA accumulations (Table 6.2), so they are not the best option from a techno-economic point of view.

6.3.3.2. Environmental impacts of System B scenarios with FPW

For SB, the processes based on circular economy have less impact than the ones based in linear economy for all categories except for marine eutrophication (Figure 6.11) due to small differences in the effluent quality. Only SB1 performs slightly worse in some categories because of the higher use of chemicals for the enrichment of the MMC, as dosages were not optimized at this stage.

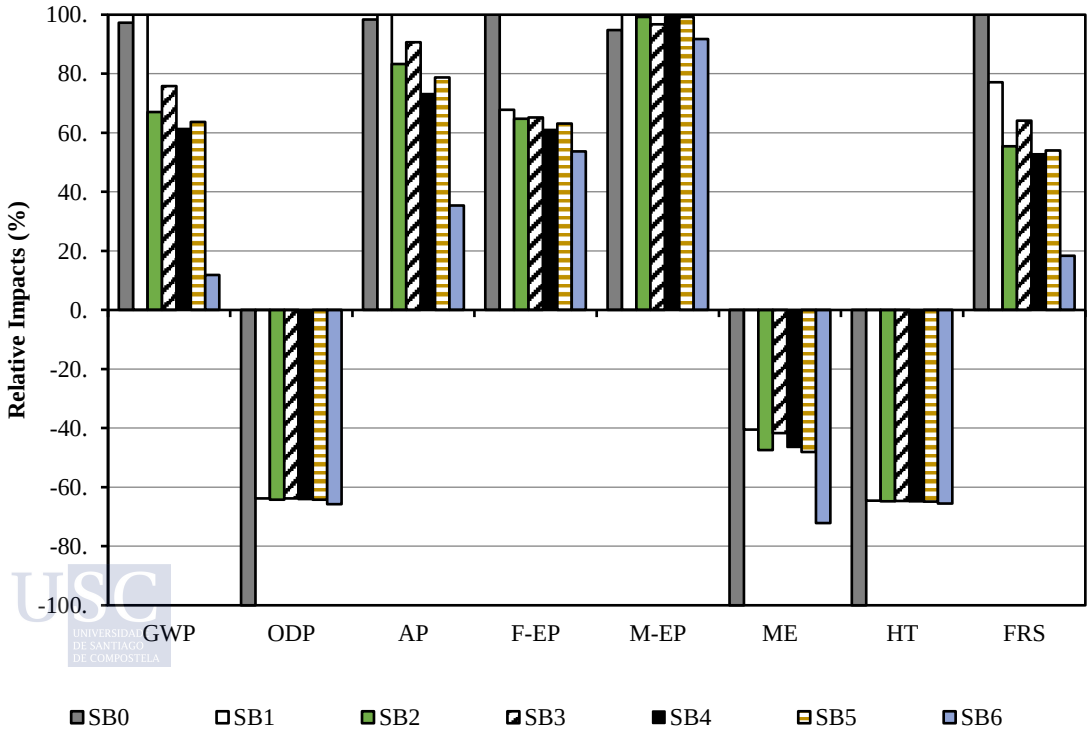


Figure 6.11. Impacts of the assessed processes for SB at small scale (DSP and PHA are not accounted). GWP = Global Warming Potential, ODP = Ozone Depletion Potential, AP = Acidification Potential, F-EP and M-EP = Freshwater and marine eutrophication potential, respectively, ME = Marine Ecotoxicity, HT = Human Carcinogenic Toxicity, FRS = Fossil Resource Scarcity.

In fact, although the processes based on circular economy have a lower impact than the conventional one, the use of chemicals have important effects. As the enrichment process in the valorisation system had uncoupled nitrogen and carbon feedings and pH control, ammonium chloride and sodium bicarbonate were used, respectively. This yields in noticeable effects, representing about 50% of the impacts for global warming, acidification, and fossil resource scarcity.

Moreover, the operation of the valorisation reactors for SB need to happen at mild temperature, and the steam needed yielded in environmental impacts (10 – 20% of the effects for global warming, acidification and marine ecotoxicity). Finally, the use of anaerobic digestion for sludge management results in environmental credits (specially for ozone depletion, marine ecotoxicity and human toxicity, but also for global warming), although it generates relevant impacts for freshwater eutrophication.

Finally, managing oil externally (SB6) by esterification for biodiesel production results in the best environmental profile of all the studied scenarios. Therefore, replacing the current CAS by a system based on biofilms and AGS would yield in lower use of electricity, heat, and chemicals, so the process would be more profitable both economically and environmentally. Hence, it seems that biologically valorise oil into PHA/TAG is not yet the most attractive option from the environmental point of view.

6.4 RESULTS AND DISCUSSION – 2ND STAGE

The results of the first stage showed that, for most process configurations, the economic performance of circular-economy and/or innovative approaches improves or has the potential to improve the current conventional approach. The analysis also showed that process configuration affects both the environmental and the economic performance, so the best alternative for each system was selected for the second stage of the study.

For the case of MCW (SA), process configuration SA3 was chosen to upscale the facilities, as: i) SA3 has the lower costs at small scale, ii) lower environmental impacts are expected if waste sludge is treated by anaerobic digestion instead composting, and chemicals addition is optimized for full-scale operation, and iii) it is the scenario with the highest net PHA accumulation.

For the case of FPW (SB), two alternatives based on two DSP approaches are considered, as two products can be generated (PHA and TAG). For PHA production only, the considered process configuration is SB3, as it has the highest production of PHA. For PHA+TAG extraction, SB2 was considered, as it is the only process approach that presented non-negligible concentrations of both products simultaneously. Besides, the environmental performance of SB6 is studied as well, as it presented the best economic performance in the first stage of the study. Note that it is not necessary to perform an economic evaluation of SB6 in the second stage of the study, as no biomaterials are produced in this scenario. Therefore, the cost of wastewater treatment was already assessed in the first stage of the evaluation, and no extra costs need to be calculated (thus, no extra benefits are included).

USC DE SANTIAGO DE COMPOSTELA

Three scales will be tested for each of the selected scenarios. First, it was considered a scale ten times higher than the current one (x10), representing ten small facilities that provide PHA/TAG-rich biomass to be extracted in a centralised facility (plus SB6 case, where the performance of ten WWTPs treating AGS is compared to the same plants using CAS). Accordingly, scenarios for twenty (x20) and fifty (x50) WWTPs are assessed, where DSP would be centralized. Besides, for all the cases, it was assumed that waste

solids are managed by centralized anaerobic digestion. Moreover, it is important to notice that, in this 2nd stage of the evaluation where DSP is included, the environmental and economic burdens of PHA/TAG-rich biomass production (thus, wastewater treatment) are allocated to the WWTPs, while the burdens and benefits of biomaterials production (PHA/TAG extraction, and PHA/TAG replacement of fossil products) are allocated to the centralized facility where DSP is carried out.

Finally, the use and end of life phases of the bioproducts life cycles are excluded of the study due to the lack of information. Note that transport costs are not considered in this study due to uncertainty, and because previous studies showed that the impact will not be very significant for short distances (Chapter 5), so it is assumed that raw materials (PHA-rich sludge) are supplied to the DSP plant directly.

6.4.1 Centralized biorefinery for PHA production: economic results

As previously presented (Figure 6.8), the cost of wastewater management was 0.48 €/kg COD for SA3 (MCW). For the scenario x10 times the current one, this centralized biorefinery performs the DSP of the received biomass from ten facilities. The production cost of PHA under this approach would be 1.18 €/kg PHA (Figure 6.12), where costs come only from extraction operations (the cost of biomass generation is allocated to the WWTPs).

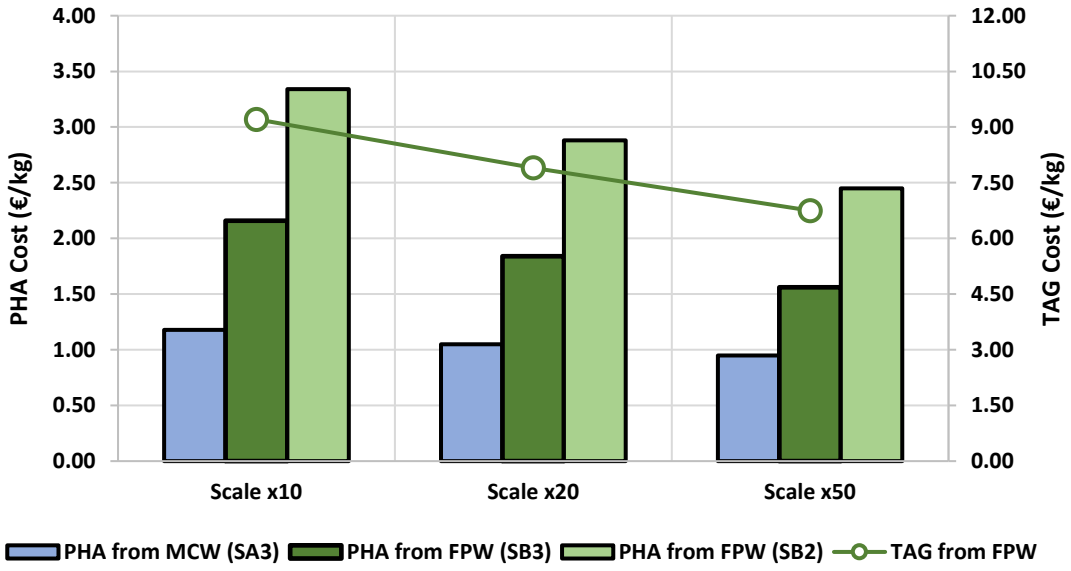


Figure 6.12. Cost of PHA and TAG production under the centralized approach where WWTPs are raw materials providers for a centralized biorefinery where PHA is extracted from the biomass. Here, the cost of PHA is only due to the DSP.

The costs of producing PHA with centralized extraction approaches for higher scales decrease accordingly. For a system with 20 facilities, costs are 1.05 €/kg PHA. For the scale of 50 facilities with centralized DSP, costs are 0.95 €/kg PHA (Figure 6.9). Considering that the cost of HDPE varied in the last four years in a range of 0.70 – 1.04 €/kg (statista, 2021), for the x20 and x50 scales, the cost of PHA produced from waste streams would yield in competitive prices, with promising results that could improve with process optimization and due to factors like the petrol availability.

For FPW (SB3), the cost of treating wastewater for each facility is 0.33 €/kg COD (Figure 6.9). Then, the cost of the centralized extraction is 2.16 €/kg PHA, and it

decreases as scale increases, yielding in 1.84 €/kg PHA and 1.56 €/kg PHA for the scales x20 and x50, respectively (Figure 6.12). The reason why DSP is more expensive for SB than for SA is linked to the lower biomass concentration for the system that uses oil. Here, prices are close to the competitive cost of HDPE for the highest scale, although they are still about 30% higher.

6.4.2. Centralized biorefinery for PHA and TAG production: economic results

To extract TAG, it is necessary to include distillation columns using hexane and methanol in the extraction process (Figure 6.7). Besides, the DSP is carried out at relatively high temperature (80 ºC, while DSP for low grade PHA only is at room temperature). Therefore, for scenarios where both TAG and PHA are produced, the cost of producing PHA increases when compared to scenarios where only PHA is extracted.

The costs of a system with centralized extraction also decrease linearly with scale, but they are considerably higher than the market price of their petrochemical competition. PHA produced by centralized DSP when also TAG is extracted costs 3.35, 2.88, and 2.45 €/kg PHA for scales x10, x20, and x50, respectively (Figure 6.12). Therefore, PHA production cost for this combined DSP approach increases with respect to one obtained for PHA extraction only.

Finally, TAG cost is 9.21, 7.90, and 6.75 €/kg TAG for the scales x10, x20, and x50, respectively. As there is still not a global market for TAG, it is difficult to stablish a threshold value to compare the results with other studies. Nevertheless, these numbers are considerably higher than the 1 €/kg that is traditionally assigned to petrochemical goods of common use. Moreover, the marketability of TAG is still uncertain, so more research is needed in this area.

6.4.3. Environmental performance of the centralized DSP approaches

The environmental performance of ten, twenty, or fifty facilities that provide sludge for a centralized biorefinery where PHA and TAG are extracted is compared with the performance of the same facilities when they only treat wastewater (i.e. the current linear economy approach). Moreover, the performance of SB6 is included, where the approach is not circular, but innovative (as AGS is used to treat wastewater, instead of CAS).

As happened with economic results, the scale had a linear effect on the results and that is why only the results of the scenario for 10 facilities are shown (Figure 6.13). Thus, only absolute values of emissions change, as they increase accordingly, but the relative impacts are the same between scales.

Here, the avoided products (HDPE for SA3, and diesel with full petrochemical origin for SB2 and SB3) are accounted for, so the circular economy-based processes generally perform better than the conventional ones (Figure 6.13), generating environmental benefits.

For MCW management, the circular economy-based process (SA3) performs better than the linear economy-based one (SA0) for all categories freshwater eutrophication potential, due to the use of chemicals, where a wide margin for improvement still exists. Here, the credits generated by the avoidance of petrochemical materials are smaller, so the impacts linked to the use of chemicals still provoke that the improvement for circular economy-based processes is not fully validated for this category. Positive effects are especially noticeable for global warming and fossil depletion categories, where the

environmental credits generated by the avoidance of a petrochemical plastic production are clearer.

For FPW management, the conventional process (SB0) has impacts higher than SB3 for all categories except for marine eutrophication potential, due to small variations in the effluent quality between scenarios, while SB2 performs worse than SB0 for some categories due to the lower bioproduct generation. Finally, small impacts are linked to scenario SB6, where biological valorisation of oil is not performed. As the amount of bioproduct generated for systems using FPW is low, the potential benefits of avoiding petrochemical materials are not enough to buffer the burdens of operating the train of biological reactors, as it was for SA3.

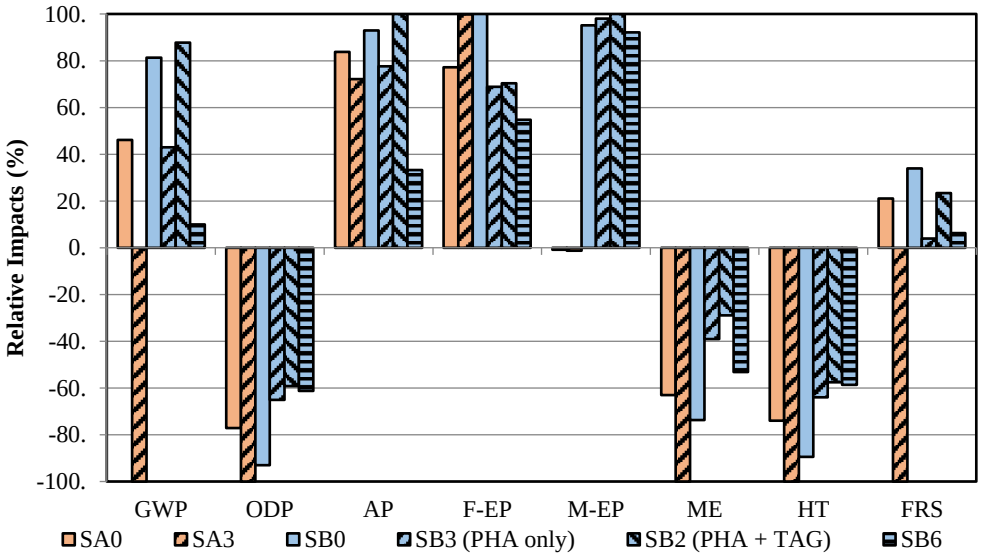


Figure 6.13. Impacts for a bigger scale considering 10 small facilities. The ones based on circular economy work as PHA/TAG-rich biomass providers for centralized DSP. GWP = Global Warming Potential, ODP = Ozone Depletion Potential, AP = Acidification Potential, F-EP and M-EP = Freshwater and marine eutrophication potential, respectively, ME = Marine Ecotoxicity, HT = Human Carcinogenic Toxicity, FRS = Fossil Resource Scarcity.

On the other hand, biomass production is lower for oil-based systems than for MCW ones. Therefore, the final process biomass yield is lower than that for the MCW-based process: 0.019 kg PHA/kg COD for SB2 and 0.024 kg PHA/kg COD for SB3, versus 0.16 kg PHA/kg COD for SA3. This makes SB2 performance worse than SB3, and, for some categories, also worse than SB0. Therefore, the margin of improvement (the impact reduction) of circular economy-based systems with oily feedstock is narrower than the one for SA, where impact reduction is clearer.

6.5 DISCUSSION AND COMPARISON WITH THE AVAILABLE LITERATURE

The use of biomass and wastes to generate biofuels and biomaterials could be a feasible solution to solve current threats faced by humans, as the climate crisis. However, different problems need to be addressed before these biotechnological processes are applied (Ahmed et al., 2021). Although there is a claim for biomaterials like PHA to be the environmentally friendly solution for the world's future economy (Shahid et al., 2021), the true is that the lack of environmental and economic validation is hindering the introduction of these materials in the value chains.

The present study showed that applying strategies based on circular economy to manage industrial effluents could decrease the cost of wastewater treatment. Moreover, the economic feasibility of these processes is not necessarily relying on the production of biomaterials, but in the implementation of a more efficient treatment scheme combined with centralized and low-cost DSP approaches. It was proved here the economic and environmental feasibility of transforming industrial WWTPs into raw materials suppliers for biorefineries. Moreover, realistic models to achieve technical, environmental, and economic viability were explored. For example, in Galicia (NW Spain), around 70 companies generate about 85% of the total Spanish canned fish products and 50% of the total European canned tuna production (González-López, 2018). Here, a system where these existing small and medium capacity WWTPs work as biomass providers for a centralized downstream facility could improve not only economic and environmental benefits, but also the viability of a sector where conservative business models have in some cases hindered the evolution of this industry (González-López, 2018).

The average price of PHA production for processes based on pure cultures reported in the las fifteen years is 3.46 €/kg PHA (Bhattacharyya et al., 2014; Leong et al., 2017; Martinez-Hernandez et al., 2018; Musonda et al., 2020; Pavan et al., 2019; Shahzad et al., 2017; Vega et al., 2020). Here, the use of mixed microbial cultures and waste streams to produce biomaterials claims to reduce those costs.

When some of the first economic evaluations were made for MMC-based PHA, costs were estimated in 3.52 $/kg PHA (2.71 €/kg PHA) for a production of 700 t PHA/year (Gurieff and Lant, 2007). Later, Fernández-Dacosta et al. (2015) achieved 1.40 – 1.95 €/kg PHA for a production of 1500 t PHA/year depending on the DSP method, while Bengtsson et al. (2017) used for the first time pilot scale data to estimate 3.40 €/kg PHA for a production of 5000 t PHA/year. Crutchik et al. (2020) also assessed the effect of scale using data from real WWTPs, where production costs were 2.26 €/kg PHA for 90 t PHA/year and 1.26 €/kg PHA for 5700 t PHA/year. Pérez et al. (2020a) discussed on how PHA economic feasibility is variable with the geographical location, and costs ranged 1.5 – 6.9 €/kg PHA when part of the methane was used to generate energy on-site for systems using methanotrophic MMC. Therefore, the cost for MMC-based processes averaged 2.58 €/kg PHA in the last fifteen years, proving that it would indeed decrease PHA price with respect to pure culture biomaterials.

Finally, Saavedra del Oso et al. (2020) investigated the cost of different DSP processes, and their cost ranged 0.2 – 2.25 €/kg PHA, where the lowest prices corresponded to low grade PHA (i.e. for low grade applications like packaging).

Under the approach applied in this study, where WWTPs are raw biomass suppliers and the low-cost DSP is centralized, WWTP holders would not directly benefit from PHA production (they do not produce PHA on-site). Indirect benefits would be the lower cost of sequential reactors, as they would treat the same flow of wastewater using less reactor volumes (thus, less aeration is needed), and the savings in sludge management. Therefore, PHA production cost would only correspond to the extraction, being 1.18, 1.05, and 0.95 €/kg PHA for productions of 580, 1200, and 3000 t PHA/year, respectively, for wastewater feedstock (MCW). For waste oil feedstock (FPW), PHA costs are 2.16, 1.84, and 1.56 €/kg PHA for productions of 230, 450, and 1100 t PHA/year (if TAG is not recovered).

Regarding the environmental effects, among the about 60 research works assessing the impacts of PHA production recently revised (Roibás-Rozas et al., 2022b) only 11

considered MMC-based processes, and global warming potential was the most studied category. Although results vary depending on different methodological or process choices, global warming-related emissions generally ranged 0.5 – 5 kg CO_2-eq/kg PHA (Vogli et al., 2020), which clearly show a great variability of the resultant impacts.

For the present study, the emissions linked to the wastewater treatment generally decreased due to the less use of energy when compared to the conventional treatments currently applied, so the processes would be validated from a wastewater management perspective. When PHA extraction is centralized, the environmental burdens of PHA-rich biomass production are allocated to the WWTP, so PHA production's burdens only account for the DSP. Here, the emissions of the extraction process are 0.25 kg CO_2-eq/kg PHA for the system using MCW, 0.29 kg CO_2-eq/kg PHA for the system using FPW with no TAG extraction, and 0.31 kg CO_2-eq/kg PHA and 1.1 kg CO_2-eq/kg TAG for the oil system that also recovers TAG, which is in the low range considering other literature studies.

It is noticeable that the feasibility of the proposed system is also relying on new DSP approaches where the use of energy and chemicals is lower than in the extraction processes traditionally employed in PHA extraction. Generally, DSP was one of the process bottlenecks. However, with the use of these low-demanding processes, this might not be the case anymore if the aim is to produce low grade PHA (Saavedra del Oso et al., 2020).

6.6 FUTURE OUTLOOK

The need of implementing more sustainable, yet economically profitable, production systems and growth models, is urgent. However, there are just a few PHA pilot-scale projects worldwide (Estévez-Alonso et al., 2021; Mannina et al., 2020), so studies on the economic and environmental validation of resource recovery processes are missing. As proved in this study, and stablished by Saavedra del Oso et al. (2020), DSP might not be a bottleneck anymore if low grade PHA is enough to meet the standards required. Therefore, special attention needs to be paid to the use of chemicals in the PHA-rich biomass production process, where pilot experiences are needed to stablish real thresholds.

Regarding PHA production with wastewater, for the system using MCW, the presence of proteins was a major problem at laboratory scale (Chapter 3). Hence, the HRT of the acidification unit was high (6.25 days) to carry out their fermentation and remove them from the wastewater. Nevertheless, if other strategies to remove proteins were tested (like coagulation), the wastewater rich in carbohydrates can be fermented into VFA in approximately two days (Yang et al., 2015). This would decrease the CSTR volume (direct costs) and operational expenses (electricity of the stirrers) yielding in a higher efficiency and lower costs. Moreover, the obtained proteins could be used for other value-added applications, like animal feeding. However, pilot and lab-scale data would be needed to confirm this.

Besides, the potential benefits linked to the end of life of biomaterials or the detrimental (and hard to quantify) effects of plastic pollution of petrochemical materials (like plastic in the oceans) have been disregarded. As stated in chapter 1, ongoing projects like MariLCA (Marine impacts in LCA) or Plastic Leak, where these effects are trying to be quantified, could bring some light into the issue.

Finally, it is important to point out that the proposed strategy would only be successful if efforts are made by different stakeholders and administrations to facilitate the generation of clusters like the ones studied here. In this sense, multidisciplinary approaches including marketability, social acceptance or stakeholder inclusion need to be included in the assessments, as future bottlenecks might not be linked anymore to technical, environmental, or economic aspects, but to societal, legal, or market issues (Kehrein et al., 2020).

6.7 CONCLUSIONS

Although the use of MMCs and waste streams to produce value-added materials is a promising solution for resource recovery processes, there is a lack of environmental and economic validation. The present research work used long-term laboratory data to up-scale several systems for industrial effluents' treatment and biomaterials (PHA/TAG) production to carry out both detailed techno-economic and environmental assessments.

Results showed that costs can be potentially reduced when treating these effluents under a circular economy approach that include sequential reactors to produce PHA-rich biomass. This would also yield in lower costs and environmental impacts, although the operational strategy chosen, and the use of chemicals, could be critical for the validation. Results also showed that some improvements are still needed if TAG recovery is included in the systems.

Moreover, the present study proved the feasibility of stablishing a clustered system where WWTPs work as raw materials providers for biorefineries, and DSP is centralized. Finally, the success of the current approach also relies on the use of low-cost extraction processes, that occur at low temperature, use less chemicals, and generate low grade PHA, avoiding the typical bottleneck of expensive extraction approaches. Under this approach, PHA costs can be lower than 1 €/kg PHA.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

7. MODELING THE IMPACT OF SALINITY VARIATIONS ON AQUATIC ENVIRONMENTS: INCLUDING NEGATIVE AND POSITIVE EFFECTS IN LIFE CYCLE ASSESSMENT

SUMMARY

Salinity is changing in aquatic systems, but little is known about the effects of this change, especially transitional waters like the Galician estuaries. The few works that addressed this topic considered these impacts using ecotoxicity models. However, these models: i) assume poisoning effects for a chemical, and ii) state that an increase in the concentration of a pollutant generates an increase in the impacts. But first, salinity does not behave as a poison. Secondly, these ecotoxic effects disregard the effects of water freshening. In this chapter, a general framework to address the impacts of salinity variations is proposed, where the classic methodologies to generate emission-related Characterization Factors (particularly, Effect Factors) are extended in their scope. The new model is validated by applying it to the *Arousa* estuary in Galicia.

The content of this chapter was published as: Alba Roibás-Rozas[a], Montserrat Núñez[b], Anuska Mosquera-Corral[a] and Almudena Hospido[a] (2022). **Modeling the impact of salinity variations on aquatic environments: including negative and positive effects in life cycle assessment**. *Environmental Science & Technology*. https://doi.org/10.1021/acs.est.1c04656

[a] CRETUS, Department of Chemical Engineering, Universidade de Santiago de Compostela, 15782 Santiago de Compostela, Spain

[b] Sustainability in Biosystems, Institute of Agrifood Research and Technology (IRTA), 08140 Caldes de Montbuí, Barcelona, Spain.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

7.1 INTRODUCTION

Climate change is altering the biogeochemical cycles on the planet (Breitburg et al., 2018) and shifting water temperature, pH, dissolved oxygen concentration, and salinity (Bramwell et al., 2021). In fact, direct relationships between anthropogenic CO_2 release and alterations in the water cycle which result into salinity variations have been already established (Durack, 2015; Helm et al., 2010; Sun et al., 2021). Other direct human activities, such as irrigation (Amores et al., 2013; Perret and Payen, 2020), industrialization and agricultural expansion (Elliott et al., 2019), effluent disposal (Böhme, 2011; Zhou et al., 2013), or dam management (Parada et al., 2012), are also behind these salinity variations. These changes and their effects can be even more important in transitional waterbodies, such as estuaries, deltas, or coastal lagoons, which constitute less than 5% of the brackish areas worldwide but provide about half of the global fish catch (Elliott et al., 2019).

However, to the best of our knowledge, just five research works have tried to model the impacts of salinity changes on the environment (Payen et al., 2016). Although a new impact category addressing salinity was proposed (Leske and Buckley, 2004a, 2004b, 2003), to the best of our knowledge, it has never been included in an LCA study (Mosquera-Corral and Campos Gómez, 2021). Two assessment methodologies focused on soils, modelling salinity impacts according to variations in soil conductivity and linking them with food production and crop diversity loss (Feitz and Lundie, 2002; Núñez and Finkbeiner, 2020). Two other methodologies focused on aquatic systems, shaping the effects of salinity variations according to ecotoxicity models for water environments (Amores et al., 2013; Zhou et al., 2013).

The latter were only partially successful for several reasons, as ecotoxicity is currently based on the observation that the sensitivities of different species to a chemical follow a normal distribution, so increased exposure will generate increased impacts (Owsianiak et al., 2019). However, the impact of salinity is not only linked to concentration increases, but also to concentration decreases (systems can become saltier or fresher), so an approach based on ecotoxic models would fail to describe these effects. Moreover, salt is not a pollutant or a toxic, but an essential element, so ecotoxic models might not be valid to describe its behaviour. Hence, a critical improvement of these methodologies is necessary. Therefore, the aim of this study is to provide a framework for the evaluation of the impacts of salinity changes in aquatic ecosystems.

Estuaries are important ecosystems from an ecological point of view (Parada et al., 2012), supporting highly productive communities of arthropods, molluscs, fish, and birds, as well as complex food webs (Kingsford et al., 2020). The Western *rías* of the northwest Spanish region of Galicia, vital for the local economic and social development, are partially mixed estuaries where partial stratification is maintained by the river discharge in the winter and by solar heating in the summer (Alvarez et al., 2005). Due to their uniqueness, it is common that geography experts call these areas by their name in Galician (*rías*) (Carral et al., 2021).

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Nevertheless, in the past few decades, events of massive shellfish deaths were reported in these estuaries due to sharp decreases in the concentration of salt in the *rías* (Domínguez et al., 2020; Parada et al., 2012; Parada and Molares, 2008; Peteiro et al., 2018). These occurrences have been linked to heavy rains, locally managed freshwater releases from river dams, and increased river runoffs, where the frequency of these climatological episodes is supposed to increase in the coming years due to climate change

(Domínguez et al., 2020). Among the Galician *rías*, the biggest and most productive one is the *Arousa ría* (Domínguez et al., 2020; Parada et al., 2012; Roibás-Rozas et al., 2020). Therefore, this *ría* was chosen to test and validate the methodology proposed in the present research work.

In sum, we describe in this paper a general model shaping the effects of salt variations in aquatic systems. Then, we apply the model to a case study for the procedure validation. By providing this framework, we expect to expand the current knowledge of transitional water systems, allowing to integrate the evaluation of impacts (positive and negative) in environmental sustainability assessments, and to improve, for example, effluent disposal or dam management control in sensitive areas.

7.2 MATERIALS AND METHODS

Salinity is defined as the concentration of several inorganic ions (including Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, SO_4^{2-}, CO_3^{2-}, NO_3^- and HCO_3^-) (Mosquera-Corral and Campos Gómez, 2021; Payen et al., 2016), where most of these elements are essential substances needed for organisms to live. In the present research work, salinity is referred to as the concentration of NaCl, in kilograms per cubic meter (kg/m^3), as it is abundant in water streams considered saline.

Regarding the methodology, impacts linked to chemical releases are measured as in Eq. 7.1, where IS is the impact score, CF is the characterization factor, and M the mass of substance emitted (see Chapter 2). Then, the CF is calculated considering the principal cause-effect chains – thus, through fate, exposure, and effect factors (FF, XF and EF, respectively), according to Eq. 7.2 (Rosenbaum et al., 2018).

$$IS = CF \cdot M \quad \text{Eq. 7.1}$$

$$CF = FF \cdot XF \cdot EF \quad \text{Eq. 7.2}$$

CFs addressing impacts on ecosystem quality (aka natural environment) at the endpoint level have units of potentially disappeared fraction of species (PDF)·m^3·time/kg. As M in Eq. 7.1 is in kilograms, IS has units of PDF·m^3·time (Rosenbaum et al., 2018). FF in Eq. 7.2 is expressed in units of time (it represents the mass of a chemical in the environment resulting after an emission flow, so units are kg/(kg/day), which yields days), XF is dimensionless, and EF is expressed as PDF·m^3/kg. The XF represents the availability of the released chemical in a system (the fraction of the pollutant that is dissolved in the water), which can be considered as 1 for salt as it is fully dissolved (Zhou et al., 2013).

7.2.1. Fate Factor (FF)

The FF is linked to the physical behaviour/distribution of the substance in the environment and normally expresses its persistence in units of time (Fantke et al., 2018; Owsianiak et al., 2019; Rosenbaum et al., 2018). However, the FF also represents the predicted mass residence of a substance in a receiving compartment per unit of emission flow into it (Owsianiak et al., 2019), so the FF is calculated by applying mass balances to the studied compartment in which degradation and transfer processes are described (Fantke et al., 2018). Salt do not degrade (i.e. salt's residence time on the environment equals millions of years (Zhou et al., 2013)), so the mass balances will describe how they are transferred, and the relevant flows entering and leaving an aquatic compartment. For the calculation of an FF for salinity, the streams considered are shown in Figure 7.1.

W_I refers to the main inlet stream, where returns from the studied compartment can take place. In a marshland, for example, the main inflow (**$W_{I\text{-}IN}$**) is the river, and the W_I outflow (**$W_{I\text{-}OUT}$**) represents infiltrations of saline water into the fluvial system.

W_O streams represent the main outflow from the system (**$W_{O\text{-}OUT}$**), where the flow can also occur in both directions; thus, returns from the main outflow to the compartment (**$W_{O\text{-}IN}$**) can occur. For example, for an open estuary, these flows represent the tidal, so this main outstream would be subjected to bidirectional flows.

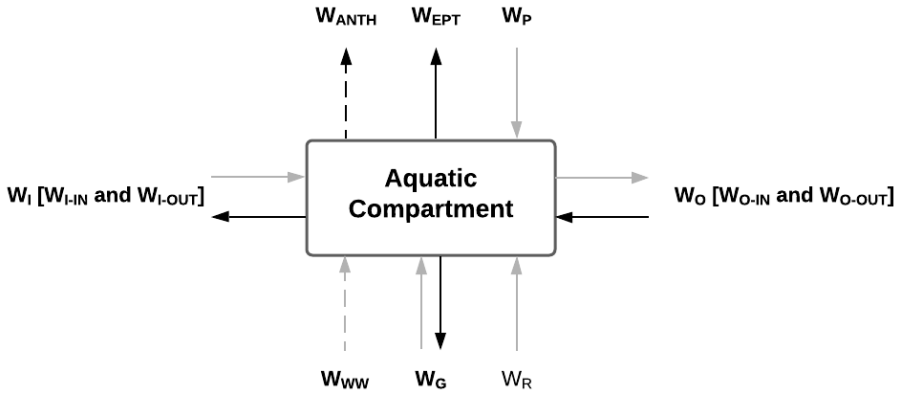


Figure 7.1. Streams to be considered in the mass balances for quantifying the FF. Gray lines represent the inflows and black lines the outflows. Dashed lines are the anthropogenic flows, and continuous lines are natural streams. Wi refers to each stream, where I = main inlet stream, O = main outlet stream (for both, I/O-OUT is the outflow and I/O-IN is the inflow of the stream). ANTH = anthropogenic outflows, WW = anthropogenic inflows (wastewater), EPT = evapotranspiration, G = groundwater, P = precipitations, and R = runoff.

W_P, **W_{EPT}**, **W_G**, and **W_R** are climate and water cycle-related streams that represent the precipitation, the evaporation, the groundwater, and the runoff of the system, respectively. Note that the groundwater flow could also be bidirectional, as it can feed the compartment, but infiltrations from the system to the groundwater network can also occur.

W_{ANTH} and **W_{WW}** are the anthropogenic outflows and inflows in the system, respectively. **W_{ANTH}** represents the water taken from the compartment for anthropogenic purposes (for example, for irrigation), and W_{WW} is the poured water into the system (normally, treated wastewater).

Once the streams are identified and quantified, the salt concentration of each flow (S_i, kg NaCl /m^3) is also needed for the calculation of the FF (Eq. 7.3, in units of time). $\bar{S}$ is the average salt concentration in the system (kg/m^3), $\bar{V}$ the average volume of the studied compartment (m^3), and $\overline{F_t}$ the average mass flow of the calculated F_{IN} and F_{OUT} (kg salt/time) (Eq. 7.4), which represent the total mass of salt in the inlet and outlet flows, respectively. F_{IN} should equal F_{OUT} as no accumulation takes place and the model is then valid for steady state, but the FF is quantified using $\overline{F_t}$ (an average) to acknowledge possible discrepancies of experimental data. Additionally, $\overline{F_t}$ can be calculated yearly, monthly, or seasonally, defining the units of the factor itself.

$$FF = \frac{\bar{S} \cdot \bar{V}}{\overline{F_t}} \qquad \text{Eq. 7.3}$$

$$\overline{F_t} = \sum W_{i-IN} \cdot S_{i-IN} = \sum W_{i-OUT} \cdot S_{i-OUT} = F_{IN} = F_{OUT} \qquad \text{Eq. 7.4}$$

7.2.3. Effect Factor (EF)

The EF quantifies the fraction of living species that are going to potentially disappear in the aquatic ecosystem by the release of a certain chemical (Fantke et al., 2018; Owsianiak et al., 2019; Rosenbaum et al., 2018). The available literature regarding salinity variation effects in aquatic ecosystems models these impacts according to ecotoxic methodologies, such as USEtox (Amores et al., 2013; Zhou et al., 2013).

Under that approach, the EF is calculated employing a species-sensitivity distribution (SSD) curve which represents the sensitivity of an entire ecosystem to a substance (Rosenbaum et al., 2018). However, it is constructed based on the premise that increased exposures will lead to increased effects (Owsianiak et al., 2019). Indeed, if the salinity of a fresh aquatic environment increases, the organisms living in the system will be negatively affected (Böhme, 2011; Kefford et al., 2011; Piscart et al., 2011; Verbrugge et al., 2012; Ziemann and Schulz, 2011). However, negative effects in aquatic ecosystems are also reported when salinity decreases, such as massive mortalities reported after exposure to low salt concentrations due to climatologic events or anthropogenic actions (Du et al., 2021; Johnson et al., 2021; Parada et al., 2012).

Moreover, some limitations of SSD curves to model ecotoxic impacts due to salinity have been pointed out (Fox et al., 2021), and other indicators, such as species richness, have been proposed for studies assessing the effects of salinity (Kefford et al., 2011).

Therefore, variations in the concentration of essential substances can provoke effects in several directions, as an increase in the salt concentration can be potentially beneficial for an ecosystem and vice versa, meaning that emission-related impacts can be positive in some cases. Moreover, currently, it is considered that a substance provokes toxic effects if it enters in an organism and causes poisoning, endocrine disruption, or other lethal effects (Owsianiak et al., 2019). This approach might be partially accurate for effects of exposure to high salt concentrations, but it fails when defining the observed effects on ecosystems for decreases in salinity. This is the reason why the current applied approach (using ecotoxic methodologies) to model the impacts due to variations in essential substances (such as salts) need to be reconsidered.

7.3. EFFECT FACTOR FOR SALINITY VARIATIONS IN AQUATIC ENVIRONMENTS

The proposed approach is based on the premise that the species in an ecosystem have an optimal range of salinity for living, and that detrimental effects will be observed if it varies below or above it (Smyth and Elliot, 2016). Figure 7.2 shows an ideal representation of a transitional water body, where there is an optimal concentration of salt at which no impact occurs (i.e., PDF = 0). Please note that this figure represents a Then, negative effects will occur if salinity increases above the optimal range or decreases below it. Moreover, positive impacts will occur for increments in the salt concentration for environmental concentrations below the optimal, and vice versa. Note that, for salinity increases in the high range, the function follows a distribution like the classic SSD curve.

USC DE SANTIAGO DE COMPOSTELA

The first step is then to define the optimal salt concentration range by gathering data of chronic effects for the species of the ecosystem regarding salinity (also needed to quantify the EF itself). If the data are expressed as acute, an acute-to-chronic ratio (ACR), which is generally 2 (Fantke et al., 2018), can be used by dividing or multiplying the salt concentration by the ACR in the high range or in the low range, respectively. Then, according to our hypothesis (effects will happen above and below a favourable range),

that data representing the chronic effects linked to salinity will be shaped like in the examples provided in Figures 7.2a, 7.2b, or 7.2c.

For water systems from oligohaline to hyperhaline (see Appendix 5), it is expected that the concentration-to-response curves have a shape like the ones shown in Figure 7.2a or 7.2b, where the optimal (environmental) salt concentration can be estimated as a range (the points for which the function has values $y < 0$, Figure 7.2a) or a point (the minimum value of y, Figure 7.2b).

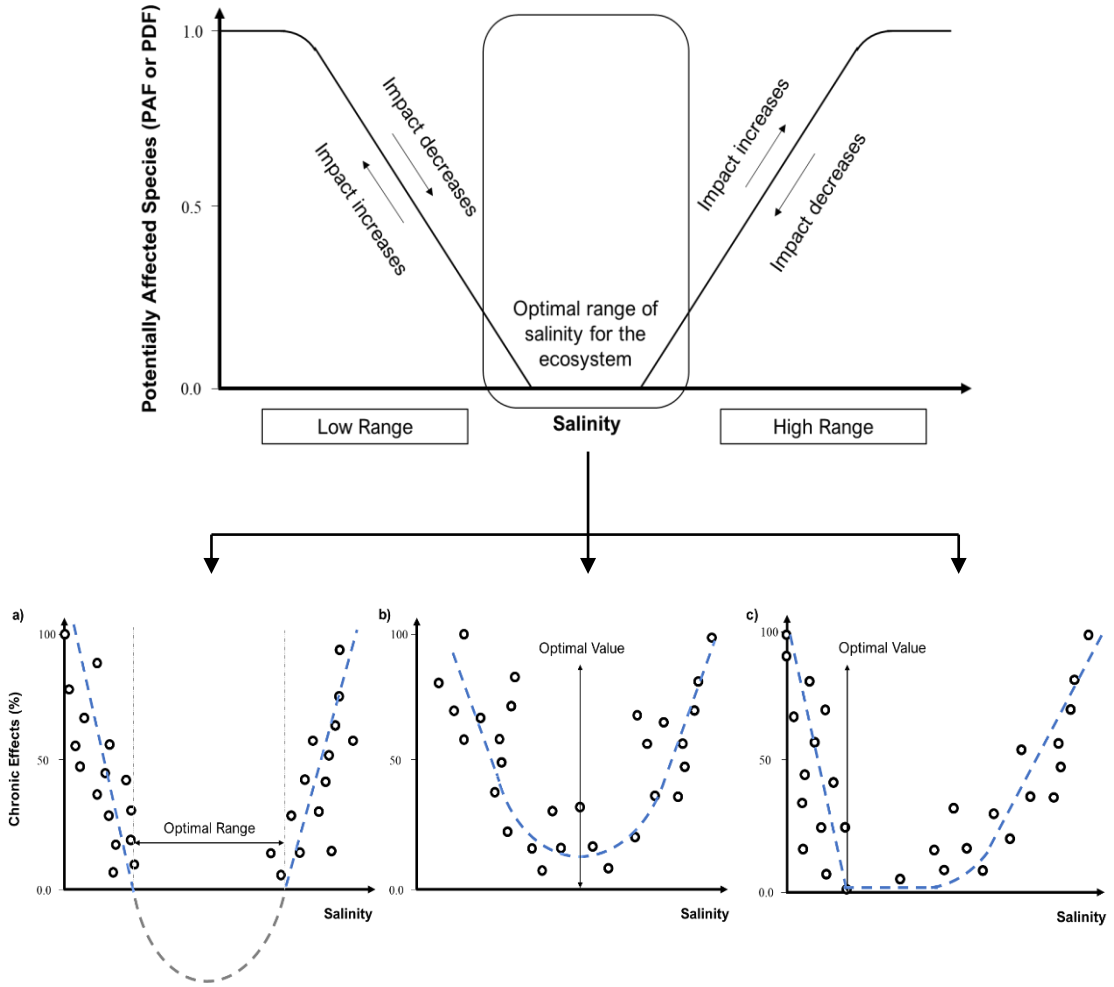


Figure 7.2. Expected (hypothetical) shape of the curve defining the optimal salinity range for aquatic ecosystems. The main plot is an ideal representation of the impacts of salt variations, and the graphs a, b, and c are theoretical representations of different types of ecosystems, where a and b shape the impacts of transitional or non-fresh compartments, and c is a freshwater system.

For freshwater environments, it is more likely that the impacts in the ecosystem are due to salt concentration increases. For the part of the curve representing salinization, a classic SSD approach might be a fair approximation. Nevertheless, the whole system profile is only provided if both ranges (high and low) are included, where the optimal (environmental) concentration can be found as the intersection of the two functions (a lineal one for the low range and SSD-like for the high range, Figure 7.2c).

After defining the optimal region, there are two EFs: EF_{LOW} and EF_{HIGH}, both in PDF·m^3/kg. They represent the amount of a substance that generates a certain effect on the ecosystem, where the EF is the slope of the concentration-response curve. $HC50_{LOW}$ and $HC50_{HIGH}$ (both in kg NaCl/m^3) represent the salt concentration that generates an

effect in 50% of the ecosystem species in the low and high range, respectively; thus, for effects in 50% of the species, EFs are expressed as in Eq. 7.5 and Eq. 7.6. HC50s are the geometric mean of the individual species EC50 (Fantke et al., 2018), which is the concentration of pollutant that generates an effect in the 50% of the individuals of a single species. In this sense, an SSD-like approach is still maintained.

$$EF_{LOW} = \frac{0.5}{HC50_{LOW}} \quad \text{Eq. 7.5}$$

$$EF_{HIGH} = \frac{0.5}{HC50_{HIGH}} \quad \text{Eq. 7.6}$$

Assuming a classic SSD-like curve (i.e., log-logistic functions), EC50s could be calculated using to mirrored sigmoidal curves. Moreover, according to our hypothesis (Figure 7.2), EC50s could be estimated by fitting the chronic effect data to a quadratic function (see Appendix 5). Note that the log-logistic and the quadratic curves only converge at intermediate ranges of effects, so this approximation might not be accurate to calculate EFs based on HC10 or HC20, as new trends on ecotoxicity modelling suggest the use of EC10s or EC20s to model the factors (Owsianiak et al., 2019).

Finally, the sign of each EF will be established depending on the direction of the change: increasing the salt concentration in the low range accounts for a negative EF_{LOW} (impacts decline), and decreasing it accounts for a positive EF_{LOW} (impacts increase), and vice versa for the high range.

7.4. APPLICATION OF THE METHODOLOGY TO A CASE STUDY

7.4.1 Fate Factor Calculation

For the case of *Arousa ría*, Figure 7.1 is simplified: W_G is neglected due to the insignificant contribution of the groundwater to the total flow of the *ría* (Ministerio de Agricultura Pesca y Alimentación, 2021), and $W_{I\text{-}OUT}$ is ignored since no infiltrations from the estuary to the river are expected (see Appendix 5). The remaining streams are quantified in cubic meters per month, covering from 2011 to 2018 according to the data availability, as detailed bellow. First, stream flows are determined:

$W_{I\text{-}IN}$ corresponds to the flows of rivers Ulla and Umia, which are monthly measured and reported by local administrations (Augas de Galicia, 2021) in several locations along the river courses, so the closest point to the *ría* was chosen.

W_{WW} can be divided into fresh wastewater (**$W_{WW\text{-}FRESH}$**) and salty one (**$W_{WW\text{-}SALT}$**). It was estimated that the area of *Arousa ría* is responsible for about 6.6% of the total (W_{WW}) Galician discharges (Ministerio de Agricultura Pesca y Alimentación, 2021; Ministerio para la transición ecológica y el reto demográfico, 2018) and that 82–92% of this amount (yearly variation in 2011-2018) corresponded to fish canning wastewater (Ministerio de Agricultura Pesca y Alimentación, 2021; Ministerio para la transición ecológica y el reto demográfico, 2018), the latter representing virtually all saline emitting sectors ($W_{WW\text{-}SALT}$, in cubic meters per year).

Then, to obtain monthly data, a distribution was defined based on the shellfish and seafood harvesting and processing seasonal pattern to estimate $W_{WW\text{-}SALT}$ in m^3/month (see Appendix 5). On the other hand, $W_{WW\text{-}FRESH}$ (including urban wastewater and fresh industrial streams, in cubic meters per year) was estimated as the subtraction of $W_{WW\text{-}}$

$_{SALT}$ from the total W_{WW}. Then, to obtain monthly data, it was assumed that fresh discharges are evenly distributed along the months of the year (also considering that freshwater streams represent a flow considerably lower than salty streams).

W_{ANTH} is not linked here to irrigation, as estuary water has high salinity and Galicia is an area with high precipitations, but the local fish canning industries use seawater for their processes with variable flows. Due to the lack of more precise data, we assumed that 75% of water input for fish canning processes was saline water and 25% was freshwater (Barros et al., 2009; Bello Bugallo et al., 2012; Dirección Xeral de Calidade e Avaliación Ambiental, 2008c; Tomczak-Wandzel et al., 2015) and that there are no water losses in the process, i.e., inputs = outputs, thus $W_{ANTH} = 0.75 \cdot W_{WW\text{-}SALT}$.

Evaporation rates were estimated using empirical correlations as indicated in Eq. 7.7 (Mcjannet et al., 2012; Risch et al., 2014), where W^*_{EPT} is stated in L/m^2·time (i.e., monthly averages in this case), u_2 is the wind speed (m/s), A is the evaporating area (m^2), v^*_w is the saturated vapor pressure at the water surface temperature (kPa), and v_a is the partial vapor pressure in the air at 2 m height (kPa) calculated by Eq. 7.8, where v^*_a is the saturated vapor pressure at air temperature (kPa) and ϕ is the relative humidity (%). Finally, to obtain W_{EPT} in equivalent units to the rest of the streams (m^3/month), Eq. 7.9 is needed.

$$W^*_{EPT} = (2.36 + 1.72 \cdot u_2) \cdot A^{-0.05} \cdot (v^*_w - v_a) \quad \text{Eq. 7.7}$$

$$v_a = v^*_a \cdot \phi \quad \text{Eq. 7.8}$$

$$W_{EPT} = \frac{W^*_{EPT} \cdot A}{1000} \quad \text{Eq. 7.9}$$

Average monthly precipitations (**W_P**) were obtained from the meteorological station located in Corón (Vilanova de Arousa) (MeteoGalicia, 2021).

W_R (runoff) was taken as the average values of the South West basins of Galicia (Fekete et al., 2002). Being the only data set available, the monthly distribution and expected runoff flow for 2002 were applied to the 2011-2018 period defined.

At this point, all the flows, except **$W_{O\text{-}IN}$** and **$W_{O\text{-}OUT}$**, which represent the tidal, are quantified, so mass balances must be solved. First, Eq. 7.10 and Eq. 7.11 present the general steady state water and the salt balances, respectively, where S_i is the salt concentration (kg NaCl/m^3) of each stream W_i.

$$W_{O-IN} + W_I + W_R + W_P + W_{WW} = W_{O-OUT} + W_{EPT} + W_{ANTH} \quad \text{Eq. 7.10}$$

$$W_{O-IN} \cdot S_{O-IN} + W_I \cdot S_I + W_R \cdot S_R + W_P \cdot S_P + W_{WW} \cdot S_{WW} = W_{O-OUT} \cdot S_{O-OUT} + W_{ANTH} \cdot S_{ANTH} \quad \text{Eq. 7.11}$$

Note that Eq. 6.10 could be written as $W_{IN} = W_{OUT}$, representing the total inflows and outflows in the estuary. Then, to accurately quantify the tidal streams, new mass balances will be stated, representing a system as natural as possible (Eq. 7.12 and Eq. 7.13), where no anthropogenic activity exists.

$$W_{O-IN} + W_I + W_R + W_P = W_{O-OUT} + W_{EPT} \quad \text{Eq. 7.12}$$

$$W_{O-IN} \cdot S_{O-IN} + W_I \cdot S_I + W_R \cdot S_R + W_P \cdot S_P = W_{O-OUT} \cdot S_{O-OUT} \quad \text{Eq. 7.13}$$

To be able to proceed, the salt concentration of each stream, $\mathbf{S_i}$, needs now to be quantified:

The salt concentration of the ocean ($\mathbf{S_{O\text{-}IN}}$) was considered constant at 36 g NaCl/L, as it is the average estimated sea surface salinity measured between 2004 and 2013 for this Atlantic area by satellite monitoring (Durack, 2015).

The salt concentration of the estuary ($\mathbf{S_{O\text{-}OUT}}$, $\mathbf{S_{ANTH}}$, but also $\bar{S}$ in Eq. 7.3) is variable according to the spatial distribution and the tidal intensity. Water is fresher near the river mouth and saltier near the ocean, but the salt concentration also increases with depth because salty water has a higher density (Venâncio et al., 2019). Data collected from two buoys (*Ribeira*, in an intermediate location between the river mouth and the ocean, and *Cortegada*, near the river mouth; see Appendix 5) (MeteoGalicia, 2021) were used to determine the monthly average salinity of the estuary.

Galician rivers are not salinized, presenting low salinity and conductivity (Estévez et al., 2019). Normally, chloride concentrations of Spanish rivers range between 0.010 and 0.030 kg Cl^-/m^3, and recent studies for Galician rivers near shore areas reported values of 0.0103 – 0.0222 kg Cl^-/m^3 (Dias et al., 2019). An average value of 0.020 kg $NaCl/m^3$ is considered for $\mathbf{S_I}$ and $\mathbf{S_R}$.

Chloride concentration in rainwater is generally very low, although it varies worldwide depending on wind intensity and seawater proximity. Measurements of the chloride concentration in rainwater in Spain and Portugal averaged 0.020 kg Cl^-/m^3, and the value reported for the Galician station, located at around 40 km from the coast, was 0.003 kg Cl^-/m^3 (Alcalá and Custodio, 2008). A slightly greater salinity of 0.005 kg $NaCl/m^3$ is applied for $\mathbf{S_P}$ to consider the effect of sea salt aerosols.

Eq. 7.14 and Eq. 7.15 are used to calculate $\mathbf{S_{WW}}$, where $W_{WW\text{-}SALT}$ is the yearly flow of industrial fish canning wastewater discharged into the estuary (m^3/year), and $\bar{S}_t$ is the average salt concentration of the estuary each year (kg/m^3). It was assumed that $S_{WW\text{-}FRESH}$ coming from drinkable sources was 0.5 kg $NaCl/m^3$ (WHO, 2006), and that $S_{WW\text{-}SALT}$ contained 75% of estuarine water and 25% of fresh (drinkable) water as previously indicated. The salinity of waste streams poured into the *ría* S_{WW} can be estimated as an average value as shown in Eq. 7.15.

$$S_{WW-SALT} = \frac{0.75 \cdot W_{WW-SALT} \cdot \bar{S}_t + 0.25 \cdot W_{WW-FRESH} \cdot 0.5}{W_{WW-SALT}} \quad \text{Eq. 7.14}$$

$$S_{WW} = \frac{W_{WW-SALT} \cdot S_{WW-SALT} + W_{WW-FRESH} \cdot S_{WW-FRESH}}{W_{WW-SALT} + W_{WW-FRESH}} \quad \text{Eq. 7.15}$$

Finally, the FF can be calculated. As already stated, both monthly and yearly results can be obtained, as well as seasonal values, the dry season spanning June, July and August in the case study area, and the remaining months are the wet season. To see detailed monthly, seasonal, and yearly results, see Appendix 5. The results for the FF are shown in Table 7.1.

Table 7.1. Results of the FF for *Arousa ría* (in units of month and year). The results are expressed as average ± standard deviation, and the confidence intervals are between brackets, where negative results were assumed zero.

Wet (month)	Dry (month)	Annual (year)
6.84 ± 1.84	4.02 ± 1.52	4.51 ± 1.51
[1.32, 12.36]	[0, 8.59]	[0, 9.05]

7.4.2. Effect Factor calculation

To calculate the EFs, data of chronic effects were gathered (for this study, only mortality was considered). Then, the collected data were represented together to find the ecosystem optimal (environmental) salt concentration (Figure 7.3).

Note that this value is not going to be employed in any other further calculation, and it is just used to determine which data will be utilized to generate EF_{LOW} and EF_{HIGH}. In this sense, several fittings could be tested, as shown in Figure 7.2a and 7.2b. Different approaches were applied, evaluated, and discussed (see Appendix 3) to identify this cut-off point. These strategies pointed out a possible environmental optimal range of 24–36 g NaCl/L, where the average point obtained after testing different fittings is 31.9 ± 1.4, which will be the cut-off to define the low and high ranges of salt concentration comprising EF_{LOW} and EF_{HIGH}, respectively.

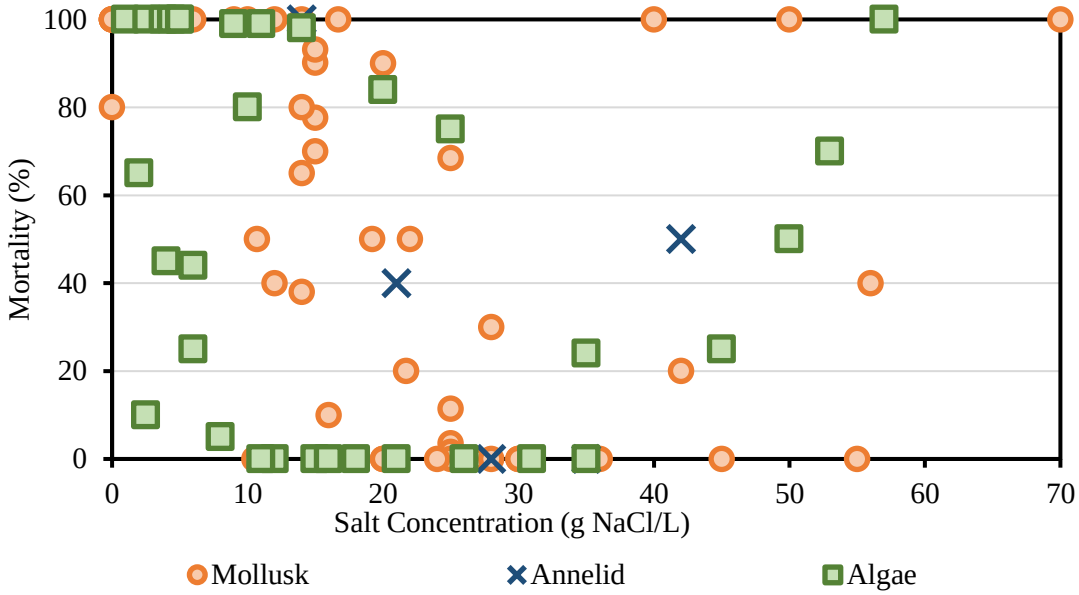


Figure 7.3. Distribution of the data regarding mortality of different species present in *Arousa ría*. The data used to generate this plot are specified in Table 7.1 and in Appendix 5.

Therefore, EC50 concentrations are calculated for each species at high and low range considering the estimated cut-off point. Most of the information gathered referred to effects at low range, so the $EC50_{LOW}$ for these species can be obtained directly using a sigmoidal curve with a profile mirrored to the classic SSD curve fittings. However, $EC50_{LOW}$ and $EC50_{HIGH}$ can be also estimated for each species by using the quadratic approach of our hypothesis, which provides effects at both ranges (see Appendix 5 for detailed information of how the quadratic approximation was used). The value of each EC50 (high and low) and both HC50s are shown in Table 7.2.

For Table 7.2, the numbers in italic indicate that the concentration was obtained by the quadratic fitting using data from the low range of salinity (for *V. senegalensis* and *S. polyschides*, the power function had a convex profile, so the data were estimated using the inverse function, see Appendix 5).

The results of EF_{HIGH} and EF_{LOW} (Table 7.3) fit with the expected system behaviour, as biotic diversity in estuaries starts to decline above a salinity of about 40 kg NaCl/m^3, with most species unable to survive in salinities above 50 kg NaCl/m^3 (Smyth and Elliot, 2016).

Table 7.2. Value of each EC50s and HC50s, and chronic data used to generate them. EC50s in italic represent the values estimated assuming quadratic distribution (see Appendix 5). The results are expressed as average ± standard deviation.

HC50s and EC50s (kg/m^3)		
Species	**$EC50_{LOW}$**	**$EC50_{HIGH}$**
Ruditapes philipinarium (Japanese clam) (Carregosa et al., 2014; Parada et al., 2012)	16.0	*34.4*
Ruditapes decussatus (Grooved carpet shell) (Gharbi et al., 2016; Parada et al., 2012)	15.5	*42.9*
Cerastoderma edule (Common cockle) (Parada et al., 2012; Verdelhos et al., 2015)	20.4	*61.7*
Vanerupis corrugata (Pullet carpet shell)(Carregosa et al., 2014; Parada et al., 2012)	21.7	*37.8*
Saccharina latissima (Sea belt, brown algae)(Fiett, n.d.; Peteiro and Sánchez, 2012)	8.6	*39.1*
Diopatra neapolitana (Polychaete) (Freitas et al., 2015)	19.0	42.3
Donax trunculus (Wedge clam) (Reyes-martínez et al., 2020)	19.3	*32.1*
Mytilus galloprovincialis (Mediterranean Mussel) (Gaag et al., 2016)	9.8	38.2
Scrobicularia plana (Peppery furrow shell) (Verdelhos et al., 2015)	10.7	*40.4*
Saccorhiza polyschides (Furbellow, brown algae) (Norton and South, 1969)	30.1	*40.1*
Zostera noltei (Dwarf eelgrass, seagrass) (Fernández-Torquemada and Sánchez-Lizaso, 2011; Salo et al., 2014)	1.3	49.6
HC50 (Geometric mean)	**12.7**	**41.1**
HC50 arithmetic mean	**15.7 ± 7.8**	**41.7 ± 8.0**

7.4.3. Characterization Factor calculation

CFs were obtained according to Eq. (7.2). Here, the EF achieves different values according to the level of salinity (high and low), while the FF, which is supposed to be the same for both ranges of salinity (the water streams will have the same physical distribution regardless of the salt concentration), varies seasonally (wet/dry season). Therefore, there are six possible CFs to use (Table 7.4).

Table 7.3. Value of each EF (PDF·m^3/kg). The results are expressed as average ± standard deviation, and the confidence intervals are between brackets, where negative results were assumed zero.

EF_{LOW}	**EF_{HIGH}**
0.04 ± 0.02 [0, 0.1]	0.01 ± 0.002 [0.005, 0.02]

For the obtained FFs, the standard deviation acknowledges the discrepancy of steady state assumption (see Appendix 5). For the EF, the confidence intervals and the standard deviation of the factor were calculated by using the arithmetic average (see Table 7.4). For all factors, the uncertainty intervals were obtained considering three times the standard deviation, and negative lower bounds were considered zero.

Table 7.4. Value of the CFs (PDF·month·m^3/kg). The results are expressed as average ± standard deviation, and the confidence intervals are between brackets, where negative results were assumed zero.

CF_{LOW} (PDF·month·m3/kg)		
Dry	Wet	Annual
0.27 ± 0.21 [0, 0.89]	0.16 ± 0.14 [0, 0.57]	0.18 ± 0.15 [0, 0.62]
CF_{HIGH} (PDF·month·m3/kg)		
Dry	Wet	Annual
0.08 ± 0.04 [0, 0.20]	0.05 ± 0.03 [0, 0.13]	0.05 ± 0.03 [0, 0.14]

CFs are reported as absolute values, but they depend on the direction of the change (i.e., increase or decrease in salt concentration at low or high range). Therefore, to quantify the effects of a dam release during the wet season which provokes a decrease in the salt concentration, CF = 0.16 PDF·month·m^3/kg (i.e. impact increases); but to quantify the impact of a saline effluent discharge in the same situation (wet season, low salinity), CF = -0.16 PDF·month·m^3/kg should be used as salinity is already below the optimal, so the increase in salt concentration has a positive effect in the receiving waters. In this example, CF_{LOW} is used instead of CF_{HIGH} because *Arousa ría* has a salt concentration below the optimal (Estévez et al., 2019).

7.5 DISCUSSION

When evaluating the effects of pollutants' release, it is logic to assume that a rise in the chemical concentration generates an increase in the impacts. However, anthropogenic activities are affecting the planetary biogeochemical cycles, and impacts are now not only due to the release of harmful substances, but also to variations in the environmental conditions of ecosystems provoked by unnatural changes. There are some substances (e.g., salt, nitrogen, dissolved oxygen) to which the classic ecotoxic impact approach might just fit partially. Indeed, a drastic increase in the substance concentration will generate adverse impacts. However, at the same time, a certain concentration in the media is needed to support the ecosystem survival.

In recent years, concern for salt variations in the different environmental compartments has increased, but the models available to describe their impacts are still scarce and the current literature uses ecotoxicity models to assess these effects (Amores et al., 2013; Berger et al., 2019; Núñez and Finkbeiner, 2020; Payen et al., 2016). For instance, the USEtox model, one of the most used methods to evaluate ecotoxic impacts in LCA, has not addressed effects of salt releases so far (Payen et al., 2016) and does not include the coastal seawater and brackish areas as environmental compartments (Owsianiak et al., 2019). Therefore, new approaches are necessary to include essential substances and these critical areas in the impact assessment models, and specific methodological choices must be implemented to develop CFs for essential elements in non-freshwater environments.

Regarding the FF, it is expressed in this work in units of time (see the fate factor section in materials and methods). This provides a suitable framework to model the fate of essential substances. The few research works that have assessed the effects of salinity variations in aquatic environments solved the FF question from different approaches. In a first study, when evaluating the impacts of brine disposal (Zhou et al., 2013), the different elements present in brine were grouped. NaCl had a high residence time, so the FF was chosen as the residence time of the second most persistent element of the salinity group (Cu^{2+}, 37 days). However, this approximation can have high errors due to the difference in the elements' residence times (from days to millions of years). In another study, when steady-state mass balances were applied to the water streams and the salt in a coastal wetland (Amores et al., 2013), the units of the FF were g·year/L, yielding in a dimension of time per concentration.

This factor was useful to directly link salt variations in the wetland not only with ecotoxic impacts, but also with social and economic effects as crop loss. However, the unit of that FF complicates the comparison of the results obtained there with the present study. Moreover, it hinders the evaluation of the impacts of salinity variations on the ecosystem linked to different impact categories as ecotoxicity. Therefore, to remain

coherent and consistent with other impact categories, it is necessary to provide factors that align with the standard units of the impact assessment stage and that are used by other methodologies recommended by the UNEP's Life Cycle Initiative, such as the USEtox. This harmonization aspect also applies to the measure of the EF, which is PDF in this study because UNEP's Life Cycle Initiative recommends to base ecosystem damage estimates on this metric (Owsianiak et al., 2019; Verones et al., 2017).

As for the EF, the proposed approach copes with the fact that impacts might be due to increases but also decreases in the concentration of essential substances, and it acknowledges potential benefits linked to emission-related impacts. In this sense, a classic SSD-based methodology (typically used in ecotoxicity) appears as a practical and useful tool to predict the possible negative effects of a pollutant's release on ecosystems. However, although some of the limitations linked to the use of SSD curves have been pointed out in the past few years, the principles shaping the methodology have remained unchanged for decades (Fox et al., 2021). Additionally, the use of classic ecotoxicity methodologies also have some limitations to model impacts due to variations in the concentration of essential substances, since the effects of these variations do not fit the classic definition of toxicity (linked to poisoning, endocrine disruption, etc.).

For the present study, a quadratic function was used to shape the effects of salinity on ecosystems instead of the typical log-normal or log-logistic distribution applied in the SSD-based methods, by applying this approximation for the calculation of EC50s (and, therefore, the EFs).

Nevertheless, log-logistic distributions were also applied to the chronic data gathered for each individual species to test the robustness of the approximation. The differences between the EC50s found applying log-logistic and quadratic fittings averaged 3.82% (data not shown), meaning that the quadratic approach is accurate to estimate effects at intermediate ranges of concentration in an LCA context (but might not be accurate for EC10s or EC20s). This approximation was used to estimate the EC50s and HC50s at both concentration ranges (low and high), but the SSD-based methodology fundamentals are maintained. Thus, the current study does not aim to question the SSD curves themselves, but to expand their scope to understand new ecological features.

In fact, this quadratic approximation can be especially useful for systems from oligohaline to hyperhaline (see Appendix 5), but it might not be as accurate for freshwater systems. Here, the expected effects are linked to salinization, while impacts linked to a sharp freshening are unlikely. In fact, the most important impacts derived from anthropogenic activities and climate change in freshwater ecosystems are expected to be linked to salinization (Mosquera-Corral and Campos Gómez, 2021), where the SSD-based methodology can be a fair approximation to determine the effects of salinity increase if the effects of freshening are neglected.

The truth is that the few studies considering the effects of salt variations in aquatic environments considered SSD approaches. In a wetland-related study (Amores et al., 2013), an SSD curve was constructed considering that anthropogenic activities (irrigation) were provoking salinization, which seems to be a fair approximation considering that the salt concentration in the water body had increased from 2.6 g/L in 1983 to 7.50 g/L in 2008. However, the salinity conditions of the wetland were oligohaline, so impacts related to freshening could also take place (due to rainfalls, for example). Therefore, the approximation was fair to evaluate salinization, but failed at evaluating the effects due to potential freshening.

In a brine disposal research work (Zhou et al., 2013), a concentration of 40 kg NaCl/m^3 was chosen as EC50. Analogously to the wetland case, this approximation might be fair considering that desalination plants would discharge their briny effluents in marine waters, and the effects of this saline disposal are expected to be negative impacts linked to salinity increase. However, the modelling of the system is still not fully comprehensive if the low ranges of salinity are no included. In fact, a case where a transitional brackish ecosystem is subjected to freshening and where brine disposal is potentially beneficial could take place under this point of view (impacts would decrease due to brine disposal), so an approach where this is not considered is lacking some relevant information for the system.

Finally, these divergent approaches hinder a discussion of the obtained CF. The wetland and brine CFs, in terms of potentially affected fraction of species (PAF), were 0.32 PAF·yr and 0.47 PAF·m^3·day/kg, respectively, which are similar to the ranges obtained here (0.05 – 0.27 PDF·m^3·day/kg). However, for the wetland, the units are not comparable, and, for both cases, the obtained CFs were at the midpoint level (measuring PAF, not PDF), so the discussion is not straightforward.

Nevertheless, it is important to point out how the quantification of CFs to measure the effects of salinity variations can be relevant for the management of anthropogenic activities in sensitive ecological areas, such as transitional waters. In fact, the method described and applied here has the potential to support decision-making processes around effluent discharge, industrial stream management, brine disposal control, and dam flow regulation, by providing useful information about when and how to discharge these anthropogenic streams with s minimum or even a positive impact.

To apply the CFs developed here, the life cycle inventory (LCI) shall record the mass of salts released to the aquatic environment per functional unit, acknowledging possible differences between the wet and the dry season when relevant and if information is available.

7.6. FUTURE OUTLOOK

The presented novel approach proposes a model to shape the effects of variations in the concentration of an essential substance in the environment (Figure 7.4). Here, the effects in the ecosystem might not be always directly proportional to the pollutant concentration. Although the methodology was applied to salt (i.e., NaCl), the same principles can be implemented for other essential substances such as macronutrients, other salts, metals, and even resources such as water.

Moreover, as the definition of salinity is broader than just sodium chloride, a comprehensive salinity assessment may shape the effects of varying the concentration of other substances. Furthermore, by including elements such as carbonate, nitrate and sulphate, a comprehensive study of the ecosystem salinity might extend the effects of the observed variations to other environmental categories (such as climate change, eutrophication, or acidification, respectively). In any case, the uniformization of the CFs for salinity impacts (expressed in the consensus units) opens a new pathway where the effects due to variations in the concentration of essential substances can be fully assessed for the first time.

This pathway has a clear bottleneck, which might hinder its extensive application, and which is not new to the LCA community, i.e., regarding data availability. Fortunately, the access to information is becoming easier as science is becoming more accessible and

data is being acquired through more sophisticated means, such as dedicated satellites, which can be more easily managed through advanced computational methods. In this sense, although the provided CF only has local applications, the methodological approach is transferable to any other region. Although some data might be difficult to find, a preliminary guidance for data acquirement in the CF development and application is provided in Appendix 3.

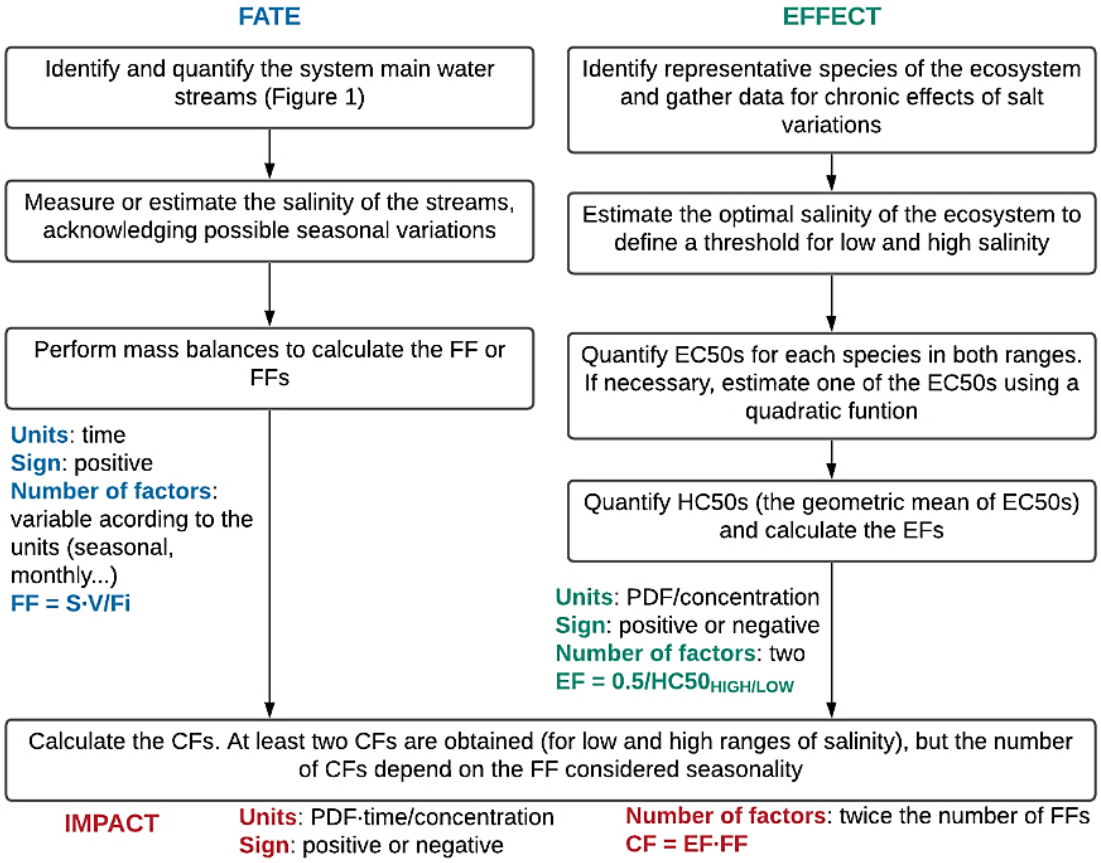


Figure 7.4 Overview of the steps that must be followed to apply the methodology to assess the effects of salinity variations in aquatic environments.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

CHAPTER 8: CONCLUSIONS

8.1 INTEGRAL DISCUSSION OF THE MAIN OUTCOMES

This final chapter provides the general conclusions of this document, where the aim was to provide a robust framework to increase the technology readiness level for circular economy emerging processes based on resource recovery as summed up in Figure 1.3, with special emphasis to the ones employing saline effluents and/or streams coming from the fish canning industry.

Therefore, the layout presented in Figure 1.3 was upgraded in Figure 8.1 to outline the main results of this document in relationship to the proposed methodology to increase technology readiness level.

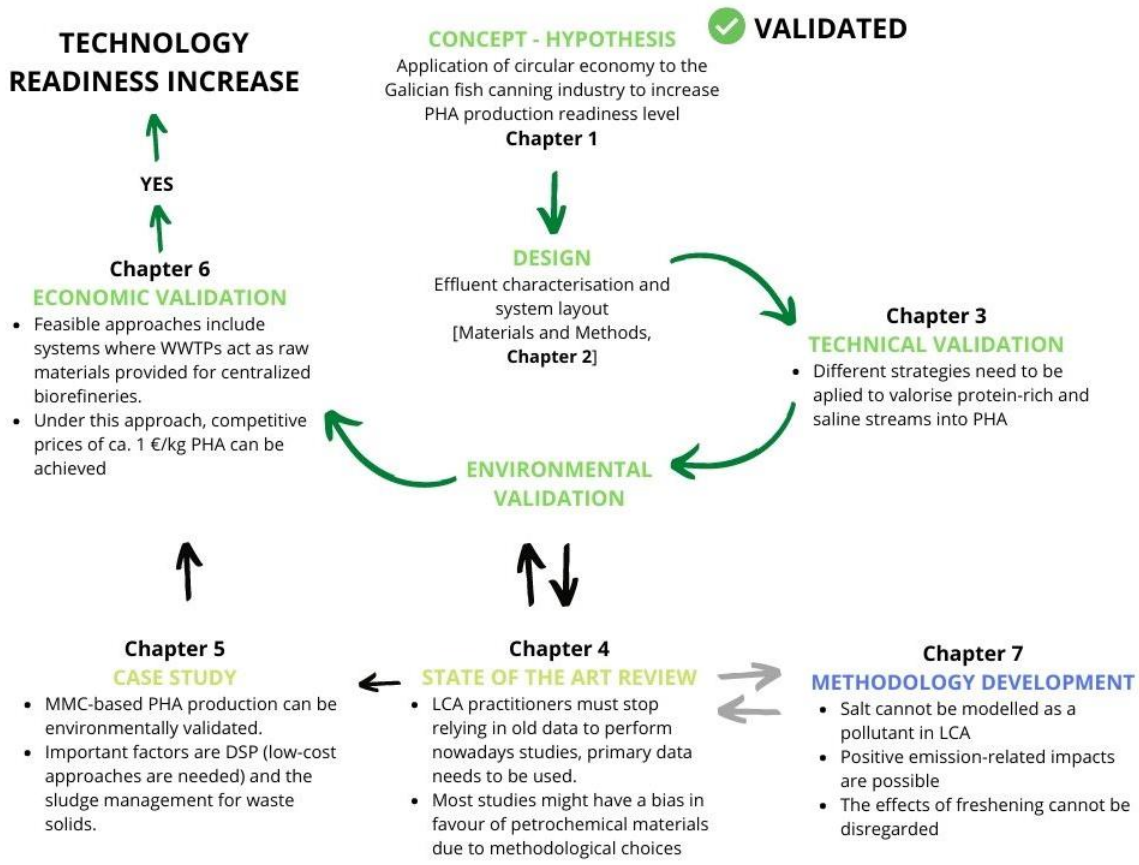


Figure 8.1. Summary of the thesis conclusions, based on Figure 1.3.

Regarding the technical validation, it was found that it is feasible to transform saline complex effluents coming from the fish canning industry into PHA and value-added biomaterials. To achieve this feasibility, it might be necessary to apply strategies specially oriented to solve the issues linked to the effluent features. In the present thesis, the strategies tested aimed to improve the valorisation possibilities of effluents with a high concentration of proteins.

The key factors to this improvement were: i) alkalinity/pH management in the acidification unit, ii) gradual acclimation of the VFA-producing biomass to the high protein content, and iii) application of a settling stage after the feast phase in the MMC enrichment reactor to ensure that proteins are consumed during the starvation period and

the released nitrogen can be used for growth. In this sense, salinity was not a barrier to achieve PHA valorisation.

Regarding the environmental analysis, the validation of biomaterials like PHA was hindered by the lack of general frameworks to perform this verification. Since recently, methodologies are stablished, but there is still a lack of studies using them, so robust analysis is still not possible.

Most studies regarding LCA of PHA provided conflicting results surely because all of them suffered from a bias in favour of conventional (petrochemical) materials. On the one hand, there is little information about the end-of-life stage of PHA, thus most of the benefits of this biomaterial are being disregarded. On the other hand, the impacts of plastic pollution are still uncertain, and their effects are not being accounted on most LCA studies.

Moreover, these LCA studies of PHA production are not using primary data (original information), but secondary one (from literature). After tracking these references, most of them lead to the same works, performed one or two decades ago. LCA practitioners need to stop relying on data generated twenty years ago to provide nowadays results, and different branches of knowledge need to be combined to generate new outputs.

As more studies using primary data are needed, the data obtained in Chapter 3 was used to perform an environmental assessment of PHA production. In this case, the outputs indicated that, when using effluents from fish canneries, centralized models are more favourable than decentralized ones, mostly considering the particularities of the market in a region like Galicia.

The DSP can still be a potential drawback for the environmental validation of PHA production systems, so low impact and low-cost approaches should be explored. The management of the waste sludge generated in the process is another key factor to ensure that the circular economy-based process (PHA production) has lower impacts than the conventional ones (treatment only), and it is recommended a treatment by anaerobic digestion, where this waste sludge can also potentially be employed to generate new Volatile Fatty Acids to be used in PHA production.

To complete the validation proposed in the methodology here suggested, an economic analysis was performed. Here, the economic evaluation of PHA and TAG production showed that, for small scales, DSP on-site was not technically feasible.

For bigger scales, the process is economically attractive if one factory centralizes a big production. However, in Galicia, a lot of small factories oversee a big production. Here, the best solution is to turn WWTPs into PHA-rich biomass providers, where a centralized biorefinery would carry out the DSP.

PHA production achieves competitive prices, around 1 €/kg PHA (so like petrochemical materials), under the centralized biorefinery approach if low-cost DSP approaches are chosen. The production of TAG is still not feasible because lower concentrations of biomass and the necessity of working with low mass loads result into higher prices.

The operational approach chosen to produce the PHA-rich biomass (thus, to treat the effluent) has an important influence on both the economic and the environmental validation of the process, where low residence times in the reactors and mild chemicals

usage are keys to achieve lower costs and environmental impacts than the conventional processes.

Finally, attention was paid to the particular requirements in LCA studies when assessing processes using saline streams as, to the best of our knowledge, there were no methodologies available to evaluate the effects of salinity variations in aquatic environments in LCA. This gap on the impact assessment methods prevent the LCA to get the complete picture of the environmental impact when evaluating processes using (saline) fish canning effluents.

As salinity is not a pollutant, its effects cannot be measured using classic ecotoxic methods (the only ones available until now in LCA). Salts are essential substances that need to be present in a certain concentration in the media, so biota can survive (like nitrogen, or some metals, for example).

A new methodology was proposed to evaluate the effects of salinity variations in aquatic environments, considering that salts are essential substances. Therefore, the effects of an emission-related impact can be positive if the water body is subjected to freshening, while a decrease in the concentration of salt can have negative impacts for water environments that suffer salinisation.

To model these features, the classic SSD curves were re-thought and extended to provide information about freshening. Therefore, the methodology traditionally used to calculate Effect Factors in LCIA was reviewed and upgraded to include essential substances.

8.2 TAKE-HOME MESSAGES

This thesis proved the feasibility of implementing circular economy-based approaches and processes in the Galician fish canning industry and provided a framework to increase the readiness level of emerging technologies. Furthermore, the results obtained led to the generation of a new methodology to assess the impacts of salinity variations in aquatic environments, opening a new field of discussion in the LCA community. Moreover, it proved the usefulness of life thinking strategies to holistically validate the feasibility of developing processes based on circular economy, like the resource recovery ones.

Nowadays, the feasibility of producing PHA using different substrates (like fish canning effluents) is already proved. Therefore, what is needed now is the generation of new holistic studies considering different vectors, like economic, environmental, societal, or policy-related ones, with the goal of increasing the technology readiness and introducing these materials in the market.

In other words, now that technical feasibility of PHA production has already been proved, the scientific community should orient their efforts to provide a robust socio-economic and environmental framework that could help policy makers to regulate the introduction of biomaterials in the value chains.

USC
DE SANTIAGO
DE COMPOSTELA

Therefore, in this thesis, a feasible model for circular economy implementation and biomaterials production was proposed and valuated. This model consists of a scheme were industrial WWTP are transformed into raw materials providers for a centralized biorefinery where downstream operations would be carried out to extract the PHA (and, potentially, TAG) from the biomass (Figure 8.2).

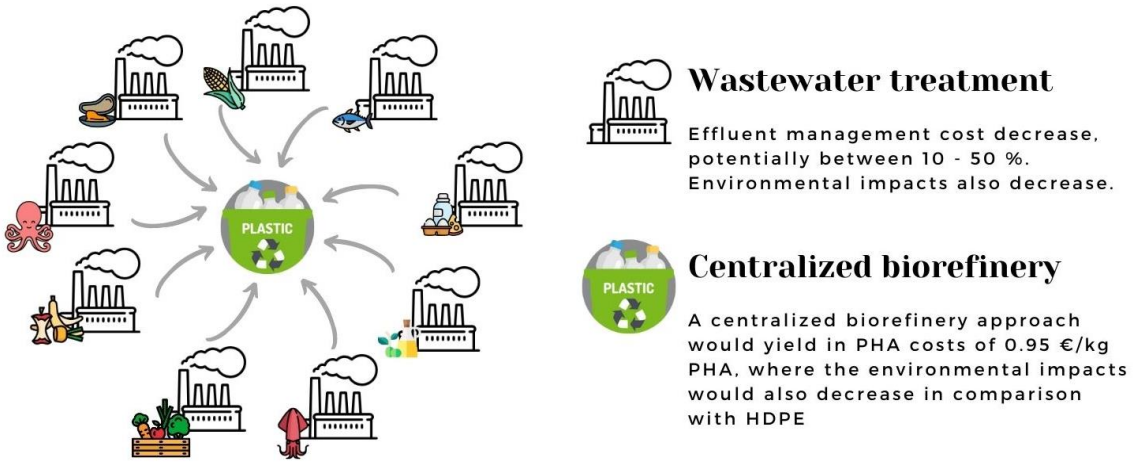


Figure 8.2. Scheme where different WWTPs of food processing industry would provide PHA-rich biomass to be extracted in a centralized biorefinery.

The current prices of petrochemical plastics were usually around 1 €/kg, so this was traditionally considered as the threshold price to achieve for bioplastics to be economically feasible. Nevertheless, events like the sudden rise in the petrol cost due to the war in Ukraine might be raising that threshold. Nevertheless, in the work carried out in this thesis process lower than 1 €/kg were achieved when a system that included several WWTPs working in a circular economy cluster was considered.

Here, on the one hand, the cost and the environmental impacts of treating wastewater under the innovative circular economy-based approach showed potential impacts. On the other hand, extracting PHA using low-cost DSP approaches yielded in competitive market prices of 0.95 €/kg if 50 factories participated of this cluster, where the environmental benefits of replacing petrochemical materials for PHA is also clear.

Therefore, the required implementation of circular economy strategies to reduce environmental impacts and face the current challenges have to be linked to the definition of policies that facilitate the generation of clusters where different companies can join, as this thesis proved that circular economy can be a reality if strategies like clustering and centralization are applied.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

APPENDIX 1: SUPPLEMENTARY INFORMATION FOR CHAPTER 3

S1.1. Description of the system

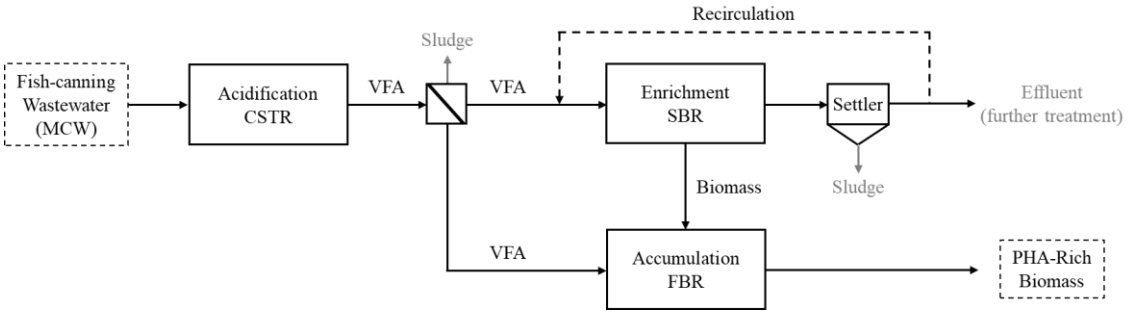


Figure S1.1. Flowchart of the system employed for the PHA

S1.2. Characterization of the Mussels Cooking Wastewater (MCW)

Table S1.1. Characterization of the raw MCW: ranges of variability in the different parameters measured.

Parameter	Units	Value
pH	---	6.1 – 8.1
Conductivity	mS/cm	33.2 – 44.4
Alkalinity	mg $CaCO_3$/L	170.0 – 275.0
VFA	mg COD_{VFA}/L	0.0 – 115.5
Carbohydrates	g COD_{CARB}/L	6.9 – 20.9
Proteins	g COD_{PROT}/L	1.8 – 5.7
Total Organic Carbon	g TOC/L	4.0 – 11.1
Inorganic Carbon	mg IC/L	20.6 – 91.4
Total COD	g COD_T/L	9.8 – 26.5
Soluble COD	g COD/L	9.0 – 23.2
Ammonium	mg NH_4^+-N/L	51.9 – 223.7
Total Nitrogen	g TN/L	0.85 – 1.2
Sodium Chloride	g NaCl/L	17.9 – 19.0
Sulfate	g SO_4^{2-}/L	1.9 – 3.4
Total Suspended Solids	g TSS/L	1.9 – 5.7
Volatile Suspended Solids	g VSS/L	1.1 – 3.2

COD: Chemical Oxygen Demand, VFA: Volatile Fatty Acids.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

S1.3. Description of the enrichment cycles

Table S1.2. Phases of the SBR cycles in the different operational strategies.

Cycle type	Distribution of the phases throughout the cycle Duration (min)					
1 (ADF)	Feeding	Reaction				Discharge
	15	690				15
2 (M-ADF)	Feeding	Reaction 1	Settling	Supernatant Discharge	Reaction 2	Discharge
	15	135	10	5	540	15

S1.4. Nitrogen concentration profile in the different streams of the enrichment reactor.

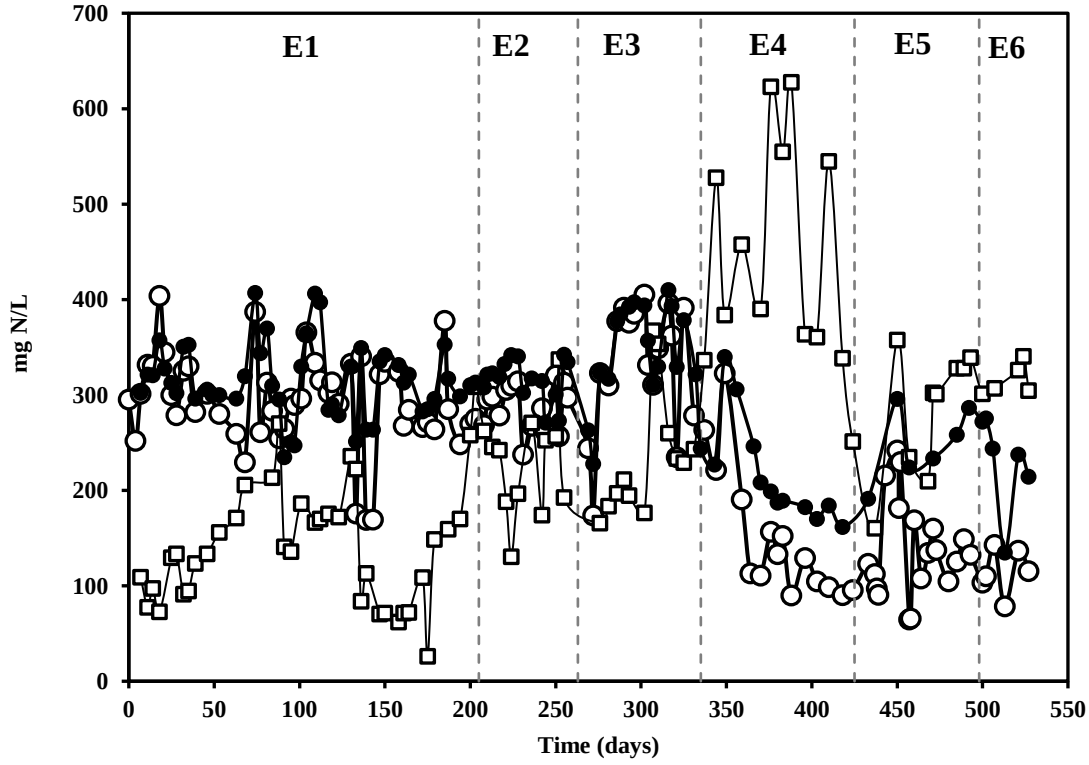


Figure S1.2. Ammonia nitrogen concentration at the beginning of the SBR cycle[1] (NH_4^+-N_0, ●) and in the effluent (NH_4^+-N_{EFF}, ○), and total nitrogen consumed in SBR cycles[2] (REM_{TN}, □), in mg N/L.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

[1] Calculated according to eq. (2) of Chapter 3, but substituting COD_i by NH_4^+-N.
[2] Calculated using the mass balance exposed in the numerator of eq. (4) in Chapter 3, but substituting COD_{PROT} by TN (the result of the mass balance is not divided by the initial TN concentration, so the units are mg/L instead of a percentage).

APPENDIX 2: SUPPLEMENTARY INFORMATION FOR CHAPTER 4

S2.1 References included in the critical review but not explicitly discussed in the text (Further Reading)

Cristóbal, J., Matos, C. T., Aurambout, J. P., Manfredi, S., & Kavalov, B. (2016). Environmental sustainability assessment of bioeconomy value chains. Biomass and Bioenergy, 89, 159–171. https://doi.org/10.1016/j.biombioe.2016.02.002

Gerngross, T. U. (1999). Can biotechnology move us toward a sustainable society? Nature Biotechnology, 17(6), 541–544. https://doi.org/10.1038/9843

Halley, P. J., & Dorgan, J. R. (2011). Next-generation biopolymers: Advanced functionality and improved sustainability. MRS Bulletin, 36(9), 687–691. https://doi.org/10.1557/mrs.2011.180

Hottle, T. A., Bilec, M. M., & Landis, A. E. (2013). Sustainability assessments of bio-based polymers. Polymer Degradation and Stability, 98(9), 1898–1907. https://doi.org/10.1016/j.polymdegradstab.2013.06.016

Koller, M., Gasser, I., Schmid, F., & Berg, G. (2011). Linking ecology with economy: Insights into polyhydroxyalkanoate-producing microorganisms. Engineering in Life Sciences, 11(3), 222–237. https://doi.org/10.1002/elsc.201000190

Patel, M., Crank, M., Dornburg, V., Hermann, B., Roes, Husing, B., Overbeek, L., Terragni, F., & Recchia, E. (2006). Medium and Long-term Opportunities and Risks of the Biotechnological Production of Bulk Chemicals from Renewable Resources The BREW Project.

Ramesh, P., & Vinodh, S. (2020). State of art review on Life Cycle Assessment of polymers. International Journal of Sustainable Engineering, 13(6), 411–422. https://doi.org/10.1080/19397038.2020.1802623

Rostkowski, K. H., Criddle, C. S., & Lepech, M. D. (2012). Cradle-to-gate life cycle assessment for a cradle-to-cradle cycle: Biogas-to-bioplastic (and back). Environmental Science and Technology, 46(18), 9822–9829. https://doi.org/10.1021/es204541w

Urtuvia, V., Villegas, P., González, M., & Seeger, M. (2014). Bacterial production of the biodegradable plastics polyhydroxyalkanoates. International Journal of Biological Macromolecules, 70, 208–213. https://doi.org/10.1016/j.ijbiomac.2014.06.001

Wampfler, B., Ramsauer, T., Rezzonico, S., Hischier, R., Köhling, R., Thöny-Meyer, L., & Zinn, M. (2010). Isolation and purification of medium chain length poly(3-hydroxyalkanoates) (mcl-PHA) for medical applications using nonchlorinated solvents. Biomacromolecules, 11(10), 2716–2723. https://doi.org/10.1021/bm1007663

Yu, J., & Chen, L. X. L. (2008). The greenhouse gas emissions and fossil energy requirement of bioplastics from cradle to gate of a biomass refinery. Environmental Science and Technology, 42(18), 6961–6966. https://doi.org/10.1021/es7032235

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

APPENDIX 3: SUPPLEMENTARY INFORMATION FOR CHAPTER 5

S3.1. Description of the three-stage system

A three-stage system (Figure S3.1) was operated for 2 years to produce PHA from Mussels Cooking Wastewater using mixed microbial cultures. The acidification reactor is a 5 L Continuously Stirred Tank Reactor described in Fra-Vázquez et al. (2020). It was fed with 0.8 L of MCW/day to achieve a Solids Retention Time equal to the Hydraulic Retention Time of 6.25 days. After solids separation through a centrifuge, the effluent of the first reactor, rich in Volatile Fatty Acids, was fed to the enrichment and the accumulation reactors. The former is a Sequencing Batch Reactor with a working volume of 2L where the feast-famine strategy was carried out to enrich the MMC where a modified dynamic feeding regime was imposed with a settling stage as described in Argiz et al. (2020). The latter is a Fed-Batch Reactor of 1 L where a pulse-wise strategy was applied to maximize the accumulated PHA in the biomass.

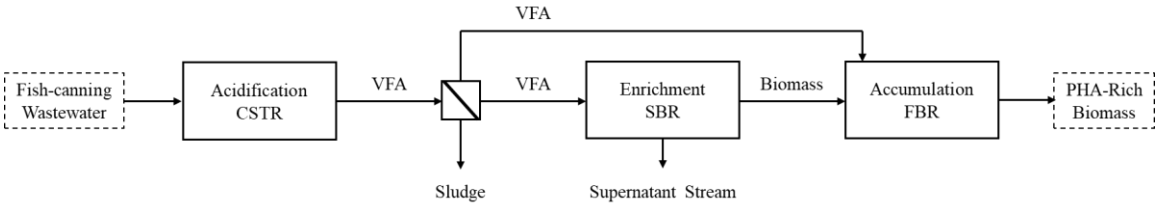


Figure S3.1. Flowchart of the three-stage system employed for PHA production

S.3.2. Process units design information

S.3.2.1. Kinetic parameters of aerobic biological systems

For both process approaches, a temperature of 20 ºC was considered, and typical kinetic parameters were used for bioreactor (Metcalf & Eddy, 2014) (Table S3.1):

Table S3.*1*. Kinetic parameters employed for the design of the treatment units of both scenarios.

	Heterotrophic Biomass	Autotrophic Biomass
μ (g VSS/(g VSS·d))	6	0.9
K_s, K_{NH4} (mg COD/L and mg N/L)	8	0.5
Y (g VSS/g substrate oxidized)	0.45	0.15
b (g VSS/(g VSS·d))	0.12	0.17
f_d (unitless)	0.15	0.15
K_{O2} (mg O_2/L)	0.2	0.5

S3.3. Main information about process units

S3.3.1. Linear Economy approach

In each of the three facilities, there is a homogenization unit of 50 m^3. The DAF unit has a volume of 2.46 m^3 and an air flow of 4.25 m^3/day at high pressure. The first pond has a volume of 102 m^3 and operates at a HRT of 87 hours with a concentration of biomass of 2.79 g VSS/L. The second pond has a volume of 7 m^3, and operates at a HRT of 6 hours and biomass concentration of 2.13 g VSS/L to remove the remaining COD and

nitrogen. The total secondary sludge production is 7.73 m^3/day at 7.5 g VSS/L, so the gravity thickener is fed with a total flow of 9.53 m^3/day (from secondary reactors and DAF). In this unit, with a volume of 15 m^3, it is generated a thickened stream of 3.20 m^3/day, with a solid concentration of 45 g TSS/L, which will be composted in a local facility. Aeration is supplied with mechanical surface aerators, and their power needs are estimated according to their small size in 7.5 kW per pond (Metcalf & Eddy, 2014). Total electricity consumption is 52.84 MJ/ m^3 of MCW, and it is detailed in Figure S3.2.

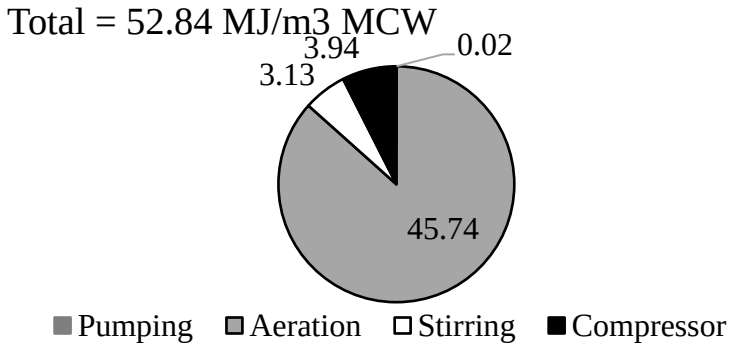


Figure S3.2. Electricity consumption of process units in the linear economy scenario (in MJ/m^3 of MCW).

S3.3.2. Circular Economy approach

The centralized facility is the one in the middle of the three considered plants, so the other ones are located at a distance of 6.6 km and 2.3 km from the centralized facility. The homogenization unit has a volume of 100 m^3 and it feeds the anaerobic digester, with a volume of 531 m^3. The effluent is centrifuged to remove the acidogenic biomass from the VFA-rich stream. Then, the clarified effluent is diverted: 31.8 m^3/d are fed to the SBR enrichment reactor, of 17 m^3, and 52.2 m^3/d are fed to a tank at higher pressure (1.5 atm approximately), to prevent VFA volatilization while this VFA-rich stream is fed by pulses to the FBR accumulation reactor (30 m^3) in the 12 hours accumulation cycle. The SBR works in cycles, and ¼ of the reactor volume (the supernatant stream after SBR settling) is removed and sent to the CAS after approximately 4 hours of the cycle (total duration of 12 hours).

Table S3.2. Compositions of every supernatant stream sent to CAS for further treatment.

	Supernatant Stream			CAS Influent
Parameter	SBR	Settler	FBR	
Q (m^3/d)	15.9	11.9	47.7	75.6
COD (g/L)	2.3	0.7	2.6	2.2
N_T (mg/L)	262.4	178.9	723.3	540.3
NH_4^+ (mg N/L)	144.2	72.8	230.6	187.5
NO_2^- (mg N/L)	0.00	42.2	0.00	6.7
TSS (g/L)	5.6	1.3	0.25	1.5
VSS (g/L)	2.3	0.56	0.17	0.68

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Then, it works with ¾ of the initial volume during the remaining 8 hours. Here, some ammonia is nitritated during the famine phase and denitritated during the feast one (as a consequence of the low oxygen concentrations in this cycle stage) as indicated in Fra-Vázquez et al. (2019). Its effluent is pumped to a settler of 10 m^3, where the supernatant (11.93 m^3/d) is removed and sent to the CAS, while the concentrated biomass (3.98 m^3/d) is pumped to the FBR as inoculum. After the accumulation cycle,

the biomass is decanted, and the supernatant (47.73 m^3/d) is sent to CAS while the high solids stream (PHA rich at 41.5 % PHA and 18.40 g VSS/day, 8.42 m^3/d) is sent to the DSP units (modelled as indicated in Fernández-Dacosta et al. (2015). In the treatment line, the three waste streams (supernatant from the SBR, from the decanter between SBR and FBR, and from the FBR) are treated together in a CAS, with compositions and flows according to Table S3.2.

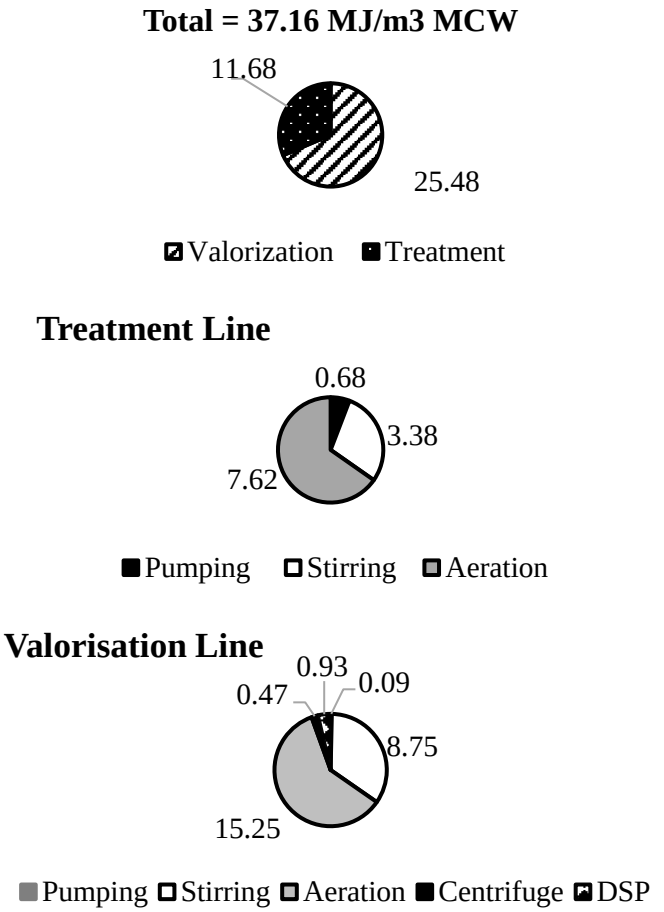


Figure S3.3. Electricity consumption of process units in the circular economy scenario (in MJ/m^3 of MCW). Note that only electricity is included in this table (steam and cooling energy are excluded from the DSP).

In the treatment line, the operational regime goes from sequential to continuous mode, so there is a settler of 6 m^3 acting as homogenization and buffer unit and in which about half of the solids and 30 % of the COD is removed. Then, a step-fed nitrification/denitrification system with four anoxic/aerobic sections is employed to remove nitrogen and organic matter. The total anoxic volume of the system is 58.83 m^3, while the aerobic one is 70.20 m^3. The SRT of the system is of 15 days, and biomass concentrations in the ponds are between 2 and 4 g VSS/L, so the generation of waste sludge is of 4 m^3/day at 6.8 g VSS/L, that is fed to the thickener. In the thickener, it reaches a concentration of 45 g TSS/L so an approximate flow of sludge of 1 m^3/day is generated to be treated in the digester. Aeration power needs are estimated as in the linear economy scenario.

The three high-solid concentrated streams are fed to the anaerobic digester with a total flow of almost 3 m^3/day and a load of 2.8 kg VSS/(m^3·d) and at a HRT of 25 days,

so the volume is about 75 m^3 and it produces 133.9 kWh/day of heat and 104.2 kWh/day of electricity. Avoided electricity is 35.5 kWh/m^3 of sludge, and consumption (basically stirring) is 3.9 kWh/m^3 sludge. The total electricity consumption is 37.16 MJ/ m^3 of MCW, as detailed in Figure S3.3.

S3.3.3. Power equations

The power needs for stirrers, pumps and blowers were calculated according to standard theoretical power equations:

Stirrers

$$P = N_P \cdot \rho \cdot N^3 \cdot D^5 \quad \text{Eq. S3.1}$$

Where:
P: Power (W)
N_P: power number (dimensionless). It is dependent on the Reynolds number (Re, dimensionless, defined below) and was estimated from diagrams available in literature (Coulson and Richardson, 1999).
ρ: density of the fluid (kg/m^3)
N: stirrer speed (s^{-1})
D: stirrer diameter (m), considered as $D_T/4$, where D_T is tank diameter (m)

Pumps

$$P = \frac{W \cdot m}{\eta} \quad \text{Eq. S3.2}$$

Where:
P: Power (W)
m: mass flow of the fluid (kg/s)
η: pump efficiency, generally 0.8, and:

$$W = g \cdot \Delta z + \frac{\Delta P}{\rho} + \frac{\Delta P_f}{\rho} \quad \text{Eq. S3.3}$$

Being:
g = 9.8 m/s^2
Δz: height difference (m)
ΔP: pressure difference (N/m^2)
ΔP_f: pressure drop due to friction (N/m^2):

$$\Delta P_f = 2 \cdot L \cdot f_F \frac{\rho \cdot v^2}{D_i} \quad \text{Eq. S3.4}$$

Where:
L is the tube equivalent length (m)
v is the fluid velocity (m/s)
D_i is the inner diameter of the tube (m)
f_F is the Fanning factor (dimensionless). It is dependent of Re number and it was estimated from diagrams in literature (Coulson and Richardson, 1999).

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Compressor:

$$P = \frac{W_S \cdot m}{\eta} \quad \text{Eq. S3.5}$$

Where:

$$W_S = \frac{\gamma \cdot Z \cdot R \cdot T_1}{(\gamma - 1) \cdot M} \cdot \left[\left(\frac{P_2}{P_1} \right)^{(\gamma-1)/\gamma} - 1 \right] \quad \text{Eq. S3.6}$$

W_s: minimum isentropic gas work (kJ/kg)
γ: adiabatic compression factor, dimensionless (considering ideal gas, **γ** = 1.4)
Z: compressibility factor (considering ideal gas, Z=1)
T_1: gas temperature before compression (K)
P_1, P_2: in and out pressure of the gas, respectively (Pa)

Reynolds number

$$Re = \frac{\rho \cdot v \cdot D_i}{\mu} \quad \text{Eq. S3.7}$$

For fluids in tanks, v = N and D_i = D (see Eq.1)
For fluids in conductions:

$$v = \frac{4 \cdot m}{\rho \cdot {D_i}^2 \cdot \pi}$$

The equivalent length of conductions (L) was calculated considering standard losses for accessories, valves etc. according to Coulson and Richardson (1999), while the size and characteristics of tubes were designed according to Schedule 40. The centrifuge power needs were estimated considering theoretical power needs indicated in the literature as a function of the solids flow and the inlet liquid flow (Coulson and Richardson, 1999).

S3.4. Detailed impacts of each scenario

The following tables show the detailed impacts of each scenario.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Impact category	Unit	Total	Direct Emissions	Sodium Bicarbonate	Sodium hydroxide	Electricity	Transport MCW	Transport Chemicals	DSP	PET	Anaerobic Digestion
Climate change	kg CO2 eq	14.1	5.1	0.054	1.2	3.9	0.86	0.010	5.6	-2.1	-0.42
Ozone depletion	kg CFC-11 eq	2.7E-06	0	2.5E-09	6.9E-07	5.4E-07	1.62E-07	2.0E-09	1.5E-06	-7.4E-08	-6.0E-08
Terrestrial acidification	kg SO2 eq	0.042	0	0.00052	0.0055	0.024	0.0015	1.9E-05	0.019	-0.0073	-0.00038
Freshwater eutrophication	kg P eq	0.00032	0	4.3E-06	6.6E-05	0.00014	2.3E-06	2.8E-08	0.00017	-4.5E-05	-1.6E-05
Marine eutrophication	kg N eq	0.023	0.023	2.7E-05	0.00018	0.00048	4.3E-05	5.2E-07	0.00044	-0.00015	8.5E-05
Human toxicity	kg 1,4-DB eq	0.64	0	0.0034	0.12	0.090	0.17	0.0021	0.30	-0.048	0.0022
Photochemical oxidant formation	kg NMVOC	0.025	0	0.00019	0.0032	0.012	0.0016	1.9E-05	0.011	-0.0059	0.0026
Terrestrial ecotoxicity	kg 1,4-DB eq	0.00072	0	4.1E-06	8.0E-05	0.00011	0.00041	5.0E-06	0.00020	-8.4E-05	-6.8E-06
Marine ecotoxicity	kg 1,4-DB eq	0.0063	0	4.7E-05	0.00074	0.0014	0.0032	3.8E-05	0.0022	-0.0011	-0.00015
Fossil depletion	kg oil eq	2.2	0	0.012	0.29	1.07	0.31	0.0037	1.84	-1.1	-0.12

Table S3.3 Absolute values of every impact category analyzed for the circular economy scenario (per m^3 of MCW).

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Impact category	Unit	Total	Direct Emissions	Sodium hydroxide	Hydrochloric acid	Transport (Chemicals)	Electricity	Composting
Climate change	kg CO_2 eq	17.5	4.2	0.61	1.8	0.0028	5.7	4.2
Ozone depletion	kg CFC-11 eq	2.05E-06	0.00	3.7E-07	8.4E-07	5.7E-10	7.9E-07	5.4E-08
Terrestrial acidification	kg SO2 eq	0.083	0.00	0.0029	0.0083	5.8E-06	0.035	0.037
Freshwater eutrophication	kg P eq	0.00036	0.00	3.5E-05	9.6E-05	1.1E-08	0.00021	1.8E-05
Marine eutrophication	kg N eq	0.025	0.022	9.3E-05	0.00024	1.7E-07	0.00070	0.0017
Human toxicity	kg 1,4-DB eq	0.79	0.00	0.062	0.59	0.00081	0.13	0.0067
Photochemical oxidant formation	kg NMVOC	0.033	0.00	0.0017	0.0047	6.7E-06	0.018	0.0090
Terrestrial ecotoxicity	kg 1,4-DB eq	0.0013	0.00	4.2E-05	0.0011	1.9E-06	0.00016	1.2E-05
Marine ecotoxicity	kg 1,4-DB eq	0.011	0.00	0.00034	0.0088	1.5E-05	0.0020	0.00013
Fossil depletion	kg oil eq	2.3	0.00	0.15	0.48	0.0011	1.6	0.11

Table S3.4. Absolute values of every impact category analyzed for the linear economy scenario (per m^3 of MCW).

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

APPENDIX 4: SUPPLEMENTARY INFORMATION FOR CHAPTER 6

S4.1. Discharged effluent quality

Discharge Limits are stablished according to the local legislation (Boletín Oficial de la Provincia de A Coruña, 2016). For the most important parameters, they are the following ones:

Table S4.1. Discharge Limits for both facilities

Parameter	Discharge Limit (mg/L)
Chemical Oxygen Demand	700
Biological Oxygen Demand	300
Suspended Solids	250
Total Nitrogen	115
Ammonia Nitrogen	50
Total Phosphorus	25
Fats and Oils	50

S4.2. Supplementary information for innovative processes in System A (MCW)

S4.2.1. Description of the stable experimental periods for System A (SA1 - SA4)

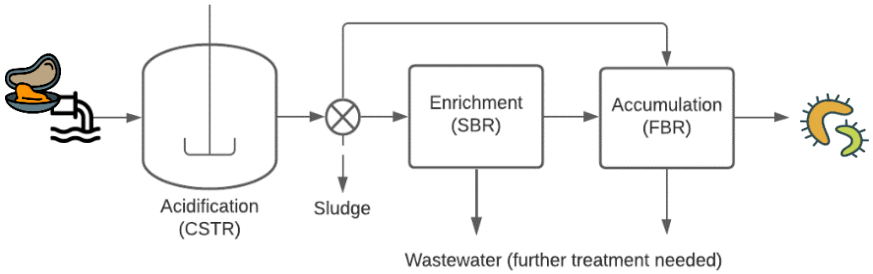


Figure S4.1. Laboratory-scale system for SA.

SA1: pH in the acidification CSTR was below 5 due to VFA production and no pH control. The operational strategy in the enrichment SBR was based on an aerobic dynamic feeding (ADF) regime where the feast/famine strategy was carried out and the reactor exchanged volume was 50%. To avoid salinity variations and the use of drinking water for load control, the settled effluent of the SBR was recirculated as a dilution water for the CSTR effluent. The remaining SBR effluent, containing biomass, was fed to the accumulation reactor (FBR) to maximise PHA production.

SA2: slight amounts of sodium bicarbonate (20 mg $NaHCO_3$/L of MCW) were added in the acidification CSTR to increase alkalinity and improve pH control, although it remained below 5. Then, the enrichment strategy operated in SA1 was modified by introducing a settling stage after the feast phase (after about 2.5 hours of SBR cycle). Therefore, after settling, a withdrawal waste stream was generated, corresponding to 25% of the reactor volume, so it operated with 75% of the initial reactor volume during the remaining SBR cycle time (about 9.5 hours). This means that the SBR effluent also 25% of the initial reactor volume, so hydraulic retention times were still 1 day. Finally, part of the settled effluent was recirculated as a dilution water for load control in the SBR as in SA1, and the remaining broth (with the enriched biomass) was used as inoculum for the FBR.

SA3: sodium bicarbonate addition was increased to 320 mg $NaHCO_3$/L of MCW in the acidification CSTR, although pH continued below 5. Then, the enrichment strategy was the same as that described in SA2 (including a settling stage that generated a withdrawal waste stream), but here VFA load was maximised, meaning that no dilution agent was used for load control (there was no recirculation). Therefore, CSTR effluent was directly fed to the SBR, so all the SBR effluent (25% of the initial reactor volume, as the settling withdrawal stream was also 25% of the volume and retention times were maintained) was used as an inoculum for the FBR.

SA4: the addition of sodium bicarbonate was maximized in the acidification CSTR (2,700 mg $NaHCO_3$/L of MCW), so pH was neutral in the reactor and the VFA concentration in the effluent was higher than in the previous operational scenarios. The enrichment strategy was the same as in SA1, so there was no settling stage, and a recirculation for load control was performed, as stability could not be maintained for high loads and neutral CSTR effluent pH.

S4.2.2. Mass balances for SA

In the mass balances here presented, each inflow Q is expressed in m^3/cycle and represents the stream flows in Figures 6.2 and 6.3. Q_{INLET} is the total flow of MCW fed to the WWTP (thus, 100 m^3/day), Q_{OUTLET} is the effluent flow of the WWTP.

Q_{ENR} and Q_{ACC} represent the flow of centrifuged CSTR effluent (VFA-rich) that is fed to the enrichment and to the accumulation reactor (the SBR and the FBR), respectively, while Q_{INOC} is the flow of the inoculum stream (enriched biomass from the SBR to the FBR).

Q_{WITHD} is the withdrawal stream generated after the settling stage of the SBR cycle (only for operational stages SA2 and SA3), and Q_R is the recirculation used for load control (only in operational stages SA1, SA2 and SA4). These streams, that only exist for some operational stages, are represented in the flowchart of Figure 6.3 as grey lines, to differentiate them from the remaining streams, existing in all the operational stages considered.

V_{ENR} and V_{ACC} are the volumes of the enrichment and accumulation reactors (m^3), respectively, while $C_{VFA\text{-}ENR}$ and $C_{VFA\text{-}ACC}$ (see Equations S4.3 and S4.4) represent the concentration of VFA in the SBR and the FBR, respectively, necessary to achieve the amount of PHA generated for each stage (in kg COD/m^3). $C_{VFA\text{-}CSTR}$ is the concentration of VFA, in kg COD/m^3, in the effluent of the acidification reactor.

Note that, for stage SA3, Eq. (S4.3) and Eq. (S4.12) are the same, indicating that, as there is no recirculation, and as the exchanged volume is 50% (HRT=1 day), the concentration of VFA at the beginning of a SBR cycle is half the concentration of VFA in the CSTR effluent. The results of the mass balances are shown in Table 6.1.

It is also noticeable that, for stages SA1 and SA4, there is no settling stage. Hence, Q_{WITHD} is 0, although there is a recirculation stream, so concentrated biomass is used as inoculum in the accumulation reactor and the supernatant of the decanter is returned to the enrichment one.

For stage SA2, there are both recirculation and settling stage, while for stage SA3 there is a settling stage but no recirculation. That means that there is no decanter after the

enrichment reactor to obtain a supernatant and the effluent of the SBR is directly fed to the FBR.

General Equations (applied for each circular economy-based scenario)

Stream balances

$$Q_{INLET} = Q_{TOTAL-SLUDGE} + Q_{DSP} + Q_{OUTLET} \quad \text{Eq. S4.1}$$

$$V_{ACC} = (Q_{ACC} + Q_{INOC}) \cdot 1 \text{ cycle} \quad \text{Eq. S4.2}$$

Stoichiometric balances (load-related)

$$Q_{ENR} = \left(\frac{C_{VFA-ENR} \cdot V_{ENR}}{C_{VFA-CSTR}}\right) \cdot \frac{1}{\text{cycle}} \quad \text{Eq. S4.3}$$

$$Q_{ACC} = \left(\frac{C_{VFA-ACC} \cdot V_{ACC}}{C_{VFA-CSTR}}\right) \cdot \frac{1}{\text{cycle}} \quad \text{Eq. S4.4}$$

Equations for SA1 and SA4 (with recirculation for load control, but no settling stage)

$$Q_{WITHD} = 0 \quad \text{Eq. S4.5}$$

$$V_{ENR} = 2 \cdot (Q_{ENR} + Q_R) \cdot 1 \text{ cycle} \quad \text{Eq. S4.6}$$

$$Q_{ENR} = Q_{INOC} \quad \text{Eq. S4.7}$$

Equations for SA2 (with recirculation and settling stage, 50% each flow)

$$Q_{ENR} = Q_{INOC} + Q_{WITHD} \quad \text{Eq. S4.8}$$

$$Q_{WITHD} = Q_{INOC} + Q_R \quad \text{Eq. S4.9}$$

$$V_{ENR} = 4 \cdot Q_{WITHD} \cdot 1 \text{ cycle} \quad \text{Eq. S4.10}$$

Equations for SA3 (with settling stage, but no recirculation)

$$Q_R = 0; \text{ so } Q_{WITHD} = Q_{INOC} \quad \text{Eq. S4.11}$$

$$Q_{INOC} = Q_{WITHD} = 0.5 \cdot Q_{ENR} \quad \text{Eq. S4.12}$$

$$V_{ENR} = 2 \cdot Q_{ENR} \cdot 1 \text{ cycle} \quad \text{Eq. S4.13}$$

Note that some of this mass balances are expressed in this way to better fit their resolution, but most equations could be reformulated in different ways. For example, Eq. S4.10 could be expressed as $V_{ENR} = 2 \cdot Q_{ENR} \cdot 1$ cycle, or $V_{ENR} = 2 \cdot (Q_{WITHD} + Q_{INOC}) \cdot 1$ cycle.

In this sense, the equations could be expressed differently, with the only conditions that Eq. S4.1 to Eq. S4.4 always need to be accomplished, and that the specific requirements of each stage are met. This means that for SA1 and SA4, $Q_{WITH} = 0$, and for SA3 $Q_R = 0$ (for SA2, both Q_{WITH} and Q_R have a value different to zero).

Moreover, it is noticeable that $Q_{WASTEWATER} \cdot 1$ cycle = $V_{ACC} \cdot 0.85$, as it is considered that 85% of the accumulation reactor volume is withdrawn after settling once the cycle is

performed to be treated in the CAS (so the remaining 15% is Q_{DSP}) (see Figure 6.3, $Q_{WASTEWATER}$ is the waste effluent resulting after the accumulation cycles in the FBR).

The influent treated by the CAS reactor (for stage SB1 and SB4, only $Q_{WASTEWATER}$, for stages SB2 and SB3 $Q_{WASTEWATER}$ + Q_{WITHD}) varies slightly for each stage according to the mass balances previously presented. However, Q_{CAS} is around 85 m^3/day with a concentration of about 1 kg COD/m^3 for all stages. Biomass concentration in the CAS reactor also varied for each scenario, but it was in the range of 2.5 – 3.5 g VSS/L.

Finally, the mass balances for the linear scenario SA0 were made according to the same considerations performed to design the linear economy-based facility of Chapter 5. Thus, the details of the mass balances and design considerations can be found in Appendix 3.

S4.3. Supplementary information for innovative processes in System B (FPW)

S4.3.1. Characterization of FPW

Table S4.2. Characterization of the raw FPW (average).

Parameter	Units	Value
pH	---	6.4
Conductivity	mS/cm	0.77
VFA	mg COD_{VFA}/L	≈ 0.0
Carbohydrates	g COD_{CARB}/L	0.21
Proteins	g COD_{PROT}/L	0.41
Fats	g COD_{FAT}/L	0.71
Total Organic Carbon	mg TOC/L	487.1
Inorganic Carbon	mg IC/L	56.8
Total COD	g COD_T/L	4.08
Soluble COD	g COD/L	0.57
Ammonium	mg NH_4^+-N/L	13.4
Total Nitrogen	mg TN/L	63.7
Sodium Chloride	g NaCl/L	0.78
Total Suspended Solids	g TSS/L	1.2
Volatile Suspended Solids	g VSS/L	1.1

COD: Chemical Oxygen Demand, VFA: Volatile Fatty Acids.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

S4.3.2. Description of the stable experimental periods for System B (SB1 - SB5)

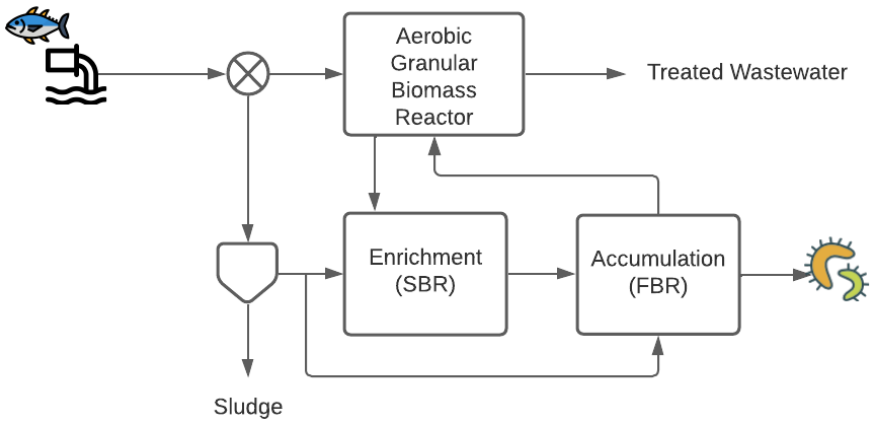


Figure S4.3. Laboratory-scale System for SB.

Regarding the AGS unit, it is scaled-up according to the results from a laboratory-scale reactor that was operated for three months fed with FPW. It eliminated 85% of the total COD, 92% of the soluble COD and 67% of the total nitrogen, where the remaining 33% of the nitrogen is in the form of nitrite. Considering the FPW composition and the process efficiency, the AGS reactor effluent accomplishes the discharge limits.

With respect to the valorisation section, the oil is recovered after the DAF, the sludge is settled, so the oil floats in the superior layer. Here, the process configurations described by Argiz et al. (2020b) define the studied scenarios **SB1, SB2, SB3, SB4, and SB5**. The valorisation section has two units: a hydrolysis plus MMC enrichment SBR and a hydrolysis plus PHA and TAG accumulation FBR, both fed with oil.

This oil is fed in pulses to the media where the MMC grows in a proportion of 0.5 L_{OIL}/m^3_{SBR}·cycle and 1.2 – 1.6 L_{OIL}/m^3_{FBR}·cycle for all the stages. This media is the effluent of the AGS reactor (Figure 6.4b), which has low concentration of COD and nitrogen and is ready to be discharged. The process happens at 22.5 ºC to avoid fat solidification, so a heat exchanger is located prior to the SBR.

SB1: the enrichment SBR was operated using the ADF regime where the feast/famine strategy was carried out for biomass selection. As oil has no nitrogen on its elemental composition, it was supplied at the beginning of the cycle as ammonia chloride to reach a concentration of 0.3 g NH_4Cl/L, along with the oil and the dilution water to avoid overload. Also, sodium bicarbonate was added to a concentration of 0.33 g $NaHCO_3$/L.

SB2: the ADF regime was modified by changing the feeding strategy. Here, the carbon source (oil) was fed at the beginning of the cycle (when the reactor's operational volume is half the total volume due to withdrawal at the end of the previous cycle). Then, after feast (about 3 hours from the beginning of the cycle), nitrogen was fed along with the dilution water (reaching the total reactor's operational volume during the remaining 9 cycle hours). As the idea is to promote biopolymer storing by limiting the nutrients concentration, nitrogen was fed to reach 0.15 g NH_4Cl/L, and sodium bicarbonate to 0.25 g $NaHCO_3$/L.

SB3: the strategy was the same as in SB2, but the dilution water was fed along with the oil (at the beginning of the cycle) and the nitrogen (0.15 g NH_4Cl/L) was fed in a concentrated spike after feast. Sodium bicarbonate was 0.18 g $NaHCO_3$/L.

SB4: the operational strategy was the same as in SB3, but in this case pH was also considered as a selective pressure, so the sodium bicarbonate dosage changed to 0.125 g $NaHCO_3$/L.

SB5: the operational strategy was the same as in SB3 (only sodium bicarbonate dosage changed to 0.2 g $NaHCO_3$/L). The aim was to test the system recovery capacity after operational changes as pH variations.

S4.3.3. Mass balances for SB

The enrichment reactor was designed considering the applied load of oil. Therefore, from the operational experience, it was known the kg COD fed to the reactor per m^3 of reactor per cycle. Thus, after that, the volumes of the SBR for the MMC enrichment and for the FBR for the PHA and TAG accumulation were calculated, knowing that the FBR will have half of the SBR volume. This happens because the exchange volume of the SBR is 50%, and the FBR feeding is supplied in spikes with a small volume. Note that the oily feedstock is provided in pulses to the reactor, with negligible volume in comparison with the dilution water volume, so the reactor can be designed using the flow of the dilution stream.

Moreover, the oil was periodically characterized to observe that its composition and density did not change with time, so knowing that the oil had a density of 0.9 kg/L and a COD of 2.5 g COD/g oil, it is possible to calculate the reactors' volume as follows.

$$V_{ENR} = 2 \cdot V_{ACC} \qquad \text{Eq. S4.14}$$

$$Q_{OIL} = 2 \cdot V_{ACC} \cdot q_E + \cdot V_{ACC} \cdot q_A \qquad \text{Eq. S4.15}$$

Where V_{ACC} and V_{ENR} are the volumes of the accumulation and enrichment reactors, respectively, and Q_{OIL} is the flow of oil in m^3/day. Then, q_E and q_A are the m^3 of oil supplied to the enrichment and accumulation reactors, respectively, per m^3 of reactor and day, knowing that there are always 2 cycles per day. q_E and q_A vary for each stage according to the data indicated in Argiz et al. (2020b):

Table S4.3. Oil supply for each operational stage in SB including biological valorisation of oil.

Stage	SB1	SB2	SB3	SB4	SB5
q_E Oil supply in the SBR (kg COD/m^3_R·day)	2.25	2.25	2.25	2.25	2.25
q_A Oil supply in the FBR (kg COD/m^3_R·day)	7.20	7.20	5.40	7.20	7.20

Then, the dilution water needs are calculated knowing that the exchanged volume in the SBR is 50%, so $Q_{DILUTION}$ ·1 cycle = $V_{ENR}/2$. This stream flow is used to recalculate the volume of the AGS reactor, that treats the wastewater resulting from the PHA/TAG production process. Therefore, $Q_{WASTEWATER}$ ·1 cycle = V_{FBR}·0.8, as it is considered that 80% of the accumulation reactor volume is withdrawal after settling once the cycle is performed (so the remaining 20% is Q_{DSP}) (see Figure 6.5, where $Q_{WASTEWATER}$ is the waste flow resulting after the accumulation cycle in the FBR).

Finally, the design of the SBR was performed considering that it must treat $Q_{BIORREACTOR}$ (m^3/day), that contains $Q_{WASTEWATER}$ and the supernatant of the centrifuge, plus Q_{INLET} minus the sludge volume removed in the DAF. Then, according to laboratory

results, the exchange volume is 40% (thus, there are 6 cycles/day in the AGS-SBR). Experimental data of the solids in the effluent were used to design the decanter after the AGS reactor, where the concentration was generally below 0.3 g TSS/L.

The AGS reactor treated a volume of 860 – 890 m^3/day, depending on the scenario, with an organic matter concentration of about 1 g COD/L. Note that both biological reactors (the CAS in SA and the AGS in SB) treated effluents with similar characteristics. However, MCW has high salinity compared to FPW, and the results performed in the lab showed that the performance of the AGS was worse for the range of salinity of MCW (meaning a lower COD uptake, and more instability in the granule formation). Thus, the AGS is considered for SB with FPW, and not for SA with MCW. The concentration of biomass was approximately 7.0 g VSS/L in the AGS.

S4.4 Energy balances for SA and SB

For the mass balances, each system has its specific characteristics. However, the equations used to calculate the energy needs of both systems are the same, as they refer to the electricity demand of equipment and the heat needs of reactors, for which general equations were used. Moreover, as indicated in Chapter 5, the temperature of MCW when leaving the process is already about 100 ºC (normally, that heat is just dissipated naturally before proceeding with treatment). Therefore, it is considered that no extra heat is needed for the process units of SA. The equations used to calculate the heat needs of SB are indicated below.

S4.4.1 Electricity and power needs of the process units

The power needs of the process units are calculated as indicated in equations from S3.1 to S3.7 in Appendix 3 for stirrers, pumps, and DAF compressor. The power needs of the centrifuges and the centrifuge type were estimated as a function of the solid concentration in the separator feeding and the unit working time as indicated in Perry (1997). Air needs are estimated as explained in Chapter 5, so as a function of the reactor volume and shape, according to Metcalf & Eddy (2014). However, for the AGS reactors, it was considered that the aeration needs were higher, so a factor of 1.5 was applied, considering approximately a 50% more aeration needs than CAS-based reactors.

S4.4.2. Heat

For SA, it was considered that the maintenance of reactors' temperature is covered by the extra heat of the boilers for the mussels (see Chapter 5). For SB, heat is provided to prevent oil solidification in the valorization reactors by maintaining the temperature over 22.5 ºC, as it was observed at lab-scale that this was a key element for the process success. It was assumed that this heat was provided for six months a year, as the remaining time, the water temperature would be enough to ensure proper process performance. Therefore, the heat demand was calculated as indicated in the following equations:

$$Q = C_P \cdot m \cdot \Delta T \qquad \text{Eq. S4.16}$$

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Where:

Q is the heat demand in W, as m, which is the mass of fluid to be heated (kg/s), is expressed as m = F/t, being F the flow of the stream to be heated (kg/day) and t the time that this stream is being pumped (s/day).

C_P is the heat capacity, considered 4184 J/kg·K

ΔT is the temperature difference (K), so $T_2 - T_1$, being T_2 the final temperature (22.5 ºC) and T_1 the initial one (20 ºC).

S4.5 Cost estimation equations

The equations for cost estimation of equipment is based on the equations published in Seider et al. (2017), and they are the ones stated in the following equations, where C_i represents the cost of each unit in $ for 2013. C_B ($) represents, for all cases, the base cost of the equipment, that is always dependent on a size factor. Then, the costs were transformed to the current price by using the Chemical Engineering Plant Cost Index (CEPCI) of 2021, and the current euro currency.

$$C_{PUMPS} = F_T \cdot F_M \cdot C_B \quad \text{Eq. S4.17}$$

$$C_B = \exp\{12.1656 - 1.1448 \cdot [\ln(S)] + [0.0862 \cdot \ln(S)]^2\} \quad \text{Eq. S4.18}$$

$$S = q \cdot H^{0.5} \quad \text{Eq. S4.19}$$

Where:

F_T is a pump-type factor dependent of the pump head (H). F_M is a material factor that was assumed 1.35 as it is considered cast steel for pump materials. Therefore, S is a size factor in gpm·ft$^{0.5}$ dependent of the flow q in gpm and the head H in ft.

$$C_{STIRRERS} = 3740 \cdot P^{0.17} \quad \text{Eq. S4.20}$$

Where P is the power of the stirrer (hp)

$$C_{BLOWERS} = F_M \cdot C_B \quad \text{Eq. S4.21}$$

$$C_B = \exp\{7.0187 + 0.7900 \cdot [\ln (P_C)]\} \quad \text{Eq. S4.22}$$

Where:

P_C is the power of the blower (in hp), and F_M is a material factor that is assumed 0.6 for cast aluminum.

$$C_{COMPRESSORS} = F_D \cdot F_M \cdot C_B \quad \text{Eq. S4.23}$$

$$C_B = \exp\{8.2496 + 0.7243 \cdot [\ln (P_C)]\} \quad \text{Eq. S4.24}$$

Where:

P_C is the power of the blower (in hp), F_D is the motor drive, considered 1.25 for a gas turbine drive, and F_M is a material factor that is assumed 2.5 for stainless steel.

$$C_{HEAT\ EXCHANGERS} = \exp\{8.2015 + 0.4343 \cdot \ln(A) + 0.3812 \cdot [\ln(A)]^2\} \quad \text{Eq. S4.25}$$

Where A is the exchange surface, in ft^2. It is calculated as follows:

$$A = \frac{Q}{F_0 \cdot U} \quad \text{Eq. S4.26}$$

Being:

Q the heat demand (W), and:

$$F_0 = \frac{T_4 - T_3}{T_2 - T_1} \quad \text{Eq. S4.27}$$

$$\frac{1}{U} = \frac{1}{h_0} + \frac{1}{h_{0d}} + \frac{d_0 \ln\left(\frac{d_0}{d_i}\right)}{2 \cdot k_w} + \frac{d_0}{d_i} \cdot \frac{1}{h_{id}} + \frac{d_0}{d_i} \cdot \frac{1}{h_i} \quad \text{Eq. S4.28}$$

Where:

U is the overall heat transfer coefficient ($W/m^2 \cdot ºC$)

h_0, h_i, h_{0d}, and h_{id} are individual transfer coefficients for: outside fluid film, inside fluid film, outside dirt, and inside dirt, respectively (in $W/ft^2 \cdot ºC$). Their values were estimated from data in Coulson and Richardson (1999).

k_w is the thermal conductivity of the material, in $W/m \cdot ºC$, and d_0 and d_i are the outside and inside diameter of the tubes, in m, also standard values were estimated from Coulson and Richardson (1999).

Regarding the temperatures, T_4 and T_3 are the outflow and inflow temperature of the hot fluid, respectively, and T_2 and T_1 are the inflow and outflow temperature of the cold fluid, respectively, all in K.

The cost of the reactors is estimated considering that the construction material is concrete. Therefore, the direct costs of the reactors were dependent of the reactor shape and geometry, considering that expenditures are due to the excavation and to the concrete itself. For the concrete costs, it was first considered a cost for the slab, with a thickness of 50 cm, and a different value for the walls, with a width of 30 cm. The excavation is 28 $\$/m^2$, the slab is 380 $\$/m^3$, and the walls 720 $\$/m^3$, as indicated in Arif et al. (2020).

$$C_{CENTRIFUGE} = 975 \cdot B_D \quad \text{Eq. S4.29}$$

B_D is the bowl diameter, which was estimated according to the solids flow (in t/h) as indicated in Perry (1997).

The cost of the elements of DSP, used in the second stage of the assessment, are estimated according to Saavedra del Oso et al. (2020), where costs are in € (the costing factors are transformed to provide results transformed from $ to €). The results also correspond to a CEPCI of 2013, so they still need to be transformed to the cost of 2021.

$$C_{MEMBRANES} = 256.6 \cdot A \quad \text{Eq. S4.30}$$

Where A is the area of the membrane in m^2.

$$C_{REACTORS} = 4.416 \cdot V^{0.153} \quad \text{Eq. S4.31}$$

For the WWTPs, the reactors were made of concrete, so the cost was calculated according to the volume of concrete needed and excavation costs. For the DSP reactors, they are made of steel, so their costs are calculated as in Eq. S4.31 where V is the volume of the reactor in m^3.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

$$C_{COLLUMNS} = F_M \cdot C_V + C_{PL}$$ Eq. S4.32

Where F_M is an unitless material factor, which equals 1 for carbon steel.

C_V is the main cost of the column (€), that depends on the dimensions:

$$C_V = 0.954\exp\{7.2977 + 0.2189[\ln(W)] + 0.02297[\ln(W)]^2\}$$ Eq. S4.33

Where:

$$W = \pi(D_i + t_s)(L + 0.8D_i)t_s\rho$$ Eq. S4.34

Being:

W the weight of the shell and the two heads (kg), t_s the thickness (m), D_i the inner diameter (m), and ρ the density of the material (kg/m^3).

C_{PL} represents the additional costs (€), calculated as follows:

$$C_{pL} = 1788(D_i)^{0.63316}(L)^{0.80161}$$ Eq. S4.35

Where D_i is the inner diameter (m) as in Eq. S4.34, and L is the length of the column (m).

The cost of the spray driers ($C_{S\text{-}D}$, in €) is calculated as follows:

$$C_{S-D} = 0.954\exp(9.2567 + 0.8960[\ln(Ev)] - 0.0561[\ln(Ev)]^2)$$ Eq. S4.36

Where Ev is the evaporation rate in kg/h.

The cost of high-pressure homogenizers (C_{HPH}, in €) is estimated as in the following equation:

$$C_{HPH} = 10^{4.85} \cdot Q^{0.43}$$ Eq. S4.37

Being Q the inflow to the homogenizer, in m^3/h.

Finally, it is assumed that a DAF unit is employed to treat the wastewater generated in the DSP. However, to estimate the indirect cost of effluent management in the DSP biorefinery, the cost of wastewater primary treatment (C_{WW}, in S) is estimated as follows:

$$C_{HPH} = 16785 \cdot Q^{0.64}$$ Eq. S4.38

Where Q is the influent of wastewater in gal/min.

The cost of the remaining process units (heat exchangers, stirrers, centrifuges…) are calculated as indicated previously (Eq. S4.20, S4.35, S4.29).

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

APPENDIX 5: SUPPLEMENTARY INFORMATION FOR CHAPTER 7

S5.1. General water bodies classification

Table S5.1. Water bodies classification according to biome distribution

Waterbody Category	Characteristics and subcategories
Rivers and streams	Freshwater
Lakes	Salt lakes (from oligohaline to hyperhaline), freshwater lakes, oases and springs (normally fed with fresh ground water).
Palustrine wetlands	Highly biodiverse, non-riverine and non-tidal freshwater ecosystems. Include tropical flooded and peat forests, subtropical/temperate forested wetlands, permanent marshes, seasonal floodplain marshes, episodic arid floodplains, boreal, temperate and montane peat bogs, and boreal and temperate fens.
Transitional waters	Combined marine and freshwater sources, varying from oligohaline to euhaline. It includes estuaries and bays, coastal inlets or fjords, and intermittently closed and open lagoons and lakes
Brackish tidal systems	Intersection of marine, terrestrial, and freshwater processes, usually euhaline. They include deltas, intertidal forests and shrublands and saltmarshes.
Subterranean waterbodies	Freshwater
Oceanic	Euhaline (averaging 35 g/L)
Artificial wetlands	Freshwater

Table S5.2. Water bodies classification according to salt concentration

Waterbody Category	Concentration (g salts*/L)
Freshwater	< 0.5
Oligohaline	0.5 – 4.0
Mesohaline	5.0 – 18.0
Polyhaline	18.0 – 30.0
Euhaline	30.0 – 40.0
Hyperhaline	> 40.0

* Salinity is defined by the concentration of several ions (Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, $SO_4^{2-}CO_3^{2-}$, NO_3^- and HCO_3^-), where the main considered substance is NaCl. However, to accurately measure it, the sum of the concentrations of all these ions needs to be considered.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

S5.2. Effect factor calculation assuming an exponential approximation

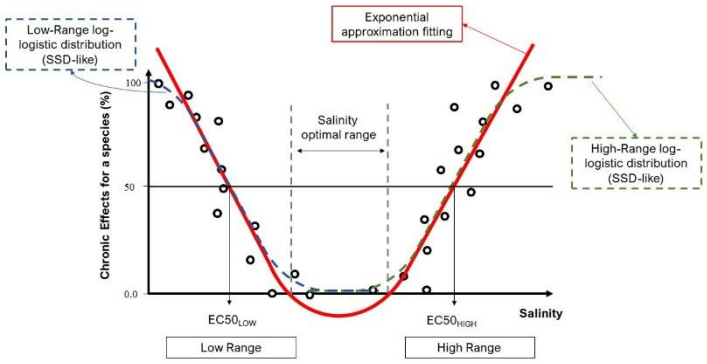


Figure S5.1 Quadratic approximation to quantify EC50s (power function).

S5.3. Fate Factor calculation for *Arousa ría*

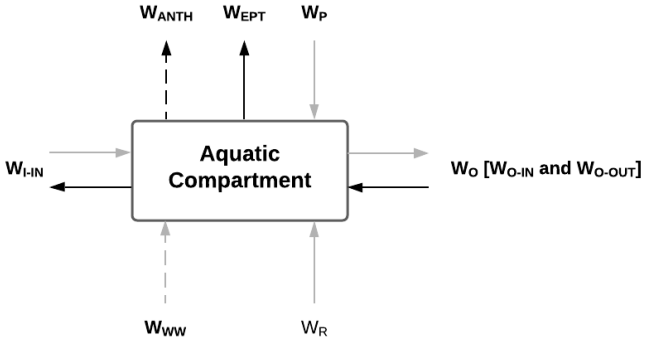


Figure S5.2 Scheme of the streams considered to solve the mass balances necessary to calculate the *Arousa* regional FF.

Table S5.3. Distribution of the shellfish and seafood captures along the year, calculated as an average of the monthly captures of three of the most representative captures (cockle, clam, and octopus)(Xunta de Galicia, 2018).

Month	Distribution (%)
January	7.5
February	6.8
March	7.9
April	5.4
May	4.8
June	3.7
July	8.0
August	6.9
September	8.0
October	12.0
November	14.5
December	14.6

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

a)



b)

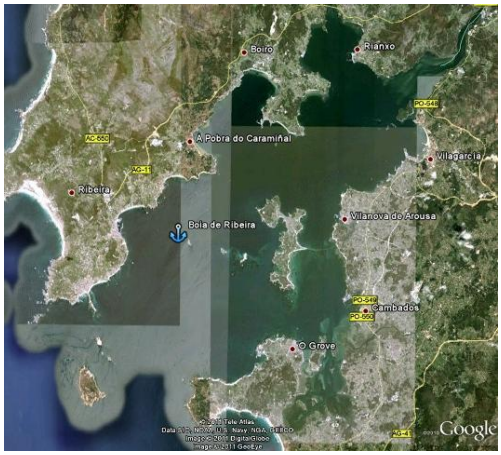


Figure S5.3 Location of the two buoys measuring salinity in *Arousa ría*. a) Cortegada buoy, location (-8° 47,03'; 42° 37,54') is marked with a red arrow. b) Ribeira buoy, location (-8° 56,87'; 42° 32,98') is marked with a blue anchor. Both pictures were provided by the Galician meteorological services(MeteoGalicia, 2021).

Due to the quantification procedure (solving mass balances for a situation as natural as possible and then summing the anthropogenic flows, where $W_{WW} > W_{ANTH}$), the total inlet flow is sometimes slightly higher than the total outlet flow ($W_{IN}>W_{OUT}$, see Table S3.4). Therefore, $W_{O\text{-}OUT}$ was recalculated monthly ($W^*_{O\text{-}OUT}$) to fit the steady state assumption ($W_{IN}=W_{OUT}$).

To calculate $W^*_{O\text{-}OUT}$, the total inlet and outlet flows (W_{IN} and W_{OUT}, respectively) are calculated summing each stream of Fig. S3.2 (being anthropogenic also included). At this point, as the volume of wastewater is higher than the withdrawals, $W_{IN} > W_{OUT}$. Therefore, $W_{O\text{-}OUT}$ was recalculated monthly to fit steady state assumption according to Eq. (S1).

$$W^*_{O-OUT} = W_{WW} + W_R + W_{I-IN} + W_{O-IN} - W_{EPT} - W_{ANTH} \qquad \text{Eq. S5.1}$$

Finally, to quantify the FF, F_{IN} and F_{OUT} are calculated as stablished in Eq. (4), where *Arousa ría* salinity ($\bar{S}$) was considered as seasonal or yearly averages. Finally, as ocean height varies widely due to the tidal ($\approx$ 5 m), the volume of the estuary also changes seasonally and yearly. To acknowledge this, average volume (4.34 km^3 of water) and area (230 km^2) of *Arousa ría*(Alvarez et al., 2005) was used to estimate an average depth of 18.9 m. Then, data of sea height variability (monthly averages) were directly taken from the buoy located in Vilagarcía de Arousa(Ministerio de Transportes Movilidad y Agenda Urbana, 2021) (depth = 2.23 ± 0.09 m). Monthly data of sea height were used to estimate the proportion in which depth deviated from the average, and that proportion was used to estimate volume variations (considering a height of 18.9 m). Finally, data were processed to obtain seasonal and yearly volume averages.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

	YEAR							
	2011	2012	2013	2014	2015	2016	2017	2018
Stream	Stream flow (m^3/year)							
$W_{I\text{-}IN}$	$1.81\cdot10^{9}$	$1.28\cdot10^{9}$	$2.55\cdot10^{9}$	$3.16\cdot10^{9}$	$1.64\cdot10^{9}$	$2.96\cdot10^{9}$	$1.20\cdot10^{9}$	$2.20\cdot10^{9}$
W_{WW}	$1.06\cdot10^{8}$	$1.06\cdot10^{8}$	$1.08\cdot10^{8}$	$1.02\cdot10^{8}$	$1.07\cdot10^{8}$	$1.07\cdot10^{8}$	$1.03\cdot10^{8}$	$1.12\cdot10^{8}$
$W_{WW\text{-}SALT}$	$9.69\cdot10^{7}$	$9.68\cdot10^{7}$	$9.94\cdot10^{7}$	$9.43\cdot10^{7}$	$9.44\cdot10^{7}$	$9.45\cdot10^{7}$	$9.41\cdot10^{7}$	$9.42\cdot10^{7}$
$W_{WW\text{-}FRESH}$	$8.89\cdot10^{6}$	$9.33\cdot10^{6}$	$8.29\cdot10^{6}$	$7.77\cdot10^{6}$	$1.25\cdot10^{7}$	$1.26\cdot10^{7}$	$8.39\cdot10^{6}$	$1.78\cdot10^{7}$
W_{ANTH}	$7.27\cdot10^{7}$	$7.26\cdot10^{7}$	$7.45\cdot10^{7}$	$7.07\cdot10^{7}$	$7.08\cdot10^{7}$	$7.09\cdot10^{7}$	$7.06\cdot10^{7}$	$7.07\cdot10^{7}$
W_{EPT}	$9.37\cdot10^{6}$	$9.58\cdot10^{6}$	$4.75\cdot10^{6}$	$4.86\cdot10^{6}$	$5.22\cdot10^{6}$	$4.97\cdot10^{6}$	$5.31\cdot10^{6}$	$4.62\cdot10^{6}$
W_{P}	$2.42\cdot10^{8}$	$2.82\cdot10^{8}$	$3.86\cdot10^{8}$	$4.70\cdot10^{8}$	$2.56\cdot10^{8}$	$3.39\cdot10^{8}$	$2.24\cdot10^{8}$	$3.40\cdot10^{8}$
W_{R}	$1.72\cdot10^{8}$	$1.72\cdot10^{8}$	$1.72\cdot10^{8}$	$1.72\cdot10^{8}$	$1.72\cdot10^{8}$	$1.72\cdot10^{8}$	$1.72\cdot10^{8}$	$1.72\cdot10^{8}$
$W_{O\text{-}IN}$	$9.69\cdot10^{9}$	$8.94\cdot10^{9}$	$1.24\cdot10^{10}$	$9.84\cdot10^{9}$	$6.75\cdot10^{9}$	$1.06\cdot10^{10}$	$7.97\cdot10^{9}$	$5.09\cdot10^{9}$
$W_{O\text{-}OUT}$	$1.19\cdot10^{10}$	$1.07\cdot10^{10}$	$1.55\cdot10^{10}$	$1.36\cdot10^{10}$	$8.81\cdot10^{10}$	$1.41\cdot10^{10}$	$9.56\cdot10^{9}$	$7.80\cdot10^{9}$
W_{IN}	**$1.21\cdot10^{10}$**	**$1.08\cdot10^{10}$**	**$1.56\cdot10^{10}$**	**$1.37\cdot10^{10}$**	**$8.92\cdot10^{9}$**	**$1.42\cdot10^{10}$**	**$9.66\cdot10^{9}$**	**$7.92\cdot10^{9}$**
W_{OUT}	**$1.20\cdot10^{10}$**	**$1.07\cdot10^{10}$**	**$1.56\cdot10^{10}$**	**$1.37\cdot10^{10}$**	**$8.88\cdot10^{9}$**	**$1.42\cdot10^{10}$**	**$9.63\cdot10^{9}$**	**$7.88\cdot10^{9}$**
	Salinity (kg/m^3)							
$S_{WW\text{-}SALT}$	22.75	23.01	21.39	20.67	20.67	21.94	22.93	18.03
$S_{O\text{-}OUT}$	30.17	30.52	28.35	27.40	27.40	29.08	30.40	23.88
	Corrected value of $W_{O\text{-}OUT}$ ($W^*_{O\text{-}OUT}$)							
$W^*_{O\text{-}OUT}$	$1.20\cdot10^{10}$	$1.07\cdot10^{10}$	$1.56\cdot10^{10}$	$1.37\cdot10^{10}$	$8.84\cdot10^{10}$	$1.41\cdot10^{10}$	$9.59\cdot10^{9}$	$7.95\cdot10^{9}$

Table S5.4 Quantification of system flows and the salt concentration of streams with yearly variable salinity for the time frame selected.

S5.4. Effect Factor calculation for *Arousa ría*

It is necessary to determine the cutoff point that will define the high and low effect factors, knowing that the concentration–response relationships for essential substances in aquatic environments might be modeled as a quadratic function. This means that there will be a range of concentration for which no effects will exist, or effects will be minimal. In this sense, the uncertainty of modeling a whole ecosystem can be very high, so we tested several approaches as shown below.

A) Optimal concentration point defined as the range of salt concentration where no effects are observed.

When plotting all the effect- related data (Figure S5.4), there is an intermediate concentration of NaCl for which no effects are observed (the experiments yielded a mortality of 0%). This range is 28 – 35 NaCl/L. The central point of this interval is 31.5 g/L, which is the value of the optimal (environmental) point of salt concentration derived after this estimation approach.

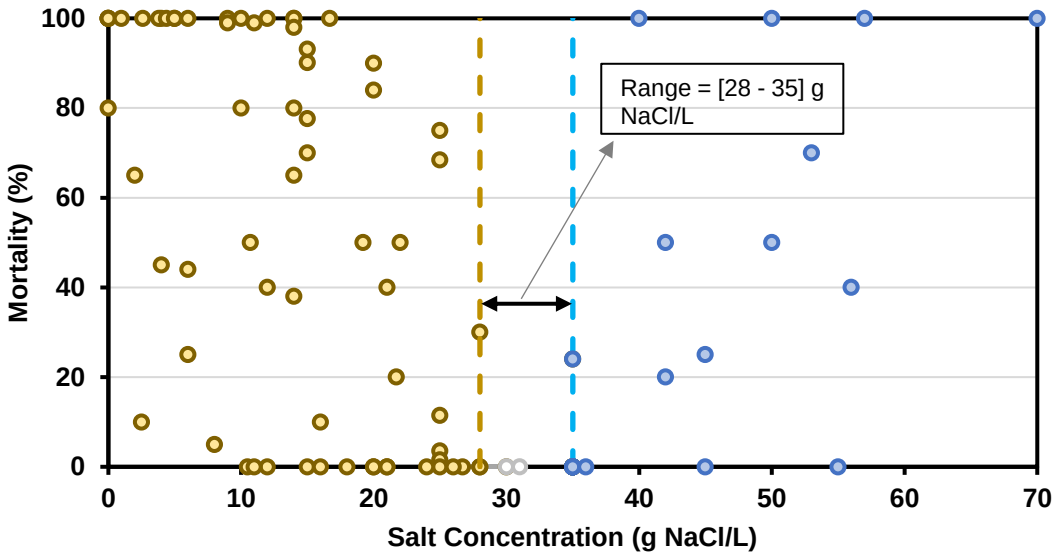


Figure S5.4. Approximation for the optimal (environmental) salinity of the aquatic ecosystem based on the observed range with no effects.

B) Optimal concentration point defined as the intersection point of two linear fittings defining low and high range of concentration.

Guided by the interval defined in the previous approach, it is possible to define a dot cloud for the high concentration, and another one for the low concentration (Figure S5.5). The cutoff point of these two linear fits is the value of the environmental concentration obtained by this estimation approach, i.e., 32.5 g NaCl/L.

The need of previously establishing the boundary that divides low and high regions (in this case, the preliminary estimation based on ranges with no effect: 31.5 g/L) is in our opinion the main weakness of this approach. In fact, as the goal of this fitting is precisely to provide a value for this cutoff, the initial hypothesis for the fitting is already partially defining the result, so this approach is not recommended for being applied individually.

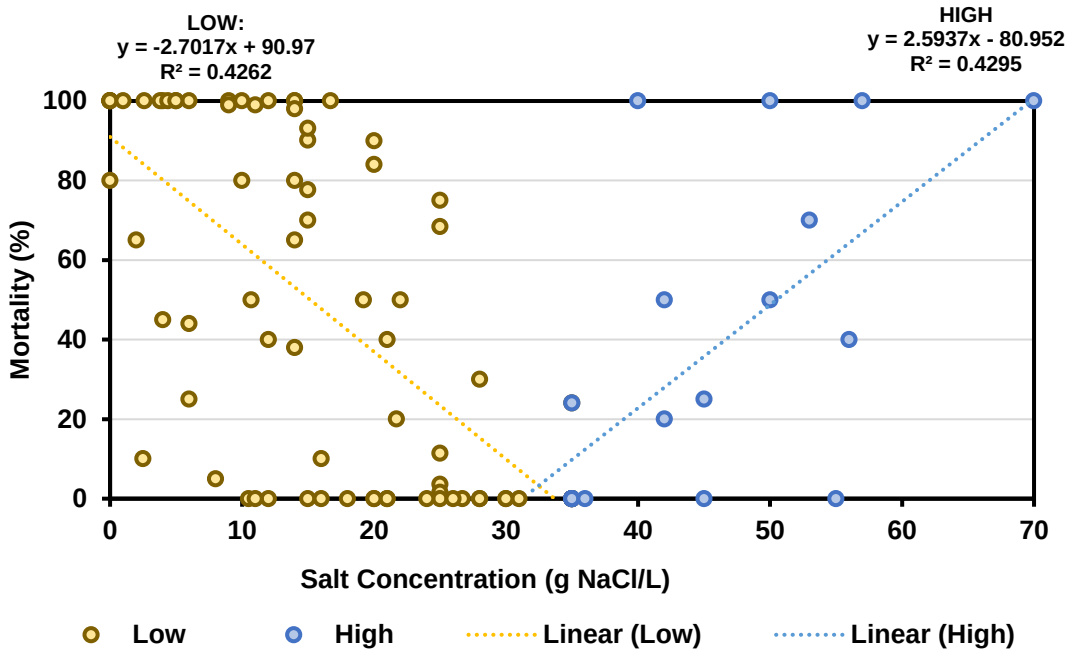


Figure S5.5. Approximation for the optimal (environmental) salinity of the aquatic ecosystem based on the cutoff point of two linear fittings.

C) Optimal concentration point defined as the minimum value of a quadratic function.

One of the core assumptions of the present work is that, for elemental substances, impacts cannot be measured considering a normal distribution, as it is necessary to acknowledge that different ecosystems will have a range of salinity that is optimal for the biota living in that environment, and therefore effects are observed below and above that range. Consequently, the application of a quadratic fitting appropriately represents the ecosystem under this approach (Figure S5.6), which yields a minimum value of 33.8 g NaCl/L.

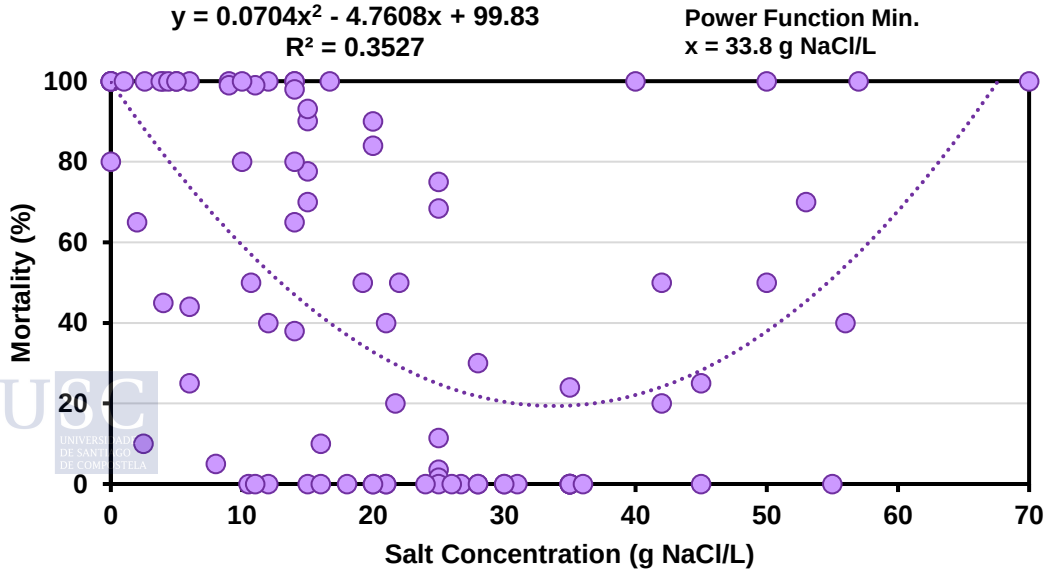


Figure S5.6. Approximation for the optimal (environmental) salinity of the aquatic ecosystem based on the minimum point of a quadratic fitting.

Although this strategy is useful because acknowledges the nature of the element studied (an essential substance, not a pollutant), it presents an important drawback. The minimum value obtained corresponds with an effect of 20%, while for the fitting to be completely accurate this effect should be around 0%. The reason behind this might be linked to the spatial approximation performed, as salt concentration varies along with depth as well as with the proximity to the river mouth. However, salt concentration of the estuary was considered as an average of measurements taken in several locations at several depths. This quadratic function disregards the fact that some marine species can move and redistribute, and that the species distribution might affect salinity tolerance (for example, biota living near the river mouth could better tolerate lower salinities). Therefore, that observed effect of 20% when applying the quadratic approach acknowledges the uncertainty linked to the chosen estimation strategy.

D) Optimal concentration point defined as a range of the minimal values for two opposite sigmoidal curves.

Sigmoidal curves define the classic Species Sensitivity Distribution (SSD) approach for concentration–response relationships in aquatic ecosystems, so it makes sense to have two opposite sigmoidal curves (for both positive and negative effects) where the optimal (environmental) value corresponds to the range where the two functions overlap (i.e., at their lower values). Note that the two curves do not have to be necessarily mirrored (Figure S5.7), as the effects of exposure to low and high salt concentrations do not necessary affect the ecosystems in the same way (which is also a drawback affecting the quadratic approximation C explained above).

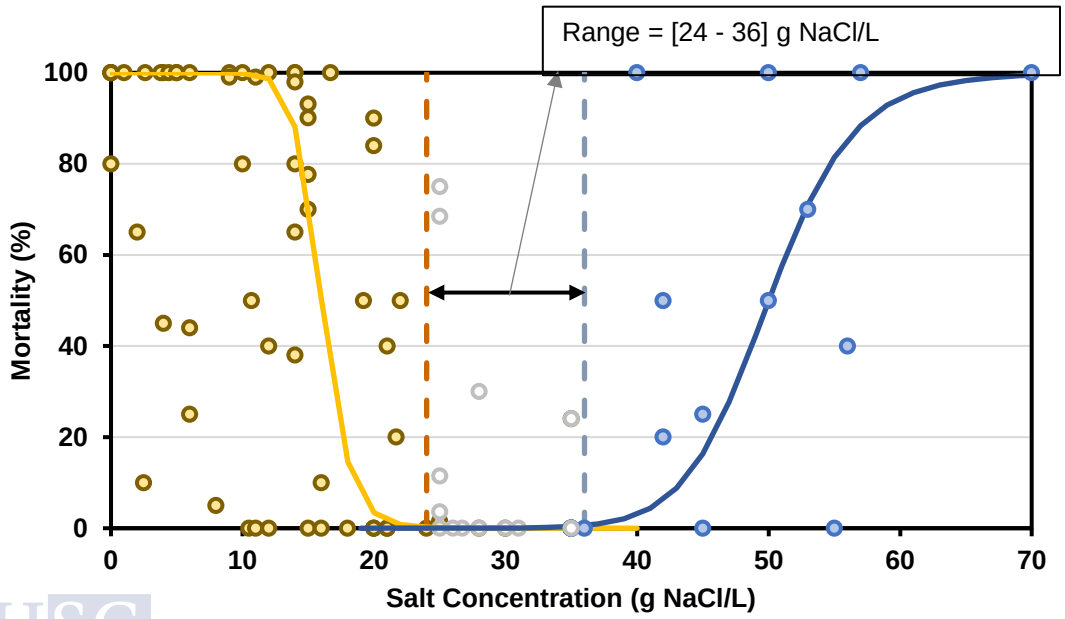


Figure S5.7 Approximation for the optimal (environmental) salinity of the aquatic ecosystem based on the coincident points of two sigmoidal curves.

For the double-sigmoidal approach (Figure S5.7), a range of 24 – 36 g NaCl/L, with a central point of 32.3 g/L (i.e., the optimal (environmental) concentration) is obtained. Also note that this approximation presents a similar problem than approximation B, as it also requires to previously define the range of data used for the high and for the low

ranges (again fixed as the value obtained by approximation A: 31.5 g/L), so the outcome of this strategy is also partially pre-defined as occurred with the double linear fitting approach.

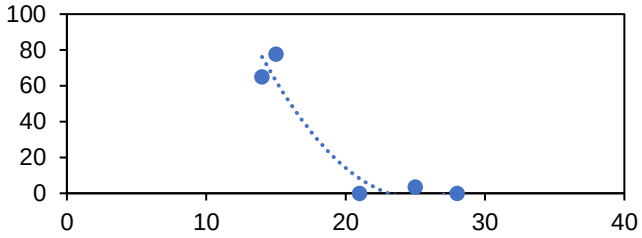
E) Optimal concentration point definition

As observed, results might vary by applying each of the proposed approaches, and the recommendation is to test several approximations and generate an average optimal value out of this testing, acknowledging the uncertainty linked to this type of studies. For the present case, the average optimal (environmental) salt concentration is 31.9 ± 1.4 g NaCl/L, calculated as the average value brought by each proposed fitting. Therefore, this value will be the cutoff point to calculate the low and high effect factors.

USC
UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Table S5.5. Data used to calculate the EFs. For each graphic, the y-axis represents Mortality (%), and the x-axis represents the salt concentration (g NaCl/L). The literature from which data was acquired are indicated in the references section.

Ruditapes philipinarium (Carregosa et al., 2014; Parada et al., 2012) **(Manila clam)**

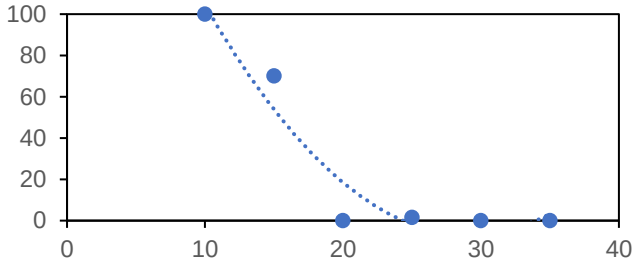


Function: $y = 0.6275 \cdot x^2 - 31.653 \cdot x + 396.27$

$R^2 = 0.923$

$EC50_{LOW}$ = 16.0 g/L, $EC50_{HIGH}$ = 34.4 g/L

Ruditapes decussatus (Gharbi et al., 2016; Parada et al., 2012) **(Grooved carpet shell)**

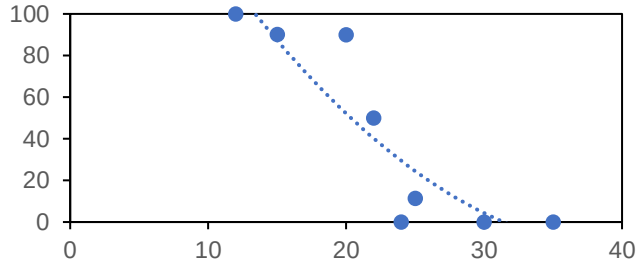


Function: $y = 0.3027 \cdot x^2 - 17.668 \cdot x + 250.83$

$R^2 = 0.931$

$EC50_{LOW}$ = 15.5 g/L, $EC50_{HIGH}$ = 42.9 g/L

Cerastoderma edule (Parada et al., 2012; Verdelhos et al., 2015) **(Common cockle)**

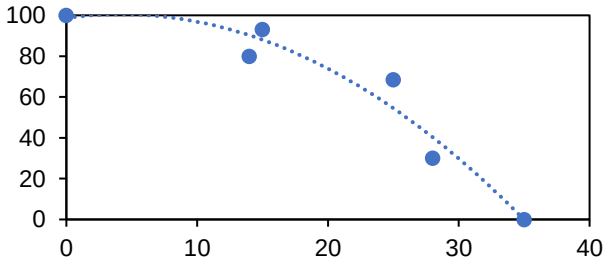


Function: $y = 0.1493 \cdot x^2 - 12.248 \cdot x + 237.42$

$R^2 = 0.803$

$EC50_{LOW}$ = 20.4 g/L, $EC50_{HIGH}$ = 61.7 g/L

Vanerupis corrugata (Carregosa et al., 2014; Parada et al., 2012) (Pullet carpet shell)



USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Function: $y = -0.1056 \cdot x^2 + 0.8701 \cdot x + 98.721$

$R^2 = 0.9434$

$EC50_{LOW}$ = 21.7 g/L

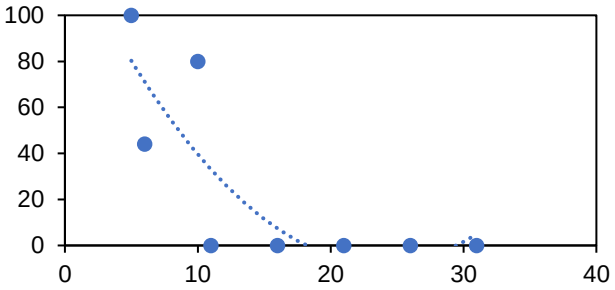
To calculate $EC50_{HIGH}$, the inverse function was used, being:

Function: $y_{HIGH} = (x - 30.7)^2$

$EC50_{LOW}$ = 37.8 g/L

Table S5.5. Continues

Saccharina latissima (Fiett, n.d.; Peteiro and Sánchez, 2012) (Sea belt, brown algae)

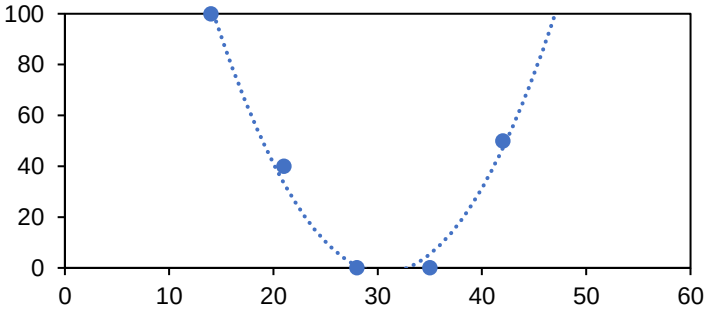


Function: $y = 0.2485 \cdot x^2 - 11.842 \cdot x + 133.27$

$R^2 = 0.668$

$EC50_{LOW}$ = 8.6 g/L, $EC50_{HIGH}$ = 39.1 g/L

Diopatra neapolitana (Freitas et al., 2015) (Polychaete)



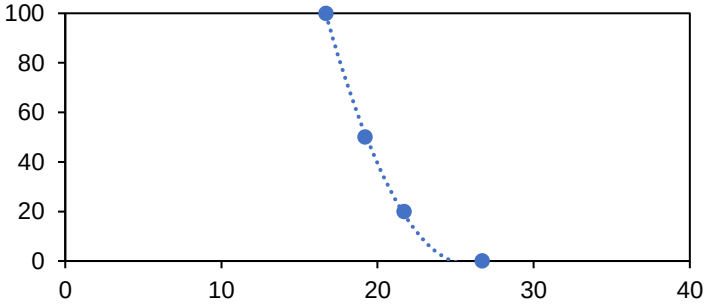
The chronic data provided information for both ranges of the curve, fitting an exponential profile.

Function: $y = 0.379 \cdot x^2 - 23.224 \cdot x + 354$

$R^2 = 0.987$

$EC50_{LOW}$ = 19.0 g/L, $EC50_{HIGH}$ = 42.3 g/L

Donax trunculus (Reyes-martínez et al., 2020) (Wedge clam)



Function: $y = 1.2364 \cdot x^2 - 63.585 \cdot x + 816.52$

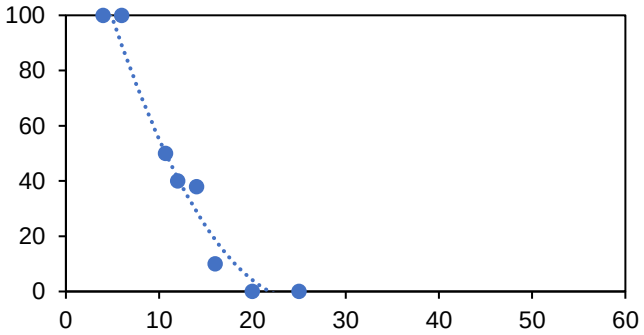
$R^2 = 0.999$

$EC50_{LOW}$ = 19.3 g/L, $EC50_{HIGH}$ = 32.1 g/L

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Table S5.5. Continues

Scrobicularia plana (Verdelhos et al., 2015) (Peppery furrow shell)

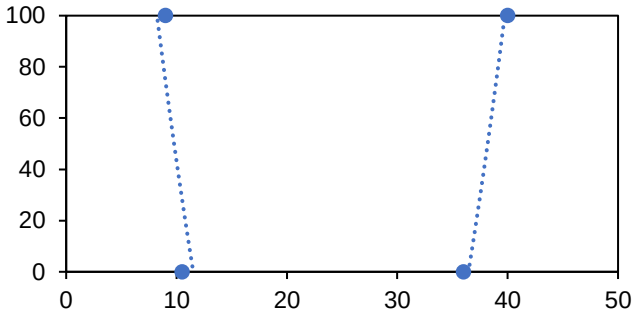


Function: $y = 0.2414 \cdot x^2 - 12.349 \cdot x + 154.57$

$R^2 = 0.966$

$EC50_{LOW}$ = 10.7 g/L, $EC50_{HIGH}$ = 40.4 g/L

Mytilus galloprovincialis (Gaag et al., 2016) (Mediterranean Mussel)



Information provided by other studies reveal that the Mediterranean mussel adapts worse to salinities around 11 (non-lethal effects observed), but mortality only increases to 10%, while it adapts good for salinity of 37(Hamer et al., 2008). This is in concordance with the lethal effects observed for M. edulis (blue mussel, not found in Galicia), as mortality sharply increases for salt concentrations of about 9 and 40 g/L. Therefore, we assumed that both mytilids present the same salt tolerance. The chronic data provided information for both ranges of the curve, fitting an exponential profile.

Function: $y = 1.074 \cdot x^2 - 51.534 \cdot x + 451.16$

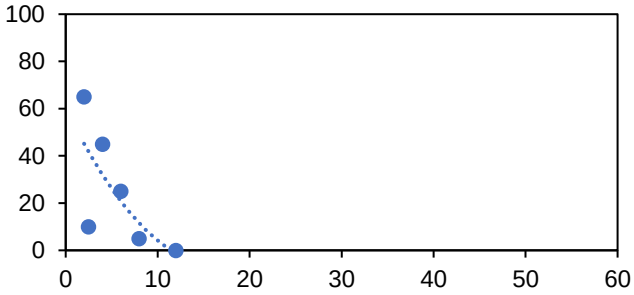
$R^2 = 0.832$

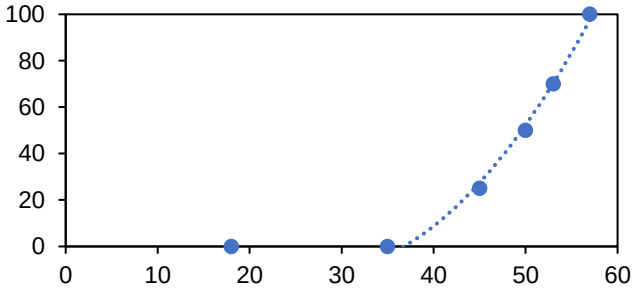
$EC50_{LOW}$ = 9.8 g/L, $EC50_{HIGH}$ = 38.2 g/L

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Table S5.5. Continues

Zostera noltei (Fernández-Torquemada and Sánchez-Lizaso, 2011; Salo et al., 2014) (Dwarf eelgrass, seagrass)





The functions provided a better fitting if they were adjusted separately for each range.

Functions: $y_{LOW} = 0.2402 \cdot x^2 - 8.003 \cdot x + 60.199$

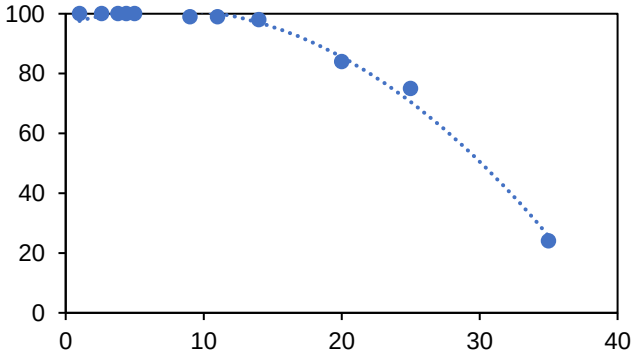
$R^2 = 0.499$

$y_{HIGH} = 0.1249 \cdot x^2 - 6.8814 \cdot x + 84.254$

$R^2 = 0.996$

$EC50_{LOW}$ = 1.3 g/L, $EC50_{HIGH}$ = 49.6 g/L

Saccorhiza polyschides (Norton and South, 1969) (Furbellow, brown algae)



Function: $y = -0.1056 \cdot x^2 + 0.8701 \cdot x + 98.721$

$R^2 = 0.9434$

$EC50_{LOW}$ = 30.1 g/L

To calculate $EC50_{HIGH}$, the inverse function was used, being:

Function: $y_{HIGH} = (x - 39.36) \cdot (x + 24.8)$

$EC50_{LOW}$ = 40.1 g/L

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

USC
UNIVERSIDADE
DE SANTIAGO
DE COMPOSTELA

REFERENCES

ACS Newsroom, 2020. Methods for microplastics, nanoplastics and plastic monomer detection and reporting in human tissues.

Ahmed, I., Zia, M.A., Afzal, H., Ahmed, S., Ahmad, M., Akram, Z., Sher, F., Iqbal, H.M.N., 2021. Socio-economic and environmental impacts of biomass valorisation: A strategic drive for sustainable bioeconomy. Sustain. 13, 1–32. https://doi.org/10.3390/su13084200

Akhbarizadeh, R., Dobaradaran, S., Nabipour, I., Tajbakhsh, S., Darabi, A.H., Spitz, J., 2020. Abundance, composition, and potential intake of microplastics in canned fish. Mar. Pollut. Bull. 160, 111633. https://doi.org/10.1016/j.marpolbul.2020.111633

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

Alcalá, F.J., Custodio, E., 2008. Using the Cl/Br ratio as a tracer to identify the origin of salinity in aquifers in Spain and Portugal. J. Hydrol. 359, 189–207. https://doi.org/10.1016/j.jhydrol.2008.06.028

Alibardi, L., Cossu, R., 2016. Effects of carbohydrate, protein and lipid content of organic waste on hydrogen production and fermentation products. Waste Manag. 47, 69–77. https://doi.org/10.1016/j.wasman.2015.07.049

Allegue, L.B., Hinge, J., 2014. Biogas upgrading Evaluation of methods for H 2 S removal. Danish Technol. Inst. 31. https://doi.org/10.1074/mcp.M110.002766

Alvarez, I., DeCastro, M., Gomez-Gesteira, M., Prego, R., 2005. Inter- and intra-annual analysis of the salinity and temperature evolution in the Galician Rías Baixas-ocean boundary (northwest Spain). J. Geophys. Res. 110, 1–14. https://doi.org/10.1029/2004JC002504

Ammendolia, J., Walker, T.R., 2022. Citizen science: A way forward in tackling the plastic pollution crisis during and beyond the COVID-19 pandemic. Sci. Total Environ. 805, 149957. https://doi.org/10.1016/j.scitotenv.2021.149957

Amores, M.J., Verones, F., Raptis, C., Juraske, R., Pfister, S., Stoessel, F., Antón, A., Castells, F., Hellweg, S., 2013. Biodiversity impacts from salinity increase in a coastal wetland. Environ. Sci. Technol. 47, 6384–6392. https://doi.org/10.1021/es3045423

Andreasi Bassi, S., Boldrin, A., Frenna, G., Astrup, T.F., 2021. An environmental and economic assessment of bioplastic from urban biowaste. The example of polyhydroxyalkanoate. Bioresour. Technol. 327, 124813. https://doi.org/10.1016/j.biortech.2021.124813

Antelava, A., Damilos, S., Hafeez, S., Manos, G., Al-Salem, S.M., Sharma, B.K., Kohli, K., Constantinou, A., 2019. Plastic Solid Waste (PSW) in the Context of Life Cycle Assessment (LCA) and Sustainable Management. Environ. Manage. 64, 230–244. https://doi.org/10.1007/s00267-019-01178-3

APHA-AWWA-WEF, 2017. Standard Methods for the examination of wastewater, 23rd ed. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC, USA.

APROMAR, 2019. Apromar Informe acuicultura 2019.

Arcos-Hernandez, M. V., Laycock, B., Pratt, S., Donose, B.C., Nikolič, M.A.L., Luckman, P., Werker, A., Lant, P.A., 2012. Biodegradation in a soil environment of activated sludge derived polyhydroxyalkanoate (PHBV). Polym. Degrad. Stab. 97, 2301–2312. https://doi.org/10.1016/j.polymdegradstab.2012.07.035

Argiz, L., Fra-Vázquez, A., Val del Río, Á., Mosquera-Corral, A., 2020a. Optimization of an enriched mixed culture to increase PHA accumulation using industrial saline complex wastewater as a substrate. Chemosphere 247. https://doi.org/10.1016/j.chemosphere.2020.125873

Argiz, L., Gonzalez-Cabaleiro, R., Correa-Galeote, D., Val del Río, A., 2021. Open-culture biotechnological process for triacylglycerides and polyhydroxyalkanoates recovery from industrial waste fish oil under saline conditions. Sep. Purif. Technol. 270, 118805. https://doi.org/10.1016/j.seppur.2021.118805

Argiz, L., González-Cabaleiro, R., Val del Río, Á., González-López, J., Mosquera-Corral, A., 2020b. A novel strategy for triacylglycerides and polyhydroxyalkanoates production using waste lipids. Sci. Total Environ. 763, 142944. https://doi.org/10.1016/j.scitotenv.2020.142944

Arif, A.U.A., Sorour, M.T., Aly, S.A., 2020. Cost analysis of activated sludge and membrane bioreactor WWTPs using CapdetWorks simulation program: Case study of Tikrit WWTP (middle Iraq). Alexandria Eng. J. 59, 4659–4667. https://doi.org/10.1016/j.aej.2020.08.023

Augas de Galicia, 2021. Rede de aforos [WWW Document]. URL https://augasdegalicia.xunta.gal/portada (accessed 3.29.21).

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

Basset, N., Katsou, E., Frison, N., Malamis, S., Dosta, J., Fatone, F., 2016. Integrating the selection of PHA storing biomass and nitrogen removal via nitrite in the main wastewater treatment line. Bioresour. Technol. 200, 820–829. https://doi.org/10.1016/j.biortech.2015.10.063

Battista, F., Frison, N., Bolzonella, D., 2021. Can bioplastics be treated in conventional anaerobic digesters for food waste treatment ? Environ. Technol. Innov. 22, 101393. https://doi.org/10.1016/j.eti.2021.101393

Begotti, S., 2017. Processes for recovering and purifying polyhydroxyalkanoates from cell cultures. US 9683076 B2.

Bello Bugallo, P.M., Stupak, A., Cristóbal Andrade, L., Torres López, R., 2012. Material flow analysis in a cooked mussel processing industry. J. Food Eng. 113, 100–117. https://doi.org/10.1016/j.jfoodeng.2012.05.014

Bengtsson, S., Karlsson, A., Alexandersson, T., Quadri, L., Hjort, M., Johansson, P., Morgan-Sagastume, F., Anterrieu, S., Arcos-Hernandez, M., Karabegovic, L., Magnusson, P., Werker, A., 2017a. A process for polyhydroxyalkanoate (PHA) production from municipal wastewater treatment with biological carbon and nitrogen removal demonstrated at pilot-scale. N. Biotechnol. 35, 42–53. https://doi.org/10.1016/J.NBT.2016.11.005

Bengtsson, S., Werker, A., Visser, C., Korving, L., 2017b. PHARIO Stepping stone to a sustainable value chain for PHA bioplastic using municipal activated sludge.

Berger, E., Frör, O., Schäfer, R.B., 2019. Salinity impacts on river ecosystem processes: A critical mini-review. Philos. Trans. R. Soc. B Biol. Sci. 374. https://doi.org/10.1098/rstb.2018.0010

Bergerson, J.A., Brandt, A., Cresko, J., Carbajales-dale, M., Maclean, H.L., Matthews, H.S., Mccoy, S., Mcmanus, M., Miller, S.A., Morrow, W.R., Posen, I.D., Seafer, T., Skone, T., Sleep, S., 2019. Life cycle assessment of emerging technologies. Evaluation techniques at different stages of market and technical maturity 1–15. https://doi.org/10.1111/jiec.12954

Bevilacqua, R., Regueira, A., Mauricio-Iglesias, M., Lema, J.M., Carballa, M., 2020. Protein composition determines the preferential consumption of amino acids during anaerobic mixed-culture fermentation. Water Res. 183, 115958. https://doi.org/10.1016/j.watres.2020.115958

Bhattacharyya, A., Saha, J., Haldar, S., Bhowmic, A., Mukhopadhyay, U.K., Mukherjee, J., 2014. Production of poly-3-(hydroxybutyrate-co-hydroxyvalerate) by Haloferax mediterranei using rice-based ethanol stillage with simultaneous recovery and re-use of medium salts. Extremophiles 18, 463–470. https://doi.org/10.1007/s00792-013-0622-9

Böhme, D., 2011. Evaluation of brine discharge to rivers and streams: Methodology of rapid impact assessment. Limnologica 41, 80–89. https://doi.org/10.1016/j.limno.2010.08.003

Boletín Oficial de la Provincia de A Coruña, 2016. BOP nº227. Corrección de erros publicación texto definitivo ordenanza de verteduras e do servizo municipal de saneamento do concello de Riveira. Spain.

Boulay, A.M., Verones, F., Vázquez-Rowe, I., 2021. Marine plastics in LCA: current status and MarILCA's contributions. Int. J. Life Cycle Assess. 2105–2108. https://doi.org/10.1007/s11367-021-01975-1

Bower, C.E., Holm-Hansen, T., 1980. A Salicylate–Hypochlorite Method for Determining Ammonia in Seawater. Can. J. Fish. Aquat. Sci. 37, 794–798. https://doi.org/10.1139/f80-106

Bramwell, G., Schultz, A., Sherman, C., Giraudeau, M., Thomas, F., Ujvari, B., Dujon, A.M., 2021. A review of the potential effects of climate change on disseminated neoplasia with an emphasis on efficient detection in marine bivalve populations. Sci. Total Environ. 775, 145134. https://doi.org/10.1016/j.scitotenv.2021.145134

Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G.S., Limburg, K.E., Montes, I., Naqvi, S.W.A., Pitcher, G.C., Rabalais, N.N., Roman, M.R., Rose, K.A., Seibel, B.A., Telszewski, M., Yasuhara, M., Zhang, J., 2018. Declining oxygen in the global ocean and coastal waters. Science (80-.). 359. https://doi.org/10.1126/science.aam7240

Browning, S., Beymer-Farris, B., Seay, J.R., 2021. Addressing the challenges associated with plastic waste disposal and management in developing countries. Curr. Opin. Chem. Eng. 32, 100682. https://doi.org/10.1016/j.coche.2021.100682

Burck, C., 2018. Techno-Economic Modeling for New Technology Development [WWW Document]. URL https://www.aiche.org/resources/publications/cep/2018/january/techno-economic-modeling-new-technology-development

Campos, J.L., Valenzuela-Heredia, D., Pedrouso, A., Val Del Río, A., Belmonte, M., Mosquera-Corral, A., 2016. Greenhouse Gases Emissions from Wastewater Treatment Plants: Minimization, Treatment, and Prevention. J. Chem. 2016. https://doi.org/10.1155/2016/3796352

Carral, L., Barros, J.J.C., Fidalgo, H.C., Fabal, C.C., Doce, A.M., 2021. Greenhouse gas emissions and energy consumption of coastal ecosystem enhancement programme through sustainable artificial reefs in Galicia. Int. J. Environ. Res. Public Health 18, 1–19. https://doi.org/10.3390/ijerph18041909

Carregosa, V., Velez, C., Soares, A.M.V.M., Figueira, E., Freitas, R., 2014. Physiological and biochemical responses of three Veneridae clams exposed to salinity changes. Comp. Biochem. Physiol. Part B 177–178, 1–9. https://doi.org/10.1016/j.cbpb.2014.08.001

Changwichan, K., Silalertruksa, T., Gheewala, S.H., 2018. Eco-efficiency assessment of bioplastics production systems and end-of-life options. Sustain. 10, 1–15. https://doi.org/10.3390/su10040952

Chanprateep, S., 2010. Current trends in biodegradable polyhydroxyalkanoates. J. Biosci. Bioeng. 110, 621–632. https://doi.org/10.1016/j.jbiosc.2010.07.014

Chen, W., Oldfield, T.L., Cinelli, P., Righetti, M.C., Holden, N.M., 2020. Hybrid life cycle assessment of potato pulp valorisation in biocomposite production. J. Clean. Prod. 269. https://doi.org/10.1016/j.jclepro.2020.122366

Colombo, B., Pereira, J., Martins, M., Torres-Acosta, M.A., Dias, A.C.R.V., Lemos, P.C., Ventura, S.P.M., Eisele, G., Alekseeva, A., Adani, F., Serafim, L.S., 2020. Recovering PHA from mixed microbial biomass: Using non-ionic surfactants as a pretreatment step. Sep. Purif. Technol. 253. https://doi.org/10.1016/j.seppur.2020.117521

Colombo, B., Villegas Calvo, M., Pepè Sciarria, T., Scaglia, B., Savio Kizito, S., D'Imporzano, G., Adani, F., 2019. Biohydrogen and polyhydroxyalkanoates (PHA) as products of a two-steps bioprocess from deproteinized dairy wastes. Waste Manag. 95, 22–31. https://doi.org/10.1016/j.wasman.2019.05.052

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Conejo-Watt, H., Luisetti, T., 2019. Tackling Marine Litter in the Atlantic Area.

Consellería de Medio Rural e Mar, 2015. DOG nº 21. Galicia.

Consello Regulador do Mexillón de Galicia, 2020. Mexillón de Galicia. [WWW Document]. URL https://www.mexillondegalicia.org/productos-dop-2/comercializadores/ (accessed 4.6.20).

Coulson, J.M., Richardson, J.F., 1999. Chemical Engineering, 6th ed. Butterworth Heinemann, Oxford.

Cristóvão, R.O., Gonçalves, C., Botelho, C.M., Martins, R.J.E., Loureiro, J.M., Boaventura, R.A.R., 2015. Fish canning wastewater treatment by activated sludge: Application of factorial design optimization. Biological treatment by activated sludge of fish canning wastewater. Water Resour. Ind. 10, 29–38. https://doi.org/10.1016/j.wri.2015.03.001

Crutchik, D., Franchi, O., Caminos, L., Jeison, D., Belmonte, M., Pedrouso, A., Val del Rio, A., Mosquera-Corral, A., Campos, J.L., 2020. Polyhydroxyalkanoates (PHAs) production: A feasible economic option for the treatment of sewage sludge in municipalwastewater treatment plants? Water (Switzerland) 12, 1–12. https://doi.org/10.3390/W12041118

de Oliveira, G.H.D., Niz, M.Y.K., Zaiat, M., Rodrigues, J.A.D., 2019. Effects of the Organic Loading Rate on Polyhydroxyalkanoate Production from Sugarcane Stillage by Mixed Microbial Cultures. Appl. Biochem. Biotechnol. 189, 1039–1055. https://doi.org/10.1007/s12010-019-03051-9

de Souza Machado, A.A., Kloas, W., Zarfl, C., Hempel, S., Rillig, M.C., 2018. Microplastics as an emerging threat to terrestrial ecosystems. Glob. Chang. Biol. 24, 1405–1416. https://doi.org/10.1111/gcb.14020

Di Bartolo, A., Infurna, G., Dintcheva, N.T., 2021. A review of bioplastics and their adoption in the circular economy. Polymers (Basel). 13. https://doi.org/10.3390/polym13081229

Dias, R. da S., Naves, A., Pisani, B., García-Tomillo, A., 2019. Comparación de la concentración y del lixiviado de cloruro y sulfato en dos cuencas con diferente distancia a la costa. Conference proceeding, vol XIV., in: Jornadas de La Zona No Saturada. Madrid, Spain, p. 10.

Dietrich, K., Dumont, M.J., Del Rio, L.F., Orsat, V., 2017. Producing PHAs in the bioeconomy — Towards a sustainable bioplastic. Sustain. Prod. Consum. 9, 58–70. https://doi.org/10.1016/j.spc.2016.09.001

Dirección Xeral de Calidade e Avaliación Ambiental, 2008a. 2007/0016_NAA/IPPC.

Dirección Xeral de Calidade e Avaliación Ambiental, 2008b. 2006/0347_NAA/IPPC.

Dirección Xeral de Calidade e Avaliación Ambiental, 2008c. Resolución de 27 de Xuño de 2008, 2006/0347_NAA/IPPC.

Domínguez, R., Vázquez, E., Woodin, S.A., Wethey, D.S., Peteiro, L.G., Macho, G., Olabarria, C., 2020. Sublethal responses of four commercially important bivalves to low salinity. Ecol. Indic. 111, 106031. https://doi.org/10.1016/j.ecolind.2019.106031

Du, J., Park, K., Jensen, C., Dellapenna, T.M., Zhang, W.G., Shi, Y., 2021. Massive oyster kill in Galveston Bay caused by prolonged low-salinity exposure after Hurricane Harvey. Sci. Total Environ. 774, 145132. https://doi.org/10.1016/j.scitotenv.2021.145132

Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric Method for Determination of Sugars and Related Substances. Anal. Chem. 28, 350–356. https://doi.org/10.1021/ac60111a017

Durack, P.J., 2015. Ocean salinity and the global water cycle. Oceanography 28, 20–31. https://doi.org/10.5670/oceanog.2015.03

EC, 2022. EU taxonomy for sustainable activities [WWW Document]. URL https://ec.europa.eu/info/business-economy-euro/banking-and-finance/sustainable-finance/eu-taxonomy-sustainable-activities_en#regulation (accessed 1.19.22).

Eleconomista.es, 2018. Ranking de Empresas del sector Fabricación de conservas de pescado [WWW Document]. URL https://ranking-empresas.eleconomista.es/sector-1022.html?_ga=2.173580897.398242025.1588167155-2135159228.1587568983# (accessed 4.29.20).

Elías, X., 2012. Reciclaje de residuos industriales, 2nd ed. Díaz de Santos, Madrid.

Elliott, M., Day, J.W., Ramachandran, R., Wolanski, E., Fang, Q., Sheehan, M.R., Seen, A.J., Ellison, J.C., 2019. A Synthesis: What Is the Future for Coasts, Estuaries, Deltas and Other Transitional Habitats in 2050 and Beyond?, in: Wolanski, E., Day, J.W., Elliott, M., Ramachandran, R. (Eds.), Coasts and Estuaries: The Future. Elsevier Inc., pp. 1–28. https://doi.org/10.1016/B978-0-12-814003-1.00001-0

EN, 2007. DIN EN 13432. Requirements for packaging recoverable through composting and biodegradation - Test scheme and evaluation criteria for the final acceptance of packaging.

Estévez-Alonso, Á., Pei, R., van Loosdrecht, M.C.M., Kleerebezem, R., Werker, A., 2021. Scaling-up microbial community-based polyhydroxyalkanoate production: status and challenges. Bioresour. Technol. 327, 124790. https://doi.org/10.1016/j.biortech.2021.124790

Estévez, E., Rodríguez-Castillo, T., González-Ferreras, A.M., Cañedo-Argüelles, M., Barquín, J., 2019. Drivers of spatio-temporal patterns of salinity in Spanish rivers: A nationwide assessment. Philos. Trans. R. Soc. B Biol. Sci. 374, 1–10. https://doi.org/10.1098/rstb.2018.0022

European Bioeconomy Alliance, 2021. EUBA position on the JRC LCA Methodology.

European Commission, 2020. A new Circular Economy Action Plan for a cleaner and more competitive Europe, COM(2020) 98 final.

European Commission, 2019a. Directive (EU) 2019/904 of the European Parliament and of the Council of 5 June 2019 on the reduction of the impact of certain plastic products on the environment.

European Commission, 2019b. The European Green Deal. Eur. Comm. 53, 24. https://doi.org/10.1017/CBO9781107415324.004

European Commission, 2018a. A European Strategy for Plastics in a Circular Economy.

European Commission, 2018b. Reducing Marine Litter: action on single use plastics and fishing gear. Brussels.

European Commission, 2015. Circular Economy Action Plan for a cleaner and more competitive Europe.

Fantke, P., Bijster, M., Guignard, C., Hauschild, M., Huijbregts, M., Jolliet, O., A., K., Magaud, V., Margni, M., TE., M., Posthuma, L., Rosenbaum, R.K., van de Meent, D., Zelm, V., 2018. USEtox 2.0 Documentation (Version 1). https://doi.org/10.11581/DTU:00000011

FAO, 2020. GLOBEFISH - Information and Analysis on World Fish Trade [WWW Document]. Eur. Mark. mussels. URL http://www.fao.org/in-action/globefish/fishery-information/resource-detail/en/c/338588/ (accessed 7.22.20).

Feitz, A.J., Lundie, S., 2002. Soil salinisation: A local life cycle assessment impact category. Int. J. Life Cycle Assess. 7, 244–249. https://doi.org/10.1007/BF02978880

Fekete, B.M., Vörösmarty, C.J., Grabs, W., 2002. UNH/GRDC Composite Runoff Fields V1.0 [WWW Document]. URL https://www.compositerunoff.sr.unh.edu/index.html (accessed 3.29.21).

Fernández-Braña, Á., Feijoo-Costa, G., Dias-Ferreira, C., 2019. Looking beyond the banning of lightweight bags: analysing the role of plastic (and fuel) impacts in waste collection at a Portuguese city. Environ. Sci. Pollut. Res. 26, 35629–35647. https://doi.org/10.1007/s11356-019-05938-w

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

Fernández-Dacosta, C., Posada, J.A., Ramirez, A., 2016. Techno-economic and carbon footprint assessment of methyl crotonate and methyl acrylate production from wastewater-based polyhydroxybutyrate (PHB). J. Clean. Prod. 137, 942–952. https://doi.org/10.1016/j.jclepro.2016.07.152

Fernández-Torquemada, Y., Sánchez-Lizaso, J.L., 2011. Responses of two Mediterranean seagrasses to experimental changes in salinity. Hydrobiologia 699, 21–33. https://doi.org/10.1007/s10750-011-0644-1

Fiett, P.P., n.d. Salinity tolerance and osmoregulatory function of mannitol in Danish ecotype of Saccharina latissima 1–24.

Ford, H. V., Jones, N.H., Davies, A.J., Godley, B.J., Jambeck, J.R., Napper, I.E., Suckling, C.C., Williams, G.J., Woodall, L.C., Koldewey, H.J., 2022. The fundamental links between climate change and marine plastic pollution. Sci. Total Environ. 806, 150392. https://doi.org/10.1016/j.scitotenv.2021.150392

Fox, D.R., van Dam, R.A., Fisher, R., Batley, G.E., Tillmanns, A.R., Thorley, J., Schwarz, C.J., Spry, D.J., McTavish, K., 2021. Recent Developments in Species Sensitivity Distribution Modeling. Environ. Toxicol. Chem. 40, 293–308. https://doi.org/10.1002/etc.4925

Fra-Vázquez, A., Pedrouso, A., Palmeiro-Sánchez, T., Moralejo-Gárate, H., Mosquera-Corral, A., 2018. Feasible microbial accumulation of triacylglycerides from crude glycerol. J. Chem. Technol. Biotechnol. 93, 2644–2651. https://doi.org/10.1002/jctb.5618

Fra-Vázquez, A., Pedrouso, A., Val del Rio, A., Mosquera-Corral, A., 2020. Volatile fatty acid production from saline cooked mussel processing wastewater at low pH. Sci. Total Environ. 732, 139337. https://doi.org/10.1016/j.scitotenv.2020.139337

Fra-Vázquez, A., Santorio, S., Palmeiro-Sánchez, T., Val del Río, Á., Mosquera-Corral, A., 2019. PHA accumulation of a mixed microbial culture co-exists with ammonia partial nitritation. Chem. Eng. J. 360, 1255–1261. https://doi.org/10.1016/j.cej.2018.10.207

Freitas, R., Pires, A., Velez, C., Almeida, Â., Wrona, F.J., Soares, A.M.V.M., Figueira, E., 2015. The effects of salinity changes on the Polychaete Diopatra neapolitana: Impacts on regenerative capacity and biochemical markers. Aquat. Toxicol. 163, 167–176. https://doi.org/10.1016/j.aquatox.2015.04.006

Frison, N., Katsou, E., Malamis, S., Oehmen, A., Fatone, F., 2015. Development of a Novel Process Integrating the Treatment of Sludge Reject Water and the Production of Polyhydroxyalkanoates (PHAs). https://doi.org/10.1021/acs.est.5b01776

Gaag, M. Van Der, Velde, G. Van Der, Wijnhoven, S., 2016. Salinity as a barrier for ship hull - related dispersal and invasiveness of dreissenid and mytilid bivalves. Mar. Biol. 163, 1–13. https://doi.org/10.1007/s00227-016-2926-7

Galloway, T.S., Cole, M., Lewis, C., 2017. Interactions of microplastic debris throughout the marine ecosystem. Nat. Ecol. Evol. 1, 1–8. https://doi.org/10.1038/s41559-017-0116

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, 25–29. https://doi.org/10.1126/sciadv.1700782

Gharbi, A., Farcy, E., Van Wormhoudt, A., Denis, F., 2016. Response of the carpet shell clam (Ruditapes decussatus) and the Manila clam (Ruditapes philippinarum) to salinity stress. Biologia (Bratisl). 71, 551–562. https://doi.org/10.1515/biolog-2016-0072

Giacomella, L., 2020. Techno economic assessment (TEA) and Life Cycle Costing analysis (LCCA): discussing methodological steps and integrability. University of Ferrara.

GlobalPetrolPrices, 2022. Spain electricity prices [WWW Document]. URL https://www.globalpetrolprices.com/Spain/electricity_prices/

González-López, M., 2018. Innovation Patterns in the Canned Fish Industry in Galicia (Spain). J. Entrep. Manag. Innov. 14, 45–64. https://doi.org/10.7341/20181413

Gupta, J., Rathour, R., Medhi, K., Tyagi, B., Thakur, I.S., 2019. Microbial-derived natural bioproducts for a sustainable environment: A bioprospective for waste to wealth, in: Kumar, R.P., Raman, J.K., Gnansounou, E., Baskar, G. (Eds.), Refining Biomass Residues for Sustainable Energy and Bioproducts: Technology, Advances, Life Cycle Assessment, and Economics. Elsevier Inc., pp. 51–85. https://doi.org/10.1016/B978-0-12-818996-2.00003-X

Gurieff, N., Lant, P., 2007. Comparative life cycle assessment and financial analysis of mixed culture polyhydroxyalkanoate production. Bioresour. Technol. https://doi.org/10.1016/j.biortech.2006.10.046

Halsband, C., Herzke, D., 2019. Plastic litter in the European Arctic: What do we know? Emerg. Contam. 5, 308–318. https://doi.org/10.1016/j.emcon.2019.11.001

Hamer, B., Jakšić, Ž., Pavičić-Hamer, D., Perić, L., Medaković, D., Ivanković, D., Pavičić, J., Zilberberg, C., Schröder, H.C., Müller, W.E.G., Smodlaka, N., Batel, R., 2008. Effect of hypoosmotic stress by low salinity acclimation of Mediterranean mussels Mytilus galloprovincialis on biological parameters used for pollution assessment. Aquat. Toxicol. 89, 137–151. https://doi.org/10.1016/j.aquatox.2008.06.015

Hao, J., Wang, X., Wang, H., 2017. Overall process of using a valerate- dominant sludge hydrolysate to produce high-quality polyhydroxyalkanoates (PHA) in a mixed culture 1–11. https://doi.org/10.1038/s41598-017-07154-3

Harding, K.G., Dennis, J.S., von Blottnitz, H., Harrison, S.T.L., 2007. Environmental analysis of plastic production processes: Comparing petroleum-based polypropylene and polyethylene with biologically-based poly-β-hydroxybutyric acid using life cycle analysis. J. Biotechnol. 130, 57–66. https://doi.org/10.1016/j.jbiotec.2007.02.012

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

Helm, K.P., Bindoff, N.L., Church, J.A., 2010. Changes in the global hydrological-cycle inferred from ocean salinity. Geophys. Res. Lett. 37, 2–6. https://doi.org/10.1029/2010GL044222

Hermann, B.G., Blok, K., Patel, M.K., 2007. Producing bio-based bulk chemicals using industrial biotechnology saves energy and combats climate change. Environ. Sci. Technol. 41, 7915–7921. https://doi.org/10.1021/es062559q

Hermann, B.G., Debeer, L., De Wilde, B., Blok, K., Patel, M.K., 2011. To compost or not to compost: Carbon and energy footprints of biodegradable materials' waste treatment. Polym. Degrad. Stab. 96, 1159–1171. https://doi.org/10.1016/j.polymdegradstab.2010.12.026

Heyde, M., 1998. Ecological considerations on the use and production of biosynthetic and synthetic biodegradable polymers. Polym. Degrad. Stab. 59, 3–6. https://doi.org/10.1016/s0141-3910(97)00017-7

Hottle, T.A., Bilec, M.M., Landis, A.E., 2013. Sustainability assessments of bio-based polymers. Polym. Degrad. Stab. 98, 1898–1907. https://doi.org/10.1016/j.polymdegradstab.2013.06.016

Huijbregts, M.A.J., Steinmann, Z.J.N., Elshout, P.M.F., Stam, G., Zelm, R. Van, 2017. ReCiPe2016: a harmonised life cycle impact assessment method at midpoint and endpoint level. Int. J. Life Cycle Assess. 138–147. https://doi.org/10.1007/s11367-016-1246-y

Hung, C.R., Ellingsen, L.A.-W., Majeau-Bettez, G., 2018. LiSET: A Framework for Early-Stage Life Cycle Screening of Emerging Technologies 1–12. https://doi.org/10.1111/jiec.12807

Illukpitiya, P., de Kof, J.P., 2014. Economics of Small-Scale Biodiesel Production. Bioenergy 7–9.

International Standards Organization (ISO), 2006. ISO 14040:2006, Environmental management. Life cycle assessment. Principles and framework.

IPCC, 2019. Chapter 6 wastewater treatment and discharge 2006, in: IPCC Guidelines for National Greenhouse Gas Inventories.

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science (80-.). 347, 768–771. https://doi.org/10.1126/science.1260352

Jefatura del Estado, 2011. Ley Orgánica 22/2011, Boletín Oficial del Estado.

Jia, Q., Wang, H., Wang, X., 2013. Dynamic synthesis of polyhydroxyalkanoates by bacterial consortium from simulated excess sludge fermentation liquid. Bioresour. Technol. 140, 328–336. https://doi.org/10.1016/j.biortech.2013.04.105

Jiang, G., Johnston, B., Townrow, D.E., Radecka, I., Koller, M., Chaber, P., Adamus, G., Kowalczuk, M., 2018. Biomass extraction using non-chlorinated solvents for biocompatibility improvement of polyhydroxyalkanoates. Polymers (Basel). 10. https://doi.org/10.3390/polym10070731

Jiang, Y., Hebly, M., Kleerebezem, R., Muyzer, G., van Loosdrecht, M.C.M., 2011. Metabolic modeling of mixed substrate uptake for polyhydroxyalkanoate (PHA) production. Water Res. 45, 1309–1321. https://doi.org/10.1016/j.watres.2010.10.009

Jin, B., Wang, S., Xing, L., Li, B., Peng, Y., 2016. The effect of salinity on waste activated sludge alkaline fermentation and kinetic analysis. J. Environ. Sci. 43, 80–90. https://doi.org/10.1016/j.jes.2015.10.011

Johnson, A.J., Shields, E.C., Kendrick, G.A., Orth, R.J., 2021. Recovery Dynamics of the Seagrass Zostera marina Following Mass Mortalities from Two Extreme Climatic Events. Estuaries and Coasts 44, 535–544. https://doi.org/10.1007/s12237-020-00816-y

Kannan, K., Vimalkumar, K., 2021. A Review of Human Exposure to Microplastics and Insights Into Microplastics as Obesogens. Front. Endocrinol. (Lausanne). 12, 1–19. https://doi.org/10.3389/fendo.2021.724989

Kefford, B.J., Marchant, R., Schäfer, R.B., Metzeling, L., Dunlop, J.E., Choy, S.C., Goonan, P., Victoria, E.P.A., 2011. The definition of species richness used by species sensitivity distributions approximates observed effects of salinity on stream macroinvertebrates. Environ. Pollut. 159, 302–310. https://doi.org/10.1016/j.envpol.2010.08.025

Kehrein, P., van Loosdrecht, M., Osseweijer, P., Posada, J., Dewulf, J., 2020. The SPPD-WRF framework: A novel and holistic methodology for strategical planning and process design of water resource factories. Sustain. 12. https://doi.org/10.3390/su12104168

Kendall, A., 2012. A life cycle assessment of biopolymer production from material recovery facility residuals. Resour. Conserv. Recycl. 61, 69–74. https://doi.org/10.1016/j.resconrec.2012.01.008

Khoo, H.H., Tan, R.B.H., 2010. Environmental impacts of conventional plastic and bio-based carrier bags: Part 2: End-of-life options. Int. J. Life Cycle Assess. 15, 338–345. https://doi.org/10.1007/s11367-010-0163-8

Khoo, H.H., Tan, R.B.H., Chng, K.W.L., 2010. Environmental impacts of conventional plastic and bio-Based carrier bags. Int. J. Life Cycle Assess. 15, 284–293. https://doi.org/10.1007/s11367-010-0162-9

Kim, S., Dale, B.E., 2008. Energy and greenhouse gas profiles of polyhydroxybutyrates derived from corn grain: A life cycle perspective. Environ. Sci. Technol. 42, 7690–7695. https://doi.org/10.1021/es8004199

Kim, S., Dale, B.E., 2005. Life cycle assessment study of biopolymers (Polyhydroxyalkanoates) derived from no-tilled corn. Int. J. Life Cycle Assess. 10, 200–210. https://doi.org/10.1065/lca2004.08.171

Kingsford, R.T., Brandis, K., Bino, G., Keith, D.A., 2020. Freshwater Biome of the World, Encyclopedia of the World's Biomes. Elsevier. https://doi.org/10.1016/b978-0-12-409548-9.12479-0

Koch, D., Mihalyi, B., 2018. Assessing the change in environmental impact categories when replacing conventional plastic with bioplastic in chosen application fields. Chem. Eng. Trans. 70, 853–858. https://doi.org/10.3303/CET1870143

Koller, M., Maršálek, L., de Sousa Dias, M.M., Braunegg, G., 2017. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. N. Biotechnol. 37, 24–38. https://doi.org/10.1016/j.nbt.2016.05.001

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

Koller, M., Sandholzer, D., Salerno, A., Braunegg, G., Narodoslawsky, M., 2013b. Biopolymer from industrial residues: Life cycle assessment of poly(hydroxyalkanoates) from whey. Resour. Conserv. Recycl. 73, 64–71. https://doi.org/10.1016/j.resconrec.2013.01.017

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

Korkakaki, E., Mulders, M., Veeken, A., Rozendal, R., van Loosdrecht, M.C.M.M., Kleerebezem, R., 2016a. PHA production from the organic fraction of municipal solid waste (OFMSW): Overcoming the inhibitory matrix. Water Res. 96, 74–83. https://doi.org/10.1016/j.watres.2016.03.033

Korkakaki, E., van Loosdrecht, M.C.M., Kleerebezem, R., 2016b. Survival of the fastest: Selective removal of the side population for enhanced PHA production in a mixed substrate enrichment. Bioresour. Technol. 216, 1022–1029. https://doi.org/10.1016/j.biortech.2016.05.125

Kosseva, M.R., Rusbandi, E., 2018. Trends in the biomanufacture of polyhydroxyalkanoates with focus on downstream processing. Int. J. Biol. Macromol. 107, 762–778. https://doi.org/10.1016/j.ijbiomac.2017.09.054

Kourmentza, C., Plácido, J., Venetsaneas, N., Burniol-Figols, A., Varrone, C., Gavala, H.N., Reis, M.A.M., 2017. Recent advances and challenges towards sustainable polyhydroxyalkanoate (PHA) production. Bioengineering 4, 1–43. https://doi.org/10.3390/bioengineering4020055

Kumar, M., Rathour, R., Singh, R., Sun, Y., Pandey, A., Gnansounou, E., Andrew Lin, K.Y., Tsang, D.C.W., Thakur, I.S., 2020. Bacterial polyhydroxyalkanoates: Opportunities, challenges, and prospects. J. Clean. Prod. 263, 121500. https://doi.org/10.1016/j.jclepro.2020.121500

Kurdikar, D., Fournet, L., Slater, S.C., Paster, M., Gruys, K.J., Gerngross, T.U., Coulon, R., 2000. Greenhouse gas profile of a plastic material derived from a genetically modified plant. J. Ind. Ecol. 4, 107–122. https://doi.org/10.1162/108819800300106410

Kutralam-Muniasamy, G., Pérez-Guevara, F., Shruti, V.C., 2022. A critical synthesis of current peer-reviewed literature on the environmental and human health impacts of COVID-19 PPE litter: New findings and next steps. J. Hazard. Mater. 422. https://doi.org/10.1016/j.jhazmat.2021.126945

Lacroix, C., Kerambrun, L., Gago Piñeiro, J., González Nuevo, G., Pérez, P., Fernández, M., Russel, J., 2020. Overview of marine litter status in the atlantic area: beach litter.

Lamberti, F.M., Román-Ramírez, L.A., Wood, J., 2020. Recycling of Bioplastics: Routes and Benefits. J. Polym. Environ. 28, 2551–2571. https://doi.org/10.1007/s10924-020-01795-8

Laycock, B., Nikolić, M., Colwell, J.M., Gauthier, E., Halley, P., Bottle, S., George, G., 2017. Lifetime prediction of biodegradable polymers. Prog. Polym. Sci. 71, 144–189. https://doi.org/10.1016/j.progpolymsci.2017.02.004

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

Leong, Y.K., Show, P.L., Lin, H.C., Chang, C.K., Loh, H.S., Lan, J.C.W., Ling, T.C., 2016. Preliminary integrated economic and environmental analysis of polyhydroxyalkanoates (PHAs) biosynthesis. Bioresour. Bioprocess. 3. https://doi.org/10.1186/s40643-016-0120-x

Leske, T., Buckley, C., 2004a. Towards the development of a salinity impact category for South African life cycle assessments: Part 2 - A conceptual multimedia environmental fate and effect model. Water SA 30, 241–251. https://doi.org/10.4314/wsa.v30i2.5070

Leske, T., Buckley, C., 2004b. Towards the development of a salinity impact category for South African life cycle assessments: Part 3 - Salinity potentials. Water SA 30, 253–265. https://doi.org/10.4314/wsa.v30i2.5071

Leske, T., Buckley, C., 2003. Towards the development of a salinity impact category for South African life cycle assessments: Part 1 – A new impact category. Water SA 30, 253–265. https://doi.org/10.4314/wsa.v30i2.5070

Lim, X.Z., 2021. Microplastics are everywhere - but are they harmful? Nature 593, 22–25. https://doi.org/10.1038/d41586-021-01143-3

Liu, S., Huang, J.H., Zhang, W., Shi, L.X., Yi, K.X., Yu, H.B., Zhang, C.Y., Li, S.Z., Li, J.N., 2022. Microplastics as a vehicle of heavy metals in aquatic environments: A review of adsorption factors, mechanisms, and biological effects. J. Environ. Manage. 302, 113995. https://doi.org/10.1016/j.jenvman.2021.113995

Liu, X., Hu, X., Wang, J., Song, Y., Wang, M., Liu, R., Duan, L., 2015. Evaluating properties of protein in waste activated sludge for volatile fatty acid production: effect of pH. Environ. Earth Sci. 73, 5047–5056. https://doi.org/10.1007/s12665-015-4194-0

Liu, Z., Liu, W., Walker, T.R., Adams, M., Zhao, J., 2021. How does the global plastic waste trade contribute to environmental benefits: Implication for reductions of greenhouse gas emissions? J. Environ. Manage. 287, 112283. https://doi.org/10.1016/j.jenvman.2021.112283

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

Lorini, L., di Re, F., Majone, M., Valentino, F., 2020. High rate selection of PHA accumulating mixed cultures in sequencing batch reactors with uncoupled carbon and nitrogen feeding. N. Biotechnol. 56, 140–148. https://doi.org/10.1016/j.nbt.2020.01.006

Lowry, O.H., Randall, R.J., 1951. Protein Measurement byt the Folin Reagent. J. Biol. Chem. 193, 265–275. https://doi.org/10.1016/0304-3894(92)87011-4

Lynd, L.R., Wang, M.Q., 2003. A product-nonspecific framework for evaluating the potential of biomass-based products to displace fossil fuels. J. Ind. Ecol. 7, 17–32. https://doi.org/10.1162/108819803323059370

Manfredi, S., Allacker, K., Chomkhamsri, K., Pelletier, N., Maia de Souza, D., 2012. Product Environmental Footprint (PEF) Guide.

Mannina, G., Presti, D., Montiel-Jarillo, G., Carrera, J., Suárez-Ojeda, M.E., 2020. Recovery of polyhydroxyalkanoates (PHAs) from wastewater: A review. Bioresour. Technol. 297, 122478. https://doi.org/10.1016/j.biortech.2019.122478

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

Marciniak, P., Mozejko-Ciesielska, J., 2021. What Is New in the Field of Industrial Wastes Conversion into Polyhydroxyalkanoates by Bacteria ? 13, 1731. https://doi.org/. https://doi.org/10.3390/polym13111731

MarILCA, 2020. MarILCA [WWW Document]. URL https://marilca.org/ (accessed 12.18.20).

Martinez-Hernandez, E., Ng, K.S., Allieri, M.A.A., Anell, J.A.A., Sadhukhan, J., 2018. Value-Added Products from Wastes Using Extremophiles in Biorefineries: Process Modeling, Simulation, and Optimization Tools, in: Sani, R.K., Rathinam, N.K. (Eds.), Extremophilic Microbial Processing of Lignocellulosic Feedstocks to Biofuels, Value-Added Products, and Usable Power. Springer, pp. 1–308. https://doi.org/10.1007/978-3-319-74459-9

Masiá, P., Mateo, J.L., Arias, A., Bartolomé, M., Blanco, C., Erzini, K., Le Loc'h, F., Mve Beh, J.H., Power, D., Rodriguez, N., Schaal, G., Machado-Schiaffino, G., Garcia-Vazquez, E., 2022. Potential microplastics impacts on African fishing resources. Sci. Total Environ. 806. https://doi.org/10.1016/j.scitotenv.2021.150671

Mcjannet, D.L., Webster, I.T., Cook, F.J., 2012. Environmental Modelling & Software An area-dependent wind function for estimating open water evaporation using land-based meteorological data. Environ. Model. Softw. 31, 76–83. https://doi.org/10.1016/j.envsoft.2011.11.017

Medina-Martos, E., Istrate, I.R., Dufour, J., 2019. Technoeconomic and environmental review of value-added products from wastewater: Bioplastic production and algal cultivation for biofuels, in: Olivares, J.A., Melero, J.A., Puyol, D., Dufour, J. (Eds.), Wastewater Treatment Residues as Resources for Biorefinery Products and Biofuels. Elsevier Inc., pp. 435–454. https://doi.org/https://doi.org/10.1016/C2017-0-03670-4

Mejillones Nidal, 2018. Nidal [WWW Document]. URL https://mejillonesnidal.com/instalaciones/ (accessed 6.4.20).

Mejillones Ría de Arosa, 2016. Mejillones ría de arosa ha ejecutado en 2016 una de las instalaciones de depuración más avanzada tecnológicamente del mercado [WWW Document]. URL http://www.mejillonesriadearosa.com/notaprensa01.html (accessed 4.29.20).

Melendez-Rodriguez, B., Castro-Mayorga, J.L., Reis, M.A.M., Sammon, C., Cabedo, L., Torres-Giner, S., Lagaron, J.M., 2018. Preparation and Characterization of Electrospun Food Biopackaging Films of Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) Derived From Fruit Pulp Biowaste. Front. Sustain. Food Syst. 2, 1–16. https://doi.org/10.3389/fsufs.2018.00038

USC DE COMPOSTELA

Meneses, M., Concepción, H., Vrecko, D., Vilanova, R., 2015. Life Cycle Assessment as an environmental evaluation tool for control strategies in wastewater treatment plants. J. Clean. Prod. 107, 653–661. https://doi.org/10.1016/j.jclepro.2015.05.057

Metcalf & Eddy, I., 2014. Wastewater Engineering Treatment and Reuse, 4th ed. McGraw Hill, New York.

MeteoGalicia, 2021. Rede Meteorolóxica [WWW Document]. URL https://www.meteogalicia.gal/observacion/estacions/estacions.action?request_locale=gl (accessed 3.30.21).

Ministerio de Agricultura Pesca y Alimentación, 2021. GeoPortal [WWW Document]. URL https://sig.mapama.gob.es/geoportal/ (accessed 3.29.21).

Ministerio de Transportes Movilidad y Agenda Urbana, 2021. Puertos del Estado [WWW Document]. URL http://www.puertos.es/es-es/Paginas/default.aspx (accessed 4.9.21).

Ministerio para la transición ecológica y el reto demográfico, 2018. Informe 2018 Autorizaciones de Vertido.

Mitra, R., Xu, T., Xiang, H., Han, J., 2020. Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. Microb. Cell Fact. 19, 1–30. https://doi.org/10.1186/s12934-020-01342-z

Moni, S.M., Mahmud, R., High, K., Carbajales-Dale, M., 2020. Life cycle assessment of emerging technologies: A review. J. Ind. Ecol. 24, 52–63. https://doi.org/10.1111/jiec.12965

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

Mosquera-Corral, A., Campos Gómez, J.L., 2021. Treatment and Valorisation of Saline Wastewater: Principles and Practice. IWA Publishing. https://doi.org/https://doi.org/10.2166/9781789060645

Mulders, M., Tamis, J., Abbas, B., Sousa, J., Dijkman, H., Rozendal, R., Kleerebezem, R., 2020. Pilot-Scale Polyhydroxyalkanoate Production from Organic Waste: Process Characteristics at High pH and High Ammonium Concentration. J. Environ. Eng. (United States) 146, 1–11. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001719

Muluneh, M.G., 2021. Impact of climate change on biodiversity and food security: a global perspective—a review article. Agric. Food Secur. 10, 1–25. https://doi.org/10.1186/s40066-021-00318-5

Musonda, F., Millinger, M., Thrän, D., 2020. Greenhouse gas abatement potentials and economics of selected biochemicals in Germany. Sustain. 12. https://doi.org/10.3390/su12062230

Nessi, S., Sinkko, T., Bulgheroni, C., Garcia-Gutierrez, P., Giuntoli, J., Konti, A., Sanye-Mengual, E., Tonini, D., Pant, R., Marelli, L., 2020. Comparative Life Cycle Assessment (LCA) of Alternative Feedstock for Plastics Production.

Nitkiewicz, T., Wojnarowska, M., Sołtysik, M., Kaczmarski, A., Witko, T., Ingrao, C., Guzik, M., 2020. How sustainable are biopolymers? Findings from a life cycle assessment of polyhydroxyalkanoate production from rapeseed-oil derivatives. Sci. Total Environ. 749. https://doi.org/10.1016/j.scitotenv.2020.141279

Norton, T.A., South, G.R., 1969. Influence of Reduced Salinity on the Distribution of Two Laminarian Algae. Nord. Soc. Oikos 20, 320–326.

Núñez, M., Finkbeiner, M., 2020. A Regionalised Life Cycle Assessment Model to Globally Assess the Environmental Implications of Soil Salinization in Irrigated Agriculture. Environ. Sci. Technol. 54, 3082–3090. https://doi.org/10.1021/acs.est.9b03334

OECD, 2013. Policies for Bioplastics in the Context of a Bioeconomy, OECD Science, Technology and Industry Policy Papers. https://doi.org/http://dx.doi.org/10.1787/5k3xpf9rrw6d-en

Oliveira, C.S.S., Silva, C.E., Carvalho, G., Reis, M.A., 2017. Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities. N. Biotechnol. 37, 69–79. https://doi.org/10.1016/j.nbt.2016.10.008

Omil, F., Méndez, R., Lema, J.M., 1995. Anaerobic treatment of saline wastewaters under high sulphide and ammonia content. Bioresour. Technol. https://doi.org/10.1016/0960-8524(95)00143-3

Owsianiak, M., Fantke, P., Posthuma, L., Saouter, E., Vijver, M., Backhaus, T., Schlekat, T., Hauschild, M., 2019. Chapter 7: Ecotoxicity, in: Frischknecht, R., Jolliet, O. (Eds.), Global Guidance on Environmental Life Cycle Impact Assessment Indicators. Volume 2. UNEP, pp. 138–172.

Palmeiro-Sánchez, T., Fra-Vázquez, A., Rey-Martínez, N., Campos, J.L., Mosquera-Corral, A., 2016. Transient concentrations of NaCl affect the PHA accumulation in mixed microbial culture. J. Hazard. Mater. 306, 332–339. https://doi.org/10.1016/j.jhazmat.2015.12.032

Palmeiro-Sánchez, T, Oliveira, C.S.S., Gouveia, A.R., Noronha, J.P., Ramos, A.M., Mosquera-Corral, A., Reis, M.A.M., 2016. NaCl presence and purification affect the properties of mixed culture PHAs. Eur. Polym. J. 85, 256–265. https://doi.org/10.1016/j.eurpolymj.2016.10.035

Palmeiro-Sánchez, T., Val Del Rio, A., Fra-Vázquez, A., Luis Campos, J., Mosquera-Corral, A., 2019. High-Yield Synthesis of Poly(3-hydroxybutyrate- co-3-hydroxyvalerate) Copolymers in a Mixed Microbial Culture: Effect of Substrate Switching and F/M Ratio. Ind. Eng. Chem. Res. 58, 21921–21926. https://doi.org/10.1021/acs.iecr.9b03514

Palmeiro-Sánchez, T., Val del Río, A., Mosquera-Corral, A., Campos, J.L., Méndez, R., 2013. Comparison of the anaerobic digestion of activated and aerobic granular sludges under brackish conditions. Chem. Eng. J. 231, 449–454. https://doi.org/10.1016/j.cej.2013.07.052

Pantaloni, D., Shah, D., Baley, C., Bourmaud, A., 2020. Monitoring of mechanical performances of flax non-woven biocomposites during a home compost degradation. Polym. Degrad. Stab. 177, 109166. https://doi.org/10.1016/j.polymdegradstab.2020.109166

Paolini, V., Petracchini, F., Segreto, M., Tomassetti, L., Naja, N., Cecinato, A., 2018. Environmental impact of biogas: A short review of current knowledge. J. Environ. Sci. Heal. - Part A Toxic/Hazardous Subst. Environ. Eng. 53, 899–906. https://doi.org/10.1080/10934529.2018.1459076

Parada, J.M., Molares, J., 2008. Natural mortality of the cockle Cerastoderma edule (L.) from the Ria of Arousa (NW Spain) intertidal zone 43, 501–511.

Parada, J.M., Molares, J., Otero, X., 2012. Multispecies Mortality Patterns of Commercial Bivalves in Relation to Estuarine Salinity Fluctuation. Estuaries and Coasts 132–142. https://doi.org/10.1007/s12237-011-9426-2

Park, J., Kim, M., Nam, B., Chang, H., Kim, D., 2021. Behavior of Surfactants in Oil Extraction by Surfactant-Assisted Acidic Hydrothermal Process from Chlorella vulgaris. Appl. Biochem. Biotechnol. 319–334. https://doi.org/https://doi.org/10.1007/s12010-020- 03426-3

Pavan, F.A., Junqueira, T.L., Watanabe, M.D.B., Bonomi, A., Quines, L.K., Schmidell, W., de Aragao, G.M.F., 2019. Economic analysis of polyhydroxybutyrate production by Cupriavidus necator using different routes for product recovery. Biochem. Eng. J. 146, 97–104. https://doi.org/10.1016/j.bej.2019.03.009

Payen, S., Basset-Mens, C., Núñez, M., Follain, S., Grünberger, O., Marlet, S., Perret, S., Roux, P., 2016. Salinisation impacts in life cycle assessment: a review of challenges and options towards their consistent integration. Int. J. Life Cycle Assess. 21, 577–594. https://doi.org/10.1007/s11367-016-1040-x

Pedrouso, A., Fra-vazquez, A., Val, A., Mosquera-Corral, A., 2020. Recovery of Polyhydroxyalkanoates from Cooked Mussel Processing Wastewater at High Salinity and Acidic Conditions. Sustainability 12. https://doi.org/10.3390/su122410386

Pérez-Rivero, C., López-Gómez, J.P., Roy, I., 2019. A sustainable approach for the downstream processing of bacterial polyhydroxyalkanoates: State-of-the-art and latest developments. Biochem. Eng. J. 150, 107283. https://doi.org/10.1016/j.bej.2019.107283

Pérez, V., Lebrero, R., Muñoz, R., 2020a. Comparative Evaluation of Biogas Valorization into Electricity/Heat and Poly(hydroxyalkanoates) in Waste Treatment Plants: Assessing the Influence of Local Commodity Prices and Current Biotechnological Limitations. ACS Sustain. Chem. Eng. 8, 7701–7709. https://doi.org/10.1021/acssuschemeng.0c01543

Pérez, V., Mota, C.R., Muñoz, R., Lebrero, R., 2020b. Polyhydroxyalkanoates (PHA) production from biogas in waste treatment facilities: Assessing the potential impacts on economy, environment and society. Chemosphere 255. https://doi.org/10.1016/j.chemosphere.2020.126929

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Perret, S.R., Payen, S., 2020. Irrigation and the Environmental Tragedy: Pathways Towards Sustainability in Agricultural Water Use. Irrig. Drain. 69, 263–271. https://doi.org/10.1002/ird.2404

Perry, R.H., 1997. Perry's chemical engineers' handbook, Seventh Ed. ed.

Peteiro, C., Sánchez, N., 2012. Comparing Salinity Tolerance in Early Stages of the Sporophytes of a Non indigenous Kelp (Undaria pinnatifida) and a Native Kelp (Saccharina latissima). J. Mar. Biol. 38, 197–200. https://doi.org/10.1134/S1063074012020095

Peteiro, L.G., Woodin, S.A., Wethey, D.S., Costas-costas, D., Martínez-casal, A., Olabarria, C., Vázquez, E., 2018. Responses to salinity stress in bivalves: Evidence of ontogenetic changes in energetic physiology on Cerastoderma edule. Sci. Rep. 1–9. https://doi.org/10.1038/s41598-018-26706-9

Piccinno, F., Hischier, R., Seeger, S., Som, C., 2016. From laboratory to industrial scale : a scale-up framework for chemical processes in life cycle assessment studies. J. Clean. Prod. 135, 1085–1097. https://doi.org/10.1016/j.jclepro.2016.06.164

Pietrini, M., Roes, L., Patel, M.K., Chiellini, E., 2007. Comparative life cycle studies on poly(3-hydroxybutyrate)-based composites as potential replacement for conventional petrochemical plastics. Biomacromolecules 8, 2210–2218. https://doi.org/10.1021/bm0700892

Pinto-ibieta, F., Serrano, A., Cea, M., Ciudad, G., Fermoso, F.G., 2021. Beyond PHA : Stimulating intracellular accumulation of added-value compounds in mixed microbial cultures. Bioresour. Technol. 337, 125381. https://doi.org/10.1016/j.biortech.2021.125381

Piscart, C., Kefford, B.J., Beisel, J., 2011. Are salinity tolerances of non-native macroinvertebrates in France an indicator of potential for their translocation in a new area ? Limnologica 41, 107–112. https://doi.org/10.1016/j.limno.2010.09.002

Plackett, D., 2011. Biopolymers - New Materials for Sustainable Films and Coatings, Biopolymers - New Materials for Sustainable Films and Coatings. https://doi.org/10.1002/9781119994312

Plastic Leak Project, 2020. Tackling plastic pollution: A prioneering methodology to measure plastic leakage and identify its pathways into the environment.

Pré Sustainability, 2021. SimaPro.

Ragusa, A., Svelato, A., Santacroce, C., Catalano, P., Notarstefano, V., Carnevali, O., Papa, F., Rongioletti, M.C.A., Baiocco, F., Draghi, S., D'Amore, E., Rinaldo, D., Matta, M., Giorgini, E., 2021. Plasticenta: First evidence of microplastics in human placenta. Environ. Int. 146, 106274. https://doi.org/10.1016/j.envint.2020.106274

Rathi, D.N., Amir, H.G., Abed, R.M.M., Kosugi, A., Arai, T., Sulaiman, O., Hashim, R., Sudesh, K., 2013. Polyhydroxyalkanoate biosynthesis and simplified polymer recovery by a novel moderately halophilic bacterium isolated from hypersaline microbial mats. J. Appl. Microbiol. 114, 384–395. https://doi.org/10.1111/jam.12083

Raza, Z.A., Abid, S., Banat, I.M., 2018. Polyhydroxyalkanoates: Characteristics, production, recent developments and applications. Int. Biodeterior. Biodegradation 126, 45–56. https://doi.org/10.1016/J.IBIOD.2017.10.001

Red Eléctrica Española, 2019. Generación eléctrica en España 2018.

Regueira, A., Bevilacqua, R., Lema, J.M., Carballa, M., Mauricio-Iglesias, M., 2020a. A metabolic model for targeted volatile fatty acids production by cofermentation of carbohydrates and proteins. Bioresour. Technol. 298, 122535. https://doi.org/10.1016/j.biortech.2019.122535

Regueira, A., Lema, J.M., Carballa, M., Mauricio-Iglesias, M., 2020b. Metabolic modeling for predicting VFA production from protein-rich substrates by mixed-culture fermentation. Biotechnol. Bioeng. 117, 73–84. https://doi.org/10.1002/bit.27177

Rey-Méndez, M., Fernández Casal, J., Lodeiros, C., Guerra, A., 2016. XVIII Foro dos recursos mariños e da acuicultura das rías galegas. Santiago de Compostela.

Reyes-martínez, M.J., Martínez-pita, I., Soler-navarro, D., García-garcía, F.J., 2020. The impact of salinity changes associated with size on the wedge clam Donax trunculus Linnaeus, 1758 (Mollusca Bivalvia): A laboratory assay. Estuar. , Coast. Shelf Sci. 241, 106838. https://doi.org/https://doi.org/10.1016/j.ecss.2020.106838

Righi, S.;, Baioli, F.;, Samorì, C.;, Galletti, P.;, Stramigioli, C.;, Tugnoli, A.;, Fantke, P., 2016. A life-cycle assessment of poly-hydroxybutyrate extraction from microbial biomass using dimethylcarbonate A life-cycle assessment of poly-hydroxybutyrate extraction from microbial biomass using dimethylcarbonate. 10th Ital. LCA Conf.

Risch, E., Loubet, P., Núñez, M., Roux, P., 2014. How environmentally significant is water consumption during wastewater treatment?: Application of recent developments in LCA to WWT technologies used at 3 contrasted geographical locations. Water Res. 7. https://doi.org/10.1016/j.watres.2014.03.023

Rodriguez-Perez, S., Serrano, A., Pantión, A.A., Alonso-Fariñas, B., 2018. Challenges of scaling-up PHA production from waste streams. A review. J. Environ. Manage. 205, 215–230. https://doi.org/10.1016/j.jenvman.2017.09.083

Rodriguez-Verde, I., Regueiro, L., Carballa, M., Hospido, A., Lema, J.M., 2014. Assessing anaerobic co-digestion of pig manure with agroindustrial wastes: The link between environmental impacts and operational parameters. Sci. Total Environ. 497–498, 475–483. https://doi.org/10.1016/j.scitotenv.2014.07.127

Roibás-Rozas, A., del Río, Á.V., Hospido, A., Mosquera-Corral, A., 2021. Strategies for the valorisation of a protein-rich saline waste stream into polyhydroxyalkanoates (PHA). Bioresour. Technol. 124964. https://doi.org/10.1016/j.biortech.2021.124964

Roibás-Rozas, A., Mosquera-Corral, A., Hospido, A., 2020. Environmental assessment of complex wastewater valorisation by polyhydroxyalkanoates production. Sci. Total Environ. 744, 140893. https://doi.org/10.1016/j.scitotenv.2020.140893

Roibás-Rozas, A., Núñez, M., Mosquera-Corral, A., Hospido, A., 2022a. Modeling the impact of salinity variations on aquatic environments: including negative and positive effects in Life Cycle Assessment. Environ. Sci. Technol. https://doi.org/https://doi.org/10.1021/acs.est.1c04656

Roibás-Rozas, A., Saavedra del Oso, M., Zarroli, G., Mauricio-Iglesias, M., Mosquera-Corral, A., Fiore, S., Hospido, A., 2022b. How can we validate the environmental profile of bioplastics? Towards the introduction of Polyhydroxyalkanoates (PHA) in the value-chains., in: Teodosiu, C., Fiore, S., Hospido, A. (Eds.), Assessing Progress toward Sustainability. Elsevier.

Roohi, Zaheer, M.R., Kuddus, M., 2018. PHB (poly-β-hydroxybutyrate) and its enzymatic degradation. Polym. Adv. Technol. 29, 30–40. https://doi.org/10.1002/pat.4126

Rosenbaum, R.K., Hauschild, M.Z., Boulay, A.-M., Fantke, P., Laurent, A., Núñez, M., Vieira, M., 2018. Life Cycle Impact Assessment, in: Hauschild, M.Z., Rosenbaum, R.K., Olsen, S.I. (Eds.), Life Cycle Assessment. Theory and Practice. Springer. https://doi.org/10.1007/978-3-319-56475-3_10

Saavedra del Oso, M., Mauricio-iglesias, M., Hospido, A., 2020. Evaluation and optimization of the environmental performance of PHA downstream processing. Chem. Eng. J. https://doi.org/10.1016/j.cej.2020.127687

Sabapathy, P.C., Devaraj, S., Meixner, K., Anburajan, P., Kathirvel, P., Ravikumar, Y., Zabed, H.M., Qi, X., 2020. Recent developments in Polyhydroxyalkanoates (PHAs) production – A review. Bioresour. Technol. 306, 123132. https://doi.org/10.1016/j.biortech.2020.123132

Saling, P., Gyuzeleva, L., Wittstock, K., Wessolowski, V., Griesshammer, R., 2020. Life cycle impact assessment of microplastics as one component of marine plastic debris. Int. J. Life Cycle Assess. https://doi.org/https://doi.org/10.1007/s11367-020-01802-z

Salo, T., Foldager, M., Boström, C., 2014. Population specific salinity tolerance in eelgrass (Zostera marina). J. Exp. Mar. Bio. Ecol. 461, 425–429. https://doi.org/10.1016/j.jembe.2014.09.010

Santorio, S., Fra-Vázquez, A., Val del Rio, A., Mosquera-Corral, A., 2019. Potential of endogenous PHA as electron donor for denitrification. Sci. Total Environ. 695. https://doi.org/10.1016/j.scitotenv.2019.133747

Schwabl, P., Köppel, S., Königshofer, P., Bucsics, T., Trauner, M., Reiberger, T., Liebmann, B., 2019. Detection of Various Microplastics in Human Stool. An. Intern. Med. https://doi.org/https://doi.org/10.7326/M19-0618

Secretaría xeral de Calidade de Avaliación Ambiental, 2013a. 2012-IPPC-I-14.

Secretaría xeral de Calidade de Avaliación Ambiental, 2013b. 2012-IPPC-M-80.

Sedlacek, P., Slaninova, E., Koller, M., Nebesarova, J., Marova, I., Krzyzanek, V., Obruca, S., 2019. PHA granules help bacterial cells to preserve cell integrity when exposed to sudden osmotic imbalances. N. Biotechnol. 49, 129–136. https://doi.org/10.1016/j.nbt.2018.10.005

Seider, W.D., Lewin, D.R., Seader, J.D., Widagdo, S., Gani, R., Ng, K.M., 2017. Product and process design principles. Wiley.

SETAC, 2008. Environmental Life Cycle Costing.

Shahid, S., Razzaq, S., Farooq, R., Nazli, Z. i. H., 2021. Polyhydroxyalkanoates: Next generation natural biomolecules and a solution for the world's future economy. Int. J. Biol. Macromol. 166, 297–321. https://doi.org/10.1016/j.ijbiomac.2020.10.187

Shahzad, K., Kettl, K.H., Titz, M., Koller, M., Schnitzer, H., Narodoslawsky, M., 2013. Comparison of ecological footprint for biobased PHA production from animal residues utilizing different energy resources. Clean Technol. Environ. Policy 15, 525–536. https://doi.org/10.1007/s10098-013-0608-4

Shahzad, K., Narodoslawsky, M., Sagir, M., Ali, N., Ali, S., Rashid, M.I., Ismail, I.M.I., Koller, M., 2017. Techno-economic feasibility of waste biorefinery: Using slaughtering waste streams as starting material for biopolyester production. Waste Manag. 67, 73–85. https://doi.org/10.1016/j.wasman.2017.05.047

Simó-Cabrera, L., García-Chumillas, S., Hagagy, N., Saddiq, A., Tag, H., Selim, S., AbdElgawad, H., Arribas Agüero, A., Monzó Sánchez, F., Cánovas, V., Pire, C., Martínez-Espinosa, R.M., 2021. Haloarchaea as Cell Factories to Produce Bioplastics. Mar. Drugs 19. https://doi.org/10.3390/md19030159

Smolders, G.J.F., van der Meij, J., van Loosdrecht, M.C.., Heijnen, J.., 1994. Stoichiometric model of the aerobic metabolism of the biological phosphorus removal process. Biotechnol. Bioeng. 44, 837–848. https://doi.org/https://doi.org/10.1002/bit.260440709

Smyth, K., Elliot, M., 2016. Effects of changing salinity on the ecology of the marine environment, in: Solan, M., Whiteley, N. (Eds.), Stressors in the Marine Environment: Physiological and Ecological Responses; Societal Implications. Oxford Scholarship, pp. 161–174. https://doi.org/DOI:10.1093/acprof:oso/9780198718826.003.0009

Soto, M., Veiga, M.C., Méndez, R., Lema, J.M., 1989. Semi-micro C.O.D. determination method for high-salinity wastewater. Environ. Technol. Lett. 10, 541–548. https://doi.org/https://doi.org/10.1080/09593338909384770

statista, 2021. Price of high-density polyethylene worldwide from 2017 to 2020 with estimated figures for 2021 to 2022 [WWW Document]. URL https://www.statista.com/statistics/1171074/price-high-density-polyethylene-forecast-globally/ (accessed 1.10.22).

Sun, Q., Du, Y., Xie, S.-P., Zhang, Y., Wang, M., Kosaka, Y., 2021. Sea Surface Salinity Change since 1950: Internal Variability versus Anthropogenic Forcing. J. Clim. 34, 1305–1319. https://doi.org/10.1175/JCLI-D-20-0331.1

Tabone, M.D., Cregg, J.J., Beckman, E.J., Landis, A.E., 2010. Sustainability Metrics: Life Cycle Assessment and Green Design in Polymers 44, 8264–8269. https://doi.org/10.1021/es101640n

Talan, A., Kaur, R., Tyagi, R.D., Drogui, P., 2020. Bioconversion of oily waste to polyhydroxyalkanoates: Sustainable technology with circular bioeconomy approach and multidimensional impacts. Bioresour. Technol. Reports 11, 100496. https://doi.org/10.1016/j.biteb.2020.100496

Tamis, J., Sorokin, D.Y., Jiang, Y., Loosdrecht, M.C.M. Van, Kleerebezem, R., 2015. Lipid recovery from a vegetable oil emulsion using microbial enrichment cultures 1–11. https://doi.org/10.1186/s13068-015-0228-9

TEN, 2020. Compost [WWW Document]. Regist. Nac. Prod. Fertil. URL https://tensl.com/compost/ (accessed 4.9.20).

Tomczak-Wandzel, R., Vik, E.A., Wandzel, T., 2015. BAT in fish processing industry, BAT in fish processing industry. https://doi.org/10.6027/tn2015-566

Tu, W., Zou, Y., Wu, M., Wang, H., 2020. Reducing the effect of non-volatile fatty acids (non-VFAs) on polyhydroxyalkanoates (PHA) production from fermented thermal-hydrolyzed sludge. Int. J. Biol. Macromol. 155, 1317–1324. https://doi.org/10.1016/j.ijbiomac.2019.11.103

Valentino, F., Morgan-Sagastume, F., Campanari, S., Villano, M., Werker, A., Majone, M., 2017. Carbon recovery from wastewater through bioconversion into biodegradable polymers. N. Biotechnol. 37, 9–23. https://doi.org/10.1016/j.nbt.2016.05.007

Vega, G.C., Sohn, J., Bruun, S., Olsen, S.I., Birkved, M., 2019. Maximizing environmental impact savings potential through innovative biorefinery alternatives: An application of the TM-LCA framework for regional scale impact assessment. Sustain. 11. https://doi.org/10.3390/su11143836

Vega, G.C., Voogt, J., Sohn, J., Birkved, M., Olsen, S.I., 2020. Assessing new biotechnologies by combining TEA and TM-LCA for an efficient use of biomass resources. Sustain. 12. https://doi.org/10.3390/su12093676

Venâncio, A., Montero, P., Costa, P., Regueiro, S., Brands, S., Taboada, J., 2019. An integrated perspective of the Operational Forecasting System in Rías Baixas (Galicia, Spain) with observational data and end-users, in: Computational Science - ICCS 2019. Springer, Faro, Portugal. https://doi.org/10.1007/978-3-030-22747-0

Verbrugge, L.N.H., Schipper, A.M., Huijbregts, M.A.J., Velde, G. Van der, Leuven, R.S.E.W., 2012. Sensitivity of native and non-native mollusc species to changing river water temperature and salinity. Biol Invasions 1187–1199. https://doi.org/10.1007/s10530-011-0148-y

Verdelhos, T., Marques, J.C., Anastácio, P., 2015. The impact of estuarine salinity changes on the bivalves Scrobicularia plana and Cerastoderma edule , illustrated by behavioral and mortality responses on a laboratory assay. Ecol. Indic. 52, 96–104. https://doi.org/10.1016/j.ecolind.2014.11.022

Verones, F., Bare, J., Bulle, C., Frischknecht, R., Hauschild, M., Hellweg, S., Henderson, A., Jolliet, O., Laurent, A., Liao, X., Lindner, J.P., Maia de Souza, D., Michelsen, O., Patouillard, L., Pfister, S., Posthuma, L., Prado, V., Ridoutt, B., Rosenbaum, R.K., Sala, S., Ugaya, C., Vieira, M., Fantke, P., 2017. LCIA framework and cross-cutting issues guidance within the UNEP-SETAC Life Cycle Initiative. J. Clean. Prod. 161, 957–967. https://doi.org/10.1016/j.jclepro.2017.05.206

Vogli, L., Macrelli, S., Marazza, D., Galletti, P., Torri, C., Samorì, C., Righi, S., 2020. Life cycle assessment and energy balance of a novel polyhydroxyalkanoates production process with mixed microbial cultures fed on pyrolytic products of wastewater treatment sludge. Energies 13. https://doi.org/10.3390/en13112706

Wagner, M., Scherer, C., Alvarez-Muñoz, D., Brennholt, N., Bourrain, X., Buchinger, S., Fries, E., Grosbois, C., Klasmeier, J., Marti, T., Rodriguez-Mozaz, S., Urbatzka, R., Vethaak, A.D., Winther-Nielsen, M., Reifferscheid, G., 2014. Microplastics in freshwater ecosystems: what we know and what we need to know. Environ. Sci. Eur. 26, 1–9. https://doi.org/10.1186/s12302-014-0012-7

Wang, C., Zhao, L., Lim, M.K., Chen, W.Q., Sutherland, J.W., 2020. Structure of the global plastic waste trade network and the impact of China's import Ban. Resour. Conserv. Recycl. 153, 104591. https://doi.org/10.1016/j.resconrec.2019.104591

Way, C., Hudson, M.D., Williams, I.D., Langley, G.J., 2022. Evidence of underestimation in microplastic research: A meta-analysis of recovery rate studies. Sci. Total Environ. 805, 150227. https://doi.org/10.1016/j.scitotenv.2021.150227

Weidema, B.P., Bauer, C., Hischier, R., Mutel, C., Nemecek, T., Reinhard, J., Vadenbo, C.O., Wenet, G., 2013. Data quality guideline for the ecoinvent database version 3. Ecoinvent Report 1 (v3). Swiss Cent. Life Cycle Invent. 3.

Wen, Q., Ji, Y., Hao, Y., Huang, L., Chen, Z., Sposob, M., 2018. Effect of sodium chloride on polyhydroxyalkanoate production from food waste fermentation leachate under different organic loading rate. Bioresour. Technol. 267, 133–140. https://doi.org/10.1016/j.biortech.2018.07.036

WHO, 2006. Guidelines for drinking-water quality, First addendum to third edition, Volume 1.

Wunderlich, J., Armstrong, K., Buchner, G.A., Styring, P., Schomäcker, R., 2021. Integration of techno-economic and life cycle assessment: Defining and applying integration types for chemical technology development. J. Clean. Prod. 287. https://doi.org/10.1016/j.jclepro.2020.125021

Xiang, Y., Jiang, L., Zhou, Y., Luo, Z., Zhi, D., Yang, J., Lam, S.S., 2022. Microplastics and environmental pollutants: Key interaction and toxicology in aquatic and soil environments. J. Hazard. Mater. 422, 126843. https://doi.org/10.1016/j.jhazmat.2021.126843

Xunta de Galicia, 2020. SIRGA [WWW Document]. URL https://sirga.xunta.gal/xestores?p_p_id=xestores_WAR_sirga&p_p_lifecycle=1&p_p_state=normal&p_p_mode=view&p_p_col_id=column-2&p_p_col_count=1&_xestores_WAR_sirga__spage=%2Fportlet_action%2Fxestores%2Fbusca&_xestores_WAR_sirga__sorig=%2Fportlet_action%2Fxe (accessed 4.6.20).

Xunta de Galicia, 2018. Anuario de Pesca de Galicia 2018 [WWW Document]. URL https://www.pescadegalicia.gal/gl/publicacions (accessed 3.30.21).

Yadav, B., Pandey, A., Kumar, L.R., Tyagi, R.D., 2020. Bioconversion of waste (water)/residues to bioplastics- A circular bioeconomy approach. Bioresour. Technol. 298, 122584. https://doi.org/10.1016/j.biortech.2019.122584

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

Yang, G., Zhang, P., Zhang, G., Wang, Y., Yang, A., 2015. Degradation properties of protein and carbohydrate during sludge anaerobic digestion. Bioresour. Technol. 192, 126–130. https://doi.org/10.1016/j.biortech.2015.05.076

Yates, M.R., Barlow, C.Y., 2013. Life cycle assessments of biodegradable, commercial biopolymers - A critical review. Resour. Conserv. Recycl. 78, 54–66. https://doi.org/10.1016/j.resconrec.2013.06.010

Zang, Y., Li, Y., Wang, C., Zhang, W., Xiong, W., 2015. Towards more accurate life cycle assessment of biological wastewater treatment plants: A review. J. Clean. Prod. 107, 676–692. https://doi.org/10.1016/j.jclepro.2015.05.060

Zarroli, G., 2020. Assessment of the production of bioplastics from industrial wastewater from fish canning industry (Master Thesis). POLITECNICO DI TORINO, Corso di Laurea Magistrale in Ingegneria per l ' Ambiente e il Territorio.

Zarus, G.M., Muianga, C., Hunter, C.M., Pappas, R.S., 2021. A review of data for quantifying human exposures to micro and nanoplastics and potential health risks. Sci. Total Environ. 756, 144010. https://doi.org/10.1016/j.scitotenv.2020.144010

Zhong, Z.W., Song, B., Huang, C.X., 2009. Environmental impacts of three polyhydroxyalkanoate (pha) manufacturing processes. Mater. Manuf. Process. 24, 519–523. https://doi.org/10.1080/10426910902740120

Zhou, J., Chang, V.W.C., Fane, A.G., 2013. An improved life cycle impact assessment (LCIA) approach for assessing aquatic eco-toxic impact of brine disposal from seawater desalination plants. Desalination 308, 233–241. https://doi.org/10.1016/j.desal.2012.07.039

Ziemann, H., Schulz, C., 2011. Methods for biological assessment of salt-loaded running waters – fundamentals, current positions and perspectives. Limnologica 41, 90–95. https://doi.org/10.1016/j.limno.2010.09.005

Zimmermann, A.W., Wunderlich, J., Müller, L., Buchner, G.A., Marxen, A., Michailos, S., Armstrong, K., Naims, H., McCord, S., Styring, P., Sick, V., Schomäcker, R., 2020. Techno-Economic Assessment Guidelines for CO2 Utilization. Front. Energy Res. 8, 1–23. https://doi.org/10.3389/fenrg.2020.00005

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

LIST OF PUBLICATIONS

JOURNAL PUBLICATIONS DERIVED FROM THE THESIS

Roibás-Rozas, A., del Río, Á.V., Hospido, A., Mosquera-Corral, A., 2021. Strategies for the valorisation of a protein-rich saline waste stream into polyhydroxyalkanoates (PHA). Bioresource Technology, 124964. doi: 10.1016/j.biortech.2021.124964. Impact factor: 9.642 (2020). Subject: Environmental Engineering; Ranking: Q1. **Chapter 3** is based on this publication. Author contribution (CRediT taxonomy): Formal analysis, Investigation, Writing – original draft, Conceptualization, Resources.

Roibás-Rozas, A., Mosquera-Corral, A., Hospido, A., 2020. Environmental assessment of complex wastewater valorisation by polyhydroxyalkanoates production. Science of the Total Environment, 744. https://doi.org/10.1016/j.scitotenv.2020.140893. Impact factor: 7.956 (2020). Subject: Environmental Science; Ranking: Q1. **Chapter 5** is based on this publication. Author contribution (CRediT taxonomy): Formal analysis, Investigation, Writing – original draft, Conceptualization, Resources.

Roibás-Rozas, A., Núñez, M., Mosquera-Corral, A., Hospido, A., 2022. Modeling the impact of salinity variations on aquatic environments: including negative and positive effects in Life Cycle Assessment. Environmental Science & Technology, https://doi.org/10.1021/acs.est.1c04656. Impact factor: 9.282 (2020). Subject: Environmental Science; Ranking: Q1. **Chapter 7** is based on this publication. Author contribution (CRediT taxonomy): Formal analysis, Investigation, Writing – original draft, Conceptualization, Resources.

BOOK CHAPTERS DERIVED FROM THE THESIS

Roibás-Rozas, A., Saavedra del Oso, M., Zarroli, G., Mauricio-Iglesias, M., Mosquera-Corral, A., Fiore, Silvia, Hospido, A., 2022. How can we validate the environmental profile of bioplastics? Towards the introduction of Polyhydroxyalkanoates (PHA) in the value-chains (Chapter 20), in: Teodosiu, C., Fiore, Silvia, Hospido, A. (Eds.), Assessing Progress toward Sustainability. ISBN: 9780323897990. Elsevier. https://www.elsevier.com/books/assessing-progress-towards-sustainability/teodosiu/978-0-323-85851-9. **Chapter 4** is based on this publication, and **Chapter 1** is also partially supported by its content. Author contribution (CRediT taxonomy): Formal analysis, Investigation, Writing – original draft, Conceptualization, Resources.

OTHER PUBLICATIONS

Correa-Galeote, D., **Roibás-Rozas, A**., Mosquera-Corral, A., Juárez-Jiménez, B., González-López, J., Rodelas, B., 2021. Revealing the dissimilar structure of microbial communities in different WWTPs that treat fish-canning wastewater with different NaCl content. Journal of Water Process Engineering, 44. https://doi.org/10.1016/j.jwpe.2021.102328. Impact factor: 5.459 (2020). Subject: Water Science and Technology; Ranking: Q1. Author contribution (CRediT): Investigation, Conceptualization, Resources, Writing - review & editing.

Correa-Galeote, D., **Roibás-Rozas, A**., Mosquera-Corral, A., Juárez-Jiménez, B., González-López, J., Rodelas, B., 2021. Salinity is the major driver of the global eukaryotic community structure in fish-canning wastewater treatment plants. Journal of Environmental Management, 290. https://doi.org/10.1016/j.jenvman.2021.112623. Impact factor: 7.099 (2020). Subject: Environmental Engineering; Ranking: Q1. Author contribution (CRediT): Investigation, Conceptualization, Resources, Writing - review & editing.

CONFERENCE PROCEEDINGS

Oral Contributions

Roibás-Rozas, A., Argiz, L., Val del Río, A. Mosquera Corral, A. Hospido, A., 2021. The value of Life Cycle Thinking strategies for the early evaluation of emerging technologies of industrial waste streams valorisation. 7th Mixed Culture PHA Workshop. Valencia, Spain.

Roibás-Rozas, A. Val del Río, A. Hospido, A. Mosquera-Corral, A., 2021. What is the best operational strategy to valorize protein-rich streams into polyhydroxyalkanoates (PHA)? 4th IWA Resource Recovery Conference. Istanbul, Turkey.

Roibás-Rozas, A., Fra-Vázquez, A., Val del Río, A., Mosquera-Corral, A., 2018. Evaluación ambiental de la producción de polihidroxialcanoatos (PHA) a partir de aguas de conservera. IV Simposio de investigación en tecnologías ambientales. Santiago de Compostela, España. [In Spanish]

Roibás-Rozas, A. Argiz, L., Pedrouso, A. Val del Río, A. Hospido, A. Mosquera-Corral, A., 2019. Valorisation of complex wastewater for the production of PHA. 3rd IWA Resource Recovery Conference. Venice, Italy.

Roibás Rozas, A., Argiz, L., Val del Río, A. Mosquera-Corral, A., 2019. Neutral pH promotes simultaneous acidification of carbohydrates and proteins in high salinity wastewater. 6th Mixed Culture PHA Workshop. Venice, Italy.

Argiz, L., Fra-Vázquez, A. **Roibás-Rozas, A.,** Mosquera-Corral, A., 2018. Optimization of an enrichment mixed culture to produce PHA from industrial saline complex wastewater. Ecotechnologies for wastewater treatment: 4th Specialized International Conference. London, Canada.

Roibás-Rozas, A., Argiz, L., Fra-Vázquez, A., Val del Río, A., Mosquera-Corral, A., 2018. Optimización de un proceso de producción de bioplásticos a partir de aguas de conservera. VI Encontro da mocidade investigadora. Santiago de Compostela, España. [In Spanish]

Roibás-Rozas, A., Fra-Vázquez, A., Val del Río, A., Mosquera-Corral, A., 2018. Producción de biopolímeros mediante la valorización de grasas y aceites residuales generados en la industria conservera. II Simposio de investigación en tecnologías ambientales. Santiago de Compostela, España. [In Spanish]

Poster contributions

Roibás-Rozas, A., Núñez, M., Mosquera-Corral, A., Hospido, A. (2022). A general framework to address the impacts of salinity variations, including emission-related positive effects in LCA. SETAC Europe 32nd annual meeting. Copenhagen, Denmark.

Roibás-Rozas, A., Hospido, A., Zarroli, G., Fiore, S. (2020). Lessons learnt from a revision of LCA studies on PHA production from food industry waste streams. 12th International Conference on Life Cycle Assessment of Food (LCA Food). Berlin, Germany.

Benedetti, V. Fra-Vázquez, A. Longo, S., **Roibás-Rozas, A**., Val del Río, A. Mosquera-Corral, A. Hospido, A. (2019). Pros and cons of the valorisation of fish canning wastewater through biopolymers production. SETAC Europe 29th annual meeting. Helsinki, Finland.

PERMISSIONS

Part of the content of this thesis is drafted after the publications hereby mentioned. They are published in this thesis with the permission of the editorials, which allowed to use the original content for the author's thesis dissertation.

- For **Elsevier** publications (Chapter 1, Chapter 3, Chapter 4, and Chapter 5):

https://www.elsevier.com/about/policies/copyright

- For **ACS** publications (Chapter 7):

Reprinted with permission from https://doi.org/10.1021/acs.est.1c04656. Copyright 2021 American Chemical Society.

USC UNIVERSIDADE DE SANTIAGO DE COMPOSTELA

The circular economy could provide answers to problems like the climate crisis or fossil resource depletion. This thesis addresses the application of circular economy in the Galician fish canning industry, an economic driving force of the region. To do so, a system of reactors was operated to valorise saline effluents from fish canneries into polyhydroxyalkanoates (PHA), a biopolymer with the potential to replace petrochemical plastics.
After technical validation, environmental and economic validation were carried out. Results showed reductions in the environmental impacts of ca. 25%, and production costs of about 1€/kg PHA, similar to the cost of conventional plastics. Finally, a new methodology was developed with the goal of including the effects of salinity in environmental evaluations by Life Cycle Assessment.