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a4f algae for future

SUSTAINABLE MICROALGAE BIOREFINERY DEVELOPMENT THROUGH PROCESS OPTIMIZATION

Thesis presented for the Doctor of Philosophy degree in Refining, Petrochemical and Chemical Engineering

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Dedico esta Tese à minha Mãe e ao meu Avô por serem os meus melhores exemplos.

Abstract

With the increase of the price of oil, the interest in finding alternative solutions to produce biofuels also grew. This led to an increased interest in the study of microalgae, since the fatty acids and carbohydrates contained in the cells could be used to produce biodiesel and bioethanol. This, allied to the smaller land area required to produce high amounts of biomass when compared to other feedstocks, such as soya and sugar cane, made microalgae a very interesting, promising and highly researched topic.

Although a large number of studies have been performed on the topic of biofuel production from microalgae, due to the high costs of harvesting and processing this biomass, the estimated cost of producing biodiesel was very high in comparison to diesel, so no industrial-sized production units have been built.

Fortunately, some of the intracellular contents of microalgae, like carotenoids and polyunsaturated fatty acids (PUFA), just to name a few, have a high market value. This led to the production of a large number of biorefinery studies and projects, similar to a chemical refinery, where not only biodiesel and/or bioethanol, but also different products with high value would be produced from microalgae, in order to compensate the previously mentioned high production costs.

As A4F – Algae for Future is a company that has a large interest in developing microalgae biorefinery projects, the objective of this thesis was to design two optimized biorefineries, based on a microalgae and a cyanobacteria, to serve as a template for future projects of A4F; one for a process representative of a downstream of a microalga with target intracellular compounds and the other for a process representative of a downstream of a microalga with target extracellular compounds.

Both were selected from EU-funded FP7 projects in which A4F was one of the partners. The first, was a genetically modified *Synechocystis* strain that produces and excretes ethanol and the second one, a *Prorocentrum* strain with a high lipid (PUFA) content. Hence, the first step of this project was to design two large-scale production plants (10 ha), one for each strain. The second step of the project was then to define the most valuable products that could be obtained from each microalgae strain. Afterwards, alternative technologies and processes were proposed, and alternative process chain routes were analyzed, creating different scenarios for each microalgae strain. In the end, the several scenarios were compared, at first based on a techno-economic analysis, and the 5 scenarios with the best economic performance were compared based on their LCA performance in several impact factors. From the results of the previously mentioned analysis, one biorefinery process route was selected for each microalga

strain as the optimal and most sustainable biorefinery. For *Synechocystis,* the best route found was using a membrane for the biomass harvesting. The ethanol was afterwards recovered using a distillation column and a pervaporation membrane while the remaining biomass was ruptured using a bead mill. The first extraction process was a diafiltration followed by an affinity extraction with chitosan to produce a phycocyanin product. The remaining biomass went through an enzymatic hydrolysis to produce a protein hydrolysate. For this process the ROI was 13% and the NPV at the end of the 10^{th} year was of €2,240,976. For the *Prorocentrum* the best route found was a harvesting step with membrane filtration, followed by a rupture step with an ultrasonicator. Two solvent extractions, with heptane and ethanol were performed sequentially, and using a lipid purification process lipid soap was produced. The remaining products were a protein hydrolysate, produced with an enzymatic hydrolysis of the proteins of the biomass, and a remaining carbohydrate rich product. For this process the ROI was 2% and the NPV in the end of the 10^{th} year was €552,566.

Moreover, several conclusions were taken, to support decision making when planning a microalgae biorefinery. In the economic aspect, the choice of microalgal biomass was observed to be a key factor to obtain an economically viable biorefinery, as the value of the biomass and the maximum final concentration can dictate the economic feasibility. In the environmental aspect, the production of microalgae has the highest negative impact especially due to electricity consumption, nutrients and carbon dioxide used. An important conclusion was that different synergies with other industries are very useful to improve the economic and environmental performance of the biorefinery. In fact, undesirable outputs of industries such as wastewater and CO_2 may be used as the inputs of the microalgae production system, contributing to reduce the environmental burden of industrial activities and therefore, to a more circular economy.

Resumo

Com o aumento do preço do petróleo, o interesse no estudo de alternativas para a produção de biocombustíveis aumentou. Isto levou a um aumento do interesse nas microalgas devido à presença de alguns ácidos gordos e hidratos de carbono na composição das microalgas, que poderiam ser usados para a produção de biodiesel e bioetanol. Esta composição, em conjunto com a baixa área necessária para a produção das microalgas quando comparada com outras matérias-primas como a cana-de-açúcar e a soja, fomentou o interesse na investigação no estudo das microalgas.

Apesar do elevado número de artigos e projetos de investigação dedicados à produção de biocombustíveis a partir de microalgas, devido ao elevado custo de produção e colheita, nenhuma unidade industrial de grande escala foi ainda construída até ao momento. Felizmente, alguns dos componentes intracelulares das microalgas, como os carotenoides e alguns ácidos gordos, têm elevado valor comercial. Isto levou ao aumento do número de estudos e projetos sobre biorrefinarias onde, tal como nas refinarias químicas, aliada à produção de biodiesel ou de bioetanol, existe a produção de diferentes produtos com valor acrescentado, de modo a compensar os custos elevados previamente mencionados.

A *A4F – Algae for Future*, é uma empresa com um grande interesse no desenvolvimento de projetos de biorrefinarias a partir de microalgas, pelo que o objetivo desta tese é a formulação de duas biorrefinarias otimizadas e sustentáveis, a partir de uma microalga e uma cianobactéria, para servirem de modelo para futuros projetos; um representativo de um processo de *downstream* para uma microalga com productos de interesse intracelulares e outro para um processo de *downstream* de uma microalga com productos de interesse extracelulares Ambas foram escolhidas tendo como base os resultados de projetos europeus FP7 onde a A4F foi um dos parceiros. A primeira é uma estirpe de *Synechocystis* geneticamente modificada que produz e excreta etanol para o seu exterior, e a segunda uma estirpe de *Prorocentrum* rica em ácidos gordos polinsaturados. Deste modo, o primeiro passo da tese consistiu no projeto de dois processos de produção à escala industrial de 10 hectares, um para cada microalga, para a produção de microalgas. Na segunda fase foram identificados quais os produtos mais valiosos que podem ser obtidos a partir de cada microalga. Após estas fase, escolheram-se diferentes equipamentos e tecnologias, necessários para desenhar diversos cenários de processamento. Foi feita a análise económica a cada cenário e aos 5 cenários com o melhor desempenho económico foi feita uma análise de ciclo de vida (ACV) para identificar qual o cenário com o melhor desempenho ambiental.

Para a *Synechocystis* o processo escolhido começa com uma filtração com membrana. O etanol foi recuperado usando uma coluna de destilação e uma membrana de pervaporação. A restante biomassa foi rompida usando um moinho de bolas. Após uma diafiltração, ficocianina foi produzida usando uma extração por afinidade, enquanto um hidrolisado proteico foi produzido através de uma hidrólise enzimática da restante biomassa. O retorno do investimento deste processo foi de 13% e o valor presente liquido ao fim de 10 anos foi de €2,240,976. Para o *Prorocentrum* o processo escolhido começa com uma filtração com membrana e um ultrasonicador para a rutura celular. Dois passos de extração com solventes convencionais é usada para separar os lípidos e carotenoides dos restantes compostos. Um processo de purificação produz um sabão com os lípidos, obtendo-se também um hidrolisado proteico produzido através de uma hidrólise enzimática e um produto rico em hidratos de carbono. O retorno no investimento deste processo foi de 2% e o Valor Presente Liquido obtido ao fim de 10 anos foi de €552,566.

Neste estudo foram extraídas várias conclusões, que permitem suportar decisões informadas. Na vertente económica foi observado que a escolha da microalga é fundamental para a viabilidade económica do projeto, pois tanto o valor da biomassa como a sua concentração final são fundamentais para obter um desempenho económico positivo. Na vertente ambiental, os maiores impactos são provenientes da produção de microalga, devido aos consumos de eletricidade, de nutrientes e dióxido de carbono. Uma conclusão importante foi que a existência de diferentes sinergias com outras indústrias é extremamente útil para a melhoria do desempenho económico e ambiental do projeto. A utilização de águas residuais e de dióxido carbono libertado por essas indústrias pode feita em substituição de matérias-primas (nutrientes) para a produção de microalgas, contribuindo para reduzir a carga ambiental negativa das atividades industriais e, portanto, para uma economia mais circular.

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Abbreviations

Amino Acid Hydr. – Amino acid hydrolysate

BM – Bead mill

C – Centrifuge

CAGR - Compound Annual Growth Rate

Capex – Capital Costs

Carbohyd. Feed – Carbohydrate Feed

CIP – Cleaning in Place

Crtn – Carotenoids

DAF – Dispersed Air Flotation

DEF – Dead end Filtration

DHA - Docosahexaenoic acid

DW – Dry weight

EDTA - Tetrasodium Ethylenediaminetetraacetate

EP –Extraction process

EPA - Eicosapentaenoic acid

FE – Freshwater eutrophication

FET – Freshwater Ecotoxicity

FLC - Flocculation

FRS – Fossil Resource Scarcity

FU – Functional Unit

GMO – Genetically Modified Organism

GW – Global Warming

HCT - Human carcinogenic toxicity

HPH – High Pressure homogenizer

IFEU - Institute for Energy and Environmental Research

IRR – Internal Rate of Return

LCA – Life Cycle Assessment

LCI – Life Cycle Inventory

LCIA – Life Cycle Inventory Analysis

LCT – Life Cycle Thinking

ME – Marine eutrophication

MET – Marine Ecotoxicity

MF – Micro Filtration

MRS – Mineral Resource Scarcity

NF – Nanno Filtration

NPV – Net Present Value

OF,H – Ozone Formation, Human Health

Opex – Operational Costs

PBP – Payback Period

PC – Phycocyanin

PFD – Process Flow Diagram

PHR – Production + Harvesting + Cellular Disruption

Protein Concent. – Protein Concentrate

PUFA – Poly Unsaturated Fatty Acid

RO – Reverse Osmosis

ROI – Return on Investment

$scCO_2$ – Supercritical CO_2

SDA - Stearidonic acid

SOD - Stratospheric ozone depletion

SQDG - Sulfoquinovosyl diacylglycerol

TA - Terrestrial acidification

TAG - Triacylglyceride

TFA – Total fatty acids

TFF - Tangential flow filtration

Tkm – Tonnes per kilometer

UF – Ultra Filtration

UHT-PBR – Unilayer Horizontal Tubular Photobioreactor

US – Ultra Sonication

WC – Water Consumption

1 Introduction

1.1 Motivation

A4F – Algae for Future is a biotechnology company, located in Portugal, focused on microalgae research & development and microalgae production. The core business of A4F is the design, building, operation and transfer (DBOT) of different solutions for the industrial production of microalgae. A4F also participates in different EU-funded projects, some solely based on microalgae production or valorization, while others are focused on the full potential of the microalgae biomass and, therefore, explore the biorefinery concept (*DEMA* Project, *PUFAChain* Project, *Biofat* Project, among others).

In line with the biorefinery concept, the objective of this thesis was to perform a technical and economic evaluation of two different biorefineries, one based on a cyanobacteria and the other based on a dinoflagellate, in order to create a standard sustainable and optimal biorefinery design for each strain for future projects.

Two European projects were selected by *A4F* to serve as a starting point and information source for this study. The first was the *DEMA* project (Lopes et al., 2019; University of Limerick, 2018), where a genetically modified *Synechocystis* strain was used to produce and excrete ethanol to the medium. The second was the *PUFAChain* Project (Friedl, 2017) in which a wild type *Prorocentrum* strain was used as a source of polyunsaturated fatty acids (PUFAs). During the remaining of this manuscript both the cyanobacteria and the dinoflagellate will be referred as microalgae.

1.2 Thesis objectives

The proposed work consists in modelling and optimizing different scenarios of industrial biorefinery of cyanobacteria and microalgae, based on real data from their production. The research was divided in three distinct stages (Figure 1):

1.2.1 Stage 1. Cyanobacteria and microalgae industrial production design and techno-economic sensitivity analysis

Work in Stage 1 consisted of:

i. Defining and modelling two base scenarios of each cyanobacteria and microalgae industrial production, based on real data of large-scale plants (two different strains were identified by the company for this study).

ii. Proposing process and technology improvements, based on the analysis performed supported on the two optimized models of biomass production, to develop and study several processing routes.

1.2.2 Stage 2. Cyanobacteria and microalgae biomass valorization and sustainable biorefinery development

Work in Stage 2 involved:

i. The identification of the most relevant added-value products of the selected strains (based on the literature and chemical analysis) and their current market value, based on available market studies or direct contact with suppliers;

ii. The detailed state-of-the-art review on the possible processes and routes to achieve the identified added-value products (dewatering, disruption, extraction, conversion);

iii. The identification of the most sustainable routes, both environmentally and economically; the identification of the scenarios where the production price is closer to the current and projected added-value products market prices from other origins (synthetic, higher plants, etc.);

iv. Designing different pathways for each strain, creating a map of the possible routes;

v. Performing a preliminary techno-economic analysis to choose the 5 scenarios with the best economic performance and LCA study of the 5 selected designed routes to choose the one with the best performance;

vi. Identifying potential clusters with other industries or technologies, which enable to close the plant's mass and energy balances;

vii. Finally, developing the business case for each selected scenario.

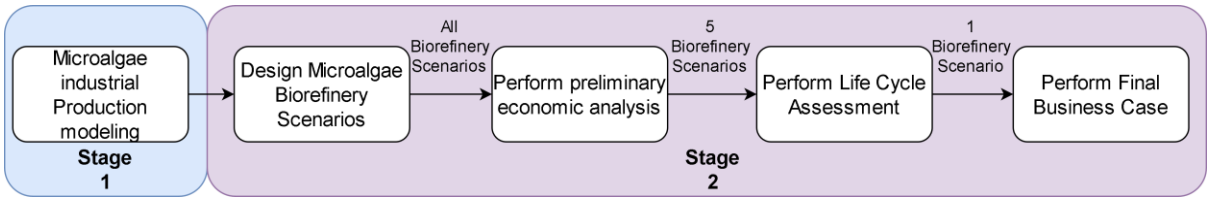

Figure 1 – Thesis objectives summary diagram.

1.3 Thesis Outline

The work performed during this PhD is organized as described below:

Chapter 1: Introduction – Describes the motivations and objectives of this thesis;

Chapter 2: Microalgae Production and Processing – Describes the state-of-the-art on microalgae (composition; possible products) to microalgae production and processing methods;

Chapter 3: Sustainability and Process Optimization – State-of-the-art on sustainability and process optimization;

Chapter 4: Biorefinery Scenarios and Process Optimization – Describes the design of the different biorefinery scenarios and the selection of the 5 best scenarios based on the economic performance;

Chapter 5: Evaluation of the Environmental Performance of Microalgae Based Biorefineries – Presents the Life Cycle Assessment of the 5 biorefinery scenarios with the best economic performance and the selection of the most sustainable scenario;

Chapter 6: Business Case – Presents a more thorough economic analysis of the scenarios chosen as well as the possible improvements and synergies that can be performed on those scenarios;

Chapter 7: Conclusion – Presents the final conclusions and results of this thesis.

2 Microalgae Production and Processing

2.1 Microalgae

Microalgae are prokaryotic and eukaryotic photosynthetic microorganisms. Due to their simple cellular constitution, they can adapt very easily to the environmental conditions that prevail in their surroundings. Microalgae are categorized into a variety of classes mostly defined by their life cycle, pigmentation and basic cellular structure. The most important classes are: green algae (Chlorophyta), red algae (Rhodophyta) and diatoms (Bacillariophyta) (Demirbas and Demirbas, 2010). Microalgae nutrition can be either autotrophic or heterotrophic. The autotrophically growing microalgae require inorganic compounds such as CO_2, salts and a light energy source for growth. They have chlorophyll-a as their primary photosynthetic pigment. For autotrophic algae, photosynthesis is a key component of their survival, whereby they convert solar radiation and CO_2 absorbed by chloroplasts into O_2 and adenosine triphosphate (ATP), the usable energy currency at cellular level, which is then used in respiration to produce energy to support growth (Davis et al., 2011). Some microalgae can be grown heterotrophically, in the absence of light and therefore require an external source of organic compounds, like sugars, as well as other nutrients as an energy source (Behera et al., 2014). Some photosynthetic algae are mixotrophic, meaning they have the ability to perform both photosynthesis and acquire exogenous organic nutrients (Liang, Y., Sarkany, N., Cui, 2009).

2.1.1 Composition

In general, microalgae are mainly composed of proteins (18-46%), carbohydrates (18-46%) and lipids (12-48%) (% dry weight), but also contain other components like minerals such as calcium, magnesium, sodium and phosphorous, and also trace elements like copper, manganese and zinc (Tibbetts et al., 2015). Other components that can be found in microalgae are pigments like chlorophyll, carotenoids, and phycobiliproteins (Spolaore et al., 2006).

2.1.1.1 Protein

Some microalgae have been identified as reliable sources of protein, as they are safe for human consumption, and due to their high protein content, when compared to the most common protein sources like soy meal, chicken, fish and beef, can replace the previously mentioned protein sources (Barka and Blecker, 2016; Koyande et al., 2019). Several strains of the microalga *Chlorella* or the cyanobacterium *Arthrospira* (former *Spirulina*)

are known for having protein contents of over 70% of their dry weight. However, it is not only the large amount of proteins that the microalgae contain that makes them so interesting, but also the fact that they contain all of the essential amino acids (EAA) that humans cannot synthesize (Bleakley and Hayes, 2017). The content depends on growth conditions. In case of nutrient starvation (N-source) the protein content of microalgae decreases (Wells et al., 2017).

2.1.1.2 Carbohydrates

Carbohydrates in microalgae can be found in the form of starch, glucose, sugars and other polysaccharides. Most of the carbohydrates are found in the inner and outer cell walls of the microalgae. *Chlorella vulgaris* along with *Chlamydomonas reinhardtii* and *Scenedesmus obliquus* strains have been described as those with the highest content of carbohydrates, going from 45% to 60% dry weight. The major player in the high production of carbohydrates in these microalgae is nitrogen. When in starvation, these microalgae have a high tendency to produce high amounts of carbohydrates (Chen et al., 2013; Priscilla de Souza et al., 2020).

2.1.1.3 Lipids

There are two main types of lipids in microalgae. One type are the phospho- and glycolipids, that contain a polar head group with two fatty acid chains, and are important for membrane function. The other type of lipids are triacylglycerols (TAGs), which are non-polar lipids containing three fatty acid chains, and are important for energy storage in the cell. In certain microalgae species like *Nannochloropsis* sp., *Porphyridium cruentum* and *Chaetoceros calcitrans* (a diatom), the TAGs content can reach between 30-40% dry weight (Georgianna and Mayfield, 2012). After separation, in the TAG fractions there can be some interesting components like polyunsaturated fatty acids (PUFAs). There are two important families of PUFAs: the omega-6 fatty acids and the omega-3 fatty acids (Wells et al., 2017).

2.1.1.4 Pigments

The pigments are the components that give color to the microorganisms. For instance, carotenoids have various colors, from yellow to red and can be divided into carotenes and xanthophylls, according to their end groups.. There are different types of chlorophyll (chlorophyll-a, b, c, d and f), which confer slightly different green colors to the microorganism, depending on the molecules attached to chlorin, a part of chlorophyll. These small differences cause differences in absorption spectrum and in tonality: chlorophyll-a - blue-green, chlorophyll-b - brilliant green, chlorophyll-c - yellow green,

chlorophyll-d - forest green and chlorophyll-f - emerald green (Takaichi, 2011). Finally, the most common phycobilins found in microalgae are phycocyanobilin (blue) and phycoerythrobilin (red). The main difference, which leads to the color difference, is the groups attached to the pyrrole rings. Phycocyanobilin is a major constituent of the phycobiliproteins phycocyanin (deep blue) and allophycocyanin (light blue), whereas phycoerythrobilin is found as the major element of phycoerythrin (orange-red) (Kraan, 2013; Mulders et al., 2014; Novoveská et al., 2019).

2.1.2 Microalgae commercial history and commercial applications

Due to their composition, microalgae are extremely interesting to produce several foods, chemical and nutritional products.

The first commercialized microalgae were *Chlorella* and *Arthrospira*, sold in Japan, Taiwan and Mexico as ´health food´. The first microalgae ingredients followed in the 1980s with the commercialization of ß-carotene from *Dunaliella salina*, astaxanthin from *Haematococcus pluvialis* and docosahexaenoic acid (DHA) from *Crypthecodinium cohnii*, in the 1990s (Borowitzka, 2013).

At present, there are a large number of products obtained from microalgae, either using the whole biomass or components produced by the microalgae. Food-grade microalgae are used as shelf-life extenders, as a source of natural colorants in food, drink mixes and beverages, and as supplements in the form of tablets, capsules, and liquids. Pharmaceutical-grade microalgae can be used in nutritional components and as food supplements. Other grades of microalgae can be used in cosmetics, paint colorants among other applications (Barkia et al., 2019; Borowitzka, 2013; Indira Priyadarshani and Rath Biswajit, 2012; Spolaore et al., 2006).

2.1.2.1 Whole microalgae products

2.1.2.1.1 Human food

The first documented use of microalgae was as food source. For more than 2000 years, and in countries as far apart as Mexico, China and the Republic of Chad, microalgae have been used as food. It was found by those diverse cultures that microalgae contained the nutrients necessary for humans to survive, and due to their simplicity and abundance, they were used by the poor as a food source. In the 1950´s with the beginning of related research, it was found that microalgae were a rich source of carbohydrates, proteins, many vitamins as well as minerals required by the human organism (Belay, 2002; Indira Priyadarshani and Rath Biswajit, 2012). The most used microalgae are *Arthrospira* (former *Spirulina*), *Chlorella*, *Dunaliella salina* and *Aphanizomenon flos-aquae* (Spolaore et al.,

2006). Currently, they are used mostly as food supplements and nutritional supplements marketed in different forms, such as tablets, capsules and liquids, or used as a source of natural food colorants and incorporated into pastas, snack foods, candy bars or gums, and beverage (Raja et al., 2018).

2.1.2.1.2 Animal Feed

In addition to human food, microalgae can also be used for animal feed, especially for aquaculture. This is due to the high amino acid content and the existence of omega-3 PUFAs, but also owing to the high availability of microalgae. Algae derived products have been a very suitable alternative for the raw material used in aquafeed and other animal feeds. Several studies have proven that small amounts, around 2.5–10%, of algae in fish diets resulted in positive effects like increase in growth performance and higher disease resistance (Norambuena et al., 2015). The most used genus are *Chlorella*, *Tetraselmis, Isochrysis, Pavlova, Phaeodactylum, Chaetoceros, Nannochloropsis, Skeletonema.* In 1999, the annual production of microalgae for aquaculture reached 1000 tons (Sathasivam et al., 2019). Microalgae are also used for farm animal diet. *Arthrospira* is largely used in this domain and concerns many types of animal (cats, dogs, aquarium fish, birds, horses and cows), since it is a source of natural vitamins, minerals, and essential fatty acids. In poultry feeds, algae can be used safely as partial replacement for conventional proteins (Indira Priyadarshani and Rath Biswajit, 2012; Spolaore et al., 2006; Sathasivam et al., 2019).

2.1.2.1.3 Other applications

Due to their composition, and nitrogen fixation capacity, microalgae biomass has potential to also be used as biofertilizer. Studies have shown that *Arthrospira platensi*s and *Chlorella vulgaris* have a positive effect on the growth and yield of rice plantations (Dineshkumar et al., 2017).

Another possible application is in the cosmetics industry. *Arthrospira* and *Chlorella* are already used in the skin care market. Microalgae components, like chlorophyll and other metabolites, protect the skin from UV radiation (Stahl and Sies, 2002) and can be used in cosmetics. Some of the most used microalgae for this purpose are *Arthrospira platensis, Nannochloropsis oculata, Chlorella vulgaris and Dunaliella salina* (Couteau and Coiffard, 2018; Mourelle et al., 2017).

2.1.2.2 High Value Products

As was mentioned before, what makes microalgae so appealing is their composition. Different components of the microalgae were found to have not only benefits for health

and could be used as food supplements (vitamins, essential amino-acids and lipids), but also some of the contents have properties that have antibiotic, antiviral, anticancer, and therapeutic applications (Levasseur et al., 2020).

Carotenoids

There are more than 400 known carotenoids, however only a few, like ß-carotene, astaxanthin, lutein and zeaxanthin are used commercially (Ambati et al., 2018). They are generally used as natural food colorants and as an additive for animal feed (poultry, fish). Some carotenoids also have applications in cosmetics owed to their photoprotective properties (Stahl and Sies, 2002). Other carotenoids are used as nutritional ingredients because of their antioxidant properties and their ability to act as provitamin A, that is, they can be converted into vitamin A. Moreover, carotenoids have intrinsic anti-inflammatory properties owing to their quenching action on relative oxygen species. In many markets, microalgae carotenoids are in competition with the synthetic form of the pigments. Although the synthetic forms are much less expensive than the natural ones, microalgal carotenoids have the advantage of supplying natural isomers in their natural ratio (Spolaore, Joannis-Cassan, Duran and Isambert, 2006; Anunciato and da Rocha Filho, 2012).

The main source of ß-carotene is *Dunaliella salina*. Three main products are obtained from *D. salina*: ß-carotene extracts, *Dunaliella* powder for human use and dried *Dunaliella* for animal feed use. The prices of these products vary from US$ 300 to US$ 3000/kg of pigment. Natural astaxanthin is mainly produced by *Haematococcus pluvialis*. Astaxanthin is principally consumed by the salmon feed industry. The annual worldwide market of this pigment is estimated at around US$ 200 million with an average price of US$ 2500/kg. Although it is more expensive than the synthetic version for certain applications, the natural product is preferred. These applications include carp, chicken and red sea bream diets. Since the 1990's, human nutraceuticals have appeared as a new market possibility because astaxanthin has antioxidant properties (Bauer and Minceva, 2019; Enzing et al., 2014).

Phycobiliproteins

The major commercial producers of phycobiliproteins are the cyanobacterium *Arthrospira* and the rhodophyte *Porphyridium*. The primary use for these molecules is as natural dyes but an increasing number of studies have shown their potential health improving properties. The first and most important application of phycocyanin is as food pigment, replacing current synthetic pigments. One of the best examples is the blue color of M&M´s

(Pondtech, 2019). In addition, phycobiliproteins have been widely used in clinical or research immunology laboratories. This is especially due to their properties like high molar absorptivity coefficients, high fluorescence quantum yield, and high photostability, which make them very powerful and highly sensitive fluorescent reagents. Due to the previous properties, they also can serve as labels for antibodies, receptors and other biological molecules in a fluorescence-activated cell sorter, fluorescence microscopy or diagnostics methods. The prices of phycobiliproteins products are US$ 3 to US$ 25/mg for native pigment but they can reach US$ 1500/mg for certain cross-linked pigments. Their global market was estimated at more than 50 million US$ in 1997 (Prasanna et al., 2007; Spolaore et al., 2006).

PUFAs

PUFAs, or Polyunsaturated fatty acids, are fatty acids that contain more than one double bond in their structure. The most interesting PUFAs are the omega-3, which are long-chained PUFAs (LC-PUFA). They are extremely interesting due to their health properties. The most important omega-3 fatty acids are docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA). The most common sources of omega–3 fatty acids include fish oils, squid oil, microalgae, nuts & seeds and vegetable oils. The main omega-3 applications include dietary supplements, pharmaceutical, infant formula, and food products (Hernandez and de Jong, 2011). Presently, the main source of omega-3 is fish oil and krill oil. However, due to the decrease of fishing quotas and consequent stagnation of fish oil production, other sources have to be used. The natural candidates are microalgae derived oils, since these are the main source of omega-3 for the fishes (Allied market research, 2017; UBIC consulting, 2014).

Different data has been published to estimate the omega-3 market revenue in recent years. The global market revenue for marine and algae EPA/DHA omega-3 ingredients market was estimated in US$ 1,806.8 million (2011) by Frost and Sullivan (2012). The market was likely to grow at a CAGR (Compound annual growth rate) of 11.8 % from 2012 to 2016. UBIC Consulting has estimated the total global market for omega-3 for all end products to be over US$ 850 million (UBIC consulting, 2014). Packaged Facts estimated the market revenue in terms of global consumer spending on EPA/DHA fortified products (Packaged Facts, 2012). This analysis estimated that the omega-3 market reached US$ 25.4 billion in 2011. The consumer demand can also be estimated by volume. Frost and Sullivan have published that the demand in 2011 increased to 103.284 metric tons (Frost & Sullivan, 2012).

The global market is still in the growth stage of its product lifecycle and it is expected to see more product innovations and possible market disruptions during the next years, especially in the pharmaceutical market (Tocher et al., 2019).

2.1.2.3 Bioethanol production

Another product that can be obtained from microalgae is bioethanol. Bioethanol is the most common biofuel used as a substitute to fossil fuels (gasoline), accounts for nearly 90% of the biofuel usage Worldwide. Currently, bioethanol is produced mainly as 1st and 2nd generation biofuels through the fermentation of biomass from agriculture crops and residues, respectively. However, the costs associated to the conventional processes (harvesting, storing and processing the biomass) as well as concerns over agriculture lands, water and cereals used for fuel, along with the high energy input associated to fermentation, led to the development of novel biological approaches where microalgae are used not only as biomass that is converted to biofuel but also as producers – cell factory concept – through the introduction of genes encoding a metabolic pathway – 3rd generation biofuel. Therefore, biofuel production from microalgae biomass will not compromise the production of food, fodder and/or other products derived from crops (Dragone et al., 2010).

As mentioned above, bioethanol is a versatile product and can have several applications in the industry beyond the use as fuel additive or as an engine fuel. Some of those applications are alcoholic beverages, chemicals, cosmetics and pharmaceuticals (ePure, 2014).

The production of ethanol by converting cereals into fuel through fermentation processes is relatively simple and it is well understood. In 2013, the USA bioethanol industry, whose feedstock is mainly corn and maize, produced and consumed around 50 billion liters, followed by Brazilian sugarcane bioethanol with approximately 23 billion liters. Europe is the World's third largest producer of bioethanol, equaling 6.7 billion liters in 2014. According to ePURE, in 2014, 86% (5.6 billion liters) of the ethanol produced in Europe was used as biofuel while 14% (1 billion liters) was for traditional markets with equal share between beverage and industrial applications. Although the EU ethanol market tripled in size between 2004 and 2009, growth rates have substantially slowed down in recent years (ePure, 2014; ePURE, 2015).

2.2 Studied species

In this project, the focus was on a cyanobacterium and a microalga. One is a genetically modified cyanobacterium from the wild type (WT) *Synechocystis* sp. PCC 6803, and the second species a non-modified Dinophyceae, *Prorocentrum* sp. These species were chosen since they were the basis of the two industrial scale study cases performed by *A4F* within the framework of EU-funded projects *DEMA* (from 2012 to 2017) and *PUFAChain* (from 2013 to 2017), respectively.

2.2.1 *Synechocystis* sp. PCC 6803

Synechocystis sp. PCC6803 (shown in Figure 2) is a unicellular fresh water cyanobacterium capable of both phototrophic growth by oxygenic photosynthesis during light periods and heterotrophic growth by glycolysis and oxidative phosphorylation during dark periods. It has a fast growth rate, high biomass yield, and simple growth requirements, which make it ideal for fast production. In addition, it is a quite simple organism, so it is frequently selected for genetic modifications.

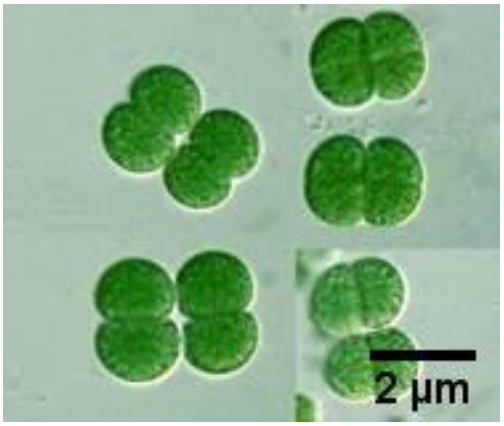

Figure 2 - Synechocystis sp. *PCC 6803 microscope view (adapted from https://alchetron.com/Synechocystis).*

In this project, this cyanobacterium was genetically modified in order to produce ethanol (which is not a native function) and to excrete the ethanol to the exterior of the cell. Studies performed at *A4F* - Algae for Future and in the available literature identified the most interesting commercial compounds present in the *Synechocystis* sp. PCC 6803 (Table 1).

Table 1 - Synechocystis sp. *most interesting components for commercial applications.*

Component	% of total biomass DW
Lipids (Sheng et al., 2011)	10.2
Sulfoquinovosyl diacylglycerol (SQDG)	3.53
Digalactosyl diacylglycerol (DGDG)	4.78
Monogalactosyl diacylglycerol (MGDG)	1.25
Phosphoglycerol (PG)	0.66
Proteins (Touloupakis et al., 2016)	67.5
Insoluble	31.40
Soluble	24.60
Phycocyanin (Deshmukh and Puranik, 2012)	11.50
Zeaxanthin	0.9
Carbohydrates *1	21.4

*1 obtained through difference calculations

2.2.1.1 Products obtained from *Synechocystis* sp.

The main product obtained from the modified *Synechocystis* is ethanol. Other interesting products are the protein phycocyanin (Eriksen, 2008; Kuddus et al., 2013) and the carotenoid zeaxanthin (Hu et al., 2011).

2.2.2 *Prorocentrum* sp.

Prorocentrum sp. (shown in Figure 3) is a microalga that belongs to the dinoflagellates group. Most frequently, it is found in marine environments, but it is also common in freshwater habitats. Many dinoflagellates are known to be photosynthetic, but a large fraction of these are in fact mixotrophic, combining photosynthesis with ingestion of other species. The *Prorocentrum* strain used in this project is a saltwater strain and therefore requires a saline medium to grow. Studies performed at *A4F* and articles published in the available literature identified the most interesting commercial compounds present in the Dinophyceae. These can be seen in Table 2.

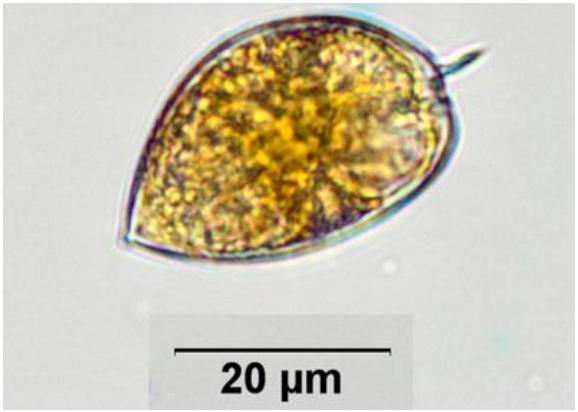

Figure 3 - Prorocentrum sp. *microscope view (from http://cfb.unh.edu).*

Table 2 – Prorocentrum sp. *component specification.*

Component	% of biomass DW
Lipids (A4F, 2018)	8.9
Triglyceride (TAG) (A4F, 2018)	0.9
Phospholipids (PL) (A4F, 2018)	6.8
Glycolipids (GL) (A4F, 2018)	1.2
Important Fatty acids	% of lipids
Docosahexaenoic acid (DHA) (A4F, 2018)	19.0
Stearidonic acid (SDA) (A4F, 2018)	19.0
Eicosapentaenoic acid (EPA) (A4F, 2018)	24.0
Proteins (Kharchuk and Pospelova, 2016)	41
Insoluble[*1]	30.0
Soluble[*1]	11.0
Pigments	0.8
Peridinin[*2]	0.5
B-Carotene (Häubner et al., n.d.)	0.1
Carbohydrates *[3]	24.3
Nucleic Acids (Kharchuk and Pospelova, 2016)	15

*[1]experimental data (Appendix 1)
*[2]Data from *PUFAChain*
*[3] Obtained through difference calculations

2.2.2.1 Products obtained from *Prorocentrum* sp.

As mentioned before, this microalga is rich in polyunsaturated fatty acids (PUFA), (van der Voort et al., 2017). Besides the PUFAs, the amount of protein is also quite high and can present an interesting product to obtain either for biofertilizers or as a protein concentrate for animal or human food. There are also interesting carotenoids like peridinin and ß-carotene present in the *Prorocentrum* biomass (Bogacz-Radomska and Harasym, 2018; Borowitzka, 2013; Carbonera et al., 2014; Ishikawa et al., 2016).

2.3 Microalgae biorefineries

A refinery is a production facility composed of a group of unit processes and unit operations that convert certain materials or raw material(s) into products of value. The most known types of refineries are the petroleum refineries where crude oil is converted into a large number of products like gasoline, diesel, and lubrication oil, among others. The concept behind a refinery is to try to obtain the largest number of products from the same raw material in order to monetize the raw material.

Due to the high prices involving the production of microalgae biomass, alternative solutions were procured and the biorefinery concept was born. The concept of biorefinery is similar to that of a traditional petroleum refinery, such that biomass is converted into

marketable chemicals, fuels and other products. According to the IEA Bioenergy Task 42 ‘‘Biorefineries” (Sonnenberg et al., 2013): ‘‘Biorefining is the sustainable processing of biomass into a spectrum of marketable products and energy”. In a microalgae biorefinery, similar to a chemical refinery, not only one product is produced from microalgae, but a panoply of different products with low/high value are obtained from the same biomass. This is used to compensate for the previously mentioned high production costs (Harun et al., 2011).

The advantage of using microalgae over lignocellulosic biomass for the extraction of high-value products is that it can be cultivated by utilizing wastewater, nutrients and atmospheric CO_2, which may be available at minimum cost. Also, it does not cause competition for land or food crops as microalgae can grow on degraded land (Chew et al., 2017). Although the biomass of microalgae can be used without further treatment, for the higher value products, there is a need for further treatment in order to extract them. This treatment can go from simple dewatering processes like filtrations or centrifugations, to complex processes like supercritical CO_2 extractions or even transesterifications. In addition, in some cases where the end product is a biofuel, a fuel producing step is included in the process.

A typical microalgae based biorefinery process can be described in five stages (Figure 4):

1. Cultivation Stage
2. Harvesting Stage
3. Disruption Stage
4. Extraction Stage
5. Conversion Stage

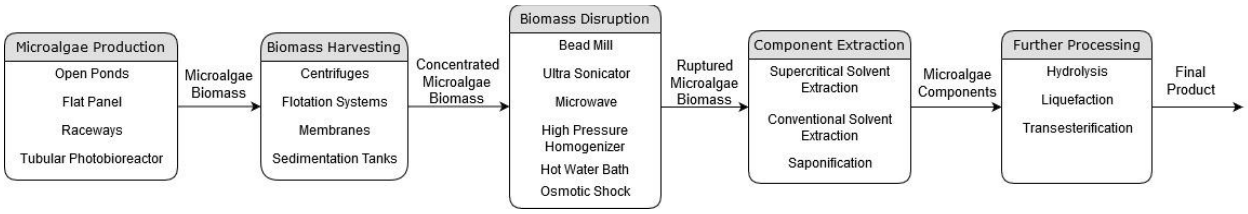

Figure 4 - Different biorefinery stages and example of possible equipment.

The first step comprises the production of microalgae biomass in a reactor. This step is performed either in open reactors (such as open ponds, raceways, cascade raceways) or in closed reactors (such as flat panels, tubular reactors) (Carvalho, A. et al., 2006).

The second step is where the biomass is concentrated and separated from the culture medium. This can go from simple nets to complex membrane systems (Muylaert et al.,

2017; Singh et al., 2013). After this step, we can also include a biomass drying step. In the end of this step one can obtain different products, either in paste or powder. In some cases, this is the last processing step before the microalgae biomass is sold to the market. For others they continue to the following step.

The third step is mostly used in processes where the final product(s) of interest is (are) a component(s) of the microalgae. As the contents are intracellular, first the cells need to be disrupted. This can be achieved through mechanical rupture by using a bead mill or by more complex processes like osmotic shock or enzymatic digestion (’t Lam et al., 2017; Qin et al., 2014).

In the fourth step, the components are extracted from the biomass and the desired components are separated from all the other components that are present in the microalgae biomass. This can be achieved by solvent extraction or supercritical fluid extraction. After purifying the desired components, these are then sold to pharmaceutical, cosmetic or food industry to be included in their products (Ventura et al., 2017).

The fifth step is used in case the final product is produced from components of the biomass, like for example polysaccharides into simple sugars and proteins into amino acids, or even the production of biodiesel or bioethanol (Bleakley and Hayes, 2017; Davis et al., 2011; Ho et al., 2013; Lopes et al., 2019).

Although to the best of the author knowledge no industrial sized microalgae biorefinery is described in the literature, a vast different number of studies have already been performed on this topic. Most studies focus the review on the different products that can be obtained from different microalgae species and processes that can be used in a biorefinery. Others focus on the production of biofuels from microalgae and other co-product, like proteins and carotenoids that can improve the economic performance of the refinery. Then, it was focused the symbiosis of microalgae biorefineries using industrial waste to produce different products from microalgae. Finally, only a few studies performed techno-economic analysis of biorefinery scenarios. Examples of some studies can be found in Table 3.

Table 3 – Example of biorefinery studies and their conclusions.

Article title	Topic	Remarks	Reference
Microalgae biorefinery: high-value products perspectives	Biorefinery Overview	• Insight on the biorefinery of microalgae for the production of high value products	(Chew et al., 2017)
Microalgae-utilizing biorefinery concept for pulp and paper industry: Converting secondary streams into value-added products	Biorefinery integrated in the Paper industry	• Production of microalgae using paper industry waste stream • Results indicate that the proposed process is technically viable • Improvement of sustainability of the paper and pulp industry	(Taelman and Sfez, 2015)
Carbon dioxide capture from flue gases using microalgae: Engineering aspects and biorefinery concept	Biorefinery Integration with Flue Gas Capture	• Discussion on approaches for making CO_2 fixation by microalgae economically competitive in comparison with CCS methodologies	(Pires et al., 2012)
Integrated microalgae biorefinery: Impact of product demand profile and prospect of carbon capture	Biorefinery Integration with Flue Gas Capture	• A biorefinery product portfolio depends on product prices and demand	(Sen Gupta et al., 2017)
A techno-economic assessment of an algal-based biorefinery	Biorefinery techno-economic assessment of 4 biorefinery scenarios	• Algal Based biorefineries can be economically viable • Price volatilization of carotenoid prices can have a large impact on the profitability of a project	(Thomassen et al., 2016)
Techno-economic assessment of the sustainability of an integrated biorefinery from microalgae and Jatropha: A review and case study	Techno-economic analysis of different biorefinery scenarios	• A biorefinery with microalgae and Jatropha as feedstock is technically feasible and economically profitable • One of the largest costs is the labour followed by the raw materials • Large scale production might bring down the cost of biofuel production	(Giwa et al., 2018)
Biorefinery of microalgae for food and fuel	Biorefinery Options and products Review	• Microalgae present a broad spectrum of possible products • Pulsed Electric Field (PEF) for rupture and ionic liquids look very promising	(Vanthoor-Koopmans et al., 2013)
Microalgae for the production of bulk chemicals and biofuels	Biorefinery Options and products Review	• Insight on the microalgae biorefinery process options and possible products	(Wijffels et al., 2010)
Zero-waste algal biorefinery for bioenergy and biochar: A green leap towards achieving energy and environmental sustainability	Zero waste biorefinery process design	• Microalgae cultivation utilizing wastewater nutrient and mitigating CO_2 emission while generating biofuels and high value products in a closed circular biorefinery results in cost reductions	(De Bhowmick et al., 2019)
A Biorefinery from *Nannochloropsis* sp. microalga – Energy and CO_2 emission and economic analyses	*Nannochloropsis* Biorefinery	• Production of oil, pigments and H_2 from *Nannochloropsis* is economically feasible	(Ferreira et al., 2013)

Article title	Topic	Remarks	Reference
Microalgae – A green multi-product biorefinery for future industrial prospects	*Biorefinery review Study*	• Biorefineries approach increases the economic feasibility of producing products from Microalgae • Microalgae production is still a bottleneck	(Bhattacharya and Goswami, 2020)
Environmental and techno-economic evaluation of ß-carotene production from Dunaliella salina. A biorefinery approach	*Dunnaliella salina* biorefinery	• Supercritical extraction exhibits low extraction yields leading to larger and more expensive equipment. • Energy (electricity and heat) can be obtained from residual biomass	(Espada et al., 2020)

Most studies agree that some microalgae strains have a large potential as a feedstock for biorefineries, due to the high number of high-value products that one can obtain from them. In addition, some studies have shown that biorefineries have a positive impact on the profitability of microalgae biomass production, especially when the main product is biodiesel or bioethanol, as both product prices are quite low in order to compete with current fossil fuel prices. However, like any other industry, the biorefinery profitability depends on the demand for the products it produces. The symbiosis between microalgae biorefineries and the industry has also demonstrated to have a high potential for treating and giving value to the waste streams produced by the industries, and at the same time decreasing biorefinery operation costs.

2.4 Microalgae production

Although microalgae grow in nature, they do not grow in sufficient amount to make their direct harvesting from the wild economically feasible. Therefore, several processes have been designed to improve and increase the yield and output of biomass production. The microalgae production complexes can go from simple open reactors, to complex closed reactors. The main purpose of these systems is to improve and supply the best growth conditions for microalgae in order to maximize their growth rate and obtain the maximum yield.

2.4.1 Open ponds production systems

This type of systems consists of Natural or Artificial Ponds, which are usually between 0.2 and 0.5 m deep with a closed recirculation loop, which is responsible for the mixing and CO_2 circulation. Artificial Ponds like the raceway ponds are usually built in concrete, while earth lined ponds are usually covered with plastic. The movement in the ponds is promoted by a paddlewheel, which is in continuous movement in order to prevent sedimentation. The CO_2 is usually obtained from the surface air although to improve CO_2 absorption, submerged aerators may be used. Cascade raceways (Figure 5) are another example of an open production system. These systems use the inclination of the raceway in order to promote the continuous movement of the culture. The culture is recirculated with assistance of a pump. Open pond systems are more useful to produce microalgae species that can be grown under highly selective environments, like high salinity, extreme pH conditions, in order to decrease the possibility of contaminations by bacteria and fungi, but also of other microalgae species and grazers. Despite the cheaper construction when compared to closed photobioreactors, they are unfortunately less efficient, with a lower productivity. The lower efficiency is attributed to several factors like, CO_2 deficiencies,

due to inefficient mixing, and light limitations, which limit the utilization of these components by the microalgae, but also temperature fluctuations, which affect the optimal growth of the microalgae and influence evaporation losses that change the optimal composition of the growth medium. Improving mixing, and therefore CO_2 diffusion, with a decrease of the layer thickness can improve the productivity of the open pond system (Brennan and Owende, 2010).

Figure 5 – A cascade raceway open pond (www.a4f.pt).

2.4.2 Closed Photobioreactor systems

Some of the previously mentioned problems can be solved by using close photobioreactor technology. These systems are better suited to cultivate microalgae species that are more susceptible to contaminations, since the risk of contamination is smaller due to their closed configuration. However, due to their higher costs, when compared to open systems, they are more suitable for microalgae with final destination high-value products like cosmetics and pharmaceuticals. Another advantage of having a higher productivity is the decrease of the harvesting cost. Closed photobioreactors can be tubular, flat plate or column systems. Tubular photobioreactors (Figure 6b) consist of an array of straight glass or plastic tubes. The tubes can be placed horizontally, vertically, inclined or as a helix and have a small diameter, usually no bigger than 0.1 m. In these systems the microalgae cultures are re-circulated with a mechanical pump. Tubular reactors are considered to be the most suitable for outdoor mass cultures since they expose a larger surface area to sunlight, however they have design limitation on the length of the tubes. This length is limited by O_2 accumulation, CO_2 depletion and pH variation (Brennan and Owende, 2010). Other types of closed systems are flat panel photobioreactors (Figure 6a). These reactors are made of transparent materials, like glass or polymethyl methacrylate plastic, for maximum solar energy capture. In addition, the thin optical path, improves the radiation absorbance in the first few millimeters thickness. The mixing is provided by an airlift system, also allowing and improving the exchange of CO_2 and O_2 between the liquid medium and the aeration gas (Slegers et al., 2011). Finally, there are also column

photobioreactors. These offer the most efficient mixing, the highest volumetric mass transfer rates and the best controllable growth conditions. Allied to these characteristics, they are cheap, compact and easy to operate. The vertical columns are aerated from the bottom and illuminated through transparent walls or internally. Although their performance can be compared to that of the tubular photobioreactors, the energy costs are higher and they have a smaller illumination surface area (Sánchez Mirón et al., 2000).

Figure 6 - a) Flat panel photobioreactor (http://www.iowaepscor.org/). b) Tubular photobioreactor (da Ponte et al., 2016) .

2.5 Biomass Harvesting

This step consists of the concentration of microalgae biomass. Usually, after the production stage, the final concentration of the biomass is around 0.1-10 g/l. The objective of this step is to increase the concentration 50 to 200 fold (Molina Grima et al., 2003). In this step, the choice of the equipment resides on the desired final concentration, but also on the properties of the microalgae, since certain harvesting processes can damage the microalgae cell walls and rupture the cells, releasing the intracellular contents into the medium. Common harvesting techniques include filtration, gravity sedimentation, flocculation, flotation or centrifugation (Barros et al., 2015).

2.5.1 Sedimentation and flocculation

The sedimentation process is influenced by the settling velocity of microalgae, which depends on the weight of the microalgae, as well as the cell dimensions. This said, it is possible to increase the settling velocity of the microalgae by adding chemicals that

promote flocculation. Flocculants can be cationic, anionic, or non-ionic and flocculate the cells without affecting their composition and/or being toxic. Since microalgae cell walls have a negative charge that prevents self-aggregation, this negative charge must be countered by the addition of polyvalent ions like Al^{3+} and Fe^{+3}. The type of flocculants must be chosen according to certain considerations such as the pH of broth, concentration of biomass, and its charge. During flocculation (Figure 7), microalgae aggregate into large bodies, increasing their size and weight, causing the large algal flocs to settle in the bottom of the container faster than single microalgae cells (Chen et al., 2015).

Since flocculation is a quite cheap process, it is frequently used in an initial stage of cellular harvesting. However, it has some drawbacks; since the gravity settling rate is quite low, it is not very useful for high rate algae harvesting. This leads to other problems like loss of biomass due to holding algal biomass for a long time under dark and static. Furthermore, flocculation may not be 100% efficient since flocs may float due to adsorption of tiny air bubbles (Al Hattab et al., 2015a).

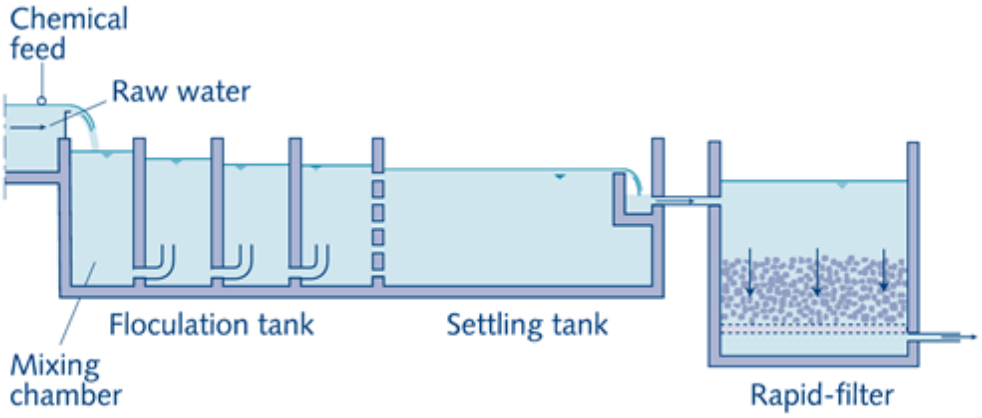

Figure 7 - Flocculation Example (Napier Reid, 2017).

2.5.2 Flotation

Flotation is a separation process that is opposed to the sedimentation. It is based on the attachment of air bubbles to the solid particles. The resulting flocs lift to the liquid surface and there they are removed by skimming and/or filtration (Figure 8). The yield of the flotation process depends on the nature and size of suspended particles. The smaller the particles, the easier it is to lift them up, and the lower instability of suspended particles results in a slightly higher air–particle contact. The attachment of air bubbles to the solids also depends on the air, solid and aqueous phase contact angle. According to the method of bubble production, there are three common techniques: Dissolved air flotation (DAF), electrolytic flotation, and dispersed air flotation. The larger the contact

angle, the greater the tendency of air to adhere to the particle. The main advantage of this method is that compared to sedimentation alone and flocculation, flotation is faster and more effective for microalgae harvesting. The main disadvantage is that it is an energy intensive process with high operational and energy costs (Ndikubwimana et al., 2016).

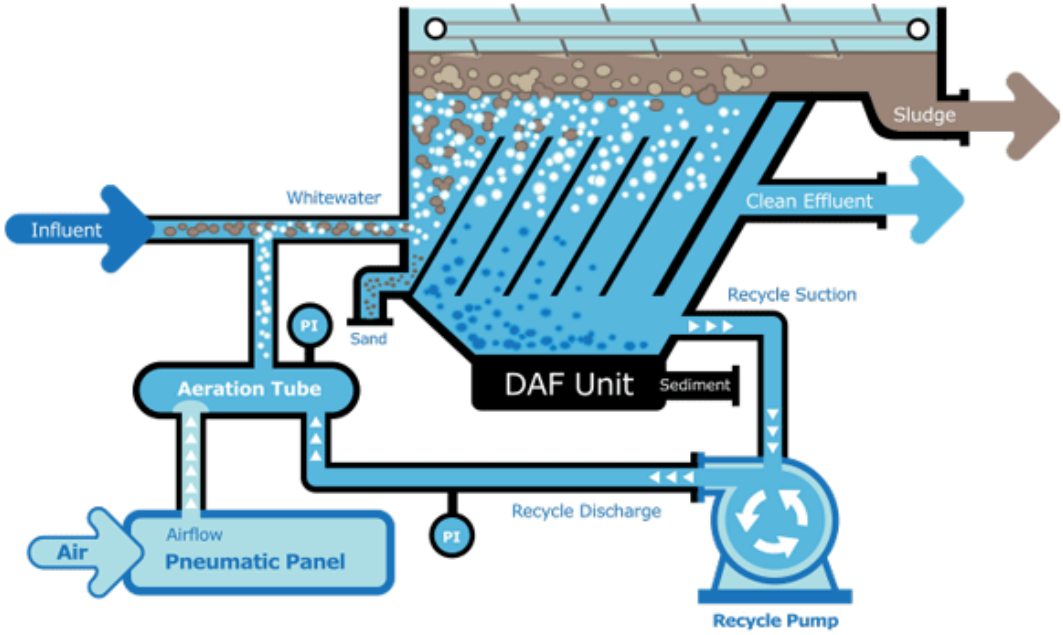

Figure 8 - Dissolved Air Flotation System (JWC Environmental, 2017).

2.5.2.1 Chemical flocculation

There are a many flocculant chemicals that can be used and therefore in order to choose the most efficient one would take a great deal of time, and another study would be required. Added to this, one of the problems of using most flocculant chemicals is that most of them prevent from using the harvested microalgae in food or pharmaceutical applications, since they are hazardous to human health, decreasing the final product options (that will occur in the second phase of this study). Since the idea of this study is to choose the best methods, and in the end, have the largest number of product options, it was decided to choose a known flocculant that allows the use of the harvested biomass in the largest number of possible products. The chosen chemical is chitosan, which is a chemical already used and that can be used for pharmaceutical and food industry. Chitosan is a cationic polyelectrolyte obtained by deacetylation of chitin. Chitin is the derivative of glucose and the second most abundant biopolymer in the world and can be obtained from the shell of crustaceans. Unlike the metal salts used as flocculants, chitosan is non-toxic, biodegradable, renewable and ecologically acceptable. Therefore, the harvested biomass can be used for pharmaceutical and food purposes and there is no problem in recycling the culture medium (Renault et al., 2009). However, if the final

scenario shows that the best refinery scenario is the use of flocculation and the manufacture of products other than for food and pharmaceutical applications, then a cheaper chemical flocculant must be chosen. This flocculant will be used in both the Sedimentation and the Flotation process (Zhu et al., 2018).

2.5.3 Membrane filtration

Membranes provide a physical barrier that limits the passage of liquids, gases, ions, molecules, colloids, cells, suspended solids, depending on their properties (size, charge, solubility, and other chemical compositions) and on membrane characteristics (pore size and distribution, charge, surface roughness, and material). Active filtration works by action of a pressure gradient, where the solute is selectively rejected and the filtrate (permeate) is drawn across the membrane. Active filtration includes dead end filtration (DEF), and tangential flow filtration (TFF) (*Figure 9*). There is also passive filtration (e.g., dialysis and forward osmosis) which relies on the transfer of a solute or solvent across the membrane as a result of concentration gradients (*Figure 9*). Passive filtration generally requires less energy than active filtration since it does not require a pressure gradient (Marcel, 1996), but the fluxes and permeabilities are much smaller.

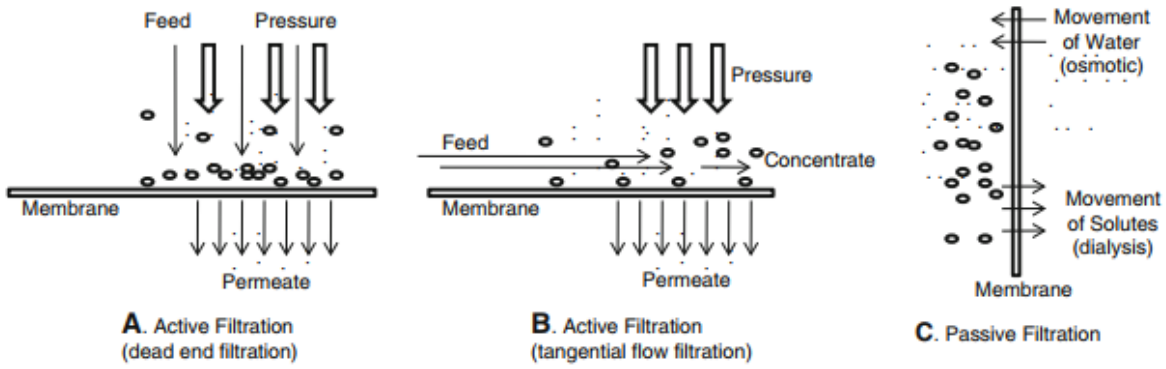

Figure 9 - Types of Filtration (Drexler and Yeh, 2014).

Filtration can also be classified according to the size of the pores. As can be seen in Figure 10, depending on the size of the rejected solutes, filtration can be qualified into macrofiltration (MaF), microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) (Drexler and Yeh, 2014).

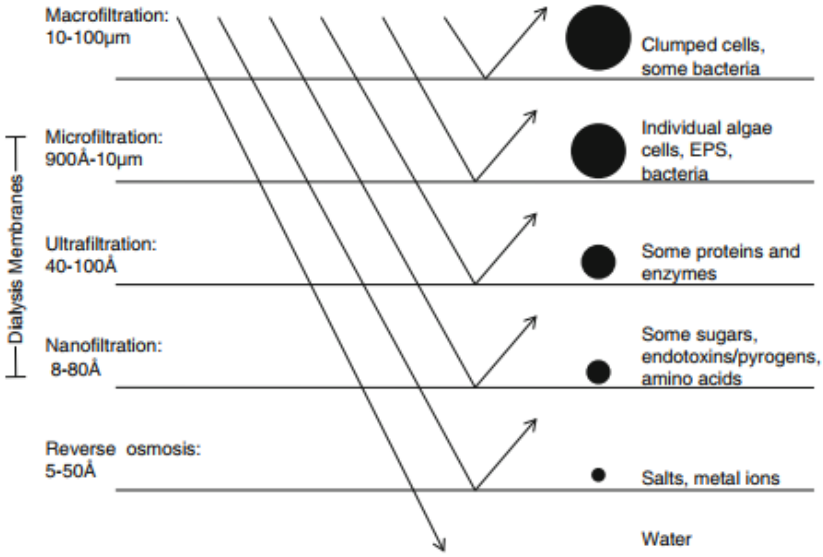

Figure 10 - Different types of filtration based on size of solute (Drexler and Yeh, 2014).

Membrane technology has in recent years become a popular option for algal harvesting, since its unique characteristics provide an efficient solid/liquid separation (cell retention, biomass concentration and dewatering) and solute/liquid separation (bioproduct recovery, feedstock preparation, and effluent recycling) that are sometimes problematic or not possible with other technologies. Due to the size of the microalgae, most membranes sizes used are for microfiltration or Ultrafiltration, with the standard type of filtration being active filtration (Drexler and Yeh, 2014).

Another positive point of membrane filtration is that it is barely disruptive to harvested biomass. Membranes cause minimal stress to the algal cells, and avoid chemical additives (e.g., flocculants or pH adjustments required for flocculation) that can degrade the quality of harvested biomass or the produced products. The main disadvantages of membrane systems are that they require high energy consumption (especially in the active filtration), therefore the operating costs are quite high, and membranes are susceptible to fouling (Drexler and Yeh, 2014).

2.5.4 Centrifugation

The principle behind centrifugation is the use of centrifugal forces to enhance the concentration of solids. The difference between particle size and density of the components are the key factors in centrifugal separation. Once separated, the algae concentrate can be obtained by simply draining the supernatant (Chaplin, 2014).

Generally, there are two types of centrifuges: Disk stack centrifuges (Figure 11) and Decanter centrifuges (Figure 12).

The first one consists of a shallow cylindrical bowl that has several stacks of metal discs, which are closely spaced together. The separation of the materials is based on densities. The mixture is placed on the center of a stack of discs and the lighter phase of the mixture remains on the inside towards the center while the denser phase is displaced outwards to the underside of the discs. This technique separates materials of different densities by layering them. The advantage of this type of centrifuge is its high-efficiency rate of algal removal. It can handle high flow rates as is able to separate small particles. Its main disadvantages are that it is hard to clean, costly and is a quite complex system (Al Hattab et al., 2015a).

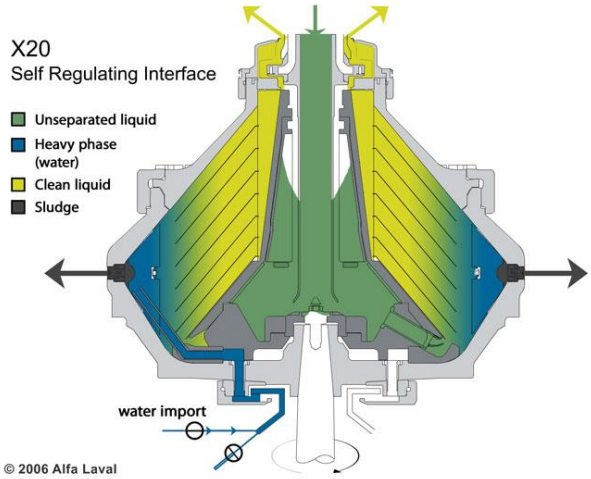

Figure 11 – Disk stack centrifuge (Geoff Gough et al., 2011).

The decanting centrifuge uses a special settling tank in which the solids in suspension are forced to fall down by action of the gravitational forces. The decanter centrifuge operates continuously by pumping the biomass into the centrifuge bowl while the suspended particles in solution are forced to the bottom of the bowl. The liquid left after removal of the particles goes through the overflow pipe (Figure 12). The main advantages of the Decanter centrifuge are the higher concentrated dewatered biomass, when compared to the disc centrifuge. However, the decanter centrifuge is more suited for suspensions with higher solid particles and is unsuitable for microalgae suspensions. The disadvantages of using this method for microalgae harvesting is the required concentrated feeds, usually in the range of 4-40% w/w. In addition, fines might be present in the liquid, which lead to inferior flow properties and can cause mechanical difficulties. Furthermore, decanting centrifuges are much more energy intensive than disc centrifuges and due to the larger processed volume it has higher operating costs associated (Shelef and Sukenik, 1984).

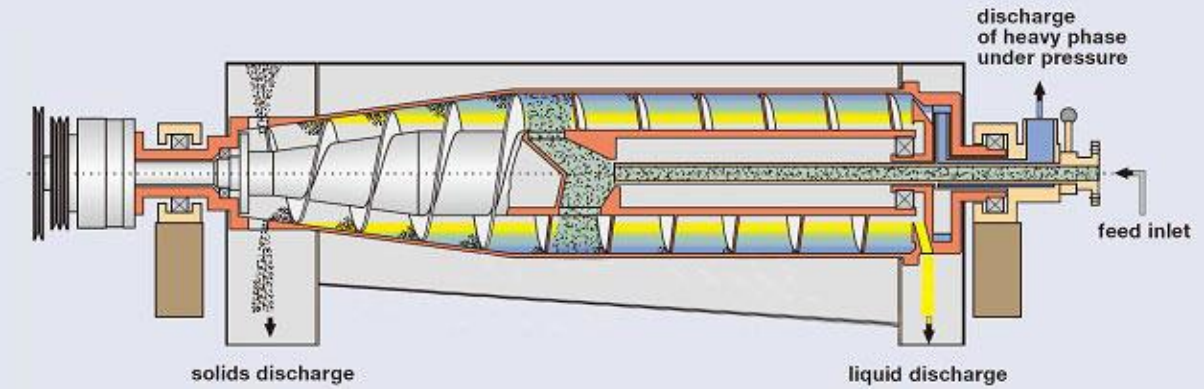

Figure 12 – Schematics of a Decanter Centrifuge operation (GN Solids Control, 2016).

Examples of the application of the different harvesting methods, including some of their operating constraints, are summarized in Table 4.

Table 4 - Examples of the different harvesting methods.

Process	Solids concentration (g/l)*	Observations	Reference
Hollow Fiber Membrane	150	Cross Flow Continuous Operation	(Bhave et al., 2012; Bilad et al., 2014; Drexler and Yeh, 2014)
flat Sheet Membrane	260	Dead End Filtration Batch Operation	(Drexler and Yeh, 2014)
Sedimentation flocculation	10 - 80	Requires addition of flocculants	(Branyikova et al., 2018; Martínez, 2016)
DAF Flotation	60 - 80	Requires addition of flocculants and air	(Branyikova et al., 2018; Ndikubwimana et al., 2016)
Decanter Centrifuge	220	Can cause cell disruption and requires high solid concentration	(Molina Grima et al., 2003)
Disk Centrifuge	200	Can cause cell disruption	(Monte et al., 2018; Shelef and Sukenik, 1984)

*values can differ depending on the microalgae species

2.6 Drying methods

In certain cases, the harvested biomass must be processed rapidly, or it can spoil. Dehydration or drying of the biomass is commonly used to extend the shelf life of the biomass, especially if whole biomass is the final product. There are several types of drying processes, from solar drying to freeze-drying. For example, spray drying is a commonly used method; however, it can cause significant deterioration of some algal components

such as pigments. On the other hand, freeze-drying is too expensive for use in large-scale commercial recovery of microalgae products, but it does not significantly damage the algal components (Molina Grima et al., 2003).

2.6.1 Solar Drying

Solar drying is the cheapest method to dry microalgae biomass, however, it requires large drying times and a large drying surface. In addition, since it is exposed to changing conditions, it is not easy to maintain the quality of the end product. The slow drying rate due to low temperature can also cause biomass degradation and increase the bacterial count. Although the quality might not be standard, this method can still be used in processes where the quality does not influence the final product. Closed solar drying devices can increase the environmental temperature from 35 to 60 ºC, and consequently decrease the drying times (Chen et al., 2015). However, too high temperatures can damage the compounds of interest.

2.6.2 Convective Drying

Convective drying is achieved by using a convective flow of hot air, such as oven drying. The drying efficiency is far better than the solar drying and, if properly optimized, the biomass FFAs content is very similar to the one obtained by freeze-drying. However, the loss of proteins can go up to 20% of the total proteins and the loss of phycocyanin can go up to 50%. Therefore, it is considered a useful method for biodiesel production, however, it is not appropriate for high value products (Chen et al., 2015; Oliveira et al., 2010).

2.6.3 Spray Drying

The spray dryer dries the biomass by atomising the suspension into fine droplets, which are thrown centrifugally into a moving stream of hot gas. The temperature is high enough to dry biomass almost instantaneously in the form of powder. The powder is then collected by a cyclone. Spray drying is a commonly used method for microalgae drying since it can dry the biomass quite fast. However, it has the disadvantage that it can cause some deterioration of some algal components, like proteins and pigments. Studies have shown that around 10% of the proteins are lost due to spray drying (Chen et al., 2015). Compared with freeze-drying, the microalgae biomass treated by spray drying is less susceptible to lipolysis upon storage, but the carotenoids in spray-dried microalgae oxidize more readily than those in freeze-dried microalgae (Molina Grima et al., 2003).

2.6.4 Freeze-drying

Freeze-drying is achieved by freezing the biomass and afterwards reducing the surrounding pressure to allow the frozen water to sublime directly from the solid phase to the gas phase. This method is very useful since all the cell constituents are preserved without rupturing the cell wall. However, if such is required, it can still cause cell disruption by slow freezing the biomass forming ice crystals that disrupt the cell wall (McGrath et al., 1978). Unfortunately, it is a very expensive method for large scale plants (Molina Grima et al., 2003).

2.7 Cell Disruption methods

In order to access the desired components, the microalgae cell wall must be ruptured. Studies have found that the extraction yield of microalgae components can be higher if the biomass is previously treated by some kind of cell rupture process (Molina Grima et al., 2003; Taucher et al., 2016). This can be done using different methods. Although the values in Table 5 are generalized values, it is important to keep in mind that the efficiency and time that it takes to achieve cell wall rupture can be significantly different depending on the microalgae. This table is meant to give a general idea of the efficiency of the different cell disruption methods.

2.7.1 Bead mill

A bead mill uses very small beads made of glass, ceramic or steel which are mixed with the biomass. The vessel is then agitated at high frequencies. The suspended biomass cells are disrupted in the bead collision zones by compaction or shear forces with energy transfer from the beads to the cells. The type of beads, the size of the beads, agitation frequency, as well as the addition of solvents are the main factors that influence the cell disintegration (Günerken et al., 2015; P.R. Postma et al., 2017).

2.7.2 Sonication or ultrasonication

Sonication produces sound waves that spread into the liquid creating alternating high-pressure and low-pressure cycles. During the low-pressure cycle, high-intensity small vacuum bubbles are created in the liquid. When the bubbles reach a certain size, they collapse violently during the high-pressure cycle. This phenomenon is called cavitation. During the implosion, very high pressures and high-speed liquid jets are produced. The resulting shear forces break the cell structure mechanically (Hielscher, 2017; Kurokawa et al., 2016).

2.7.3 Osmotic shock

This process can only be used with microalgae that grow in saline environments. The microalgae are placed in contact with a freshwater environment and due to the difference in salt concentration, water enters the cell in large quantities, swelling the cell until the wall ruptures. This method is quite inexpensive since it only requires water, however it can only be used with microalgae that grow in saline environments (Byreddy et al., 2015).

2.7.4 Microwave

When a suspension is exposed to microwaves, the microwaves interact with the dielectric or polar molecules, such as water, and cause heating as a result of frictional forces from inter- and intramolecular movements. The water exposed to microwaves reaches the boiling point, expanding within the cell and increasing the internal pressure. The advantages of microwave are effectiveness and easy scale-up because of the simplicity of the technique. Since the temperature increase is more homogeneous compared to other conventional heating methods, the heat-related denaturation is less likely to occur. The disadvantage is that, because the disruptive effect is mainly based on the absorption of microwave energy by water molecules, the effect of microwave treatment is higher on diluted suspensions in comparison with concentrated suspensions. Further, since only a fraction of the water is held inside the cells, the majority of the radiation energy is absorbed by the surrounding medium and lost as heat creating protein aggregation and denaturation (Bleakley and Hayes, 2017; Theegala, 2015).

2.7.5 Hot water bath

The hot water bath causes cell disruption by thermally induced pressure much like the microwave method. The rise of temperature increases the internal pressure of the cells and since cells cannot handle the elevated pressure, they rupture. The advantage of this method is that most of disrupted cells break into large debris from cracked and split cells, which is an advantage in large scale operation for ease of handling and separating from soluble products. However, this method has a drawback since it causes an increase in viscosity leading to non-Newtonian viscoelastic behavior, which for large scale processing may complicate pumping processes and reduce centrifuge efficiency (McMillan et al., 2013).

2.7.6 High Pressure homogenizer

A high-pressure homogenizer produces a homogeneous size distribution of particles suspended in a liquid, by forcing the liquid under the effect of pressure through a homogenization valve. The sheer stress produced by the impact of the cells in the

homogenizer wall causes the disruption of the cells. The mechanism of cell disruption through the homogenizer is still not fully understood, but has been attributed variously to fluid shear stress, inertial forces, impingement and cavitation. The cell rupture efficiency depends on impact ring bore diameter and impact distances and valve geometry (Patrignani and Lanciotti, 2016). This method is mostly used in the food industry were it successfully results in cell disruption of dense microbial cultures. An advantage of high-pressure homogenization is the scalability. For example, there are industrial scale systems that have throughput capacities over 50,000 L/h at moderate pressure and up to 5,750 L/h at high pressure (up to 310.264 MPa) (Yap et al., 2015).

Table 5 – Different cellular disruption methods and their efficiency (% of ruptured cells).

Microalgae Strain	Process	Efficiency (% of ruptured cells)	Reference
H. pluvialis, C. vulgaris	bead mill	80-95	(Postma et al., 2015; Taucher et al., 2016)
N. oculata, C. gracilis	Sonication	65-100	(Kurokawa et al., 2016; McMillan et al., 2013)
Schizochytrium sp. S31	osmotic shock	48	(Byreddy et al., 2015)
N. oculata	blender (grinder)	92	(McMillan et al., 2013)
N. oculata	microwave	92	(McMillan et al., 2013)
N. oculata	hot water bath	82	(McMillan et al., 2013)
Chlorella* sp., *T. suecica	High Pressure Homogenization	90-96	(Spiden et al., 2013; Yap et al., 2015)

2.8 Extraction and purification methods

The extraction step of the process is one of the most important stages of the biorefinery. In this step, the different components of the microalgae are separated from each other. Therefore, the selection of the method is of great importance. Currently, the most used method is solvent extraction with conventional or supercritical solvents (Table 6).

2.8.1 Conventional solvent extraction

The principle of this method is to separate compounds, based on their solubility in two different immiscible liquids, usually water and an organic solvent. There are two types of solvent extraction; liquid-solid extraction if the biomass is dried, and liquid-liquid extraction if it involves the transfer of one (or more) solute(s) contained in a feed solution to another immiscible liquid (solvent). The solvent that is enriched in solute(s) is(are)

called extract. The feed solution from where the solute(s) is(are) removed is called the raffinate (Kislik, 2012).

In the case of microalgae, this method can be used to remove lipids and pigments (Cuellar-Bermudez et al., 2015; Mubarak et al., 2015) from the ruptured biomass. The choice of solvent is usually based on the solubility of the component that is desired; however, sometimes these solvents are harmful (DMF, methanol) to health and so, depending on the application (e.g. food, pharmaceutical or chemical) others less harmful, but also less efficient solvents have to be used (e.g. ethanol, heptane).

2.8.2 Supercritical fluid extraction

Supercritical Fluid Extraction (SFE) is a process where one component is extracted from another (the matrix) using supercritical fluids as the extracting solvent. A supercritical fluid is any substance at a temperature and pressure above its critical point, where distinct liquid and gas phases do not exist. Due to these properties, it can flow through solids like a gas, while dissolving the desired component like a liquid. Supercritical fluids are used as a substitute for organic solvents in a range of industrial and laboratory processes. The desired component is usually removed from a solid matrix, but can also be removed from liquids (Ahmad et al., 2019).

The most commonly used supercritical fluid is carbon dioxide. It is very useful for the extraction of heat-sensitive components, since it has a moderate critical pressure, a high critical density, and a critical temperature close to ambient temperature. The main advantage is that by changing pressure the supercritical solvent dissipates as gas, however since large pressures are required, the operational and capital costs are quite high (Lozowski, 2010). Another advantage is that carbon dioxide is non-flammable, non-corrosive and non-toxic. However, the disadvantage of this method is that currently the biomass has to be previously dried for the method to be efficient (Ghasemi Naghdi et al., 2016).

2.8.3 Other extraction methods

Although the previously presented methods are the most used, there are other methods that have been used for the extraction of biomass components. Methods like Adsorption chromatography with activated carbon, affinity precipitation with chitosan, diafiltration, mechanical presses and a combination of methods like microwave with solvent extraction have also shown positive results. However, they still have not been used in large scale. Table 6 summarizes different examples of extraction methods applied to several microalgae strains to recover compounds of interest.

Table 6 - Examples of extraction and purification methods.

Microalgae strain	Process	Solvent or separation	Product recovered	Product Recovery	Reference
Lipids					
Pavlova sp.	Solvent extraction	hexane/ethanol	Lipid oil	50%	(Mubarak et al., 2015)
Synechocystis PCC 6803	Supercritical CO_2	-	Lipid oil	34%	(Mubarak et al., 2015)
-	Mechanical press	-	Lipid oil	75%	(Mubarak et al., 2015)
Scenedesmus obliquus	Microwave	hexane	Lipid oil	77%	(Mubarak et al., 2015)
Pigments					
Dunaliella salina	Solvent extraction	DMF	Carotenoids	14 mg/g dw	(Macías-Sánchez et al., 2008)
Dunaliella salina	Supercritical CO_2	ethanol	Carotenoids	9 mg/g dw	(Macías-Sánchez et al., 2008)
Limnothrix sp.	Chromatography with active carbon and affinity precipitation with chitosan	-	C-phycocyanin	90%	(Cuellar-Bermudez et al., 2015)
Haematococcus pluvialis	Solvent extraction	acetone	Astaxanthin	87%	(Cuellar-Bermudez et al., 2015)

3 Sustainability and Process Optimization

3.1 Sustainability

Sustainability has been a hot topic in recent years, more with the increase of climate changes. There are several definitions for sustainability, however they all agree that for a business or process to be sustainable it must protect the environment and avoid the depletion of natural resources. According to Brundtland, (1987) the definition of sustainable development is “development that meets the needs of the present generation without compromising the ability of future generations to meet their own needs”. Further, according to businessdictionary.com, the definition of sustainability is “Continued development or growth, without significant deterioration of the environment and depletion of natural resources on which human well-being depends. This definition measures income as flow of goods and services that an economy can generate indefinitely without reducing its natural productive capacity”. Furthermore, according to dictionary.cambridge.org, the environmental definition of sustainability is “the idea that goods and services should be produced in ways that do not use resources that cannot be replaced and that do not damage the environment”.

One good example of an attempt to improve the sustainability of processes is the attempt to replace petroleum-based products by lignocellulosic or microalgae-based products. The reason behind this choice is the fact that the lignocellulosic material and microalgae-based material are less likely to be depleted, unlike fossil-based fuels, and have a lower impact on the environment (Chew et al., 2017).

But, how do we know that the microalgae processes are more sustainable than the currently used ones? Using biodiesel as an example, microalgae require a large amount of water to be produced and lignocellulosic materials require a large amount of land usage, unlike fossil fuels; on the other hand fossil fuel production releases more CO_2 into the atmosphere than the previous two. Each process has its downside, thus it is necessary to analyze and compare the different environmental impacts of the different processes.

As one of the most used methods, and one of the most accepted methods for the analysis of environmental impacts of processes, the method selected for the environmental sustainability evaluation in this project was the Life Cycle Assessment (Julio et al., 2017). Another important factor for the choice of this method was the fact that this method is used in a large number of studies on the environmental sustainability of microalgae

production, products and other related studies, which would permit the possibility of results comparison.

3.2 Life cycle assessment (LCA)

The previous method is englobed in the concept of Life Cycle Thinking (LCT). The objective and goal of LCT is to reduce a process' use of resources and emissions to the environment, as well as to improve its socio-economic performance, through its life cycle.

The quantitative analysis of the LCT is performed using the Life Cycle Sustainability Assessment (LCSA) which includes the environmental Life Cycle analysis, the Social Life Cycle Assessment, which studies the social impact of sustainability and the Life Cycle Costing, which analyses the economic sustainability. In this study, we will only be looking at the Environmental Life Cycle Assessment.

The Life Cycle Assessment (LCA) studies the products life cycle, from the raw material acquisition and the material used in the construction of the production equipment, to the final disposal of the product. This analysis allows studying the environmental impacts of the production process. It measures a range of environmental impact factors, including climate change, toxicity, eutrophication, water use, land use change and other important factors. However, the impact on the previous list has different units, some are length units, other weight, and others volume. Therefore, a unique unit must be standardized in order to be able to compare the different impacts. One of the methods to study the LCA can be just to study the greenhouse gases (GHG) emission throughout the process, from emissions of the production of the raw material, to the emissions of the production of the product and the emissions caused by the electric energy used, among other. In order to compare the scenarios, it is also necessary to establish similar boundaries for all processes.

When looking at the industrial sector, a product´s life cycle can begin with the extraction of raw materials from natural resources in the ground and the energy generation. Materials and energy are thus considered part of the production process, along with packaging, distribution, use, maintenance, and eventually, reuse, recycling, recovery, or final disposal of the products. This means that the entire production chain is connected via the product´s life cycle (Koroneos et al., 2013; Life Cycle Initiative et al., 2019). The Life Cycle Assessment (LCA) is an internationally standardized method as ISO 14040 that permits the quantification of an environmental analysis.

The ISO 14040 was created by the International Organization for standardization (ISO) to standardize and ensure consistency between studies(The International Standards Organisation, 2006). According to ISO 14040, an LCA can be defined as follows:

> "*LCA studies the environmental aspects and potential impacts throughout a product's life (i.e. cradle-to-grave) from raw material acquisition through production, use and disposal. The general categories of environmental impacts needing consideration include resource use, human health, and ecological consequences.*"

The LCA combines the data collection of emissions and resource consumptions along the life cycle (Life Cycle Inventory (LCI)), with the Life Cycle Impact Assessment (LCIA) of these emissions and resource consumptions. It is a method that has been used for product and process development but also for policy development (Life et al., 2007).

The ISO 14040 standard defines 4 phases in an LCA study (Figure 13):

1. Goal Definition
2. Scope Definition
3. Inventory Analysis (LCI)
4. Impact assessment (LCIA)

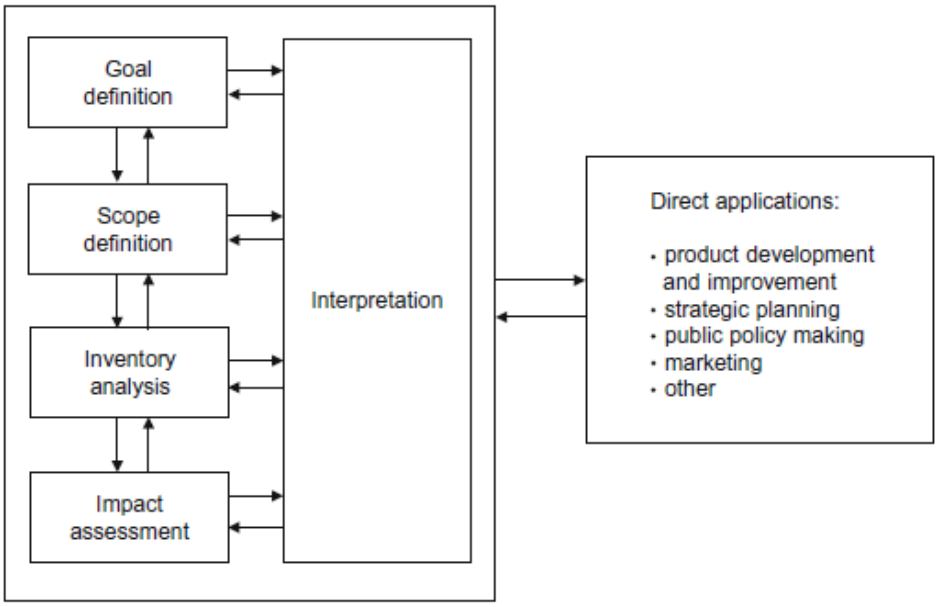

Figure 13- Framework of LCA from ISO 14040 Standard (The International Standards Organisation, 2006).

However, as seen in Figure 13, although they are in a sequence, they interact with each other. During the execution of one study, the hypothesis assumed during the initial stages (1 and 2) may change, as for example data may not be available or the existing environmental impact evaluation methodologies are still not adequate. Moreover, the LCA

methodology has an iterative nature, as the constant evolution of the production processes, the development of better environmental impact evaluation methods, the availability of more accurate data, among others, may render the LCA study outdated and impose the making of a new study.

3.2.1 Goal and Scope definition

An LCA always starts with the definition of the goal and scope of the study. The goal in this stage is to elucidate the background of the study and explain how and to whom the results are to be communicated. It is important that the goal and scope of an LCA are very clear since these will define the following steps. It is during this step that some of the most important definitions are also formed.

3.2.1.1 Function

When comparing two or more product systems, some of these product systems offer more than one function. It is therefore necessary to define a precise, quantitative description of the function provided by the product system in order to compare the systems.

3.2.1.2 Functional Unit

In order to quantitatively compare different functions/processes, it is required to define a functional unit. A functional unit defines the qualitative aspects and quantifies the quantitative aspects of the function/process. This functional unit should be formed by a function and not simply be a physical quantity, since different products can have different functions, which leads to non-comparable results.

3.2.1.3 Reference Flows

After the functional unit is defined, it is necessary to determine the reference flow. The reference flow is the amount of product that is required to perform the functional unit. Therefore, the reference flow should be the product flow to which all input and output flows of the processes in the product system are quantitatively related. The reference flow is the starting point for the ensuing LCI analysis because it determines all the product flows required throughout the life cycle of the product system studied and their associated elementary flows (resource uses and emissions).

3.2.1.4 System Boundaries

System boundaries define the boundaries between the studied product system and the surrounding economy and the environment. The boundary conditions must be the same for all the processes in order to obtain a comparable result (Maga et al., 2014). An example of such boundaries can be seen in Figure 14.

The system boundaries should contain all the unit processes required to produce the reference flow(s) defined by the functional unit. System boundaries should ideally be set so that all flows crossing them are elementary flows (resources and emissions). This means that all energy, products and waste should not cross the boundaries. This means all energy and products should be produced from the resources (inputs) while the only outputs are the emissions.

Sometimes it is required to divert from the previously mentioned parameters if a full study of the life cycle is not desired. One such example is the "cradle-to-gate" study where the system boundary ends at the gate of the factory where the studied product is produced.

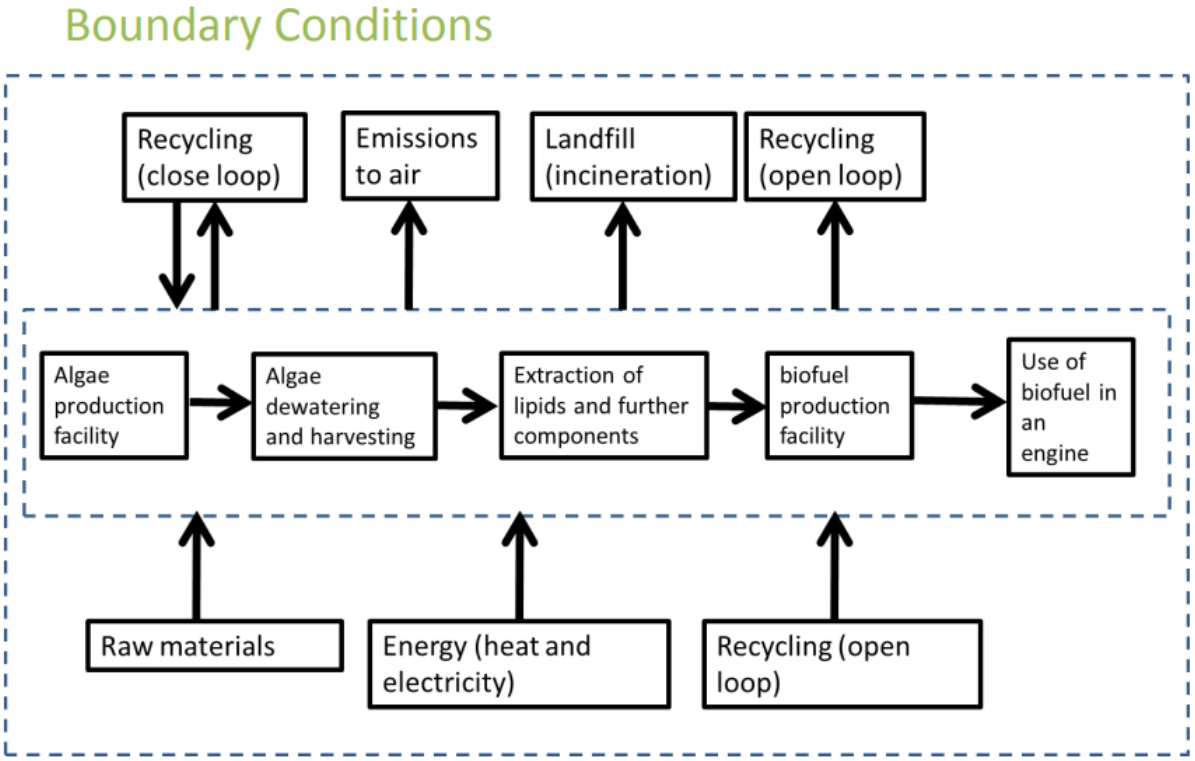

Figure 14 - Example of boundary conditions for an algae biorefinery (Maga et al., 2014).

3.2.2 Life Cycle Inventory (LCI)

After the goal and scope are defined, it is necessary to collect information on all the flows that go in/out of the process system.

The Life Cycle Inventory (LCI) is the list/inventory of flows from and to nature for a product system. Inventory flows include inputs of water, energy, raw materials, and releases to air, land, and water.

3.2.2.1 Identify the process flows

In this step, it is important to identify the various processes since there are different levels of processes in a product value chain.

The process starts with the reference flow and from there the system is constructed, process by process:

0. Is the unit process that has the reference flow as product output and should be the first to be identified (or unit processes, in the case of more than one reference flow). This is called a 'level 0' process;

1. These are the processes that deliver flows that will be physically transformed into the reference flow. These are termed "level 1" processes;

2. These processes deliver flows that perform a supporting function to the level 0 process (i.e. are not physically transformed in its output) and should then be identified. These are termed 'level 2' processes. One example is the electricity used by the 'level 0' process;

3. These are the processes required to deliver services to the level 0 processes. These are termed 'level 3' processes. Examples of level 3 processes are administration and marketing;

4. Finally the processes required to produce and maintain the infrastructure that enables the level 0. These are termed 'level 4' processes. These processes are production and maintenance (oiling, replacing and repairing parts) of the assembly machines.

After having identified level 1, 2, 3 and 4 processes belonging to the reference flow, Step 1–4 is then repeated for each of the other identified processes.

3.2.2.2 Planning and Collection of Data

3.2.2.2.1 Representativeness of LCI Data

The LCA model should portrait what occurs or has occurred in a process, to the best extent possible. Therefore, the unit processes applied to model the product system must be representative of the processes, which are used in the analyzed product system.

In order to obtain the most reliable data possible, a major part of the information used should be based on data collected first-hand by the company commissioning/performing the study.

The remaining information regarding parts of the foreground system and the entire background system is obtained from other data sources. However, it is important to consider how representative the chosen or constructed unit processes are regarding the

actual unit processes that they are modelling. Therefore, that data should be representative in three dimensions: geographical, time-related and technological.

3.2.3 Life Cycle Inventory Analysis (LCIA)

After the Life Cycle Inventory (LCI) is established and all elementary flows relevant for the product system are identified, it is necessary to compare and translate the contribution of each elementary flow (i.e. emissions or resource use of a product system) to an impact on the environment or human health. This is necessary since the elementary flows are just quantities emitted or used and cannot be directly compared to each other in terms of the importance of their impact. This is done in the Life Cycle Impact Assessment (LCIA) stage, where the objective is to examine the product system from an environmental perspective using impact categories and category indicators in conjunction with the results of the inventory analysis. For this step, it is necessary to choose a method.

LCIA characterization methods model the environmental mechanisms that cause each of the impact categories as a cause–effect chain starting from the environmental intervention (emission or physical interaction) all the way to its impact. There are different LCIA methods available in LCA software under names such as ReCiPe, CML, TRACI, EDIP, LIME, IMPACT 2002+, etc. (Menoufi, 2011), that combine a number of category indicators, based on specific characterization models. Some of the methods most frequently used in microalgae LCA studies are ReCiPe (Collet et al., 2013; Collotta et al., 2017; Jez et al., 2017) and CML (Dickinson et al., 2017; Spiden et al., 2013).

3.2.4 Impact Indicators

There are two types of Impact indicators: Midpoint indicators and the endpoint indicators.

Midpoint impact indicators are parameters in a cause-effect chain or network and are clustered into groups of substance flows that have the ability to contribute to the same environmental effect. Unlike the endpoint impact indicators, they can have a very broad specter.

Sub-categories/impact pathways are:

- Climate change
- Stratospheric ozone depletion
- Acidification (terrestrial, freshwater)
- Eutrophication (terrestrial, freshwater, marine)
- Photochemical ozone formation
- Ecotoxicity (terrestrial, freshwater, marine)

- Human toxicity (cancer, non-cancer)
- Particulate matter formation
- Ionizing radiation (human health, aquatic and terrestrial ecosystems)
- Land use (biotic productivity, aquifer recharge, carbon sequestration, albedo, erosion, mechanical and chemical filtration capacity, biodiversity)
- Water use (human health, aquatic ecosystems, terrestrial ecosystems, ecosystem services)
- Abiotic resource use (fossil and mineral)
- Biotic resource use (e.g. fishing or wood logging)
- Noise
- Pathogens.

3.2.5 Endpoint Impact Indicators

By linking several midpoint indicators, based on their impact in a stricter category (e.g. related to their impact on human health, the ecosystems or natural resources) it is possible to create one or more endpoint indicators (sometimes also referred to as damage or severity). Sometimes it is possible that the same midpoint indicator can affect two endpoint indicators (Figure 15) (Hauschild et al., 2017). These endpoint indicators represent different topics that relate to the impact on human health, the ecosystems or natural resources. The endpoint indicators are usually chosen further down the cause–effect chain of the environmental mechanism. The typical endpoint indicators are:

- Human health.
- Ecosystem quality or natural environment.
- Natural resources and ecosystem services.

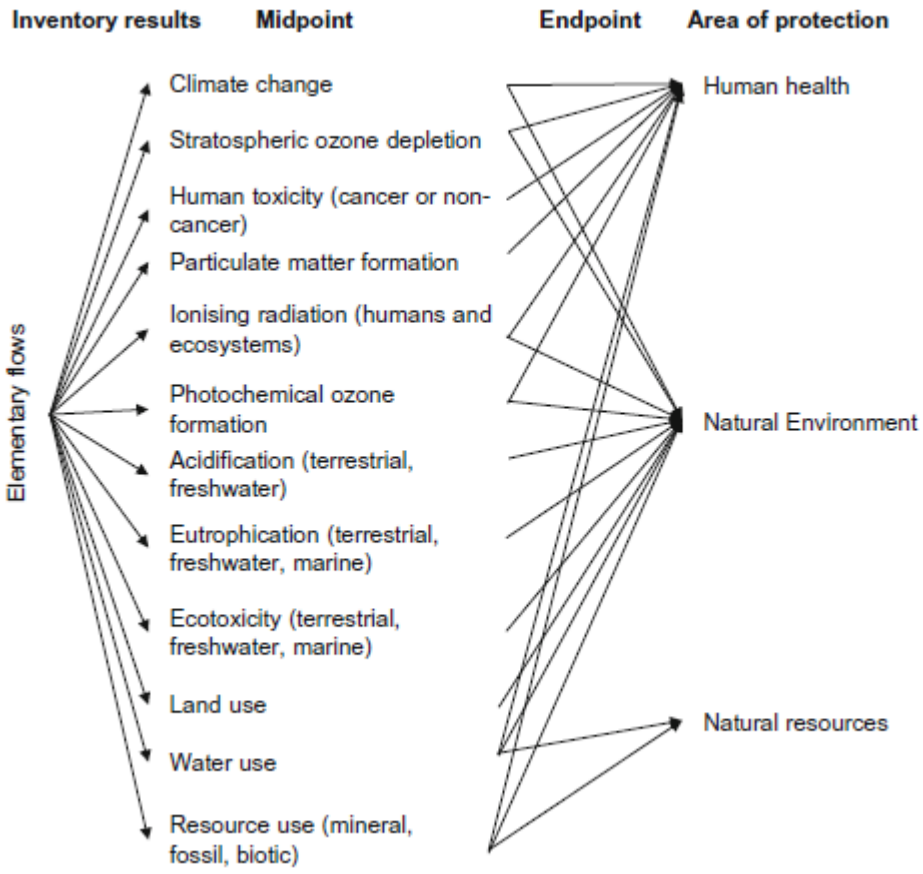

Figure 15 – Link between elementary flows from the inventory to midpoint and endpoint indicators (Hauschild et al., 2017).

3.2.6 LCA Studies

As microalgae are considered an adequate and more sustainable substitute for the current feedstock used for biofuel production, due to being a non-food feedstock, having a high amount of free fatty acids per dry weight and requiring low land use (Romagnoli et al., 2017), several microalgae LCA studies have been performed on numerous topics, from comparison and analysis of cultivation systems (Pérez-López et al., 2017; Soratana and Landis, 2011), to harvesting methods (Collotta et al., 2017). the production of biodiesel (Mata et al., 2013; Sills et al., 2013), to the analysis of full biorefineries (Keller et al., 2017; Norambuena et al., 2015). Most of these studies use the ReCiPe (Huijbregts et al., 2016) and CML methods (de Bruijn et al., 2002) midpoint impact categories to analyze the impacts of the processes studied, but also other methods more specific like the cumulative energy demand and cumulative exergy demand (Frischknecht et al., 2007). A small list of articles and some remarks can be found in Table 7.

Table 7 – List of articles related to the LCA studion on Microalgae related topics.

Article title	Topic	Remarks	Reference
Comparative life cycle assessment of real pilot reactors for microalgae cultivation in different seasons	Microalgae cultivation	• Tubular reactors had lower impacts per unit of biomass produced than open pond. • Meteorological conditions on the reactors played a critical role in LCA results.	(Pérez-López et al., 2017)
Environmental Life Cycle Assessment (LCA) of algae production in North West Europe (NWE)	Microalgae cultivation	• Microalgae Production has high energy demands and subsequent fossil fuel decrease • Microalgae production has high water consumption when compared to terrestrial crops and seaweed production	(Taelman and Sfez, 2015)
Evaluating industrial symbiosis and algae cultivation from a life cycle perspective	Microalgae cultivation	• GWP and eutrophication can be avoided by utilizing nutrients and CO_2 from waste streams to cultivate microalgae. • High environmental impact of the production of the materials used to construct the PBR	(Soratana and Landis, 2011)
Life cycle assessment of microalgae production in a raceway pond with alternative culture media	*Chlorella vulgaris* Cultivation	• Fertilizer usage and energy consumption are major components of environmental impact • Wastewater is a good alternative for NPK culture media	(Schneider et al., 2018)
Comparative LCA of flocculation for the harvesting of microalgae for biofuels production	Biomass harvesting	• Sole centrifugation has higher environmental impacts than centrifugation with flocculation.	(Collotta et al., 2017)
Microalgal biomass production pathways: evaluation of life cycle environmental impacts	Microalgae biomass production for biofuel production	• Production of microalgae biomass is energy intensive the process can be net GHG negative. • Location is important due to impact in water resources • Dewatering process has high energy demands	(Zaimes and Khanna, 2013)
Lifecycle assessment of microalgae to biofuel: Comparison of thermochemical processing pathways	*Scenedesmus dimorphus* Biomass Production for biofuel production	• Hydrothermal liquefaction has lower GHG impacts than conventional diesel production and corn ethanol	(Bennion et al., 2015)
Life cycle assessment of microalgae based biodiesel production to evaluate the impact of biomass productivity and energy source	Biofuel production from microalgae	• Production has the highest energy consumption • High biomass productivities reduce energy consumption per kg of produced biomass	(Togarcheti et al., 2017)
Life Cycle Assessment of Algal Biorefinery	Microalgae biorefinery	• Microalgae biorefinery have a positive impact on GHG • Microalgae biorefineries have a slightly negative impact on fossil oil consumption due to higher energy needs	(Gnansounou and Raman, 2017)
Evaluating microalgal integrated biorefinery schemes: Empirical controlled growth studies and life cycle assessment	Microalgae biorefinery	• Environmental impact depends on algal species and growth conditions • Higher lipid productivity rather than lipid content decreases GHG effects	(Soh et al., 2014)

Article title	Topic	Remarks	Reference
Life-cycle assessment of biofuel production from microalgae via various bioenergy conversion systems	Bioenergy production from microalgae	• Microalgae cultivation and harvesting have a high energy requirement • The major contributor to the GHG is the energy consumption • Anaerobic Digestion to produce gas for bioenergy has the lowest environmental impacts	(Sun et al., 2019)
The environmental sustainability of microalgae as feed for aquaculture: A life cycle perspective	*Nannochloropsis* sp. as feed for aquaculture	• In large scale microalgae can be more sustainable than traditional qua feed production • drying and cultivation are the biggest bottlenecks in terms of exergy	(Taelman et al., 2013)
Energy balance and life cycle assessment of a microalgae-based wastewater treatment plant: A focus on alternative biogas uses	Wastewater treatment with microalgae	• Lower energy consumption when compared to conventional wastewater treatment plants • Spent microalgae biomass can be used to produce bio-gas	(Colzi Lopes et al., 2018)
Environmental assessment of algae-based polyunsaturated fatty acid production	*Prorocentrum* sp. based polyunsaturated fatty acid production	• The largest contributions to the GWP are energy for drying of the biomass and cultivation, and nutrients production • The choice of microalgae strain is important to decrease environmental impacts • Process improvement can decrease environmental impact	(Keller et al., 2017)
Environmental assessment of industrial production of microalgal biodiesel in Central-south Chile	Diesel production from *Phaeodactylum tricornutum*	• Reactor Construction and energy consumption are the main contributors to the environmental impacts • Water reuse can improve environmental performance	(Branco-Vieira et al., 2020)
Environmental impact of phycocyanin recovery from *Spirulina platensis* cyanobacterium	*Spirulina platensis* based phycocyanin production	• Ultrasound assisted extraction has a low environmental impact • Drying the biomass produces more biomass, leading to a lower impact per kg of phycocyanin than when using wet biomass	(Papadaki et al., 2017)
Environmental and techno-economic evaluation of ß-carotene production from *Dunaliella salina*. A biorefinery approach	*Dunaliella salina biorefinery*	• Although supercritical extraction is more environmentally friendly, the low yields increase the nutrient consumption and therefore its negative environmental impacts • Energy can be recovered from residual biomass	(Espada et al., 2020)

Most of these studies conclude that microalgae cultivation has a positive impact on the greenhouse gas (GHG) emissions, as microalgae consume CO_2 as carbon source. Therefore, most processes involving microalgae production have a lower GHG impact than their chemically produced counterparts do. However, most studies also conclude that in a microalgae biorefinery, most of the negative impacts are due to microalgae cultivation. This is because microalgae cultivation has high water and energy demands, therefore decreasing the amount of available hydric and fossil resources. Another negative aspect is the use of fertilizers as nutrient sources, which also leads to an increase of negative environmental impacts.

On the other hand, most agree that different solutions are available, from using wastewater with nutrients and cultivation water reuse in order to reduce water and fertilizer consumption, combined with an efficient energy integration and the use of renewable energy sources in order to decrease the impact of the high energy consumption. In terms of products obtained from microalgae, studies have shown promising results when using microalgae as source of fish feed and as raw material for biofuels, as these present less impacts (especially in the GHG emissions), than currently used processes (Taelman et al., 2013; Bennion et al., 2015).

3.3 Process optimization

Process optimization is a very important tool for business and industrial use. The most common goals of process optimization are to minimize production costs and maximize throughput and/or efficiency. This can be achieved with process synthesis and design, which uses predictive models to create a process that is technically and economically feasible. The synthesis step is largely focused on the design of the process flowsheet and looking into particular subsystems like reaction, separation, energy management, and waste reduction. It is in this part of process optimization that this thesis will be focused. The design step is more concerned with establishing equipment parameters and nominal operating conditions for the flowsheet which require non-linear programming and more complex models (Terlaky et al., 2017).

For process optimization, the problem is usually as follows in Figure 16:

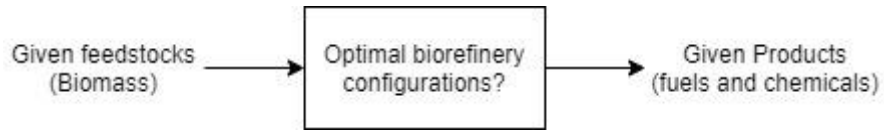

Figure 16 - Typical process optimization problem (Pham and El-Halwagi, 2012).

In the design stage, the objective is to find the most economically attractive production process. Since the initial feedstocks are given, it is necessary to discover what products can be obtained from this feedstock and what production steps and equipment are necessary. Afterwards, the analysis and optimization can be done using two possible methods: the synthesis problem and the optimization problem (Pham and El-Halwagi, 2012). The first one (Figure 17) focuses only on a few steps (five) and involves the identification of possible intermediates for the feedstock (forward branching tree) and the pathways required to produce them, and the identification of the desired products and the required pathways and species required to produce them (Backward branching tree). Afterwards, the objective is to match the intermediates (Matching) with the species or find the required production process to obtain (Interception).

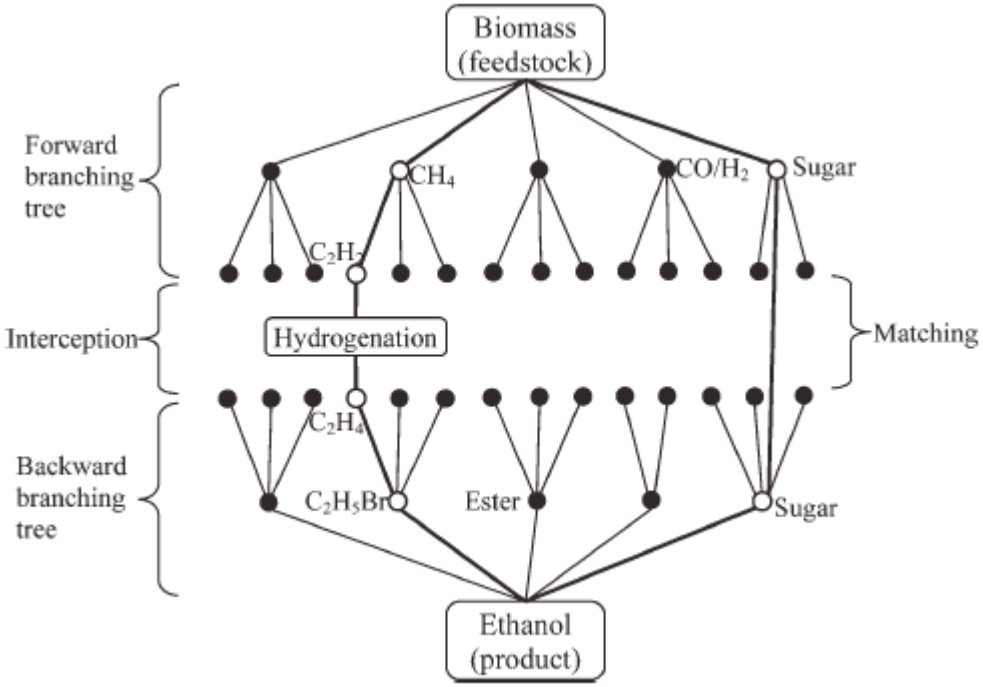

Figure 17 – Example of a synthesis method resolution (Pham and El-Halwagi, 2012).

The second process (Figure 18) is a parameter optimization process which tries to optimize the following parameters:

- Key performances of processing technologies: yield, conversion recovery.
- Mass and Energy balances
- Capital Cost
- Operating Cost

The goal is to create as many production scenarios, with different production units, end products, and as many routs as possible, to obtain the best economically attractive production process by optimizing the previously mentioned parameters. As can be seen in Figure 18 each optimization removes a path, until the best and most economically attractive configuration is achieved.

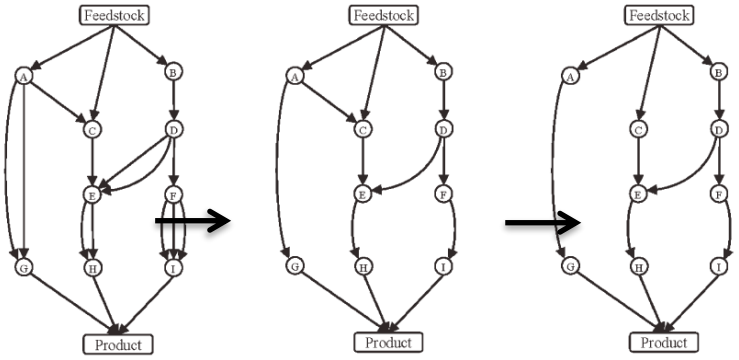

Figure 18 – Example of a parameter optimization resolution (Pham and El-Halwagi, 2012).

4 Preliminary Economic Analysis

As mentioned before, the objective of this thesis is to develop an optimal biorefinery setup for two distinct microalgae: the first, a genetically modified cyanobacteria strain that produces and excretes ethanol into the culture medium; and the second one, a non-modified microalgae with a high PUFA content, with the desired component found inside the microalgae cells.

For the production step, data from two European projects was used. For the first scenario the *DEMA* project (University of Limerick, 2018), based on a genetically modified *Synechocystis* strain that produces and excretes ethanol, was used. For the second scenario, the *PUFAChain* project (Friedl, 2017) based on a *Prorocentrum* strain was used to obtain PUFAs.

The first step of the optimization process was to create different processes with different equipment and products. A total of 136 scenarios for *Synechocystis* and 98 scenarios for *Prorocentrum* based biorefineries were created and analysed. The 5 best combinations based on economic performance calculated by 3 simplified economic parameters (the return on Investment (ROI), payback period (PBP) and net present value (NPV)), were chosen. As the objective was to obtain a sustainable biorefinery, the best process combination was chosen based on the life cycle analysis of the 5-best previously mentioned combinations.

4.1 Case Study 1 - Microalgae Production Step – *Synechocystis* based biorefinery

4.1.1 Microalgae biomass

In *Synechocystis* sp. biomass, besides the bioethanol, the most interesting component is the water soluble protein phycocyanin (PC), due to the high market value for some of its applications (Eriksen, 2008) and due to its abundance in the cyanobacteria. Another interesting component is the zeaxanthin, that although exists in a low concentration, has a high market value (Reports And Data, 2019). In addition, due to its large concentration and large application range, proteins are also interesting components (Bleakley and Hayes, 2017).

4.1.1.1 Production process considerations

From the results obtained by the *DEMA* project, the following process was considered to be the most efficient production setup, for it was the one that yielded the highest biomass and ethanol production. As the objective of the *DEMA* project was to create a 10 ha plant, the final ethanol and biomass values were the maximum values achieved with that constraint. Another objective was to use flue gas from a neighboring industry as CO_2 source for microalgae production. The results and production rates were all obtained either in laboratory experiments or in the Pilot Plant at *A4F* (University of Limerick, 2018).

In this scenario, the production and pre-production reactors were considered to be UHT-PBR reactors. For these reactors it was considered that: (1) for the biomass production, the microalgae strain has a productivity of biomass of 0.142 g/L/d and of ethanol of 0.334 g/L/d. The culture reaches a concentration of 0.5 g/L of biomass (this concentration was assumed to be the best to achieve maximum productivity) at day 3 of culture and then around 27% of the biomass is daily harvested to maintain a constant concentration. On the 120th day, the whole reactor is harvested, cleaned and prepared for new inoculation and 90% of the culture medium is recycled; (2) for the ethanol production, in the DEMA project that was used as an example, the final ethanol concentration achieved was 5 g/L (Lopes et al., 2019; University of Limerick, 2018) on the 30th day of production. However, this value was too small to be recovered by distillation, leaving the pervaporation membrane the only viable option. As the pervaporation membrane cannot handle biological material like biomass, this option would limit the harvesting methods to membrane harvesting. Since the objective of this project is to obtain as many scenarios possible, the final ethanol concentration considered was raised to 10 g/L, along with the

ethanol productivity, in order to use distillation as a method to recover the ethanol, and be able to use other harvesting methods. Thus, 10 g/L were selected because they were also considered as a possible option in the *DEMA* project, and are under the ethanol tolerance of the *Synechocystis* strain in use, of 15 g/L (Lopes et al., 2019; University of Limerick, 2018). A summary of the biomass production parameters considered in this study is shown in Table 8.

Table 8 - Biomass Production Parameters for *Synechocystis* based biorefinery.

Parameters	Value	Unit
Number of reactors	36	-
Reactor volume	97.5	m^3
Run time	120	days
Inoculations	3	Per year
Biomass Productivity	0.142	g/L/d
Final biomass concentration	0.52	g/L
Ethanol Productivity	0.334	g/L/d
Final ethanol concentration	10	g/L
Medium recycle	90	%
Total biomass produced	180.5	t/year
Total ethanol produced	421.7	t/year

4.1.1.2 Harvesting and disruption equipment choices

Although there is a large number of equipment that can perform the steps of harvesting and disruption of microalgal biomass, some are only available at laboratory scale and are still far away from being used at industrial scale. Therefore, only the ones already used in microalgae or similar industries were chosen. The chosen processes can be found in *Table 9*. The efficiencies of each equipment and the conditions can be found in Appendix 2.

Table 9 - Harvesting and Cell Disruption Equipment.

Harvesting Equipment	Abbreviation	Cell Disruption Equipment	Abbreviation
Membrane	M	**Bead Mill**	BM
Centrifuge	C	**High Pressure Homogenizer**	HPH
Dissolved Air Flotation (with flocculant)	DAF	**Ultrasonication**	US
Flocculation	FLC		

4.1.1.3 Production, harvesting and disruption

In this scenario, one of the products is a bioethanol, which is produced intracellularly and excreted into the medium. The harvesting step will concentrate the biomass but will also separate the biomass from the medium containing the ethanol. Afterwards, it is necessary to include in the process an equipment to recover ethanol from the medium. In the project used as an example, ethanol was recovered through pervaporation (Peng et al., 2010; University of Limerick, 2018). However, due to operational constrains, only the culture medium obtained through a membrane can be directly used by the pervaporation membrane. This is due to the fact that biomass is harmful to the pervaporation membranes (information provided by supplier and project partner Pervatech BV), and the membrane is the only harvesting method that completely removes the biomass from the medium. Currently, the most used options to obtain pure ethanol are distillation or pressure swing adsorption (PSA). However, in order to obtain pure ethanol with distillation, one must use extractive distillation in order to overcome the azeotrope, and to use PSA, a very high concentration of ethanol is required. Since the study of ethanol recovery options is out of the scope of this project, and in order to simplify the study, two ethanol recovery process sequences were created; one where the pervaporation is preceded by a conventional distillation; and another where a membrane is used for harvesting, where only pervaporation was used. The results of ethanol recovery, using distillation were obtained using the McCabe–Thiele method (appendix 4) and corroborated using the program Chemsep (http://www.chemsep.org/). The values for the pervaporation were calculated with the help of information provided by a supplier of pervaporation membranes (Pervatech BV, 2016; University of Limerick, 2018). After the extraction of ethanol, the remaining biomass also contains components of high value. These are found inside the cell, and unlike ethanol, have to be extracted from inside of the cell. Therefore, the last

step of this process (from production of biomass to the rupture of biomass) requires a final disruption process.

4.1.1.4 Mass balances

The first step of the project was to find the optimal equipment sequence, and therefore different sequences, with different equipment. Afterwards it was necessary to perform the mass balances on all the process sequences. The values obtained can be found in Table 10 to Table 13. This information provided the amount of product obtained in each process sequence, but also the amount of water, chemicals and utilities used in the process, and wastewater that required treatment. These values are necessary for the economic analysis of the process, and also for the LCA analysis.

Table 10 - Mass balance results for centrifuge and membrane filtration biomass harvesting with ethanol extraction by distillation and pervaporation (part 1).

	Process sequence	S.1.1	S.1.2	S.1.3	S.2.1	S.2.2	S.2.3
Microalgae Production	Biomass Production (t/year)	180.5					
	Concentration (g/L)	0.52					
	Water Consumed (m^3/year)	34,630.9	34,630.9	34,549.7	34,540.7	34,540.7	34,540.7
	Ethanol Concentration in Culture Medium (g/L)	10.0					
	Ethanol Production (t/year)	421.7					
Harvesting Step	Harvesting Process	C			M		
	Harvesting Capacity (m^3/h)	42.2					
	Harvesting Loss (%)	10.0			5.0		
	Harvested Biomass (t/year)	162.5			171.5		
	Final Concentration (g/L)	200	200	100*	100.0	100.0	100.0
	Wastewater produced (m^3/year)	34,630.9	34,630.9	34,549.7	34,540.7	34,540.7	34,540.7
Ethanol recovery	Recovery Process	Dest + Perv					
	Ethanol lost (%)	10					
	Ethanol recovery capacity (m^3/h)	4.2					
	Ethanol removed (m^3/year)	369.4	369.4	368.5	368.5	368.5	368.5
Cell Disruption Step	Cell Disruption process	BM	HPH	US	BM	HPH	US
	Cell Disruption Capacity (m^3/h)	0.1	0.1	0.2	0.2	0.2	0.2
	Cell Disruption efficiency (%)	99.0	96.0	100.0	99.0	96.0	100.0
	Cell Disruption Loss (%)	5.0					
	Final ruptured Biomass (t/year)	152.8	148.2	154.3	161.3	156.4	162.9

*-the final concentration corresponds to the maximum concentration of the cell disruption method

C – Centrifuge; M-Membrane; BM – Ball mil; HPH – High Pressure Homogenizer; US – Ultrasonicator

Table 11 - Mass balance results for flotation and sedimentation biomass harvesting with ethanol extraction by distillation and pervaporation (part 2).

	Process sequence	S.3.1	S.3.2	S.3.3	S.4.1	S.4.2	S.4.3
Microalgae Production	Biomass Production (t/year)	180.5					
	Concentration (g/L)	0.52					
	Water Consumed (m^3)	34,456.5	34,456.5	34,456.5	34,387.3	34,387.3	34,387.3
	Ethanol Concentration Culture Medium (g/L)	10.0					
	Ethanol Production (t/year)	421.7					
Harvesting Step	Harvesting Process	DAF			FLC		
	Harvesting Capacity (m^3/h)	42.2					
	Harvesting Loss (%)	15.0			10.0		
	Harvested Biomass (t/year)	153.4			162.5		
	Final Concentration (g/L) *	60.0			50.0		
	Wastewater produced (m^3/year)	34,456.5	34,456.5	34,456.5	34,387.3	34,387.3	34,387.3
Ethanol recovery	Recovery Process	Dest + Perv					
	Ethanol Lost (%)	10					
	Ethanol recovery capacity (m^3/h)	4.2					
	Ethanol removed (m^3/year)	367.2			366.5		
Cell Disruption Step	Cell Disruption process	BM	HPH	US	BM	HPH	US
	Cell Disruption Capacity (m^3/h)	0.4					
	Cell Disruption efficiency (%)	99.0	96.0	100.0	99.0	96.0	100.0
	Cell Disruption Loss (%)	5.0					
	Final ruptured Biomass (t/year)	144.3	139.9	145.8	152.8	148.2	154.3

*-the final concentration corresponds to the maximum concentration that can be achieved by the process (DAF – 60 g/l and FLC – 50 g/l)

DAF –Dissolved Air Flotation; FLC - Flocculation; BM – Ball mil; HPH – High Pressure Homogenizer; US – Ultrasonication

Table 12 - Mass balance results for scenario of biomass membrane harvesting with ethanol extraction by pervaporation membrane only.

	Process sequence	S.2.1p	S.2.2p	S.2.3p
Microalgae Production	Biomass Production (t/year)		180.5	
	Concentration (g/L)		0.52	
	Water Consumed (m^3)		34,540.7	
	Ethanol Concentration Culture Medium (g/L)		10.0	
	Ethanol Production (t/year)		421.7	
Harvesting Step	Harvesting Process		M	
	Harvesting Capacity (m^3/h)		42.2	
	Harvesting Loss (%)		5.0	
	Harvested Biomass (t/year)		171.5	
	Final Concentration (g/L)		100.0	
	Wastewater produced (m^3)		34,540.7	
Ethanol recovery	Recovery Process		Pervaporation	
	Ethanol lost (%)		15	
	Ethanol recovery capacity (m^3/h)		4.2	
	Ethanol removed (m^3/year)		348.0	
Cell Disruption Step	Cell Disruption process	BM	HPH	US
	Cell Disruption Capacity (m^3/h)		0.2	
	Cell Disruption efficiency (%)	99.0	96.0	100.0
	Cell Disruption Loss (%)		5.0	
	Final ruptured Biomass (t/year)	161.3	156.4	162.9

M-Membrane; BM – Ball mil; HPH – High Pressure Homogenizer; US – Ultrasonication

Table 13 - Mass balance results for Scenario 1 with membrane and centrifuge combination.

	Process sequence	S.5.1	S.5.2
Microalgae Production	Biomass Production (t/year)	180.5	
	Concentration (g/L)	0.52	
	nutrients (ton)	98.6	
	Water Consumed (m^3)	34,635.0	
	Ethanol Concentration Culture Medium (g/L)	10	
	Ethanol Production (t/year)	421.7	
First Harvesting Step	Harvesting Process	M	
	Harvesting Capacity (m^3/h)	42.2	
	Harvesting Loss (%)	5.00	
	Harvested Biomass (t/year)	171.5	
	Final Concentration (g/L)	10.4	
	Wastewater produced (m^3/year)	33,063.3	
Second Harvesting Step	Harvesting Process	C	
	Harvesting Capacity (m^3/h)	2.11	
	Harvesting Loss (%)	10	
	Harvested Biomass (t/year	154.3	
	Final Concentration (g/L)	200	
	Wastewater produced (m^3/year)	1,571.7	
Ethanol recovery	Recovery Process	Pervaporation	
	Ethanol lost (%)	15%	
	Ethanol recovery capacity (m^3/h)	4.0	
	Ethanol removed (m^3/year)	333.2	
Cell Disruption Step	Cell Disruption process	BM	HPH
	Cell Disruption capacity (m^3/h)	0.1	
	Cell Disruption efficiency (%)	99.00	96.00
	Cell Disruption Loss (%)	5.00	
	Final Ruptured Biomass (t/year)	145.1	140.7

C – Centrifuge; M-Membrane; BM – Ball mil; HPH – High Pressure Homogenizer

As can be seen in Table 10 to Table 13, the process combinations where the harvesting process was performed with a membrane system corresponded to the highest ruptured biomass recovery. This is anticipated since this harvesting process is the one with the highest harvesting efficiency. Furthermore, it can be seen that the impact of the cell disruption step on final value of produced ruptured biomass was not very high since the

disruption efficiencies of the equipment were considered to be quite similar, even though ultrasonication and bead mill are the most efficient. However, this does not mean that this was the most economically efficient process.

The following step was to design the extraction process and compare all scenarios to understand the impact of the harvesting processes in the production costs, due to the different concentrations that can be achieved by each harvesting process.

4.1.2 Extraction Process

After the cell disruption, a mixture of components is obtained. This can already constitute a product; however, if higher value products are desired, it is necessary to separate the different components and further purify them.

The extraction section of the biorefinery was designed to fulfil the main objective of a refinery, to obtain the largest possible economic value from the feedstock. Since there are different components in each microalga that can be obtained and used in different products, several processes are required in order to extract all the components.

In this scenario, although the product of interest is ethanol that was excreted into the culture medium, the microalga also produces other high value components like a water-soluble protein, phycocyanin, and the carotenoid zeaxanthin. As phycocyanin has a high value and is quite abundant, the extraction process of biomass in this scenario was designed to extract the highest amount of that component first, followed by the extraction of zeaxanthin.

4.1.2.1 Assumptions

To proceed with the proposal of the following process steps, several assumptions were made, as described below:

- In cases where no information on zeaxanthin was available, information on lutein was used, since both have similar properties (Nath et al., 2016);
- All membrane operations have a 95% recovery efficiency to account for the biomass lost in the process (A4F, 2018);
- The saponification process values were obtained from information provided by a specialist company IOI Oleo (Friedl, 2017);
- All solvents (ethanol and heptane) have a recovery rate of 90% (Peng et al., 2010; Tres et al., 2012) (Note: in this reference the amount recovered was higher. However, since this was performed in lab scale, it was assumed the losses are higher and therefore a lower recovery value was assumed),

- Phycocyanin purity is calculated based on ratio of Abs_{620} to Abs_{280} where Abs_{620} is the maximum absorbance of phycocyanin and Abs_{280} is the absorbance of total proteins (Patil et al., 2006).

4.1.2.2 Extraction process description

The extraction process starts with an aqueous stream containing the ruptured biomass, which enters the process and goes through a diafiltration process[1] (Table 14). This step will be performed using water in order to remove most of the water-soluble components, specially phycocyanin, and water-soluble proteins. In this process a microfiltration membrane (Millipore, 2003) will be used and it was assumed that only the water soluble contents were removed.

Table 14 – *Process parameters and results for the phycocyanin extraction process.*

	Process	Parameters	Results	References
1	Diafiltration	5 volumes of water (diafiltration volume equal to 5)/ volume of culture	5% biomass loss	(A4F, 2018)
			1 g of phycocyanin / L of water extracted	(Herrera et al., 1989)
			2 g of soluble proteins /L of water extracted	(Pace et al., 2004)
2	Phycocyanin purification	2 % wt. of chitosan	Efficiency 95% and purity 2.78	(Chamorro-Cevallos, 2016; Wang et al., 2012)

The reason why a diafiltration was used instead of another type of solid-liquid separation process was due to diafiltration ability to keep the upstream compartment at constant volume, maintaining the concentration of the remaining biomass components constant, without increasing the viscosity, which would increase the complexity of the following extraction steps. Furthermore, this method was considered to be more efficient in removing the water-soluble content, since, if the water was just separated from the biomass by normal membrane separation or centrifugation, some water and solutes would still remain in the biomass, retaining some high value components that would be very hard to remove later on in the process. With diafiltration it was assumed that most of the water-soluble components were removed, since the amount of water used was higher than the amount of water required to solubilize all the water-soluble components.

The water stream was then further treated using chitosan to perform an affinity precipitation in order to obtain a purer stream of phycocyanin for higher value

1 **Diafiltration is a mode of operation in membrane processing where water is added to the upstream compartment of the membrane at the same permeate flowrate, in a way that the volume at the upstream compartment (the retentate) is kept constant.**

applications, like pharmaceutical or food industry (Fekrat et al., 2018; Kumar et al., 2014; Wang et al., 2012) or just used without any treatment, for textile dye applications (Kuddus et al., 2015).

The remaining biomass contains the lipids, non-water-soluble proteins and carbohydrates, as well as some zeaxanthin. This biomass can be used for animal feed or can be further treated.

In order to treat the remaining biomass stream, two options were considered: one option *(Figure 19)* was to perform a conventional solvent extraction, using a polar solvent, since *Synechocystis* lipid fraction is mostly composed of DAGs, which are polar (Sheng et al., 2011). Ethanol was a good option to be used as a solvent since it is considered a safe solvent for food applications (Yang et al., 2014). Ethanol was then used as the solvent, to remove zeaxanthin and the polar lipids (Breil et al., 2016; Koo et al., 2012). This could already be a product since the stream would be rich in lipids with palmitic acid and zeaxanthin, however it could still be further purified. The zeaxanthin was then recovered by a series of purification and saponification steps (Design by IOI Oleo (Friedl, 2017)) saponifying the lipids, to produce a metal soap and a stream of almost pure zeaxanthin. The process parameters are shown in Table 15.

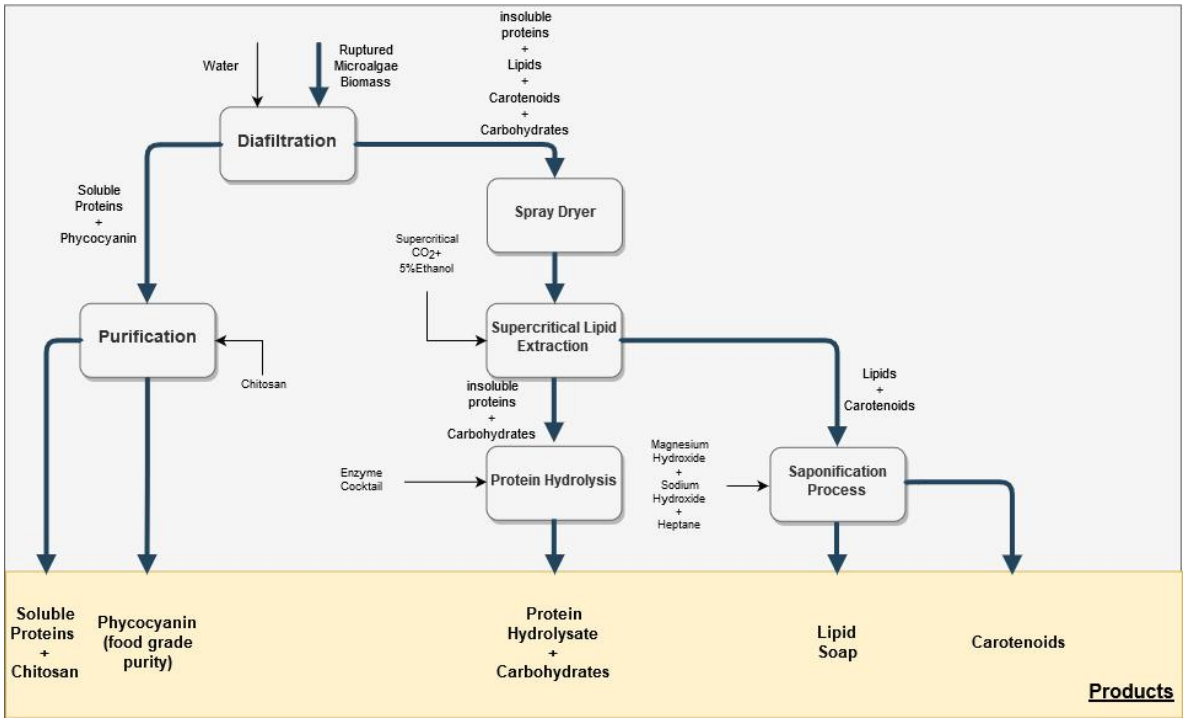

Figure 19 – Synechocystis processing option 1.

Table 15 - Process parameters and results for the spent biomass process 1 described in Figure 19.

Step	Process	Parameters	Results	References
3	Ethanol extraction	20 L of ethanol/kg of biomass	Glycolipids – 60% extracted Phospholipids – 44% extracted	(Breil et al., 2016)
			Zeaxanthin – 1 (mg/g biomass) extracted	(Cha et al., 2009; Li and Engelberth, 2018)
4	Enzymatic Hydrolysis of proteins	4 % w/w of enzyme Cocktail*1 and 100-200 g/L*2	59 % of protein hydrolysis	(De Farias Silva and Bertucco, 2017; Romero García et al., 2012)
5	Saponification	Company confidentiality	89% of efficiency	(Ibáñez et al., 1998; Li et al., 2016)

*1 - Composed of Alcalase and Flavourzyme

*2-due to process constraints

The second option (Figure 20) was to use a supercritical solvent extraction step using supercritical CO_2 ($scCO_2$) with 5% ethanol as co-solvent to remove the zeaxanthin and a small amount of lipids (Cardoso et al., 2012; Terme et al., 2017).

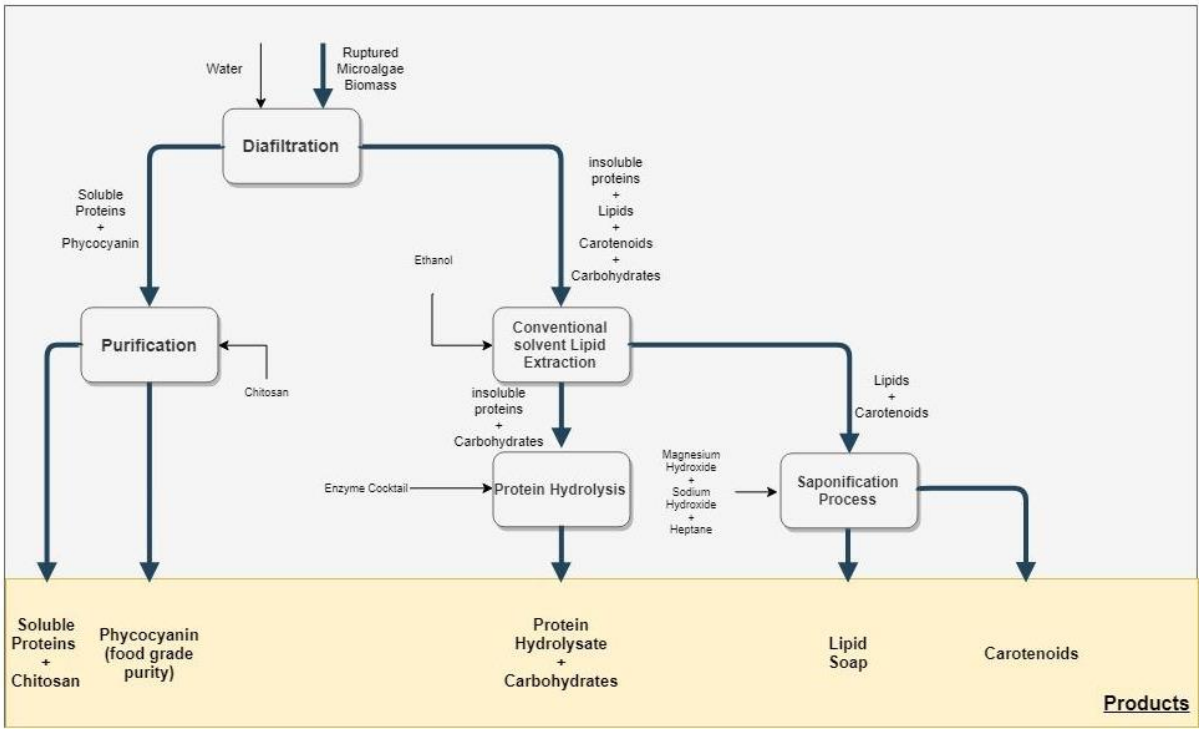

Figure 20 - Synechocystis processing option 2.

The remaining biomass, without the zeaxanthin and polar lipids, could once again be used as fertilizer, or as animal feed, but was treated, by hydrolyzing the proteins present in the remaining biomass by enzymatic conversion producing a protein hydrolysate with bio-

fertilizer potential (Romero García et al., 2012). The lipid and zeaxanthin stream were further purified through a series of filtrations and saponification steps, to separate the zeaxanthin from the lipids and obtaining two purer streams. The process parameters for this processing option are shown in Table 16.

Table 16 - Process parameters and results for the spent biomass process 1 described in Figure 3.

Step	Process	Parameters	Results	References
3	Supercritical extraction with CO_2 and 5% Ethanol	200 bar and 60 °C*1	TAGs – 61% extracted	(Terme et al., 2017)
			Glycolipids – 36% extracted	
			Phospholipids – 27% extracted	
			Beta-Carotene – 0.6 (mg/g of biomass) extracted	(Cardoso et al., 2012)
			zeaxanthin – 1.6 (mg/g biomass) extracted	
4	Enzymatic Hydrolysis of proteins	4 % w/w of enzymes cocktail*2 and 100 to 200 g/L*3	59 % of protein hydrolysis	(De Farias Silva and Bertucco, 2017)
5	Saponification	Company confidentiality	89% of efficiency	(Ibáñez et al., 1998)

*1-the parameters are different in (Terme et al., 2017) however since the objective was to extract the carotenoids, it was considered that the extraction values are the same

*2 – Composed of Alcalase and Flavourzyme

*3-Due to process limitations

4.1.2.3 Possible conformations

In the previous section, a full extraction process was designed to obtain the best possible separation of all high value components in the biomass. However, due to the high prices of some of the extraction and conversion methods, the full scenario might not be the best economic option. In order to study this hypothesis, the full process was divided into smaller processes with different conformations. This also means that for different conformation of the biorefinery there are different products that can be obtained, with different values.

All the calculations were performed for an hourly ruptured biomass production of 20 kg/h. The reason behind this choice was that this value is the average value of all production, harvesting and disruption scenarios, since due to the different harvesting and rupture process efficiencies, the ruptured biomass produced per year is slightly different in each scenario due to the different combinations of processes.

The time considered was 350 days of operation, with 22 hours of production and 2 hours of CIP per day, and 15 days of maintenance. All values can be found in Appendix 5.

4.1.2.3.1 Process option 1 + conformation 1

In this conformation, the extraction process is composed of only two operations (Figure 21). The first is a diafiltration, in a membrane system, where 5 volumes of water per volume of ruptured biomass stream are used (A4F, 2018) and two streams are obtained; stream 2 with phycocyanin and soluble proteins, and stream 3 with the non-soluble proteins, the lipids and the carbohydrates.

The second step is a purification step, with chitosan, needed to remove some proteins and impurities from stream 2 to produce food grade phycocyanin. In this process 2% w/w of chitosan is used (Fekrat et al., 2018; Wang et al., 2012). *Table 17* shows the composition of process streams for process 1 + conformation 1.

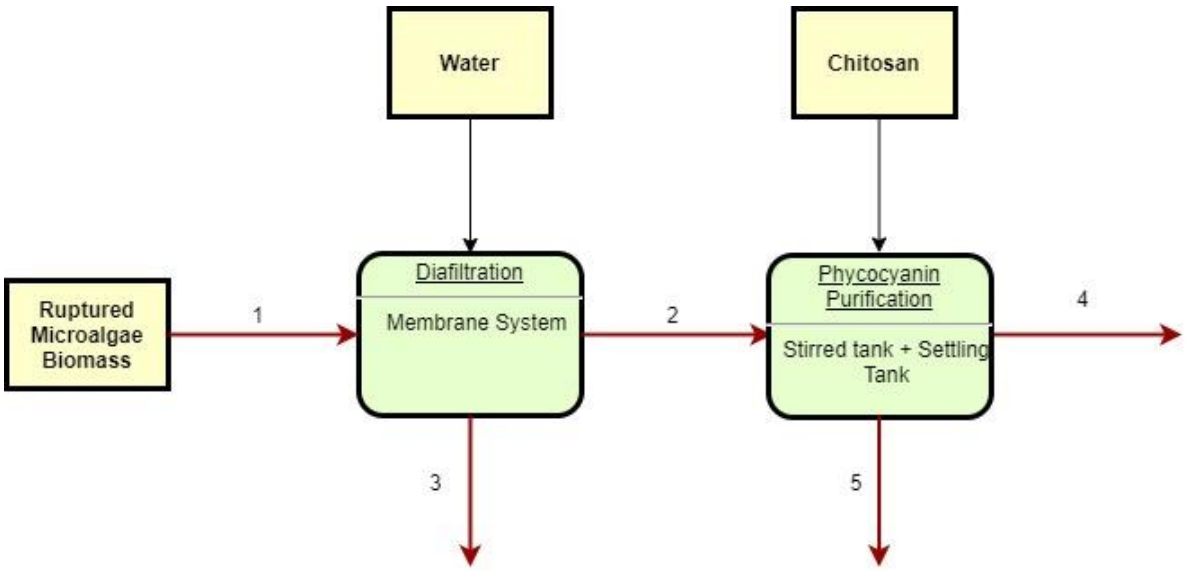

Figure 21 – Process flowsheet for process 1 + conformation 1.

Table 17 – Process streams for extraction process option 1 + conformation 1.

Component (kg/h)	Stream	1	2	3	4	5
SQDG		0.7	0.0	0.7	0.0	0.0
Glycolipids		1.2	0.0	1.2	0.0	0.0
Phospholipids		0.1	0.0	0.1	0.0	0.0
Insoluble protein		6.3	0.0	6.0	0.0	0.0
Soluble protein		4.9	4.7	0.0	4.4	0.2
Amino acids		0.0	0.0	0.0	0.0	0.0
Phycocyanin		2.3	2.2	0.0	2.0	0.2
Zeaxanthin		0.2	0.0	0.2	0.0	0.0
Carbohydrates		4.3	0.0	4.3	0.0	0.0
total mass		20.0	6.9	12.4	6.4	0.5

Products (more details in appendix 6)

In this conformation the two products obtained are phycocyanin, present in stream 4 with purity >0.4 to be used for food applications, and the spent biomass, in stream 3, which has similar characteristics to fish meal and can be used for fish feed (Table 18).

Table 18 - Products obtained in Process option 1 + conformation 1.

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Spent Biomass	3	0.62	Animal Feed

4.1.2.3.2 Process option 1 + conformation 2

In this conformation, stream 3 from the previous conformation, goes through an enzymatic hydrolysis step, using cocktail of two commercially available enzymes, *Alcalase* and *Flavourzyme*, to hydrolyze the non-soluble proteins in the stream (Romero García et al., 2012) (Figure 22). The hydrolysis is performed in a stirred tank with temperature control, to obtain the best conditions for the enzymes. In this step, only the proteins will be hydrolyzed and cleaved into amino acids, due to the specificity of the enzymes. *Table 19* shows the composition of process streams for process 1 + conformation 2.

Products (more details in appendix 6)

In this conformation, the two products obtained are, phycocyanin with food grade purity in stream 4, and a protein hydrolysate in stream 6, that can be used as a bio-stimulant for plants (Table 20).

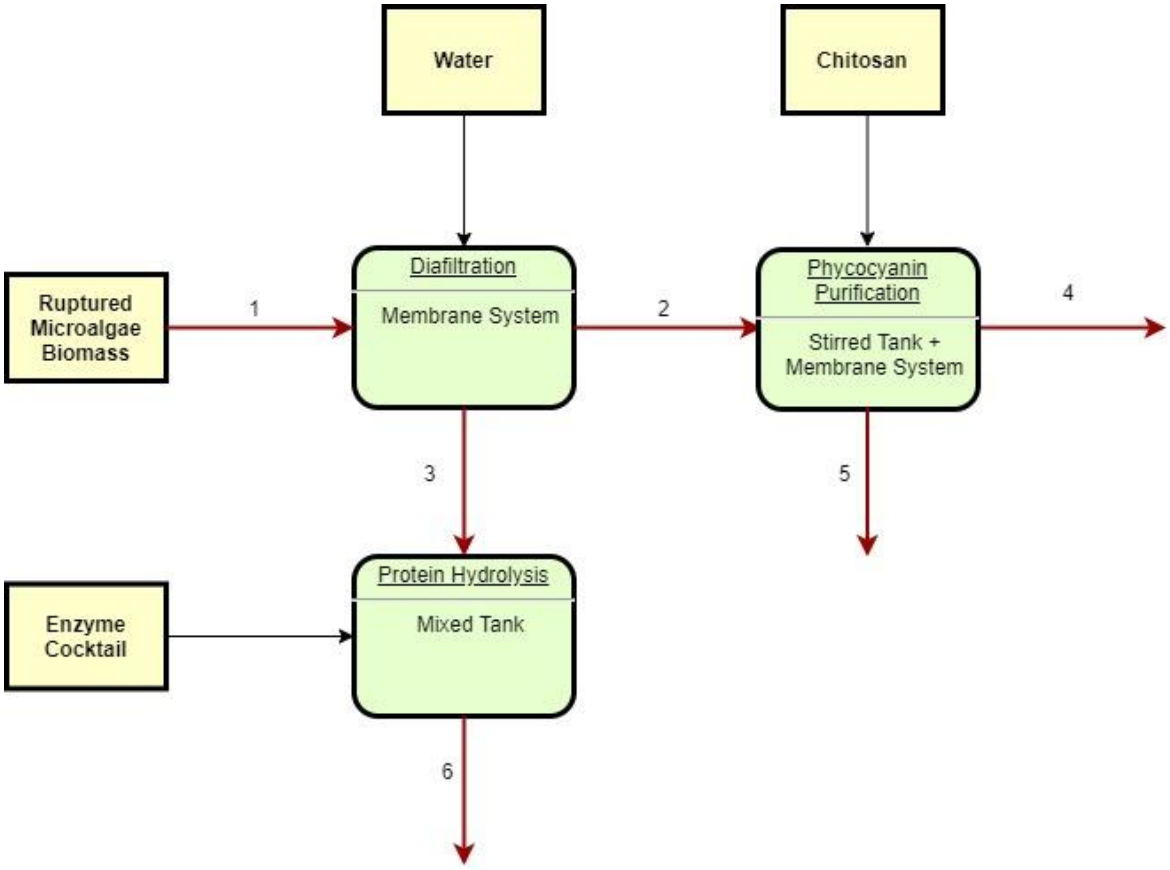

Figure 22 – Process flowsheet for option 1 + conformation 2.

Table 19 - Process streams for Process option 1 + conformation 2.

Component (kg/h) Stream	1	2	3	4	5	6
SQDG	0.7	0.0	0.7	0.0	0.0	0.7
Glycolipids	1.2	0.0	1.2	0.0	0.0	1.2
Phospholipids	0.1	0.0	0.1	0.0	0.0	0.1
Insoluble protein	6.3	0.0	6.0	0.0	0.0	6.0
Soluble protein	4.9	4.7	0.0	4.4	0.2	0.0
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0
Phycocyanin	2.3	2.2	0.0	2.0	0.2	0.0
Zeaxanthin	0.2	0.0	0.2	0.0	0.0	0.2
Carbohydrates	4.3	0.0	4.3	0.0	0.0	4.1
total mass	20.0	6.9	12.4	6.4	0.5	12.2

Table 20 - Products obtained in Process option 1 + conformation 2.

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Protein Hydrolysate	6	0.61	Bio-stimulant

4.1.2.3.3 Process option 1 + conformation 3

In this conformation (*Figure 23*), instead of hydrolyzing stream 3 as in the previous conformation, a solvent extraction using ethanol is performed to separate the polar lipids and zeaxanthin from the remaining components (Non-Polar Lipids, Proteins and Carbohydrates) present in the stream (Cardoso et al., 2012; Cha et al., 2009). This is done using a stirred tank, where ethanol is added. Afterwards, stream 6 goes through a membrane system, separating the dissolved polar lipids and zeaxanthin into stream 7, from the spent biomass. Stream 7 is then further purified, by way of a purification process with filtrations and saponification processes (Friedl, 2017; Nagappan et al., 2018) to produce two products, a dried powder of zeaxanthin (stream 10) and a stream rich in palmitic acid (stream 9). Stream 8, composed of the remaining biomass components not extracted by ethanol, can be used as animal feed. Table 21 shows the composition of process streams for process 1 + conformation 3.

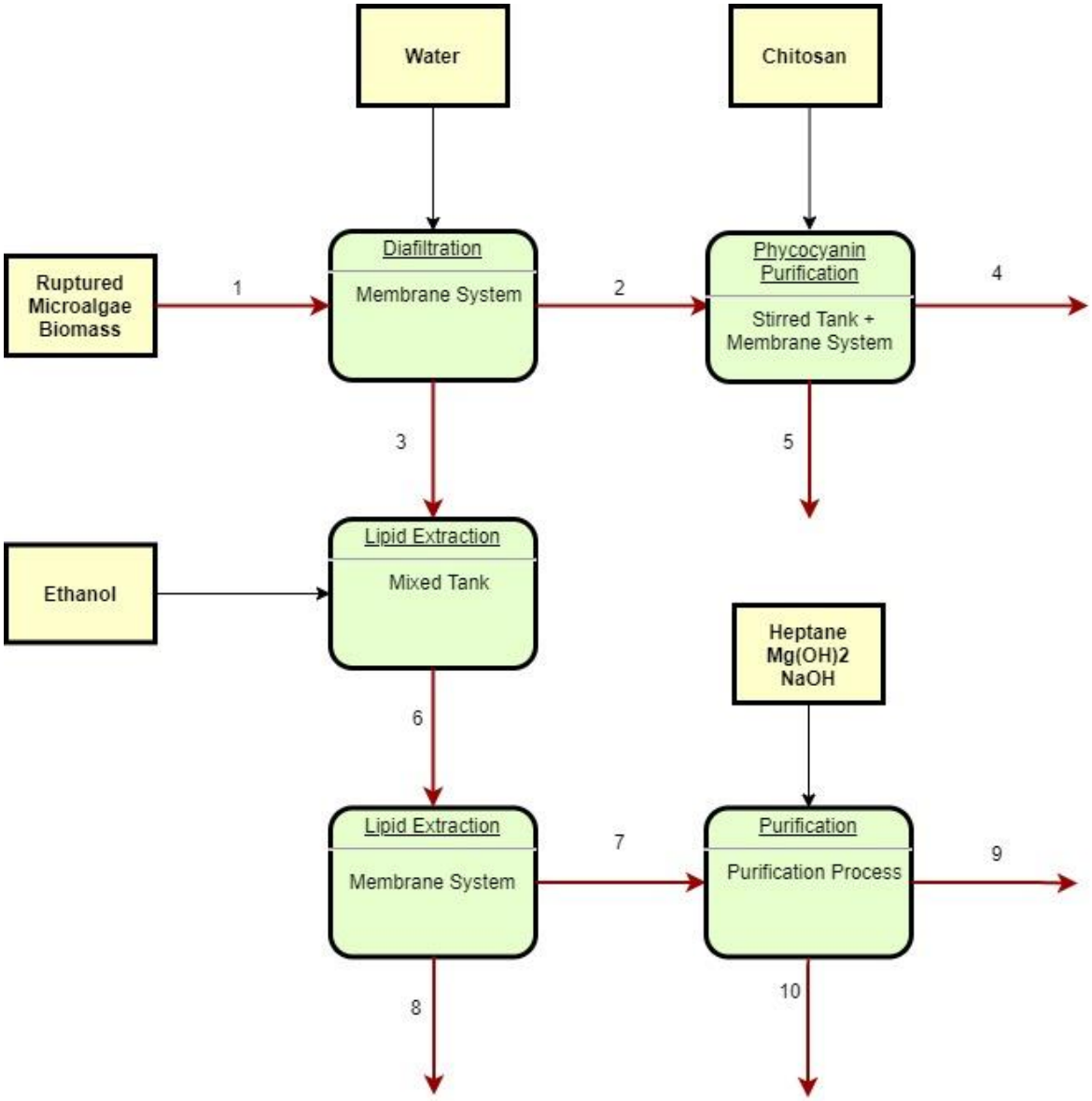

Figure 23 - Process flowsheet for option 1 + conformation 3.

Table 21 - Process streams for Process option 1 + conformation 3.

Component (kg/h) Stream	1	2	3	4	5	6	7	8	9	10
SQDG	0.7	0.0	0.7	0.0	0.0	0.7	0.3	0.4	0.2	0.7
Glycolipids	1.2	0.0	1.2	0.0	0.0	1.2	0.7	0.5	0.4	1.2
Phospholipids	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.1
Insoluble protein	6.3	0.0	6.0	0.0	0.0	6.0	0.0	5.7	0.0	6.3
Soluble protein	4.9	4.7	0.0	4.4	0.2	0.0	0.0	0.0	0.0	4.9
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phycocyanin	2.3	2.2	0.0	2.0	0.2	0.0	0.0	0.0	0.0	2.3
Zeaxanthin	0.2	0.0	0.2	0.0	0.0	0.2	0.2	0.0	0.0	0.2
Carbohydrates	4.3	0.0	4.3	0.0	0.0	4.1	0.0	3.9	0.0	4.3
Total mass	20.0	6.9	12.4	6.8	0.1	12.1	1.2	10.4	0.7	0.3

Products (more details in appendix 6)

In this conformation the four products obtained are a food grade phycocyanin in stream 4 that can be used for food applications, spent biomass in stream 8 with similar composition to fish meal, that can be used for fish feed, a lipid stream rich in palmitic acid that could replace palm oil (stream 9), and a zeaxanthin stream that can be used in nutraceuticals and pharmaceuticals (stream 10) (Table 22).

Table 22 - *Products obtained in Process option 1 + conformation 3.*

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Spent Biomass	8	0.52	Animal Feed
Lipids	9	0.03	Palm oil replacement
Zeaxanthin	10	0.01	Nutraceuticals

4.1.2.3.4 Process option 1 + conformation 4

In this conformation (Figure 24), an enzymatic hydrolysis step is added to the previous conformation, to treat the remaining biomass from stream 8, by hydrolyzing the protein into amino acids, in a tank where an enzyme cocktail, containing *Alcalase* and *Flavourzyme*, is added producing a stream (11) which is rich in amino acids. Table 23 shows the composition of process streams for process 1 + conformation 4.

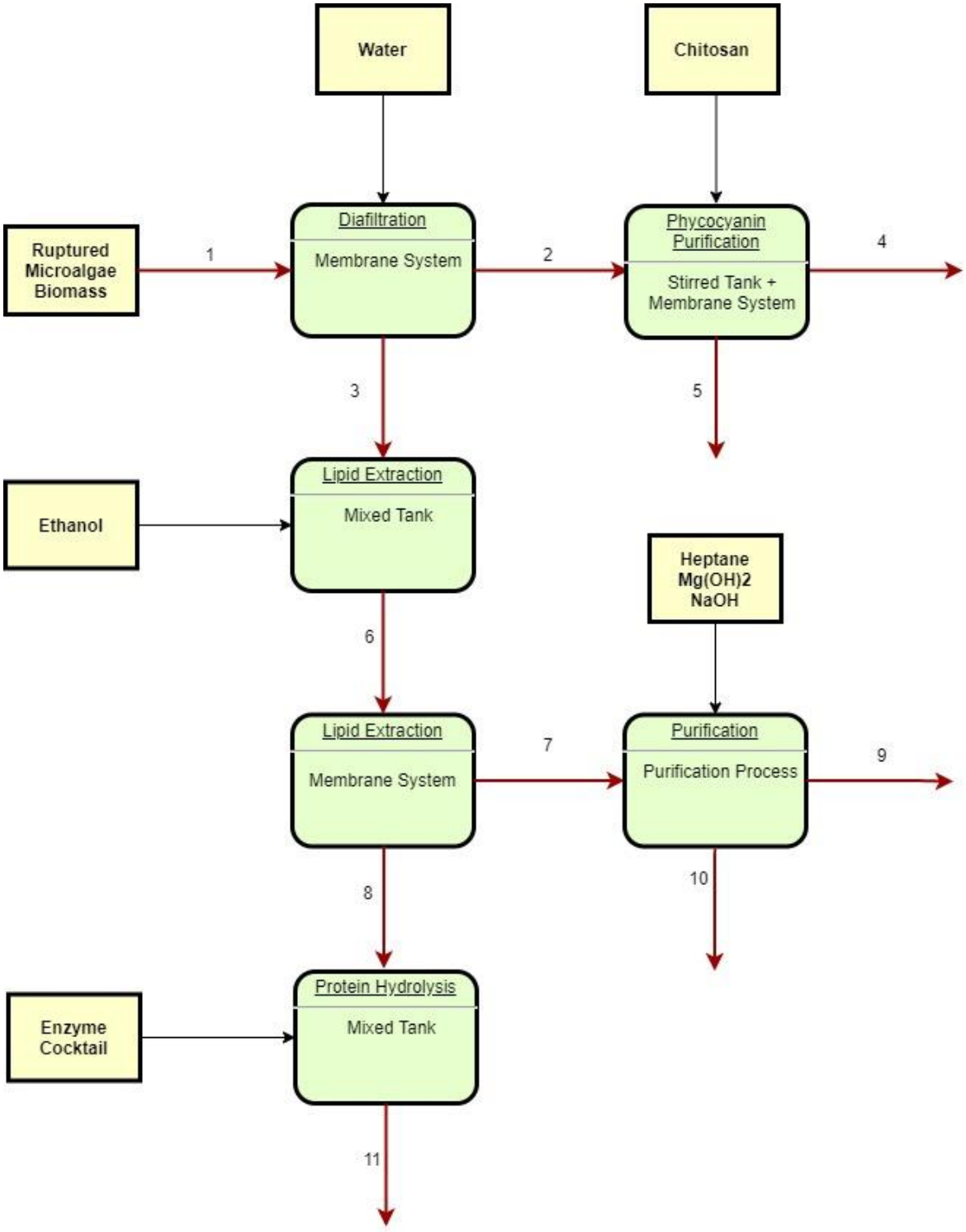

Figure 24 - Process flowsheet for option 1 + conformation 4.

Table 23 - Process streams for Process option 1 + conformation 4.

Component (kg/h) Stream	1	2	3	4	5	6	7	8	9	10	11
SQDG	0.7	0.0	0.7	0.0	0.0	0.7	0.3	0.4	0.2	0.0	0.4
Glycolipids	1.2	0.0	1.2	0.0	0.0	1.2	0.7	0.5	0.4	0.1	0.5
Phospholipids	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1	0.0	0.0	0.1
Insoluble protein	6.3	0.0	6.0	0.0	0.0	6.0	0.0	5.7	0.0	0.0	2.3
Soluble protein	4.9	4.7	0.0	4.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.3
Phycocyanin	2.3	2.2	0.0	2.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Zeaxanthin	0.2	0.0	0.2	0.0	0.0	0.2	0.2	0.0	0.0	0.2	0.0
Carbohydrates	4.3	0.0	4.3	0.0	0.0	4.1	0.0	3.9	0.0	0.0	3.9
Total mass	20.0	6.9	12.4	6.4	0.5	12.2	1.2	10.4	0.7	0.3	10.4

Products (more details in appendix 6)

As in the previous conformation, the final products are phycocyanin produced in stream 4, to be used for food applications, a lipid stream rich in palmitic acid that could replace palm oil (stream 9), and a zeaxanthin stream that can be used in nutraceuticals and pharmaceuticals. The only difference from the previous conformation is the protein hydrolysate in stream 11 that can be used as a bio-stimulant for plants (Table 24).

Table 24 - Products obtained in Process option 1 + conformation 4.

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Lipids	9	0.03	Palm oil replacement
Zeaxanthin	10	0.01	Pharmaceuticals, Nutraceuticals
Protein Hydrolysate	11	0.52	Bio-stimulant

4.1.2.3.5 Process option 2 + conformation 1

In this process option, the first step is a diafiltration, in a membrane system, where two streams are obtained: stream 2 with phycocyanin and soluble proteins, and stream 3 with the insoluble proteins, the lipids and the carbohydrates. Stream 3 containing the ruptured biomass is dried in a Spray drier and afterwards a supercritical solvent extraction, using

$scCO_2$ with 5% ethanol as co-solvent, is performed in a reactor (Cardoso et al., 2012; Terme et al., 2017) to separate the lipids and zeaxanthin into stream 7, from the remaining components that go to stream 8 (Figure 25). Table 18 shows the composition of process streams for process 2 + conformation 1.

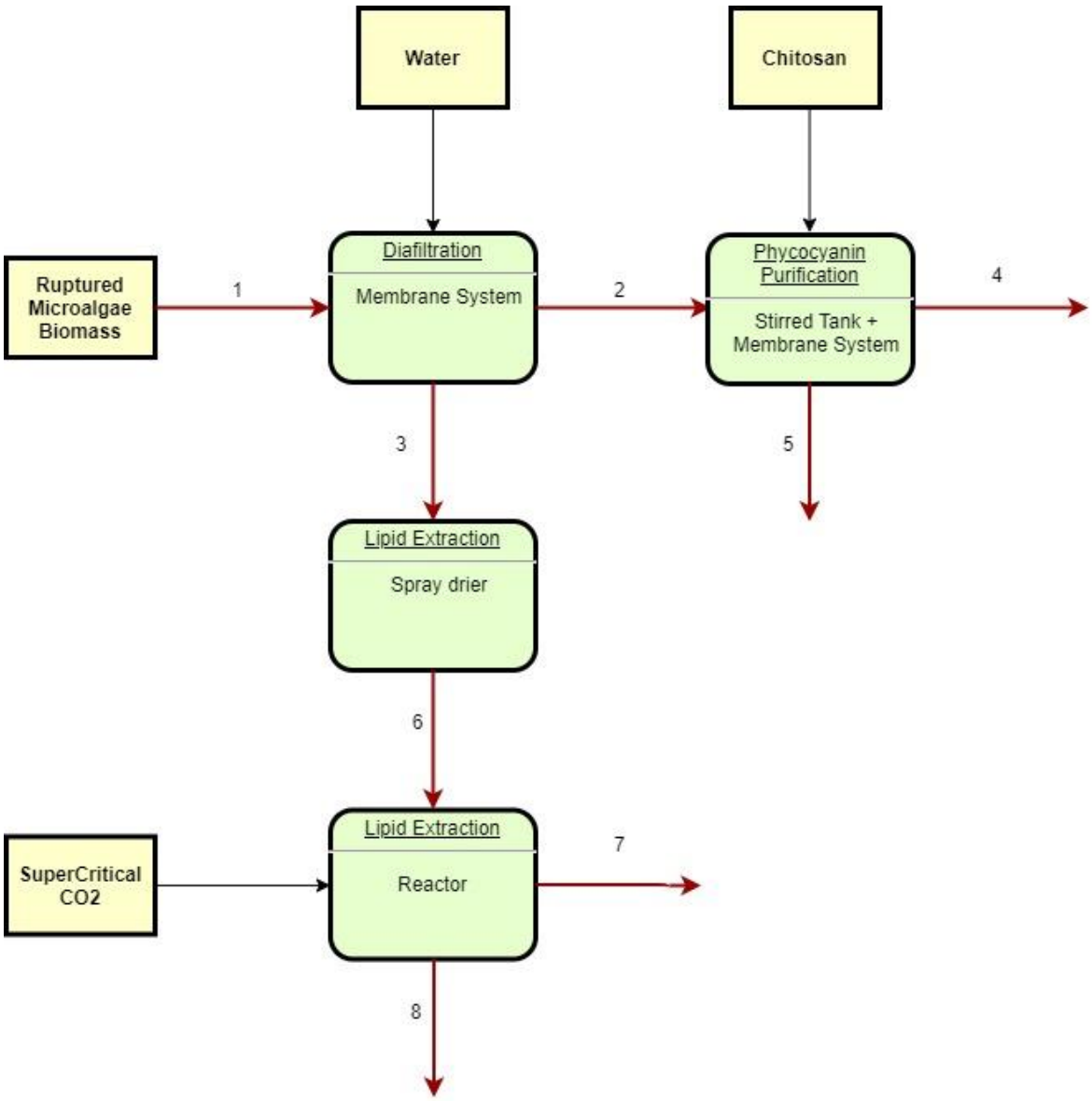

Figure 25 - Process flowsheet for option 2 + conformation 1.

Table 25 - Process streams for Process option 2 + conformation 1.

Component (kg/h)	Stream	1	2	3	4	5	6	7	8
SQDG		0.7	0.0	0.7	0.0	0.0	0.6	0.2	0.4
Glycolipids		1.2	0.0	1.2	0.0	0.0	1.1	0.4	0.7
Phospholipids		0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.1
Insoluble protein		6.3	0.0	6.0	0.0	0.0	5.4	0.0	5.4
Soluble protein		4.9	4.7	0.0	4.4	0.2	0.0	0.0	0.0
Amino acids		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phycocyanin		2.3	2.2	0.0	2.0	0.2	0.0	0.0	0.0
Zeaxanthin		0.2	0.0	0.2	0.0	0.0	0.2	0.2	0.0
Carbohydrates		4.3	0.0	4.3	0.0	0.0	3.6	0.0	3.6
Total mass		20.0	6.9	12.4	6.4	0.5	11.0	0.8	10.2

Products (details in appendix 6)

The final products in this conformation will be phycocyanin produced in stream 4 to be used for food applications, stream 7 that is composed of an oil product with zeaxanthin that can be used in nutraceuticals, and stream 8 which is composed of the remaining components (carbohydrates, proteins and a few lipids) and that can be used as fish feed (Table 26).

Table 26 - Products obtained in Process option 2 + conformation 1.

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Lipids + carotenoid	7	0.04	Palm oil replacement
Spent biomass	8	0.51	Animal feed

4.1.2.3.6 Process option 2 + conformation 2

In this conformation (Figure 26), the microalgae biomass that remains after the supercritical extraction (stream 8) is treated with enzymes to hydrolyze the proteins into amino acids, in a stirred tank where an enzyme cocktail, containing *Alcalase* and *Flavourzyme*, is added producing stream 11 containing proteins, amino acids, lipids and carbohydrates (Romero García et al., 2012). Table 27 shows the composition of process streams for process option 2 + conformation 2.

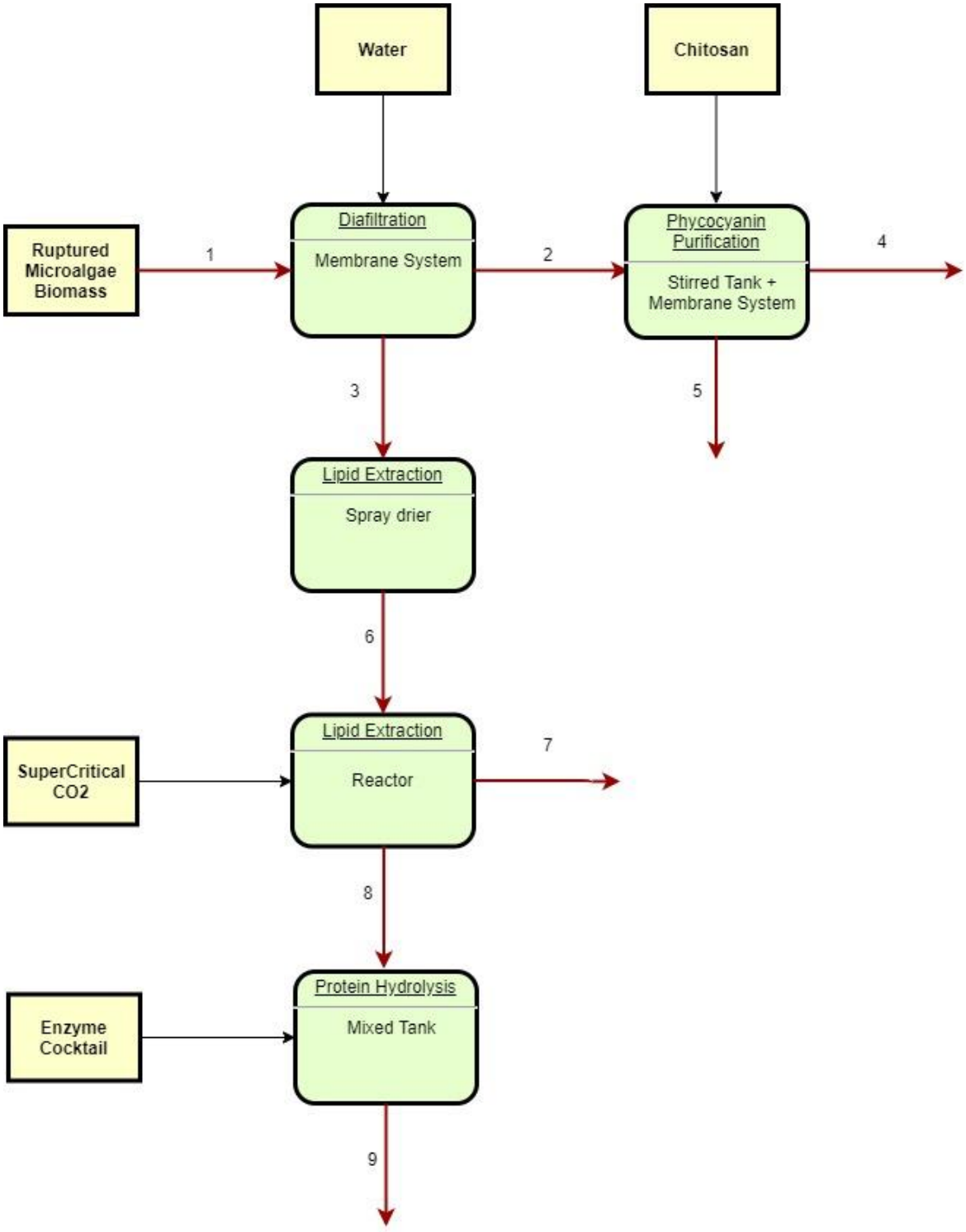

Figure 26 - Process flowsheet for option 2 + conformation 2.

Table 27 - Process streams for Process option 2 + conformation 2.

Component (kg/h) Stream	1	2	3	4	5	6	7	8	9
SQDG	0.7	0.0	0.7	0.0	0.0	0.6	0.2	0.4	0.4
Glycolipids	1.2	0.0	1.2	0.0	0.0	1.1	0.4	0.7	0.7
Phospholipids	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.1
Insoluble protein	6.3	0.0	6.0	0.0	0.0	5.4	0.0	5.4	2.2
Soluble protein	4.9	4.7	0.0	4.4	0.2	0.0	0.0	0.0	0.0
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2
Phycocyanin	2.3	2.2	0.0	2.0	0.2	0.0	0.0	0.0	0.0
Zeaxanthin	0.2	0.0	0.2	0.0	0.0	0.2	0.2	0.0	0.0
Carbohydrates	4.3	0.0	4.3	0.0	0.0	3.6	0.0	3.6	3.6
Total mass	20.0	6.9	12.4	6.4	0.5	11.0	0.8	10.2	10.2

Products (details in appendix 6)

The final products will be phycocyanin, produced in stream 4 that has the purity (>0.4) to be used for food applications; stream 7, composed of an oil product with zeaxanthin that can be used in nutraceuticals; and stream 9, that is a protein hydrolysate that can be used as a bio-stimulant for plants, and contains the remaining components (carbohydrates, proteins and a few lipids) (Table 28).

Table 28 - Products obtained in Process option 2 + conformation 2.

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Lipids + carotenoid	7	0.04	Palm oil replacement
Protein Hydrolysate	9	0.51	Bio-stimulant

4.1.2.3.7 Process option 2 + conformation 3

In this conformation (Figure 27), the lipid oil with zeaxanthin in stream 7 will go through a purification process in a reactor to separate the zeaxanthin (stream 10) from the lipids (stream 9) (IOI Oleo Data (Friedl, 2017)). Table 29 shows the composition of process streams for process option 2 + conformation 3.

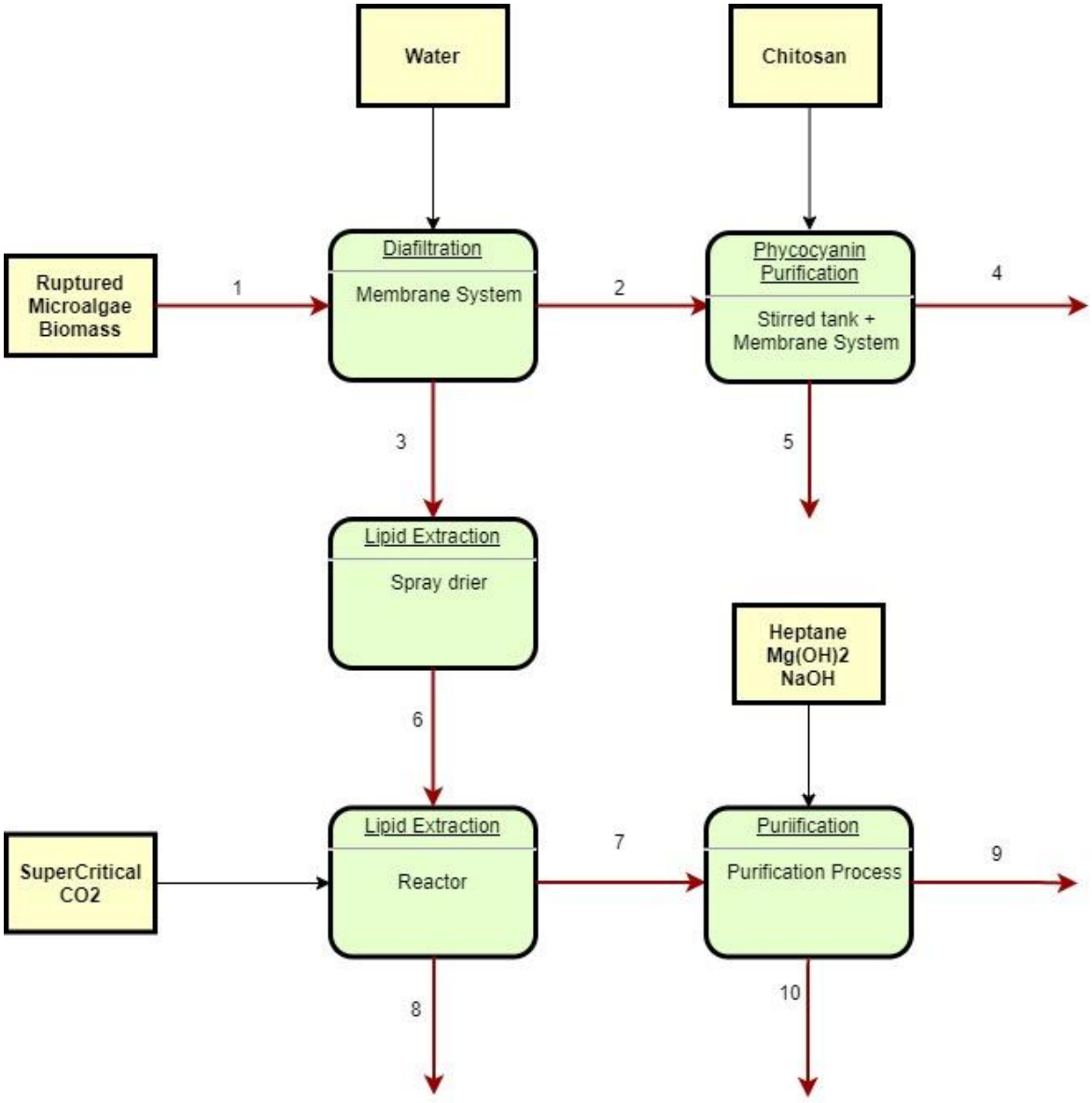

Figure 27 - Process flowsheet for Process option 2 + conformation 3.

Table 29 - Process streams in Process option 2 + conformation 3.

Component (kg/h) Stream	1	2	3	4	5	6	7	8	9	10
SQDG	0.7	0.0	0.7	0.0	0.0	0.6	0.2	0.4	0.2	0.0
Glycolipids	1.2	0.0	1.2	0.0	0.0	1.1	0.4	0.7	0.2	0.0
Phospholipids	0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0
Insoluble protein	6.3	0.0	6.0	0.0	0.0	5.4	0.0	5.4	0.0	0.0
Soluble protein	4.9	4.7	0.0	4.4	0.2	0.0	0.0	0.0	0.0	0.0
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Phycocyanin	2.3	2.2	0.0	2.0	0.2	0.0	0.0	0.0	0.0	0.0
Zeaxanthin	0.2	0.0	0.2	0.0	0.0	0.2	0.2	0.0	0.0	0.2
Carbohydrates	4.3	0.0	4.3	0.0	0.0	3.6	0.0	3.6	0.0	0.0
Total mass	20.0	6.9	12.4	6.4	0.5	11.0	0.8	10.2	0.3	0.2

Products (details in appendix 6)

The final products will be stream 4, composed of phycocyanin for food applications, stream 8, which is a stream with lipids, carbohydrates and proteins that can be used for fish feed, stream 9, which is a stream of lipids rich in palmitic oil that can be used to replace palm oil, and stream 10, a stream of zeaxanthin that can be used for nutraceuticals application (Table 30).

Table 30 - Products obtained for Process option 2 + conformation 3.

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Spent Biomass	8	0.51	Animal feed
Lipids	9	0.02	Palm oil replacement
Zeaxanthin	10	0.01	Nutraceuticals and Pharmaceuticals

4.1.2.3.8 Process option 2 + conformation 4

In this conformation (Figure 28), an enzymatic hydrolysis step is added to the previous conformation, to treat the remaining biomass from stream 8, by hydrolyzing the protein into amino acids, in a tank where an enzyme cocktail, containing Alcalase and Flavourzyme, is added producing a stream 11, which is rich in amino acids.

Table 31 shows the composition of process streams for process option 2 + conformation 4.

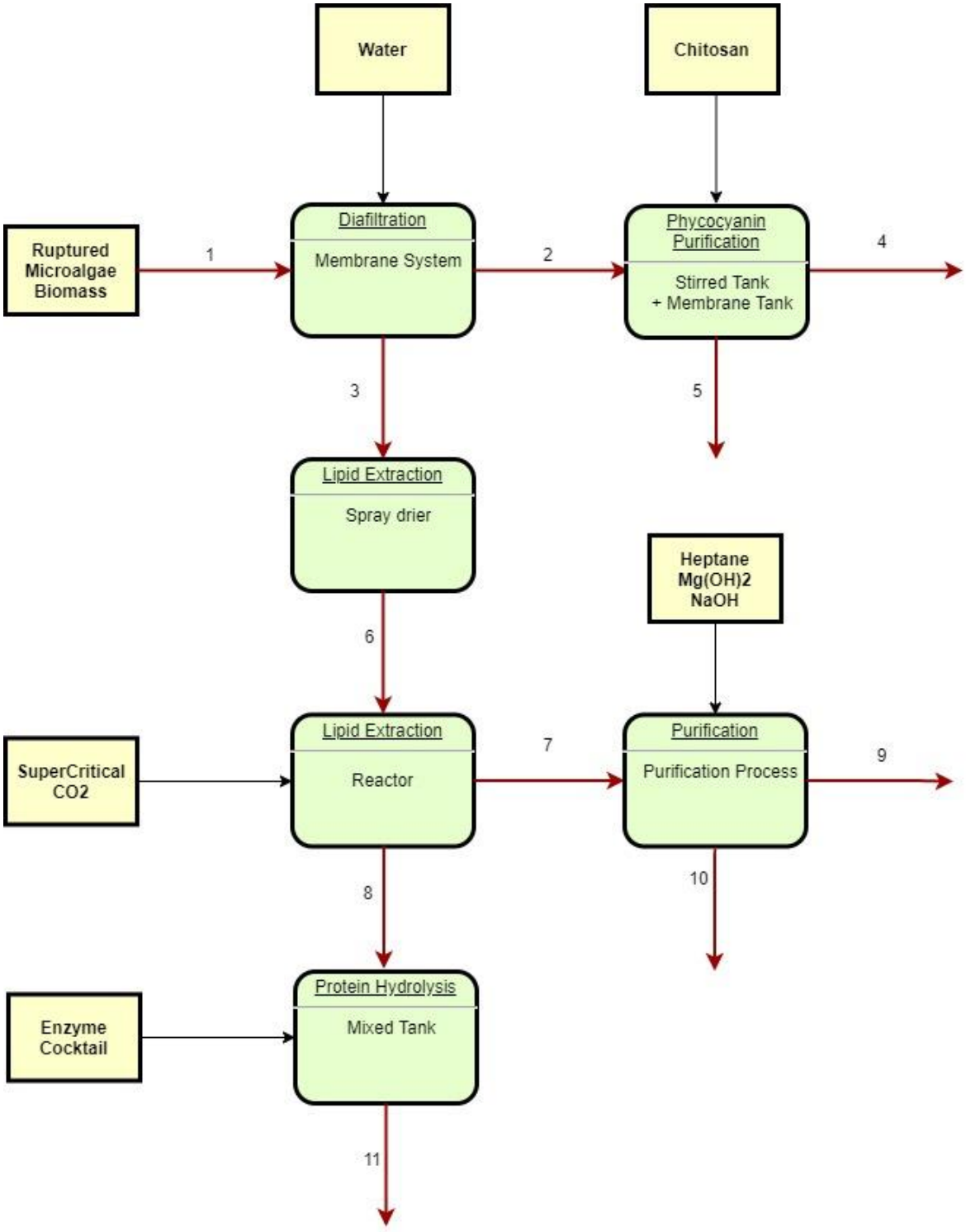

Figure 28 - Process flowsheet for option 2 + conformation 4.

Table 31 - Process streams for option 2 + conformation 4.

Component (kg/h)	Stream	1	2	3	4	5	6	7	8	9	10	11
SQDG		0.7	0.0	0.7	0.0	0.0	0.6	0.2	0.4	0.2	0.0	0.4
Glycolipids		1.2	0.0	1.2	0.0	0.0	1.1	0.4	0.7	0.2	0.0	0.7
Phospholipids		0.1	0.0	0.1	0.0	0.0	0.1	0.0	0.1	0.0	0.0	0.1
Insoluble protein		6.3	0.0	6.0	0.0	0.0	5.4	0.0	5.4	0.0	0.0	2.2
Soluble protein		4.9	4.7	0.0	4.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Amino acids		0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2
Phycocyanin		2.3	2.2	0.0	2.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Zeaxanthin		0.2	0.0	0.2	0.0	0.0	0.2	0.2	0.0	0.0	0.2	0.0
Carbohydrates		4.3	0.0	4.3	0.0	0.0	3.6	0.0	3.6	0.0	0.0	3.6
Total mass		20.0	6.9	12.4	6.4	0.5	11.0	0.8	10.2	0.3	0.2	10.2

Products (details in appendix 6)

The final products will be stream 4, composed of phycocyanin for food applications, stream 9, which is a stream of lipids rich in palmitic oil that can be used to replace palm oil, stream 10, a stream of zeaxanthin that can be used for nutraceuticals and stream 11, which contains proteins and amino acids that can be used as a bio-stimulant for plants (Table 32).

Table 32 - Products obtained for Process option 2 + conformation 4.

Product	Stream	kg/kg of biomass	Applications
Phycocyanin	4	0.32	Food applications
Lipids	9	0.02	Palm oil replacement
Zeaxanthin	10	0.01	Nutraceuticals
Protein Hydrolysate	11	0.51	Bio-stimulant

4.1.3 Economic analysis

To perform an economic analysis, one must consider not only all the investments made, but also the operational costs and the potential revenues resulting from selling the goods produced.

4.1.3.1 Definitions and assumptions

The **Capex** or **Capital expenditure** represents the investments made by a company to purchase production equipment, properties and/or industrial buildings. These costs are considered fixed costs, since they do not change with an increase or decrease of the

produced goods. These costs are expenses must be paid by a company, even if there is no production. The costs for the equipment can be found in appendix 7, while the distillation column can be found in Appendix 8.

The **Opex** or **Operational expenses** are the costs associated with the operation. These costs include rent of land or equipment, inventory costs, raw material costs, marketing, human resources, insurance, and research and development. Unlike the Capex, these costs are related to the production of goods. The Opex values for each equipment can be found in Appendix 7. The prices of utilities can be found in Appendix 9.

The **Revenue** is calculated by multiplying the price at which goods or services are sold by the number of units or amount sold. The price of the products can be found in Appendix 6.

Therefore, before an economic analysis is performed, a number of details must be known or specified. In this study, the following assumptions and conditions were considered:

- ☐ Year zero is considered to be 2020
- ☐ Capex is 100% utilized in year zero
- ☐ Capex value has a 10% margin of safety
- ☐ Production is assumed to be already 100% in the year 1
- ☐ No inflation on Opex is considered
- ☐ Opex value has a 5% margin of safety
- ☐ Land is considered to be no value and non-arable and does not represent a cost
- ☐ No financing rate was considered
- ☐ No inflation on product prices is considered
- ☐ Discount rate – 5%
- ☐ Payback period is 8 years

For each process sequence, the most important economic values are specified in Table 33 to Table 35. These are the values that were inserted in an optimization software called GAMS (https://www.gams.com/), to calculate the economic parameters of all the possible biorefinery conformations in order to choose the ones with the best economic performance.

4.1.3.2 Production, harvesting and biomass disruption economic values (Capex and Opex values and explanations in Appendix 7)

The economic results for the *Synechocystis* based biorefinery are shown in Table 33 to Table 35.

Table 33 – Economic results for Synechocystis based biorefinery part 1.

Process Sequence	S.1.1	S.1.2	S.1.3	S.2.1	S.2.2	S.2.3
Capex	€ 14,953,401	€ 14,964,730	€ 14,922,222	€ 14,851,493	€ 14,868,665	€ 14,772,222
Opex	€ 2,392,460	€ 2,394,222	€ 2,416,387	€ 2,405,669	€ 2,409,357	€ 2,423,564
Ethanol revenue	€ 141,950	€ 141,950	€ 141,580	€ 141,580	€ 141,580	€ 141,580

Table 34 – Economic results for Synechocystis based biorefinery part 2.

Process Sequence	S.3.1	S.3.2	S.3.3	S.4.1	S.4.2	S.4.3
Capex	€ 14,362,188	€ 14,385,518	€ 14,232,222	€ 14,351,670	€ 14,377,698	€ 14,199,504
Opex	€ 2,347,181	€ 2,355,030	€ 2,345,543	€ 2,383,940	€ 2,393,222	€ 2,375,420
Ethanol revenue	€ 141,087	€ 141,087	€ 141,087	€ 140,840	€ 140,840	€ 140,840

Table 35 – Economic results for Synechocystis based biorefinery part 3.

Process Sequence	S.2.1p	S.2.2p	S.2.3p	S.5.1	S.5.2
Capex	€ 14,935,690	€ 14,952,862	€ 14,856,418	€ 14,654,629	€ 14,665,958
Opex	€ 2,298,389	€ 2,302,078	€ 2,316,285	€ 2,252,857	€ 2,254,618
Ethanol revenue	€ 133,714	€ 133,714	€ 133,714	€ 127,693	€ 127,693

4.1.3.3 Extraction Values (Capex and Opex values and explanation in appendix 7)

For each harvesting and rupturing method selected in the first stage, different concentration and final ruptured biomass quantities were obtained. Therefore, the extraction (Second stage) costs were calculated for different concentrations, and the final revenue was calculated per kg of ruptured biomass produced by the first stage processes (Table 36).

Table 36 – Capex and Opex values for different final ruptured biomass concentrations.

	Capex	Opex	Capex	Opex	Capex	Opex	Capex	Opex	Revenue (€/kg)
	Final biomass concentration g/L								
	50		100		150		200		
Process 1	€ 427,866	€ 1,524,410	€ 314,988	€ 1,478,442	€ 267,354	€ 1,461,770	€ 239,718	€ 1,452,920	€ 20.6
Process 2	€ 475,412	€ 2,029,474	€ 362,534	€ 1,983,506	€ 314,900	€ 1,966,833	€ 287,264	€ 1,957,984	€ 47.4
Process 3	€ 1,419,031	€ 1,851,271	€ 1,306,153	€ 1,805,303	€ 1,258,520	€ 1,788,631	€ 1,230,884	€ 1,779,781	€ 23.4
Process 4	€ 1,412,099	€ 2,357,643	€ 1,299,221	€ 2,311,676	€ 1,251,587	€ 2,295,003	€ 1,223,951	€ 2,286,153	€ 49.9
Process 5	€ 1,220,551	€ 1,921,512	€ 1,107,080	€ 1,875,472	€ 1,058,986	€ 1,858,744	€ 1,030,946	€ 1,849,845	€ 21.6
Process 6	€ 1,236,631	€ 2,308,683	€ 1,123,160	€ 2,262,644	€ 1,075,066	€ 2,245,915	€ 1,047,026	€ 2,237,017	€ 46.5
Process 7	€ 1,929,690	€ 2,079,879	€ 1,816,219	€ 2,033,839	€ 1,768,125	€ 2,017,111	€ 1,740,085	€ 2,008,212	€ 23.6
Process 8	€ 1,945,770	€ 2,467,050	€ 1,832,299	€ 2,421,011	€ 1,784,205	€ 2,404,283	€ 1,756,165	€ 2,395,384	€ 48.5

To better compare the results of Capex estimated for the process configurations proposed and analyzed, a graph was built and is shown in Figure 29.

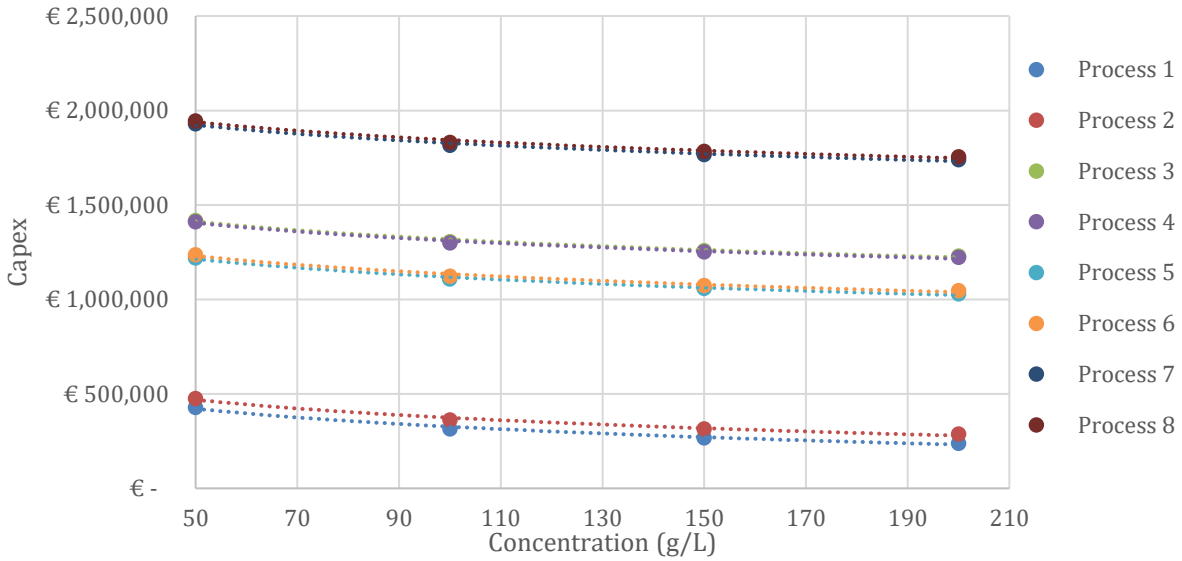

Figure 29 - Variation of the Capex of the extraction process with final ruptured biomass concentration.

From the graph in Figure 29, a logarithmic equation for the Capex, dependent on the final concentration was fitted to the results obtained for each extraction process (eq. 1 to eq. 8), as shown below:

$$Capex_{process\ 1} = -137{,}162 \times ln\ (concentration) + 958{,}038 \quad (1)$$

$$Capex_{process\ 2} = -137{,}162 \times ln\ (concentration) + 1{,}005{,}584 \quad (2)$$

$$Capex_{process\ 3} = -137{,}162 \times ln\ (concentration) + 1{,}949{,}204 \quad (3)$$

$$Capex_{process\ 4} = -137{,}162 \times ln\ (concentration) + 1{,}942{,}271 \quad (4)$$

$$Capex_{process\ 5} = -138{,}197 \times ln\ (concentration) + 1{,}754{,}821 \quad (5)$$

$$Capex_{process\ 6} = -138{,}197 \times ln\ (concentration) + 1{,}770{,}901 \quad (6)$$

$$Capex_{process\ 7} = -138{,}197 \times ln\ (concentration) + 2{,}463{,}960 \quad (7)$$

$$Capex_{process\ 8} = -138{,}197 \times ln\ (concentration) + 2{,}480{,}040 \quad (8)$$

Similarly, to better compare the results of Opex estimated for the process configurations proposed and analysed, a graphic was built and is shown in Figure 30.

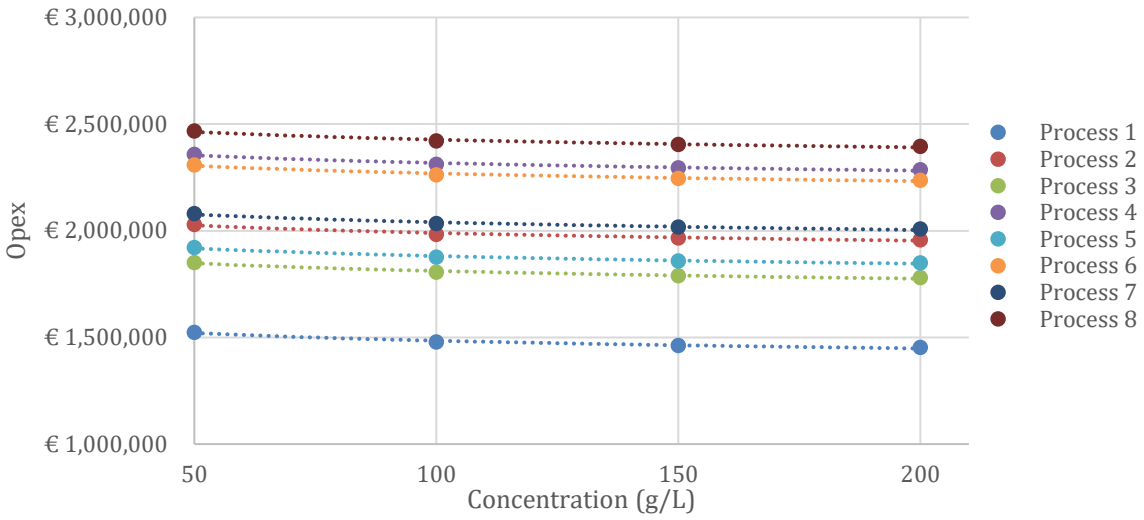

Figure 30 - Variation of the Opex of the extraction process with final ruptured biomass concentration.

From the previous graph in Figure 30, an equation for the Opex, dependent on the final concentration was obtained for each process (eq. 9 to eq. 16), as shown below:

$$Opex_{process\ 1} = -52{,}292 \times ln\,(concentration) + 1{,}725{,}501 \quad (9)$$

$$Opex_{process\ 2} = -52{,}292 \times ln\,(concentration) + 2{,}230{,}565 \quad (10)$$

$$Opex_{process\ 3} = -52{,}292 \times ln\,(concentration) + 2{,}052{,}362 \quad (11)$$

$$Opex_{process\ 4} = -52{,}292 \times ln\,(concentration) + 2{,}558{,}734 \quad (12)$$

$$Opex_{process\ 5} = -52{,}418 \times ln\,(concentration) + 2{,}121{,}099 \quad (13)$$

$$Opex_{process\ 6} = -52{,}418 \times ln\,(concentration) + 2{,}510{,}271 \quad (14)$$

$$Opex_{process\ 7} = -52{,}418 \times ln\,(concentration) + 2{,}281{,}466 \quad (15)$$

$$Opex_{process\ 8} = -52{,}418 \times ln\,(concentration) + 2{,}668{,}638 \quad (16)$$

The previously mentioned equations were introduced in the software GAMS and were calculated in order to obtain the different economic parameters necessary for the economic comparison. The code for the GAMS can be found in the Appendix 10.

Already some conclusions can be taken from the previously displayed graphs (Figure 29 and Figure 30). It is possible to conclude that the higher the final concentration of ruptured biomass, the lower the Capex and Opex. This occurs because the higher the biomass concentration, less volume is required to produce the same amount of biomass. This leads to smaller equipment size, which in turn means cheaper equipment, and therefore, lower Capex. The fact that a smaller volume is obtained also means that some equipment, like membranes, mixing tanks and spray dryer, require less energy to work. And since the maintenance and consumable costs are also dependent on the equipment

size, these will also decrease with the size of the equipment. Since these two costs are lower, a lower Opex is obtained.

4.1.3.4 Economic values for the different Scenario combinations

Capex

The Capex for the whole process (Stage 1 and Stage 2) was calculated based on eq. 17 and the values can be found in Table 37; the 5 lowest values are highlighted in green and the 5 highest values are highlighted in red.

$$Capex_{whole\ process} = Capex_{PHR} + a_{CAPEX\ DNS} \times ln\ (Final\ Concentration)\ + b_{CAPEX\ DNS} \quad (17)$$

Opex

The Opex of the whole process (Stage 1 and Stage 2) was calculated based on the eq .18 and can be found in Table 38. The 5 lowest values are highlighted in green and the 5 highest values are highlighted in red

$$Opex_{whole\ process} = Opex_{PHR} + a_{OPEX\ DNS} \times ln\ (Concentration)\ + b_{OPEX\ DNS} \quad (18)$$

Revenues

The revenue for each combination of processes (Stage 1 + Stage 2) was calculated based on the eq. 19 and can be found in Table 39; the 5 highest revenues are highlighted in green and the 5 lowest are highlighted in red.

$$Revenue = Ethanol\ revenue + DNSrevenue \times biomass\ produced\ per\ year \quad (19)$$

Table 37 - Capex for all process combinations.

Production, harvesting and Disruption Scenarios	Extraction Scenarios							
	EP 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7	EP 8
S.1.1	€ 16,703,170	€ 16,755,480	€ 17,793,460	€ 17,785,840	€ 17,573,610	€ 17,591,300	€ 18,353,660	€ 18,371,350
S.1.2	€ 16,715,630	€ 16,767,950	€ 17,805,930	€ 17,798,300	€ 17,586,070	€ 17,603,760	€ 18,366,130	€ 18,383,810
S.1.3	€ 16,773,450	€ 16,825,770	€ 17,863,750	€ 17,856,120	€ 17,644,680	€ 17,662,370	€ 18,424,740	€ 18,442,420
S.2.1	€ 16,695,650	€ 16,747,970	€ 17,785,950	€ 17,778,320	€ 17,566,880	€ 17,584,570	€ 18,346,940	€ 18,364,620
S.2.2	€ 16,714,540	€ 16,766,850	€ 17,804,840	€ 17,797,210	€ 17,585,770	€ 17,603,460	€ 18,365,830	€ 18,383,510
S.2.3	€ 16,608,450	€ 16,660,770	€ 17,698,750	€ 17,691,120	€ 17,479,680	€ 17,497,370	€ 18,259,740	€ 18,277,420
S.2.1p	€ 16,788,270	€ 16,840,580	€ 17,878,560	€ 17,870,940	€ 17,659,500	€ 17,677,190	€ 18,439,550	€ 18,457,240
S.2.2p	€ 16,807,160	€ 16,859,470	€ 17,897,450	€ 17,889,830	€ 17,678,390	€ 17,696,080	€ 18,458,440	€ 18,476,130
S.2.3p	€ 16,701,070	€ 16,753,380	€ 17,791,360	€ 17,783,740	€ 17,572,300	€ 17,589,990	€ 18,352,350	€ 18,370,040
S.3.1	€ 16,234,490	€ 16,286,800	€ 17,324,780	€ 17,317,160	€ 17,106,300	€ 17,123,990	€ 17,886,350	€ 17,904,040
S.3.2	€ 16,260,150	€ 16,312,470	€ 17,350,450	€ 17,342,820	€ 17,131,960	€ 17,149,650	€ 17,912,020	€ 17,929,710
S.3.3	€ 16,091,530	€ 16,143,840	€ 17,181,820	€ 17,174,190	€ 16,963,340	€ 16,981,030	€ 17,743,390	€ 17,761,080
S.4.1	€ 16,250,430	€ 16,302,740	€ 17,340,720	€ 17,333,100	€ 17,122,450	€ 17,140,140	€ 17,902,500	€ 17,920,190
S.4.2	€ 16,279,060	€ 16,331,370	€ 17,369,350	€ 17,361,730	€ 17,151,080	€ 17,168,770	€ 17,931,130	€ 17,948,820
S.4.3	€ 16,083,050	€ 16,135,360	€ 17,173,340	€ 17,165,710	€ 16,955,070	€ 16,972,750	€ 17,735,120	€ 17,752,810
S.5.1	€ 16,374,520	€ 16,426,830	€ 17,464,820	€ 17,457,190	€ 17,244,960	€ 17,262,650	€ 18,025,020	€ 18,042,700
S.5.2	€ 16,386,980	€ 16,439,300	€ 17,477,280	€ 17,469,650	€ 17,257,420	€ 17,275,110	€ 18,037,480	€ 18,055,170

Table 38 - Opex for all process combinations.

Production, harvesting and Disruption Scenarios	Extraction Scenarios							
	EP 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7	EP 8
S.1.1	€ 4,032,947	€ 4,563,264	€ 4,376,151	€ 4,907,841	€ 4,447,624	€ 4,856,254	€ 4,616,009	€ 5,022,540
S.1.2	€ 4,034,797	€ 4,565,114	€ 4,378,001	€ 4,909,691	€ 4,449,474	€ 4,858,104	€ 4,617,859	€ 5,024,390
S.1.3	€ 4,096,128	€ 4,626,445	€ 4,439,332	€ 4,971,023	€ 4,510,897	€ 4,919,527	€ 4,679,282	€ 5,085,813
S.2.1	€ 4,084,874	€ 4,615,191	€ 4,428,078	€ 4,959,768	€ 4,499,642	€ 4,908,273	€ 4,668,028	€ 5,074,558
S.2.2	€ 4,088,747	€ 4,619,064	€ 4,431,951	€ 4,963,641	€ 4,503,515	€ 4,912,146	€ 4,671,901	€ 5,078,431
S.2.3	€ 4,103,664	€ 4,633,982	€ 4,446,869	€ 4,978,559	€ 4,518,433	€ 4,927,064	€ 4,686,818	€ 5,093,349
S.2.1p	€ 3,972,230	€ 4,502,548	€ 4,315,434	€ 4,847,125	€ 4,386,999	€ 4,795,630	€ 4,555,384	€ 4,961,915
S.2.2p	€ 3,976,103	€ 4,506,421	€ 4,319,308	€ 4,850,998	€ 4,390,872	€ 4,799,503	€ 4,559,257	€ 4,965,788
S.2.3p	€ 3,991,021	€ 4,521,338	€ 4,334,225	€ 4,865,916	€ 4,405,790	€ 4,814,420	€ 4,574,175	€ 4,980,706
S.3.1	€ 4,051,509	€ 4,581,826	€ 4,394,713	€ 4,926,404	€ 4,466,345	€ 4,874,976	€ 4,634,731	€ 5,041,261
S.3.2	€ 4,059,751	€ 4,590,069	€ 4,402,955	€ 4,934,646	€ 4,474,588	€ 4,883,218	€ 4,642,973	€ 5,049,504
S.3.3	€ 4,049,790	€ 4,580,107	€ 4,392,994	€ 4,924,684	€ 4,464,626	€ 4,873,256	€ 4,633,011	€ 5,039,542
S.4.1	€ 4,100,117	€ 4,630,434	€ 4,443,321	€ 4,975,012	€ 4,514,977	€ 4,923,608	€ 4,683,363	€ 5,089,893
S.4.2	€ 4,109,863	€ 4,640,180	€ 4,453,067	€ 4,984,757	€ 4,524,723	€ 4,933,354	€ 4,693,108	€ 5,099,639
S.4.3	€ 4,091,171	€ 4,621,488	€ 4,434,375	€ 4,966,065	€ 4,506,031	€ 4,914,662	€ 4,674,416	€ 5,080,947
S.5.1	€ 3,886,363	€ 4,416,680	€ 4,229,567	€ 4,761,258	€ 4,301,040	€ 4,709,670	€ 4,469,425	€ 4,875,956
S.5.2	€ 3,888,213	€ 4,418,530	€ 4,231,417	€ 4,763,107	€ 4,302,890	€ 4,711,520	€ 4,471,275	€ 4,877,806

Table 39 - Revenue for all process combinations.

Production, harvesting and Disruption Scenarios	Extraction Scenarios							
	EP 1	EP 2	EP 3	EP4	EP 5	EP 6	EP 7	EP 8
S.1.1	€ 3,289,362	€ 7,384,053	€ 3,717,165	€ 7,766,021	€ 3,442,149	€ 7,246,545	€ 3,747,723	€ 7,552,119
S.1.2	€ 3,193,986	€ 7,164,595	€ 3,608,826	€ 7,534,988	€ 3,342,143	€ 7,031,254	€ 3,638,457	€ 7,327,568
S.1.3	€ 3,320,784	€ 7,456,836	€ 3,752,909	€ 7,842,661	€ 3,475,114	€ 7,317,938	€ 3,783,775	€ 7,626,599
S.2.1	€ 3,463,848	€ 7,786,022	€ 3,915,418	€ 8,189,210	€ 3,625,123	€ 7,640,874	€ 3,947,673	€ 7,963,425
S.2.2	€ 3,363,173	€ 7,554,372	€ 3,801,060	€ 7,945,342	€ 3,519,561	€ 7,413,623	€ 3,832,337	€ 7,726,399
S.2.3	€ 3,497,406	€ 7,863,239	€ 3,953,538	€ 8,270,499	€ 3,660,310	€ 7,716,625	€ 3,986,119	€ 8,042,433
S.2.1p	€ 3,455,982	€ 7,778,157	€ 3,907,553	€ 8,181,344	€ 3,617,258	€ 7,633,009	€ 3,939,808	€ 7,955,559
S.2.2p	€ 3,355,308	€ 7,546,507	€ 3,793,194	€ 7,937,477	€ 3,511,696	€ 7,405,758	€ 3,824,472	€ 7,718,534
S.2.3p	€ 3,489,541	€ 7,855,373	€ 3,945,672	€ 8,262,634	€ 3,652,445	€ 7,708,759	€ 3,978,253	€ 8,034,568
S.3.1	€ 3,113,642	€ 6,980,851	€ 3,517,679	€ 7,341,598	€ 3,257,941	€ 6,850,982	€ 3,546,539	€ 7,139,579
S.3.2	€ 3,023,565	€ 6,773,585	€ 3,415,358	€ 7,123,400	€ 3,163,491	€ 6,647,652	€ 3,443,343	€ 6,927,504
S.3.3	€ 3,143,668	€ 7,049,939	€ 3,551,786	€ 7,414,330	€ 3,289,424	€ 6,918,758	€ 3,580,937	€ 7,210,271
S.4.1	€ 3,288,252	€ 7,382,943	€ 3,716,055	€ 7,764,910	€ 3,441,039	€ 7,245,435	€ 3,746,613	€ 7,551,009
S.4.2	€ 3,192,876	€ 7,163,485	€ 3,607,715	€ 7,533,878	€ 3,341,033	€ 7,030,144	€ 3,637,347	€ 7,326,458
S.4.3	€ 3,320,044	€ 7,456,096	€ 3,752,169	€ 7,841,921	€ 3,474,374	€ 7,317,198	€ 3,783,035	€ 7,625,859
S.5.1	€ 3,117,734	€ 7,007,691	€ 3,524,147	€ 7,370,560	€ 3,262,882	€ 6,877,058	€ 3,553,177	€ 7,167,353
S.5.2	€ 3,027,127	€ 6,799,206	€ 3,421,224	€ 7,151,079	€ 3,167,876	€ 6,672,532	€ 3,449,374	€ 6,954,030

A first conclusion is that process combinations with the highest Capex and Opex (Table 37 and Table 38) are the ones with the highest number of products. This is expected since they also require more equipment to produce these products. However, in Table 39, the scenario combinations with the largest revenue are also the ones that exploit the highest number of products from the microalgal biomass. Therefore, it is necessary to perform further economic studies, by resorting to economic indicators, to see if the extra revenue from those products is sufficient to compensate the extra Capex and Opex these scenarios incur.

4.1.3.5 Economic indicators

To select the best scenarios, three indicators were used: Payback period, Return on Investment and Net Present Value.

The **payback period (PBP)** is the length of time required to recover the initial investment. A desired payback period should be under 5 years (Asselbergs, 2014). The payback period ignores the time value of money, unlike other methods such as the net present value (NPV), internal rate of return (IRR), and discounted cash flow. This might mean that the real payback period can be superior to the one calculated. As can be seen in Table 40, the scenarios with the lowest payback period all have a biorefinery approach. However, even for the scenario combinations with the lowest payback period of 6 years, it is over the 5 years mentioned previously. Nevertheless, this does not mean a process is not viable.

Table 40 - Payback period (years) for all scenario combinations.

	Extraction Scenarios							
Production, harvesting and Disruption Scenarios	EP 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7	EP 8
S.1.1	0	6	0	7	0	8	0	8
S.1.2	0	7	0	7	0	9	0	8
S.1.3	0	6	0	7	0	8	0	8
S.2.1	0	6	0	6	0	7	0	7
S.2.2	0	6	0	6	0	8	0	7
S.2.3	0	6	0	6	0	7	0	7
S.2.1p	0	6	0	6	0	7	0	7
S.2.2p	0	6	0	6	0	7	0	7
S.2.3p	0	6	0	6	0	7	0	7
S.3.1	0	7	0	8	0	9	0	9
S.3.2	0	8	0	8	0	10	0	10
S.3.3	0	7	0	7	0	9	0	9
S.4.1	0	6	0	7	0	8	0	8
S.4.2	0	7	0	7	0	9	0	9
S.4.3	0	6	0	6	0	8	0	7
S.5.1	0	7	0	7	0	8	0	8
S.5.2	0	7	0	8	0	9	0	9

The following indicator is the Return on investment.

The **Return on Investment (ROI)** evaluates the efficiency of an investment. ROI tries to directly quantify the amount of return on a particular investment, relative to the investment's cost.

The ROI (eq. 20) was calculated for a period of 8 years and does not account for the depreciation of money value over time. It is shown in Table 41, the ROI for all process combinations.

$$ROI = \frac{Profit_{8\ years} - Investment}{Investment} \times 100\% \quad (20)$$

Table 41 - *ROI values for all process combinations.*

Production, harvesting and Disruption Scenarios	Extraction Scenarios							
	EP 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7	EP 8
S.1.1	<0%	9%	<0%	4%	<0%	<0%	<0%	<0%
S.1.2	<0%	0%	<0%	<0%	<0%	<0%	0.00	<0%
S.1.3	<0%	9%	<0%	4%	<0%	<0%	0.00	<0%
S.2.1	<0%	22%	<0%	17%	<0%	0%	0.00	2%
S.2.2	<0%	13%	<0%	8%	<0%	<0%	0.00	<0%
S.2.3	<0%	25%	<0%	20%	<0%	3%	0.00	4%
S.2.1p	<0%	26%	<0%	21%	<0%	4%	0.00	5%
S.2.2p	<0%	17%	<0%	12%	<0%	<0%	0.00	<0%
S.2.3p	<0%	29%	<0%	23%	<0%	6%	0.00	7%
S.3.1	<0%	<0%	<0%	<0%	<0%	<0%	0.00	<0%
S.3.2	<0%	<0%	<0%	<0%	<0%	<0%	0.00	<0%
S.3.3	<0%	<0%	<0%	<0%	<0%	<0%	0.00	<0%
S.4.1	<0%	9%	<0%	4%	<0%	<0%	0.00	<0%
S.4.2	<0%	0%	<0%	<0%	<0%	<0%	0.00	<0%
S.4.3	<0%	14%	<0%	8%	<0%	<0%	0.00	<0%
S.5.1	<0%	2%	<0%	<0%	<0%	<0%	0.00	<0%
S.5.2	<0%	<0%	<0%	<0%	<0%	<0%	0.00	<0%

Highlighted in green in Table 41 are the 10 scenario combinations with the highest return on investment. Once again, the best results correspond to the ones with the biorefinery approach. In this analysis, it is also possible to observe that only the scenarios with some biorefinery approach have positive ROI. Another observation is that 9 scenarios use a membrane system for biomass harvesting. This observation can be explained by the higher harvesting efficiency of the membrane that leads to a higher revenue. Another observation is that a scenario with extraction process 2 has a higher ROI than a scenario with extraction process 4, which has more high purity products. This means that although biorefineries can have a positive impact on the exploration of microalgae biomass, some purification steps have high operation costs and can be more expensive than the income obtained from the products obtained. It is the case of the Lipid purification process. In this process, the costs for the production of the lipid soap and carotenoids are slightly higher than the return those products bring in.

Finally, the last indicator is the Net Present Value of the projects.

The **Net Present Value (NPV)** is the difference between the present value of cash inflows and the present value of cash outflows over a period of time (eq. 21). NPV is used to analyze the operating income ability of a projected investment or project. A positive net present value indicates that the projected earnings generated by a project or investment exceeds the anticipated costs. Usually, an investment with a positive NPV will have a positive income, and an investment with a negative NPV will result in a net loss.

$$NPV = \sum_{t=1}^{T} \frac{C_t}{(1+r)^t} - C_0 \tag{21}$$

Where:

C_t = net cash inflow during the period t (Operating income = Revenue – Opex)

C_o = total initial investment costs Capex

r = discount rate, and

t = number of time periods

The NPV for all the scenario combinations proposed is shown in Table 42.

Table 42 - *NPV for all process combinations.*

Production, harvesting and Disruption Scenarios	Extraction Scenarios							
	EP 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7	EP 8
S.1.1	-€ 21,509,100	€ 1,475,877	-€ 22,052,600	€ 687,181	-€ 24,072,200	-€ 2,142,340	-€ 23,965,600	-€ 2,022,140
S.1.2	-€ 22,150,000	€ 33,057	-€ 22,777,300	-€ 830,449	-€ 24,743,000	-€ 3,558,230	-€ 24,696,200	-€ 3,497,880
S.1.3	-€ 21,784,700	€ 1,467,649	-€ 22,300,200	€ 703,890	-€ 24,339,200	-€ 2,160,930	-€ 24,212,600	-€ 2,020,780
S.2.1	-€ 20,709,500	€ 3,745,791	-€ 21,099,400	€ 3,094,247	-€ 23,219,100	€ 76,814	-€ 23,002,700	€ 306,735
S.2.2	-€ 21,404,100	€ 2,204,668	-€ 21,882,400	€ 1,474,158	-€ 23,945,300	-€ 1,435,880	-€ 23,792,100	-€ 1,269,130
S.2.3	-€ 20,526,800	€ 4,210,609	-€ 20,887,200	€ 3,585,387	-€ 23,025,900	€ 532,156	-€ 22,788,500	€ 783,134
S.2.1p	-€ 20,124,900	€ 4,330,377	-€ 20,514,800	€ 3,678,833	-€ 22,634,500	€ 661,399	-€ 22,418,200	€ 891,320
S.2.2p	-€ 20,819,500	€ 2,789,253	-€ 21,297,800	€ 2,058,743	-€ 23,360,700	-€ 851,296	-€ 23,207,500	-€ 684,548
S.2.3p	-€ 19,942,200	€ 4,795,194	-€ 20,302,700	€ 4,169,972	-€ 22,441,300	€ 1,116,741	-€ 22,203,900	€ 1,367,719
S.3.1	-€ 22,296,100	-€ 781,398	-€ 22,993,200	-€ 1,707,250	-€ 24,916,500	-€ 4,352,640	-€ 24,919,600	-€ 4,342,170
S.3.2	-€ 22,957,200	-€ 2,199,930	-€ 23,733,500	-€ 3,196,440	-€ 25,605,900	-€ 5,745,740	-€ 25,665,500	-€ 5,791,790
S.3.3	-€ 21,948,000	-€ 180,786	-€ 22,618,700	-€ 1,083,080	-€ 24,558,900	-€ 3,760,510	-€ 24,543,200	-€ 3,731,190
S.4.1	-€ 21,497,700	€ 1,487,309	-€ 22,041,200	€ 698,613	-€ 24,063,500	-€ 2,133,680	-€ 23,956,900	-€ 2,013,480
S.4.2	-€ 22,205,700	-€ 22,712	-€ 22,833,000	-€ 886,219	-€ 24,801,500	-€ 3,616,770	-€ 24,754,700	-€ 3,556,410
S.4.3	-€ 21,067,000	€ 2,185,314	-€ 21,582,600	€ 1,421,555	-€ 23,622,900	-€ 1,444,650	-€ 23,496,300	-€ 1,304,500
S.5.1	-€ 21,342,300	€ 319,419	-€ 22,024,100	-€ 592,714	-€ 23,954,800	-€ 3,254,360	-€ 23,946,900	-€ 3,232,920
S.5.2	-€ 21,952,400	-€ 1,052,480	-€ 22,713,700	-€ 2,035,680	-€ 24,593,300	-€ 4,600,680	-€ 24,642,200	-€ 4,636,090

The 5 best scenarios are highlighted in green in Table 42. As can be observed, the ones with a positive NPV have a common denominator, a biorefinery approach. This proves that a biorefinery has a positive impact on economic performance of a microalgae production facility. The main reason behind this result is that biorefineries produce a higher number of different high values products bringing in higher operating income.

Furthermore, the extraction section of 4 out of the 5 scenarios with the higher NPV are not the ones with the highest number of high purity products produced. This shows that although biorefineries can have a positive impact on the exploitation of microalgae biomass, some purification steps are still more expensive than the operating income attained from the products obtained. Moreover, all the 10 scenarios with the highest NPV are using membranes as a harvesting method. Only the 11th best NPV has a different harvesting method, flocculation with chitosan. This demonstrates that, although the efficiency and final concentration of the flocculation are lower than those of the centrifugation or dissolved air flotation, since the Capex and Opex of the flocculation tank are much lower, these compensate for the lower efficiency. In addition, as expected, the scenarios with the pervaporation are the ones with the highest NPV since the operation costs, especially the energy costs, are lower than the combination distillation with pervaporation. However, the cost of removing ethanol from the medium is higher than the profit obtained from ethanol (Pervaporation Opex is ±170 k€ and Distillation + Pervaporation ~280 k€ while maximum ethanol profit is ±140 k€).

The scenarios with a negative NPV fail for two main reasons, either the operating income is very low and cannot compensate for the Capex, or the operating income is negative due to the revenues being lower than the Opex. The low or negative operating income values are associated to the low value and/or low amount of the products obtained, and/or due to high Opex of certain operations like the biomass production process, the enzymatic hydrolysis or the saponification process (these values are shown in Appendix 7). Other high costs are the high manpower costs which are around 20% of the Opex and the bioethanol production. Due to the low value of ethanol, the revenue from ethanol is not enough to pay for its production, and therefore, money is lost in this process.

4.1.3.6 Scenario choices

As could be observed in the previous section, the 5 scenarios combinations with the best economic performances, based on PBP, ROI and NPV, are S.2.3 + EP 2, S.2.1p + EP 2, S.2.3p + EP 2, S.2.1 + EP 2 and S.2.3 + EP 2. As the process was divided by operations, it is possible to create a process by selecting each individual operation to design the optimal sustainable biorefinery. However, to perform such task one must choose the most

environmentally friendly operations. Still, the best economic scenarios are very similar in all the stages and have few different equipment and operations. So, in order to discover which scenario stages are the most sustainable and perform a broader study of the environmental impact of different equipment and operations on a microalgae biorefinery, 2 scenarios of the 5 best were replaced by the two scenarios from the top 15, with significant equipment difference. Therefore, the combinations of scenarios proposed for LCA analysis are S.2.3 + EP 2, S.2.1 + EP 2, S.2.3p + EP 2, S.1.1 + EP 2 and. S.5.3 + EP 2. The scenarios can be seen in Table 43.

Table 43 - 5 scenarios with the best economic performance and the chosen scenarios for the LCA analysis in the Synechocystis scenario.

Combination	Best scenario	Chosen Scenario	Harvesting Step	Disruption Step	Ethanol Recovery Step	Products obtained
S2.3 + EP 2	**X**	**X**	Membrane	Ultrasonication	Distillation + Pervaporation	Phycocyanin Protein Hydrolysate
S2.1p+ EP 2	**X**		Membrane	Bead mill	Pervaporation	Phycocyanin Protein Hydrolysate
S2.3p + EP 2	**X**	**X**	Membrane	Ultrasonication	Pervaporation	Phycocyanin Protein Hydrolysate
S2.1 + EP 2	**X**		Membrane	Ultrasonication	Distillation + Pervaporation	Phycocyanin Protein Hydrolysate
S 2.3 + EP 4	**X**	**X**	Membrane	Ultrasonication	Distillation + Pervaporation	Phycocyanin Lipids Zeaxanthin Protein Hydrolysate
S1.1 + EP 2		**X**	Membrane	Bead mill	Distillation + Pervaporation	Phycocyanin Protein Hydrolysate
S 5.3 + EP 2		**X**	Flocculation	Ultrasonication	Distillation + Pervaporation	Phycocyanin Protein Hydrolysate

4.1.3.7 Sensitivity analysis

As any economic study, the economic values obtained today, can change tomorrow, sometimes for better and sometimes for worst, due to different factors that will be explained further on. Therefore, although an industrial process can be profitable the day it is designed, this does not mean that when it is built it will still be profitable. This sensitivity analysis will study the robustness of the project with several what-if situations.

There are different factors that can change this outcome (Yilmaz Balaman, 2019). These factors are:

- Market demand and price
- Supply of biomass and other materials
- Processes and technology
- Transportation and logistics
- Governmental and regulatory policies
- Natural conditions

In this study, the emphasis was on the three first factors, as these are the ones that are more likely to have a serious impact on the final outcome of this project in the near future.

4.1.3.7.1 Market demand and price

The price of utilities, products and services fluctuates due to the law of demand and supply; low supply and high demand bring prices up, while low demand and high supply bring the prices down (Hayes, 2019). While supply fluctuates with demand, demand can change due to factors like availability of cheaper substitutes, consumer preferences, and the shifts in the price of complementary products.

All the products and utilities in this study also abide by this law therefore, a study must be performed on the impact that alterations of prices can have on the production process feasibility.

Phycocyanin

Besides ethanol, phycocyanin is also produced in all process conformations. According to Future Market Insights (Insights, 2018) the market for phycocyanin will increase in the next ten years. This means demand will continue rising and the prices should increase even if slightly. However, due to this high demand, more companies can become interested in this product. This could lead to more production and a higher investment on

research, that could lead to a decrease in demand due to competition and a surplus of supply (Pettinger, 2017). Therefore, one must analyze the impact of changes in the prices of the product. Changes from -20% to 20% increase in the price of phycocyanin were analyzed. The results are shown in Table 38, and the corresponding NPV for the best 10 scenarios is represented in Figure 14.

As is expected and observed in Table 44 and Figure 31, since phycocyanin is a product common to all scenarios, any change in its price will have a large impact on the economic values of all scenarios but will not have an impact on the ranking. As phycocyanin is one of the products with the highest revenue, and present in all scenarios, by decreasing the phycocyanin prices, the number of conformations with a positive economic performance decreases due to the decrease in revenue. The opposite is also observed. Also as can be observed, as long as the prices of phycocyanin do not drop under 10% the 10 scenarios with the best performance still have a positive NPV, although with low value. These results show that all conformations viability are linked to the variations of the phycocyanin market.

Table 44 – NPV and number of profitable scenarios for different phycocyanin prices.

Phycocyanin cost variation	-20%		-10%		initial values		10%		20%	
NPV Rank										
1	2.3b + EP 2	€ 899,522	2.3b + EP 2	€ 2,794,714	2.3b + EP 2	€ 4,795,194	2.3b + EP 2	€ 6,795,675	2.3b + EP 2	€ 8,796,155
2	2.1b + EP 2	€ 473,661	2.1b + EP 2	€ 2,349,901	2.1b + EP 2	€ 4,330,377	2.1b + EP 2	€ 6,310,852	2.1b + EP 2	€ 8,291,328
3	2.3 + EP 2	€ 314,936	2.3 + EP 2	€ 2,210,128	2.3 + EP 2	€ 4,210,609	2.3 + EP 2	€ 6,211,089	2.3 + EP 2	€ 8,211,570
4	2.3b + EP 4	€ 274,300	2.3b + EP 4	€ 2,169,492	2.3b + EP 4	€ 4,169,972	2.3b + EP 4	€ 6,170,453	2.3b + EP 4	€ 8,170,933
5	2.1 + EP 2	-€ 110,925	2.1 + EP 2	€ 1,765,316	2.1 + EP 2	€ 3,745,791	2.1 + EP 2	€ 5,726,267	2.1 + EP 2	€ 7,706,743
6	2.1b + EP 4	-€ 177,883	2.1b + EP 4	€ 1,698,357	2.1b + EP 4	€ 3,678,833	2.1b + EP 4	€ 5,659,308	2.1b + EP 4	€ 7,639,784
7	2.3 + EP 4	-€ 310,286	2.3 + EP 4	€ 1,584,907	2.3 + EP 4	€ 3,585,387	2.3 + EP 4	€ 5,585,868	2.3 + EP 4	€ 7,586,348
8	2.1 + EP 4	-€ 762,469	2.1 + EP 4	€ 1,113,772	2.1 + EP 4	€ 3,094,247	2.1 + EP 4	€ 5,074,723	2.1 + EP 4	€ 7,055,199
9	2.2b + EP 2	-€ 950,592	2.2b + EP 2	€ 868,792	2.2b + EP 2	€ 2,789,253	2.2b + EP 2	€ 4,709,715	2.2b + EP 2	€ 6,630,176
10	2.2 + EP 2	-€ 1,535,180	2.2 + EP 2	€ 284,207	2.2 + EP 2	€ 2,204,668	2.2 + EP 2	€ 4,125,129	2.2 + EP 2	€ 6,045,590
Nº of Profitable scenarios	4 out of 136		12 out of 136		30 out of 136		45 out of 136		65 out of 136	

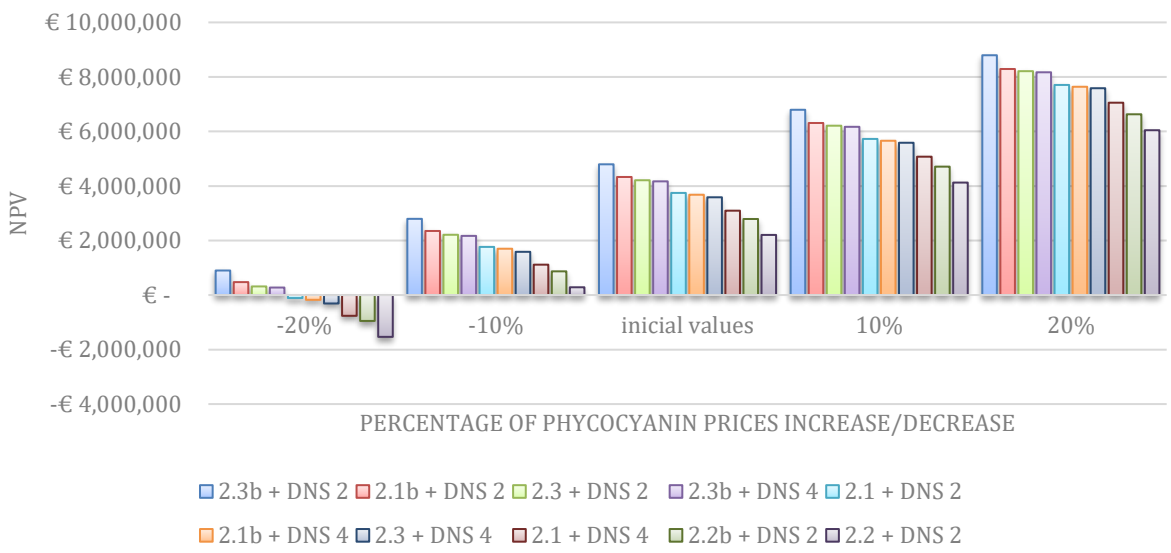

Figure 31 - Variation of the NPV of the top 10 scenarios with changes in Phycocyanin prices.

4.1.3.7.2 Zeaxanthin and Bio-Stimulant

Besides phycocyanin, there are two other main products that can be produced in these scenarios: zeaxanthin and bio-stimulant.

Currently the demand for carotenoids like zeaxanthin is increasing every year. The carotenoids market is expected to reach 2.19 billion USD by 2026. This will also bring about an increase in investment in R&D (Grand View Research, 2016; Reports And Data, 2019). Likewise, the global bio-stimulant market is expected to reach 4.9 billion USD by 2025, with a CAGR of around 13% from 2015 to 2025. The main reasons for this increase will be the necessity to feed a larger population and the negative effects of climate change therefore, an increase in the productivity of agricultural crops in a sustainable manner is required. Thus, bio-stimulants will become an important solution in the plan to increase the yield level (Intelligence, 2017).

As shown in different reports, the demand for both products is increasing. As in the phycocyanin, a higher demand can mean a slight increase in the products price. However, if the market is flooded with the same product or cheaper alternative products, the prices can decrease. As not all scenarios produce both products, the increase and decrease of the price of both products was studied, to understand what impact would these changes have in the ranking of the scenarios producing zeaxanthin and bio stimulant, comparing to the ones that only produce bio-stimulant. The results are represented in Figure 32.

The first conclusion from the analysis of Figure 32 is that, as expected, the decrease in prices of the bio stimulant has a much higher impact in all the top 10 scenarios, than the decrease of zeaxanthin due to the high amount produced (over 50% of the biomass). This impact is observable because if the price of bio stimulant decreases 15%, only one of the 10 scenarios will have a positive NPV, although very low, which means all other 135 scenarios are negative. Likewise, with the decrease of 10 % the price of the biofertilizer, only 8 scenarios have a positive impact while if the zeaxanthin decreases 10%, none of the 10 scenarios still has a negative NPV. Further, this statement is corroborated by the fact that even with the increase of the price of zeaxanthin by 10%, the scenarios with zeaxanthin will not overcome the ones that only produce phycocyanin and bio-stimulant. This shows that the amount of zeaxanthin produced and sold has very small impact on the feasibility of the process, as the amount is very small (around 1% of the biomass) although the price is higher (from around 500 €/kg of zeaxanthin compared to the 7.2 €/kg of fertilizer). These results show that besides phycocyanin, the top 10 scenarios are also very reliant on the bio-stimulants market to be viable, but not so much on the zeaxanthin market.

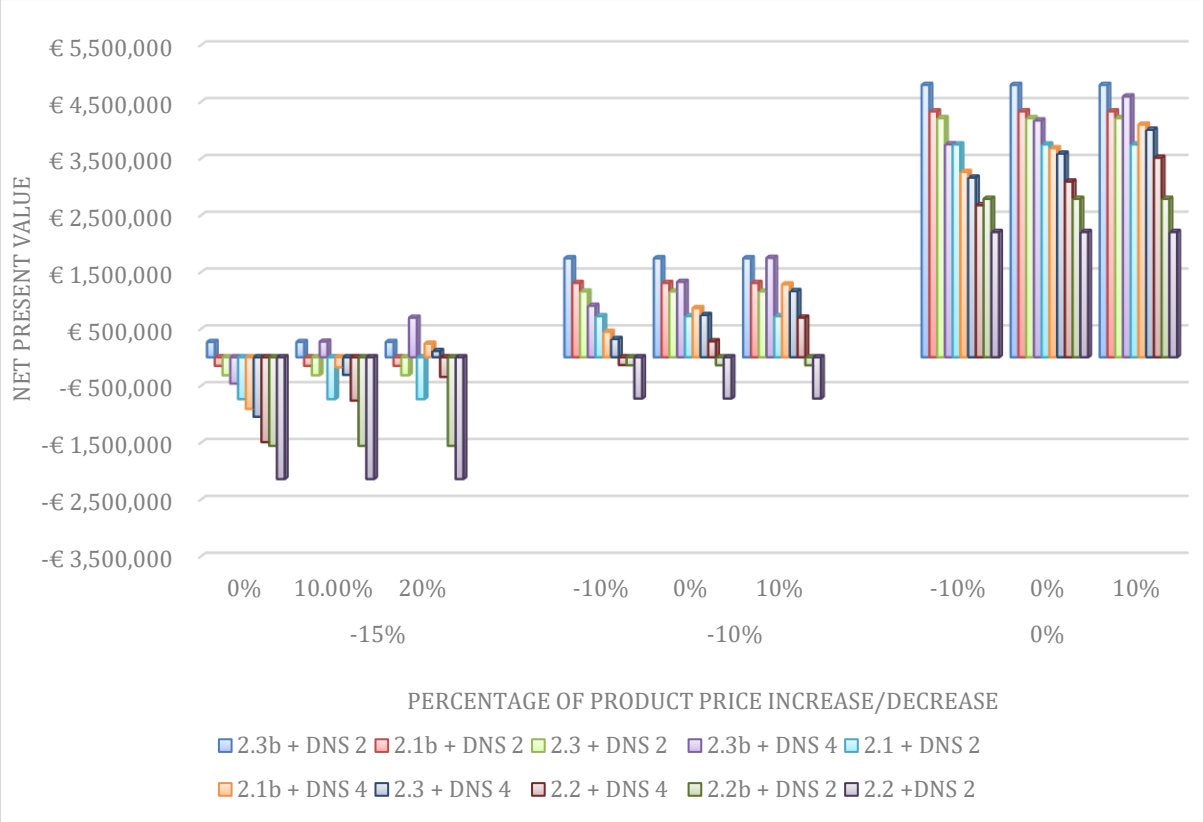

Figure 32 – Variation of the NPV of the top 10 scenarios with changes in zeaxanthin and bio-stimulant prices (for more detail see Appendix 11).

Energy prices

With the increase of oil prices and CO_2 emissions costs, there is usually an increase on energy prices (EC, 2019; Perez-Linkenheil and Energy Brainpool, 2017), as almost 50% of the energy in Portugal is still produced via fossil fuels (EDP, 2019). This increase can have a significant impact on the production process. In order to mimic this event, three scenarios were analyzed: one with an increase of 10% of the current price, and another with the increase of 25%.

The results of NPV are shown in Table 45 and represented in Figure 33. As can be seen, the energy price has little impact on the ranking of the 10 top scenarios. Only when increasing the energy costs over 25%, change in the rankings can be seen (scenario 2.1p + EP 4 switches places with scenario 2.3 + EP 4). The main reason behind this change of rank is especially due to the increase of the pervaporation energy costs, as this consumes more energy than the distillation column. Furthermore, as the extraction process 4 has more equipment, it consumes more energy, and the NPV decreases faster that the scenarios of extraction process 2 with the increase of energy costs.

Table 45 – NPV changes with electricity prices increase.

Energy cost increase	Initial case		10%		25%	
NPV Rank						
1	**2.3p + EP 2**	€ 4,795,194	**2.3p + EP 2**	€ 4,352,267	**2.3p + EP 2**	€ 3,687,848
2	**2.1p + EP 2**	€ 4,330,377	**2.1p + EP 2**	€ 3,903,360	**2.1p + EP 2**	€ 3,262,807
3	**2.3 + EP 2**	€ 4,210,609	**2.3 + EP 2**	€ 3,826,826	**2.3 + EP 2**	€ 3,251,124
4	**2.3p + EP 4**	€ 4,169,972	**2.3p + EP 4**	€ 3,689,130	**2.3b + EP 4**	€ 2,967,841
5	**2.1 + EP 2**	€ 3,745,791	**2.1 + EP 2**	€ 3,377,919	**2.1 + EP 2**	€ 2,826,082
6	**2.1p + EP 4**	€ 3,678,833	**2.1p + EP 4**	€ 3,213,900	**2.3 + EP 4**	€ 2,531,116
7	**2.3 + EP 4**	€ 3,585,387	**2.3 + EP 4**	€ 3,163,689	**2.1p + EP 4**	€ 2,516,478
8	**2.1 + EP 4**	€ 3,094,247	**2.1 + EP 4**	€ 2,688,459	**2.1 + EP 4**	€ 2,079,753
9	**2.2p + EP 2**	€ 2,789,253	**2.2p + EP 2**	€ 2,360,549	**2.2p + EP 2**	€ 1,717,465
10	**2.2 + EP 2**	€ 2,204,668	**2.2 + EP 2**	€ 1,835,108	**2.2 + EP 2**	€ 1,280,740
N° of Profitable scenarios	30 out of 136		25 out of 136		17 out 136	

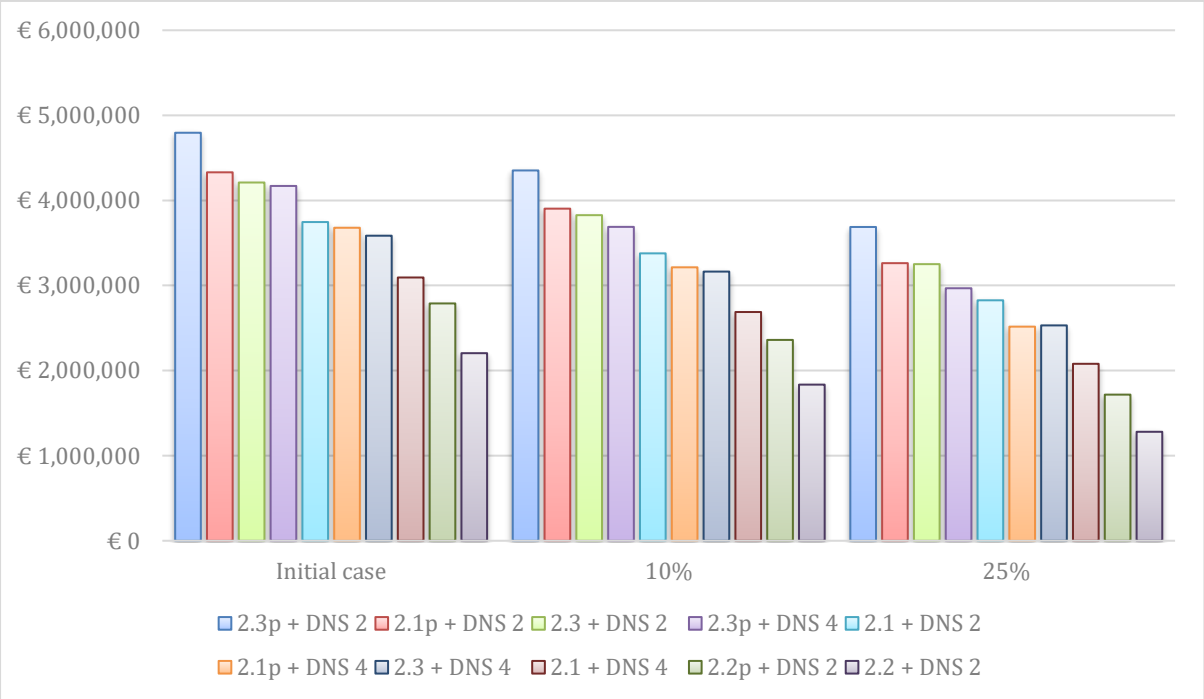

Figure 33 – NPV variation with variation in energy costs.

4.1.3.7.3 Technological improvement

Ethanol

In the current conditions, to extract ethanol from the medium costs more money than the revenue obtained from the sale of ethanol. There are several scenarios that can help improve this situation. One of such possibilities is the improvement of the genetic modification or production conditions to improve the maximum amount of ethanol produced (increase final concentration to 15 g/L which is the maximum amount not toxic to the *Synechocystis* strain).

In the DEMA project, the maximum concentration achieved by the project was 5 g/L. With further optimization, maybe it would be possible to achieve 15 g/L, which was seen to be the maximum amount tolerated by the cyanobacterium (University of Limerick,2018). The advantages of having higher concentration of ethanol are a lower €/L of ethanol produced, due to the decrease of energy per L of ethanol produced, as well as a higher revenue obtained from the sale of ethanol as more ethanol is obtained. The variation of NPV with increase of concentration of ethanol can be seen in Table 46.

Table 46 – Variation of the NPV with increase of ethanol productivity.

Extraction scenario	2			4		
Harvesting and Rupture scenario	10 g/L	15 g/L	%	10 g/L	15 g/L	%
S.2.1	€ 3,745,791	€ 4,265,015	12%	€ 3,094,247	€ 3,613,471	14%
S.2.2	€ 2,204,668	€ 2,723,892	19%	€ 1,474,158	€ 1,993,382	26%
S.2.3	€ 4,210,609	€ 4,729,833	11%	€ 3,585,387	€ 4,104,611	13%
S.2.1p	€ 4,330,377	€ 4,714,643	8%	€ 3,678,833	€ 4,063,099	9%
S.2.2p	€ 2,789,253	€ 3,173,520	12%	€ 2,058,743	€ 2,443,010	16%
S.2.3p	€ 4,795,194	€ 5,179,461	7%	€ 4,169,972	€ 4,554,239	8%

As expected, the NPV of the top 12 scenarios improved, along with an increase of profitable scenarios from 30 to 32 scenarios. The increase of NPV is higher in the scenarios with distillation and pervaporation as the amount of ethanol lost is lower, but also because the cost to produce ethanol decreases more in the pervaporation + distillation combination than in the pervaporation only option (Table 47).

Table 47 - Price of ethanol recovery (€/L of ethanol).

	10 g/L	15 g/L	% of decrease
Pervaporation	€ 1.24	€ 0.86	31%
Distillation + Pervaporation	€ 2.02	€ 1.30	36%

Renewable energy

Another possibility to improve the economic results is the implementation of renewable energy that will decrease the energy costs, but will increase the capex due to the costs of setting up the equipment. According to Lazard (Lazard Ltd, 2018) and U.S. Energy Information Administration (U.S. Energy Information Administration, 2019), the price of setting up a Photovoltaic power plant is around 1700 €/kW.

The impact of using 10%, 25%, 50% and 100% of renewable energy generated onsite to power the operation was analyzed and compared. As the best economic scenario, Conformation 2.3p with extraction process 2 will be used as an example to explain the variation of economic values with the implementation of the photovoltaic power station. The results are shown in Figure 34 and in Table 48. The electric energy required to start up the plant will not be considered in this study.

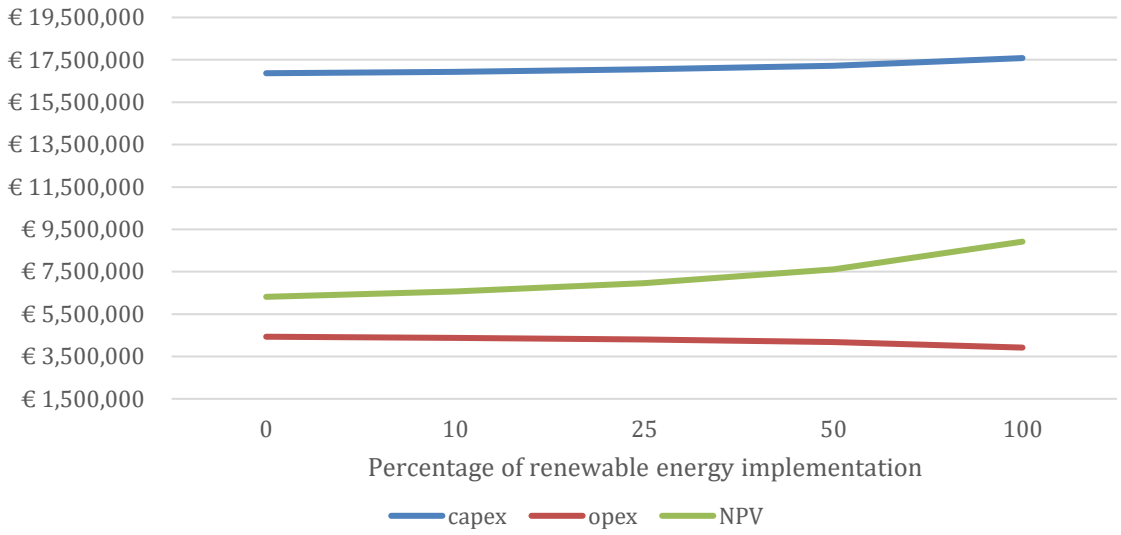

Figure 34 - Variation of the economic parameters of scenario 2.3p + EP 2 with implementation of locally produced renewable energy.

Table 48 - Variation of the economic parameters with implementation of renewable energy.

Renewables (%)	0	10	25	50	100
Capex	€ 16,868,050	€ 16,960,380	€ 17,098,890	€ 17,329,740	€ 17,791,420
Opex	€ 4,431,413	€ 4,384,629	€ 4,313,477	€ 4,194,882	€ 3,957,699
NPV	€ 6,314,621	€ 6,524,657	€ 6,846,018	€ 7,381,685	€ 8,452,960
Capex change		1%	1%	3%	5%
Opex change		-1%	-3%	-5%	-11%
NPV change		3%	8%	17%	34%

From Figure 34 and Table 48 it is possible to see that the installation of photovoltaic panels, although incurring in a small increase of capital costs, helps improve the NPV value, as total amount saved due to operational costs decrease, due to a reduction in electricity costs, is higher in 8 years than the increase in capex. In Figure 35 it is possible to see the effect the installation of photovoltaic panels has on the top 10 scenarios.

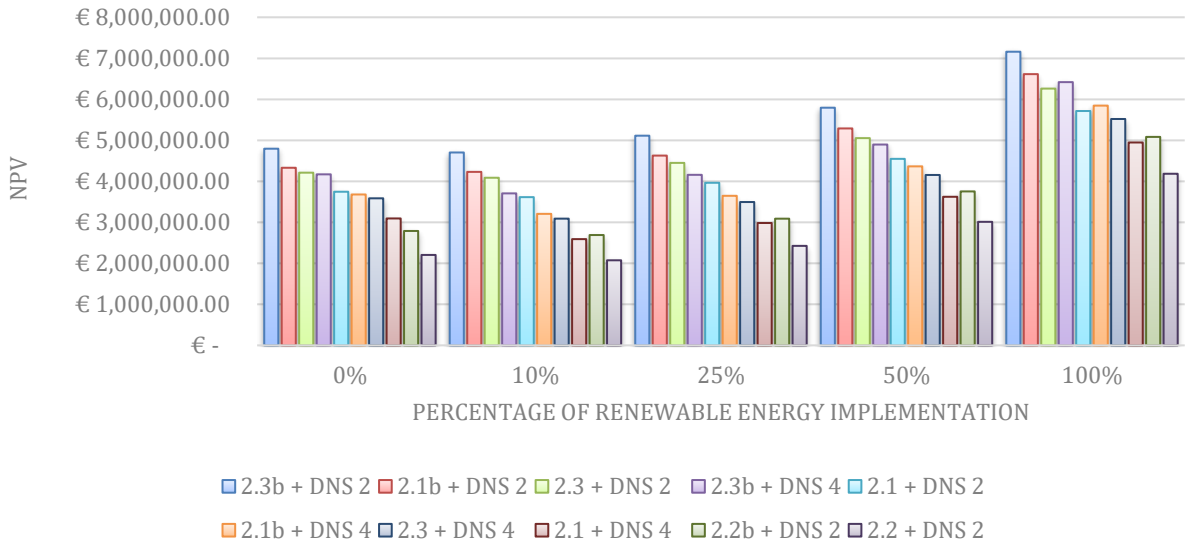

Figure 35 – Effect of renewable energy share in NPV variation.

In Figure 35 it is possible to observe that the scenarios where the use of renewable energy has the highest impact are the ones using extraction process 4. This is due to the fact that the electricity costs play a larger role (8.1%) in the operational costs of those scenarios than in the extraction scenario 2 (6.0%).

4.1.3.7.4 Supply of biomass and other materials

Biomass

Although the microalgal biomass is produced "in house", there are different factors that can decrease the amount of biomass produced and delivered for further processing. These factors can go from:

- Natural conditions (bad weather; less exposure to sunlight)
- Contaminations (appearance of bacteria, other microalgae, etc.)
- Equipment faults or human errors (leaks, process occurrences, etc.).

Nevertheless, there is also the possibility that more biomass is produced. This can be due to technological and R&D improvements and/or due to very satisfactory weather conditions.

As the biomass is the main source of raw material, any impact there is on the amount of biomass produced will have impact on the operating income ability and even sustainability of the process. However, in this analysis only the losses will be taken into account, as any improvement will have a positive impact in the NPV. The graph in Figure 36 shows the behavior of the NPV if the accumulated loss of biomass was of 10% and 5%. Although the change of biomass can have some impact in the operational costs, as these were as low as 1%, they were not taken into account in the calculations.

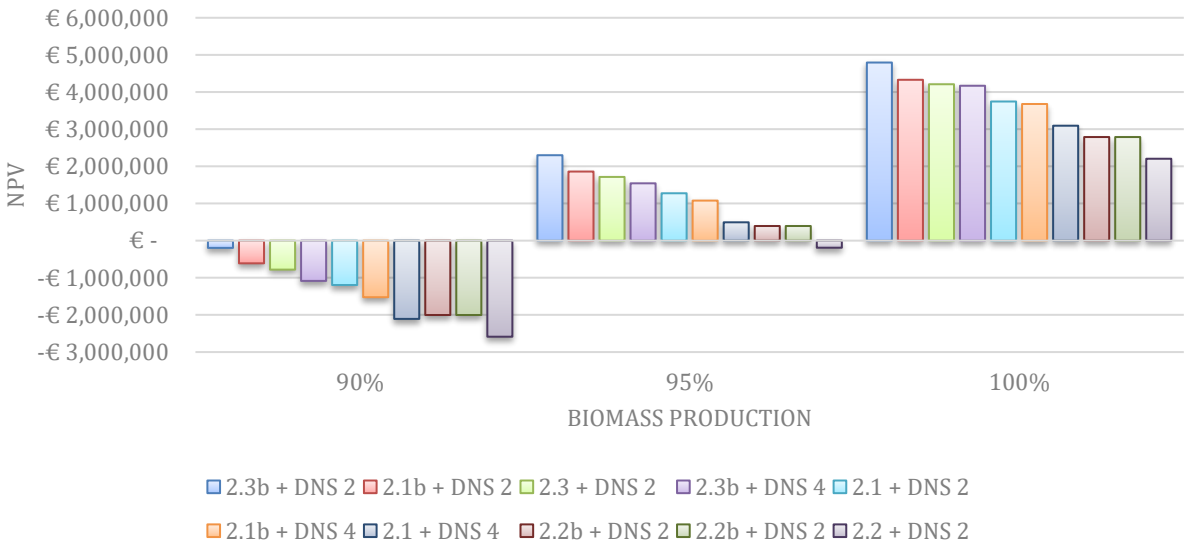

Figure 36 – NPV variation with the variation of biomass produced (for more detail see Appendix 11).

As can be observed in Figure 19 if any of the previous factors would occur and effect the production, decreasing the amount of biomass produced in the 8 years assumed for the

economic analysis to below 10% of the initially proposed value, then no scenario would be economically feasible. This would improve to 9 profitable scenarios for a loss of 5% of the biomass. However, one must take into account that this is an accumulated loss. If this would occur only in one year then the impact could be softened by a better year.

4.1.3.7.5 Other options

No ethanol production

One of the products obtained from this process is bioethanol. However, due to the low prices of ethanol, the amount of ethanol produced is not enough to compensate for operational cost required for the extraction from the medium. Therefore, how would the industrial process look like if a native *Synechocystis* was used and no ethanol was produced? If no ethanol were produced, the capital and operating costs of the scenarios would decrease as well as the revenue. However, this would still have a positive impact on the NPV of process as the Opex would decrease much more than the revenue. This would have an even bigger impact on the scenarios where the distillation and pervaporation membranes were used (Table 49). In Table 50 the results of the 12 best scenarios with and without ethanol production are compared.

Table 49 - *NPV value for no Ethanol production.*

Production, harvesting and Disruption Scenarios	Extraction Scenarios							
	EP 1	EP 2	EP 3	EP 4	EP 5	EP 6	EP 7	EP 8
S.1.1	-€ 20,231,600	€ 2,753,442	-€ 20,775,100	€ 1,964,746	-€ 22,794,600	-€ 864,779	-€ 22,688,000	-€ 744,580
S.1.2	-€ 20,872,400	€ 1,310,621	-€ 21,499,700	€ 447,115	-€ 23,465,400	-€ 2,280,670	-€ 23,418,600	-€ 2,220,320
S.1.3	-€ 20,504,700	€ 2,747,605	-€ 21,020,300	€ 1,983,845	-€ 23,059,200	-€ 880,975	-€ 22,932,600	-€ 740,826
S.2.1	-€ 19,429,500	€ 5,025,747	-€ 19,819,400	€ 4,374,203	-€ 21,939,100	€ 1,356,770	-€ 21,722,800	€ 1,586,691
S.2.2	-€ 20,124,100	€ 3,484,624	-€ 20,602,500	€ 2,754,114	-€ 22,665,300	-€ 155,926	-€ 22,512,100	€ 10,822
S.2.3	-€ 19,246,900	€ 5,490,565	-€ 19,607,300	€ 4,865,343	-€ 21,746,000	€ 1,812,112	-€ 21,508,600	€ 2,063,090
S.3.1	-€ 21,013,000	€ 501,747	-€ 21,710,100	-€ 424,101	-€ 23,633,300	-€ 3,069,500	-€ 23,636,400	-€ 3,059,020
S.3.2	-€ 21,674,100	-€ 916,789	-€ 22,450,400	-€ 1,913,290	-€ 24,322,700	-€ 4,462,600	-€ 24,382,300	-€ 4,508,650
S.3.3	-€ 20,664,800	€ 1,102,358	-€ 21,335,600	€ 200,062	-€ 23,275,800	-€ 2,477,370	-€ 23,260,000	-€ 2,448,050
S.4.1	-€ 20,212,900	€ 2,772,048	-€ 20,756,500	€ 1,983,352	-€ 22,778,800	-€ 848,936	-€ 22,672,200	-€ 728,737
S.4.2	-€ 20,921,000	€ 1,262,027	-€ 21,548,300	€ 398,520	-€ 23,516,800	-€ 2,332,030	-€ 23,470,000	-€ 2,271,680
S.4.3	-€ 19,782,300	€ 3,470,053	-€ 20,297,800	€ 2,706,294	-€ 22,338,100	-€ 159,909	-€ 22,211,600	-€ 19,760
S.5.1	-€ 20,608,000	€ 1,053,709	-€ 21,289,800	€ 141,576	-€ 23,220,500	-€ 2,520,070	-€ 23,212,600	-€ 2,498,620
S.5.2	-€ 21,218,100	-€ 318,191	-€ 21,979,400	-€ 1,301,390	-€ 23,859,000	-€ 3,866,390	-€ 23,907,900	-€ 3,901,800

Table 50 – Comparison of NPV of the top 12 scenarios with and without ethanol production.

Extraction scenario	2			4		
Harvesting and Rupture scenario	Ethanol produced	no ethanol produced	%	Ethanol produced	no ethanol produced	%
S.2.1	€ 3,745,791	€ 5,025,747	25%	€ 3,094,247	€ 4,374,203	29%
S.2.2	€ 2,204,668	€ 3,484,624	37%	€ 1,474,158	€ 2,754,114	46%
S.2.3	€ 4,210,609	€ 5,490,565	23%	€ 3,585,387	€ 4,865,343	26%
S.2.1p	€ 4,330,377	€ 5,025,747	14%	€ 3,678,833	€ 4,374,203	16%
S.2.2p	€ 2,789,253	€ 3,484,624	20%	€ 2,058,743	€ 2,754,114	25%
S.2.3p	€ 4,795,194	€ 5,490,565	13%	€ 4,169,972	€ 4,865,343	14%

As can be seen in Table 50 all the top 12 scenarios have an increase in the NPV. The ones with the smallest NPV increase are the ones where the pervaporation membrane was used. This is due to the fact that the Opex of the pervaporation process is lower than that of the distillation and pervaporation membrane combination and therefore the operational cost decrease is lower. Another aspect that can be observed is that without ethanol production the number of scenarios with a positive NPV increases from 22% of profitable scenarios to 25%, which is an increase of 12% of profitable scenarios.

Another advantage of using a non-modified species is that there would be less problems in obtaining licenses for products, as GMO are much more controlled and scrutinized.

4.2 Case Study 2 - Microalgae Production Step – *Prorocentrum* based biorefinery

4.2.1 Microalgae biomass

The most interesting components in the *Prorocentrum* biomass are the omega-3 fatty acids, DHA and EPA that are found in the lipids (Allied market research, 2017; Swanson et al., 2012). Other interesting components are the carotenoid peridinin, which is similar to zeaxanthin, and ß-carotene which has a large market value (Guedes et al., 2011). Due to its large concentration and the large application range, proteins are also interesting (Bleakley and Hayes, 2017).

4.2.1.1 Process Description

In this scenario, based on the *PUFAchain* project (Friedl, 2017), it was considered that: the production and pre-production were done in UHT-PBR and that the total area occupied by these reactors is 10 ha. The microalgae productivity of biomass was considered to be of 0.341 g/L/d. The culture was considered to reach a final concentration of 2.4 g/L of biomass (this concentration was assumed to be the best to achieve maximum productivity) at day 7 of cultivation and that it was daily harvested to maintain a constant concentration. In the end of the 110th day the whole culture is harvested and the reactor cleaned and prepared for new inoculation and 90% of the culture medium is recycled. The values of productivity and production are based on results from cultivations conducted at A4F pilot plant using tubular PBRs, and the total biomass produced is the maximum that can be achieved in the 10 ha with the previously mentioned constraints. A summary of the *Prorocentrum* sp. production parameters considered in this study is shown in Table 51.

Table 51 - Biomass Production Parameters for Prorocentrum based biorefinery.

Parameters	Value	Unit
Number of Reactors	36	-
Reactor Volume	97.5	m^3
Run Time	110	days
Inoculations	3	Per year
Biomass Productivity	0.341	g/l/d
Final biomass Concentration	2.4	g/l
Medium Recycle	90	%
Total Biomass produced	408.2	t/year

4.2.1.2 Harvesting and Disruption equipment choices

Although there is a large number of equipment that can perform the harvesting and disruption processes, some are only available in laboratory scale and are still far away from being used at industrial scale. Therefore, only the ones already used in microalgae or similar industries were chosen. The chosen processes can be found in Table 9. The efficiencies of each equipment and the conditions can be found in Appendix 2.

4.2.1.3 Production, Harvesting and Disruption Mass balances

In the example used, the *PUFAChain* project, the final biomass concentration in the culture was 2.4 g/L and 408.2 tons of *Prorocentrum* biomass were produced per year. In In this scenario, the target components, EPA and DHA Fatty acids, are found inside the cell of the microalgae. Therefore, after harvesting, to obtain higher value products, a disruption step is required.

4.2.1.4 Mass balances

As in the previous scenario, the first step is to create different process sequences and to perform the mass balances on all the process sequences. The mass balances corresponding to the different sequences can be found in Table 52 to Table 54.

Table 52 - Mass Balance Results for centrifugation and membrane harvesting processes.

	Process Sequence	P.1.1	P.1.2	P.1.3	P.2.1	P.2.2	P.2.3
Microalgae Production	Biomass Production (t/year)	408.2					
	Concentration (g/L)	2.4					
	Water Consumed (m^3/year)	16,613.5	16,613.5	16,429.8	16,538.7	16,538.7	16,409.4
Harvesting Step	Harvesting Process	C			M		
	Harvesting Capacity (m^3/h)	20.9					
	Harvesting Losses (%)	10.0			5.0		
	Harvested Biomass (t/year)	367.4			387.8		
	Final Concentration (g/L) *	200.0	200.0	100.0	150.0	150.0	100.0
	Wastewater produced (m^3/year)	16,613.5	16,613.5	16,429.8	16,538.7	16,538.7	16,409.4
	Harvested Volume (m^3/h)	0.2	0.2	0.4	0.3	0.3	0.5

Cell Disruption Step	Cell Disruption process	BM	HPH	US	BM	HPH	US
	Cell Disruption Capacity (m^3/h)	0.2	0.2	0.4	0.3	0.3	0.5
	Cell Disruption efficiency (%)	99.0	96.0	100.0	99.0	96.0	100.0
	Cell Disruption Loss (%)	5.0					
	Final ruptured Biomass (t/year)	345.5	335.0	349.0	364.7	353.6	368.4

*-the final concentration corresponds to the maximum concentration of the cell disruption method

C – Centrifuge; M-Membrane; BM – Ball mil; HPH – High Pressure Homogenizer; US – Ultrasonicator

*Table 53- Mass Balance Results for flocculation and flotation harvest*ing *processes.*

	Process Sequence	P.3.1	P.3.2	P.3.3	P.4.1	P.4.2	P.4.3
Microalgae Production	Biomass Production (t/year)	408.2					
	Concentration (g/L)	2.4					
	Water Consumed (m^3/year)	16,062.5	16,062.5	16,062.5	16,218.9	16,218.9	16,218.9
Harvesting Step	Harvesting Process	FLC			DAF		
	Harvesting Capacity (m^3/h)	20.9					
	Harvesting Loss (%)	10.0			15.0		
	Harvested Biomass (t/year)	367.4			346.9		
	Final Concentration (g/L) *	50.0			60.0		
	Wastewater produced (m^3/year)	16,062.5			16,218.9		
	Harvested Volume (m^3/h)	0.9			0.7		
Cell Disruption Step	Cell Disruption equipment	BM	HPH	US	BM	HPH	US
	Cell Disruption Capacity (m^3/h)	0.9	0.9	0.9	0.7	0.7	0.7
	Cell Disruption efficiency (%)	99.0	96.0	100.0	99.0	96.0	100.0
	Cell Disruption Loss (%)	5.0					
	Final ruptured Biomass (t/year)	345.5	335.0	349.0	326.3	316.4	329.6

*-the final concentration corresponds to the maximum concentration achieved by the harvest method (DAF -60 g/l and FLC – 50 g/l)

DAF –Dissolved Air Flotation; FLC - Flocculation; BM – Ball mil; HPH – High Pressure Homogenizer; US – Ultrasonicator

Table 54 - Mass Balance Results for Centrifuge and membrane combination harvesting.

	Process sequence	P.5.1	P.5.2
Microalgae Production	Biomass Production (t/year)	408.2	
	Concentration (g/L)	2.4	
	Water Consumed (m^3/year)	13,692.2	
Harvesting Step	Harvesting Process	M	
	Harvesting Capacity (m^3/h)	20.9	
	Harvesting Loss (%)	5.0	
	Harvested Biomass (t/year)	387.8	
	Final Concentration (g/L) *	5.0	
	Wastewater produced (m^3/year)	9,041.9	
Harvesting Step	Harvesting Process	C	
	Harvesting Capacity (m^3/h)	9.2	
	Harvesting Loss (%)	10.0	
	Harvested Biomass (t/year)	349.0	
	Final Concentration (g/L)	200.0	
	Wastewater produced (m^3/year)	4,650.3	
Cell Disruption Step	Cell Disruption process	BM	HPH
	Cell Disruption Capacity (m^3/h)	0.2	0.2
	Cell Disruption efficiency (%)	99.0	95.0
	Cell Disruption Loss (%)	5.0	
	Final ruptured Biomass (t/year)	328.2	315.0

*-the final concentration corresponds to the maximum concentration of the cell disruption method

C – Centrifuge; M-Membrane; BM – Ball mil; HPH – High Pressure Homogenizer;

As can be seen in Table 52 to Table 54, the process combinations where the recovery of ruptured biomass is the highest are the process sequences where the harvesting process is performed with a membrane system. This is expectable since this process is the one with the highest harvesting efficiency. However, this does not mean that this is the most economically efficient process. Further, it can be seen that the impact of the cell disruption step on final value of produced ruptured biomass is not very high since the disruption efficiencies of the process were considered to be quite similar, although ultrasonication and bead mill are the most efficient. The following step is to design different extraction scenarios for the extraction of the high value components from the ruptured microalgae biomass.

4.2.2 Extraction Process

As was mentioned before, in this scenario the products of interest are DHA and EPA, which are fatty acids present in the lipid content of the microalga. Therefore, the main goal of this biorefinery scenario was to start with the extraction of lipids from the ruptured biomass and afterwards perform further separation steps to obtain the remaining possible products from the other microalga components.

4.2.2.1 Assumptions

- In cases where no information on peridinin was available, information on lutein or zeaxanthin was used since both have similar properties (Nath et al., 2016; Takaichi, 2011);
- All membrane operations have a 95% recovery efficiency to account for the biomass lost in the equipment (A4F, 2018);
- The saponification process values were obtained from information provided by a specialist company and PUFAChain project partner IOI Oleo;
- In cases where no information on heptane was available, information on hexane was used since both have similar properties (Farag and McConnell, 2013);
- All solvents (ethanol and heptane) have a recovery rate of 90% (Peng et al., 2010 Tres et al., 2012; University of Limerick, 2018) (in this reference the amount recovered was higher; however, since this was performed in lab scale, it was assumed the losses at industrial scale are higher and so a lower recovery value was assumed).

4.2.2.2 Process

For the lipid extraction step two processes were selected: conventional solvent extraction or supercritical solvent extraction.

The conventional solvent extraction was performed using organic solvents. The solvents were chosen based on their interaction with the desired component (Sheng et al., 2011). These interactions reflect the ability of the solvent to extract the desired components from the microalga. Solvents commonly used for extraction of lipids are hexane, methanol, acetone, chloroform, however, they are all toxic. Since the goal of this project was to obtain as much end products or end product utilizations as possible, in this study the previously mentioned solvents were replaced with heptane (Jeevan Kumar et al., 2017) and ethanol. The reason behind the choice of these two solvents was that, as was explained before, each has a higher affinity towards certain components in the microalgae. While heptane has a higher affinity for neutral lipids, like TAGs, ethanol has

a higher affinity towards phospholipids, glycolipids (polar lipids) and carotenoids (Wu et al., 2017).

As can be seen in Figure 37, the first step was the extraction with heptane. In this step most of the non-polar components were extracted. Since heptane forms a separate phase from water, the separation step in this stage is a decanter. Two streams were produced: one with most of the non-polar lipids and non-polar proteins, and another with the more polar components like proteins, polar lipids and carbohydrates. This second stream then went through a new solvent extraction, with ethanol, removing the most polar components. Again, two streams are produced: one with the proteins and lipids, while the carbohydrates will be present in a second stream.

Both streams coming from the solvent extractions were further purified, using a series of purification and saponification steps (Friedl, 2017; Grima et al., 1994), producing metal soap. The stream coming out of the heptane extraction also produced one stream rich in non-soluble proteins that was further treated to produce a protein hydrolysate, while the stream coming from the ethanol extraction produced one stream composed of proteins, peridinin and beta-carotene (Table 55).

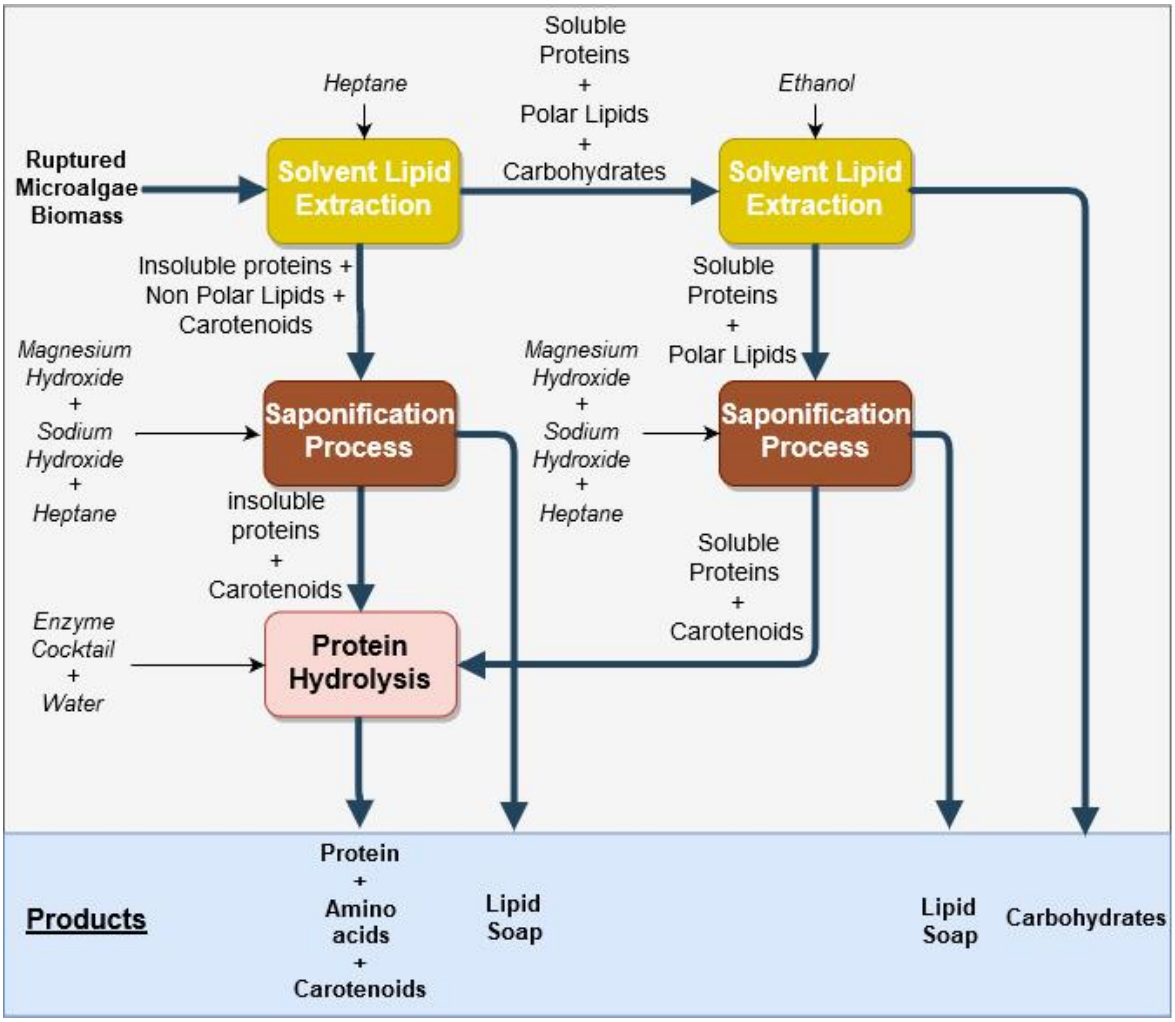

Figure 37 – Prorocentrum lipid extraction option 1.

Table 55 - Prorocentrum processing option 1 process parameters and results.

Step	Process	Parameters		References
1	Solvent extraction with Heptane	2 L of heptane/ kg of biomass*1	TAGs – 86% extracted	(Olmstead et al., 2013; Terme et al., 2017)
			Glycolipids – 20% extracted	
			Phospholipids – 35% extracted	
			50% - soluble proteins	Experimental values (Appendix 3)
2	Solvent extraction with Ethanol	20 L of ethanol / kg of biomass	TAGs – 24% extracted	(Soares et al., 2016; Wu et al., 2017)
			Glycolipids – 60% extracted	
			Phospholipids – 43% extracted	
			100% - soluble proteins extracted	Experimental values (Appendix 3)
			Peridinin – 0.98 mg/g biomass extracted	(Cardoso et al., 2012)
4	Saponification	Company confidentiality	87% efficiency	(Ibáñez et al., 1998)
5	Enzymatic Hydrolysis of proteins	4% w/w of enzyme cocktail*2	59 % of protein hydrolysis	(Romero García et al., 2012)

*1 The amount of heptane was doubled from the amount used in the literature as in the article 2 hours were needed to obtain the selected results. It was assumed that the double of the heptane would reduce the extraction time by half

*2 Contains Alcalase and Fluorzyme

A second possibility to extract the lipids was using supercritical extraction. As seen in Figure 38, this process requires dried biomass, so a dryer is required before the supercritical extraction occurs. Different settings and co-solvents can be used to enhance the selectivity towards different components. In this process, 5% ethanol was added as a co-solvent in order to remove the peridinin and beta-carotene as well (Cardoso et al., 2012). After the supercritical extraction, two streams were produced: one with ethanol, lipid oil and carotenoids, and another with the remaining biomass mostly composed of protein, carbohydrates and lipids that are not extracted. The stream containing the lipids and carotenoids can already be a product. However, to obtain a purer EPA + DHA product, a saponification was performed obtaining a DHA+EPA metal soap and a carotenoid mixture. The remaining biomass was treated using enzymes to hydrolyze the proteins into amino acids (Romero García et al., 2012). After the protein hydrolysis, the remaining biomass is rich in carbohydrates. Parameters and results of this process are described in Table 56.

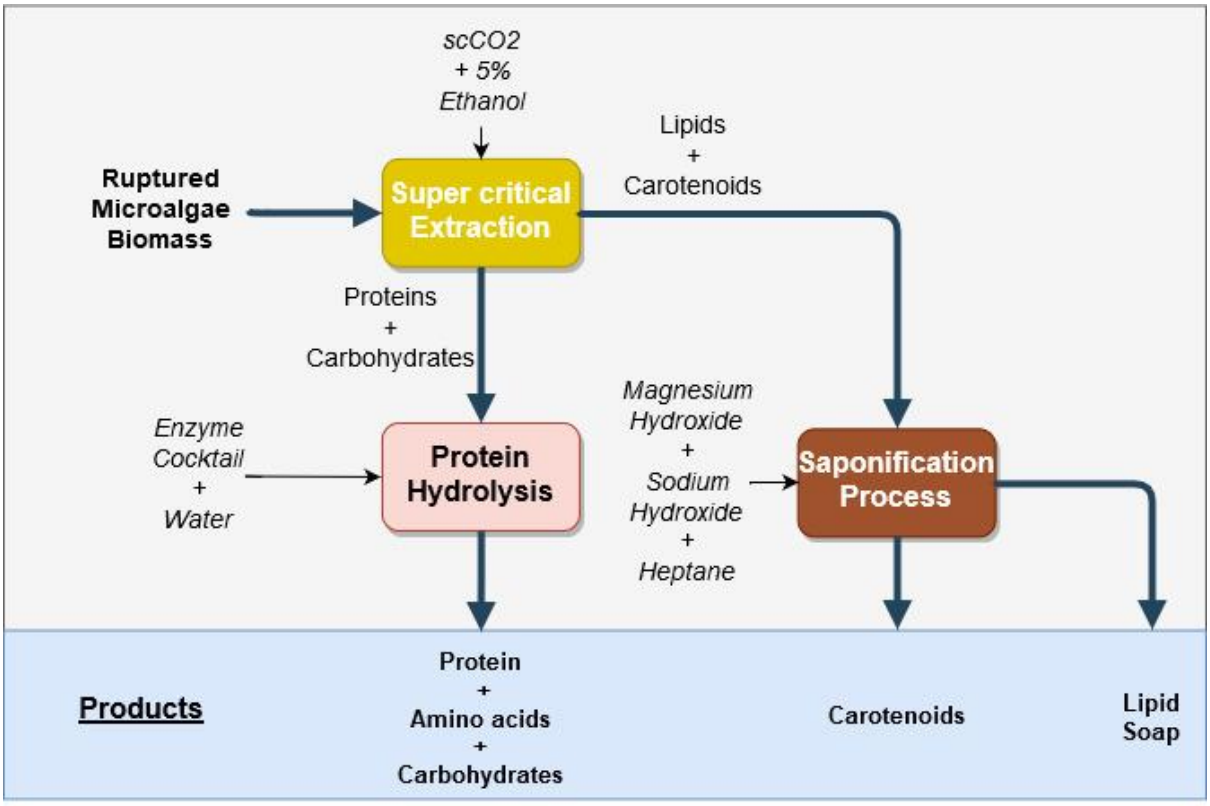

Figure 38 – Prorocentrum lipid extraction option 2.

Table 56 - Prorocentrum processing option 2 process parameters and results .

Step	Process	Parameters	Results	References
3	Supercritical extraction with CO_2 and 5% Ethanol	200 bar and 60 °C*	TAGs – 61% extracted Glycolipids – 36% extracted Phospholipids – 27% extracted	(Terme et al., 2017)
			Beta-Carotene – 0.6 mg/g of biomass Peridinin – 1.6 mg/g biomass	(Cardoso et al., 2012; Cha et al., 2009)
4	Saponification	Company confidentiality	89% of efficiency	(Ibáñez et al., 1998)
5	Enzymatic Hydrolysis of proteins	4% w/w of enzyme cocktail*2	59 % of protein hydrolysis	(Romero García et al., 2012)

*1-the parameters are different in (Terme et al., 2017) however since the objective was to extract the carotenoids and there was lack of information it was considered that the extraction values are the same

*2 – Composed of Alcalase and Flavourzyme

4.2.2.3 Possible conformations

As in the previous case study, the extraction operation was designed to obtain the highest purity possible of all high value components in the biomass. However, due to the high costs of some of the extraction methods, the full scenario might not be the most economically feasible. With this in mind, the full process was divided into smaller processes with different conformations. This also means that for different conformation of the biorefinery, there are different products that can be obtained, with different prices.

All the calculations were performed for a ruptured biomass production of 45 kg/h. The reason behind this choice was that this is the average value of all production, harvesting and disruption scenarios.

The time considered was 350 days of operation, with 22 hours of production and 2 hours of CIP per day, and 15 days of maintenance.

4.2.2.3.1 Process option 1 + conformation 1

In this first conformation (Figure 39), the extraction is performed in a stirred tank with heptane to remove the non-polar lipids like TAGs and half of the non-soluble proteins (Jeevan Kumar et al., 2017). Then, heptane and the remaining biomass are separated by decantation since heptane is not miscible with water. After the extraction, heptane is removed using a membrane and afterwards, the remaining components that were present in the heptane phase go through a purification process to separate the lipids as a metal soap, and obtain a stream containing the remaining components (proteins and beta carotene) (Table 57).

Table 57 - *Process streams for process option 1 + conformation 1.*

Component (kg/h) Stream	1	2	3	4	5	6	7
TAG	0.4	0.4	0.1	0.4	0.4	0.3	0.0
Phospholipids	3.1	3.1	2.0	1.1	1.1	0.7	0.1
Glycolipids	0.5	0.5	0.4	0.1	0.1	0.1	0.0
Water non-Soluble protein	13.5	13.5	6.5	7.0	7.0	0.0	7.0
Water Soluble protein	4.5	4.5	4.5	0.0	0.0	0.0	0.0
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carotenoids	0.3	0.3	0.3	0.0	0.0	0.0	0.0
Carbohydrates	15.5	15.5	15.5	0.0	0.0	0.0	0.0
Nucleic Acids	6.8	6.8	6.8	0.0	0.0	0.0	0.0
total mass	45.0	45.0	36.4	8.5	8.5	1.1	7.2

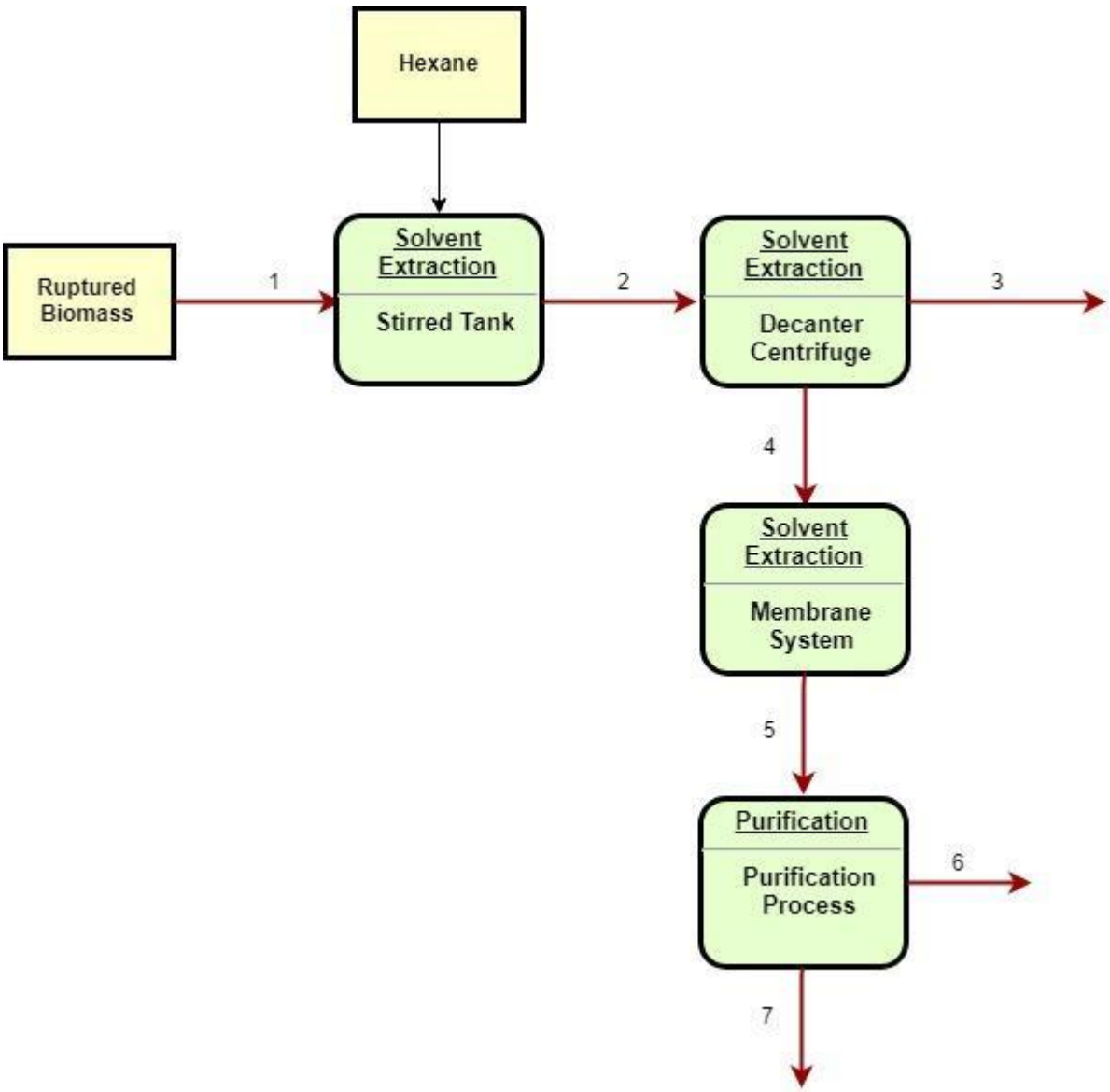

Figure 39 - Flowsheet for process option 1 + conformation 1.

Products (details in appendix 6)

The final products of this process are stream 3, which is a stream that can be sold as a feed rich in carbohydrates, containing carbohydrates and some lipids and carotene. Another product is stream 6, a stream rich in EPA and DHA, that can be used for nutraceuticals and finally stream 7 that, due to its rich protein content, can be sold as a protein concentrate (*Table 58*).

Table 58 - Products obtained in process option 1 + conformation 1.

Product	Stream	kg/kg of biomass	Applications
Carbohydrates	3	0.81	Paper industry, Animal Feed
Metal Soap	6	0.02	Nutraceuticals
Protein	7	0.16	Animal Feed

4.2.2.3.2 Process option 1 + conformation 2

In this second conformation (*Figure 40*) the stream containing the soluble components is added to a stirred tank where an enzyme cocktail containing *Alcalase* and *Flavourzyme* (Romero García et al., 2012) is added to hydrolyze the proteins and produce a protein hydrolysate (Table 59).

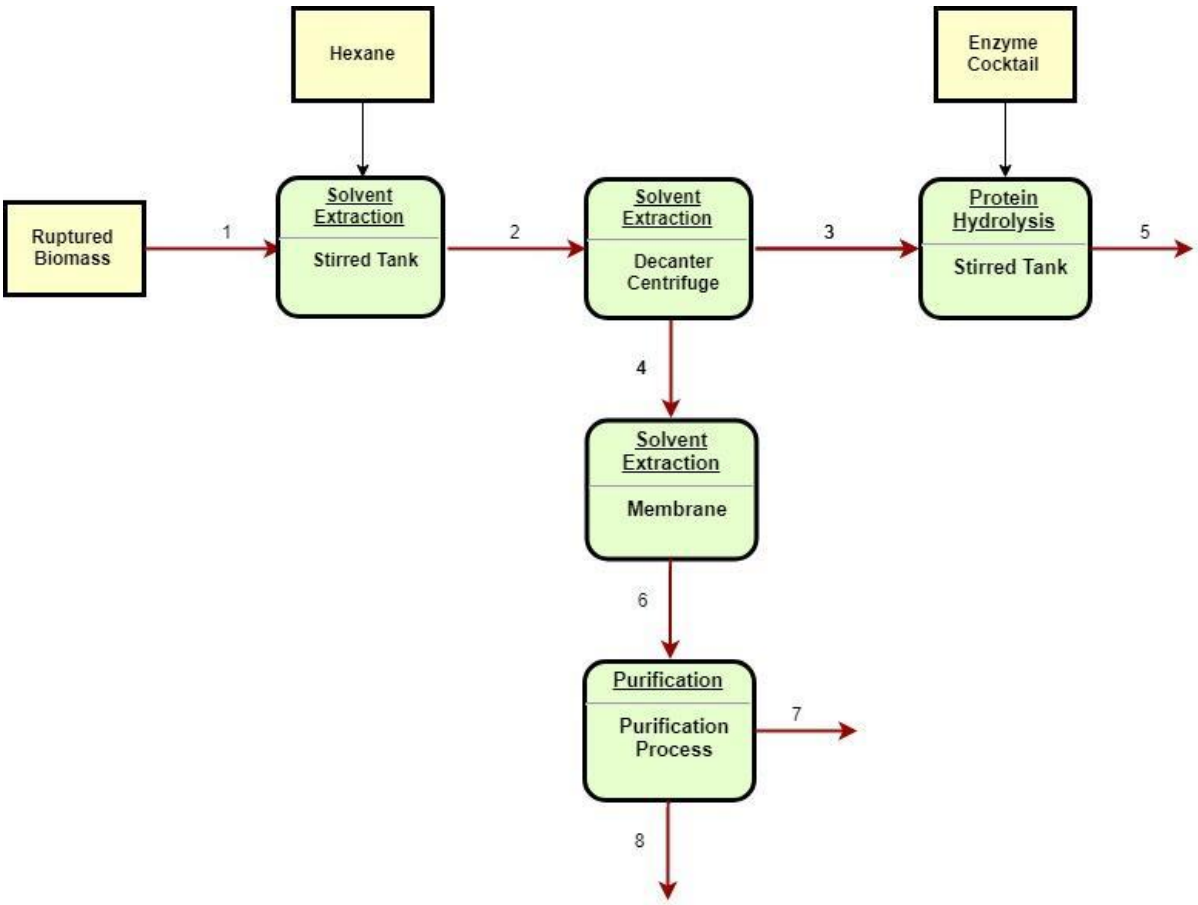

Figure 40 - Flowsheet for process option 1 + conformation 2.

Table 59 - Process streams for process option 1 + conformation 2.

Component (kg/h) Stream	1	2	3	4	5	6	7	8
TAG	0.4	0.4	0.1	0.4	0.1	0.4	0.3	0.0
Phospholipids	3.1	3.1	2.0	1.1	2.0	1.2	0.7	0.1
Glycolipids	0.5	0.5	0.4	0.1	0.4	0.1	0.1	0.0
Water non-Soluble protein	13.5	13.5	6.5	7.0	2.7	7.0	0.0	7.0
Water Soluble protein	5.0	4.5	5.0	0.0	2.0	0.0	0.0	0.0
Amino acids	0.0	0.0	0.0	0.0	6.8	0.0	0.0	0.0
Carotenoids	0.3	0.3	0.3	0.0	0.3	0.0	0.0	0.0
Carbohydrates	15.5	15.5	15.5	0.0	15.5	0.0	0.0	0.0
Nucleic Acids	6.8	6.8	6.8	0.0	6.8	0.0	0.0	0.0
total mass	45.0	45.0	36.4	8.5	36.4	8.5	1.1	7.2

Products (details in appendix 6)

The final products of this process (Table 60) are stream 5, a possible bio-stimulant for plants rich in amino acids, stream 7, a stream rich in EPA and DHA that can be used for nutraceuticals and finally stream 8, that due to its rich protein content can be sold as a protein concentrate.

Table 60 - Products obtained in process option 1 + conformation 2.

Product	Stream	kg/kg of biomass	Applications
Amino acid stream	5	0.81	Bio-stimulant
Metal Soap	7	0.02	Nutraceuticals
Protein	8	0.16	Animal Feed

4.2.2.3.3 Process option 1 + conformation 3

In this conformation (Figure 41), the extraction process is composed of two solvent extraction steps: in the first extraction step, a stirred tank is filled with heptane to remove the non-polar lipids like TAGs and half of the non-soluble proteins (Jeevan Kumar et al., 2017). Heptane and the remaining biomass are separated by decantation, since heptane is not miscible with water. The spent biomass is then added to a second stirred tank filled with ethanol to remove the more polar lipids along with polar components like proteins and peridinin (Koo et al., 2012). The spent biomass is separated from ethanol by membrane filtration. After the extraction, both the heptane and ethanol streams go

through a purification process in order to precipitate the lipids as a metal soap, and obtain a stream containing the remaining components (proteins and peridinin) (Table 61).

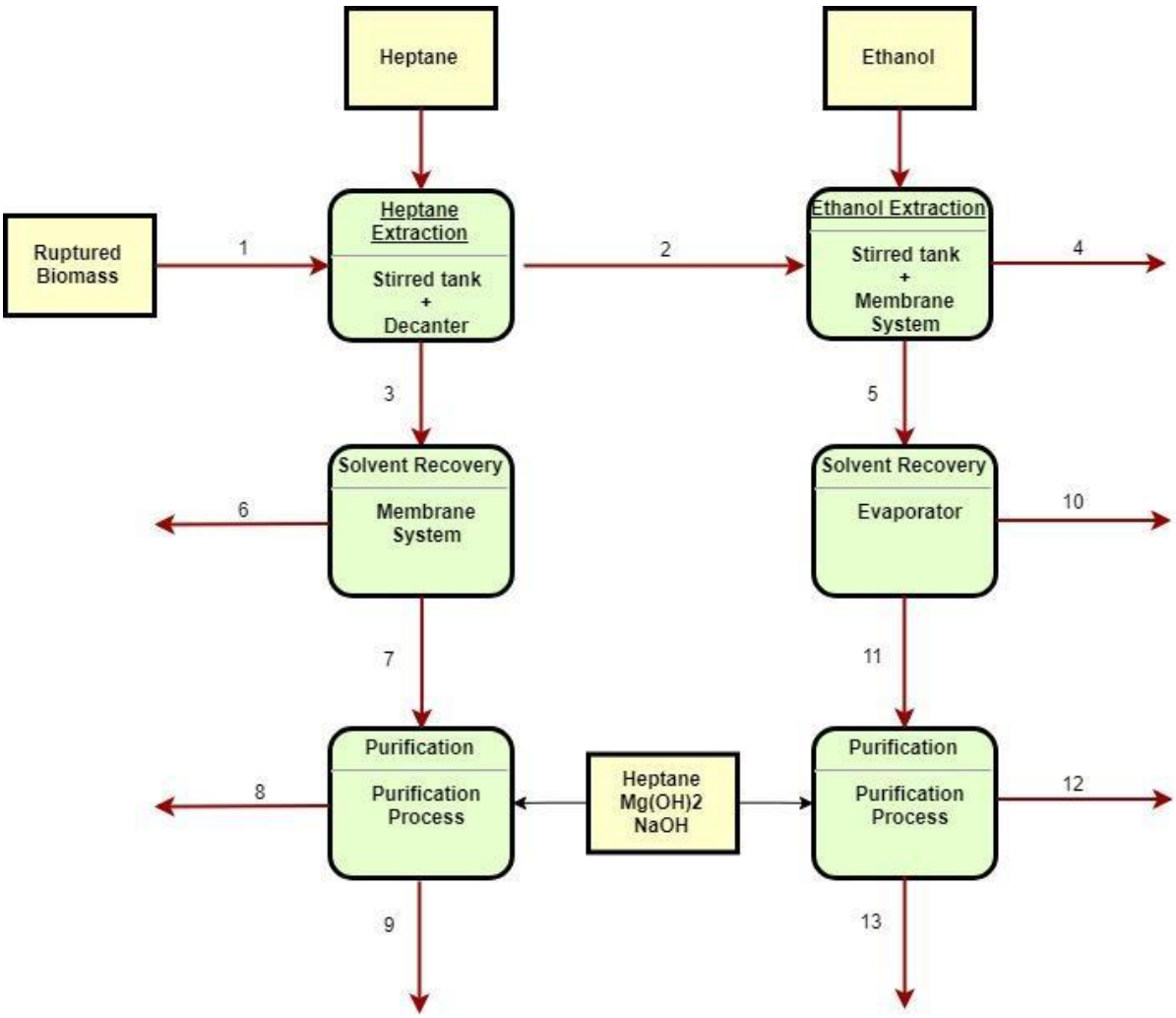

Figure 41 – Flowsheet for process option 1 + conformation 3.

Table 61 – Process streams for option 1 + conformation 3.

Component Stream (kg/h)	1	2	3	4	5	6	7	8	9	10	11	12	13
TAG	0.4	0.0	0.4	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0
Glycolipids	3.0	2.3	0.6	0.9	1.3	0.0	0.6	0.4	0.0	0.0	1.3	0.9	0.1
Phospholipids	0.5	0.5	0.2	0.3	0.2	0.0	0.2	0.1	0.0	0.0	0.2	0.1	0.0
Water non-Soluble protein	13.5	6.5	7.0	6.2	0.0	0.0	6.7	0.0	6.7	0.0	0.0	0.0	0.0
Water Soluble protein	5.0	5.0	0.0	0.9	3.9	0.0	0.0	0.0	0.0	0.0	3.4	0.0	3.4
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carotenoids	0.3	0.3	0.0	0.1	0.3	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3
Carbohydrates	15.5	15.5	0.0	14.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nucleic Acids	6.8	6.8	0.0	0.0	6.8	0.0	0.0	0.0	0.0	0.0	5.4	0.0	5.4
Total mass	45.0	36.8	8.1	23.0	12.4	0.0	7.7	0.7	6.7	0.0	10.6	1.0	9.3

Products (details in appendix 6)

The final products of this process (*Table 62*) are stream 8 and 12, which are two streams of metal soap with EPA and DHA that can be used for Nutraceuticals, and stream 9 and stream 13, which are two streams rich in proteins that can be used for animal feed, and also contain carotenoids. The final product is the remaining biomass in stream 4, very rich in carbohydrates, that can be used for animal feed or paper industry applications (Ververis et al., 2007).

Table 62 - Products for process option 1 + conformation 3.

Product	Stream	kg/kg of biomass	Applications
Carbohydrates	4	0.50	Paper industry, Animal Feed
Metal Soap	8 and 12	0.04	Nutraceuticals
Proteins stream	9	0.15	Animal Feed
Protein + carotenoids	13	0.21	Animal Feed

4.2.2.3.4 Process option 1 + conformation 4

In this conformation (Figure 42), a final stirred tank is added to perform hydrolysis of the proteins, contained in the stream coming out of the saponification (stream 10) (Table 63). This step is performed using a cocktail containing *Alcalase* and *Flavourzyme*. Thus, this process is similar to the previously described with an additional hydrolysis step.

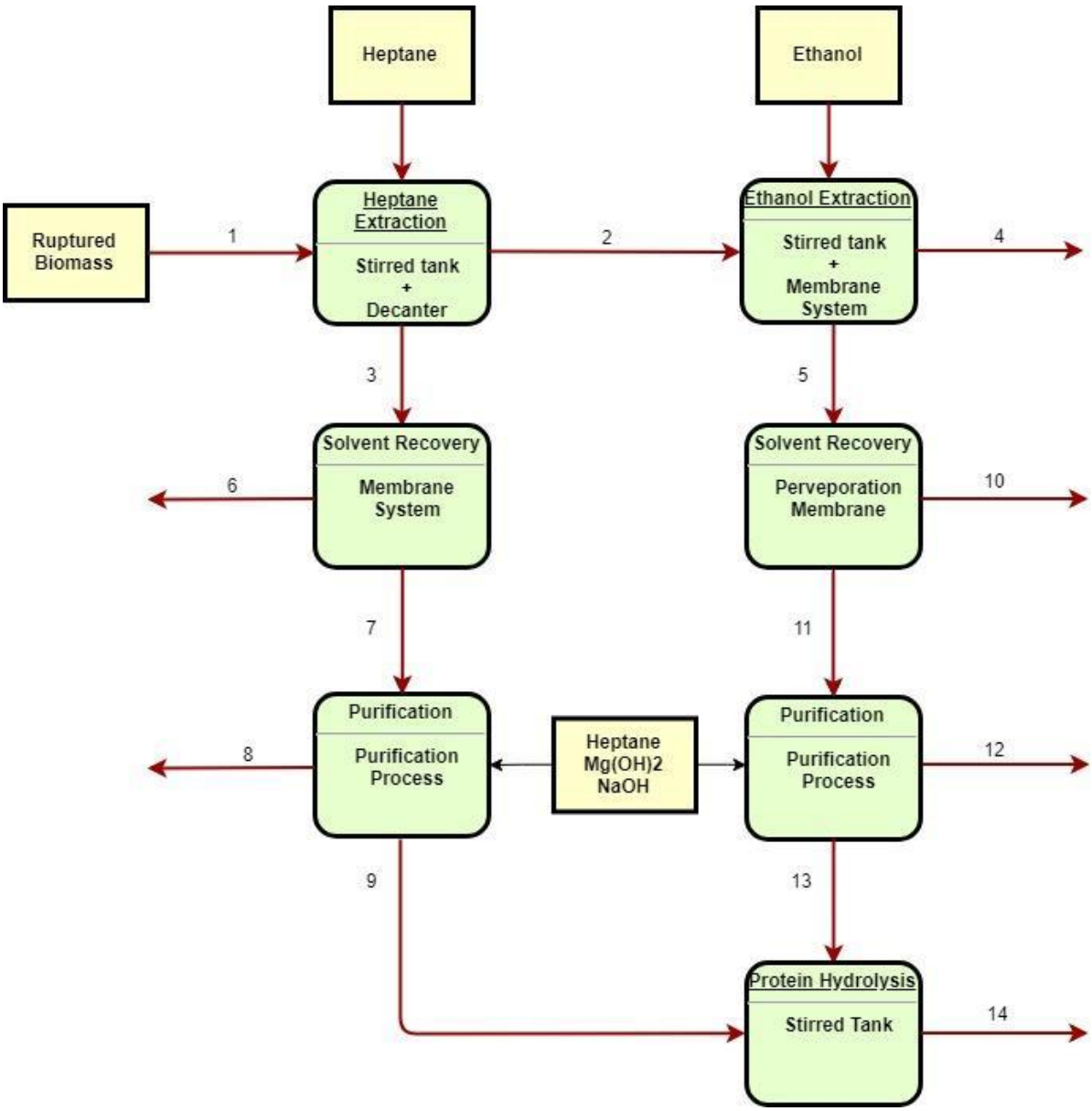

Figure 42 – Process flowsheet for process option 1 + conformation 4.

Table 63 - Process streams for process option 1 + conformation 4.

Component (kg/h) Stream	1	2	3	4	5	6	7	8	9	10	11	12	13	14
TAG	0.4	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Glycolipids	3.0	2.3	0.9	1.3	0.0	0.6	0.4	0.0	0.0	1.3	0.9	0.1	0.9	1.3
Phospholipids	0.5	0.5	0.3	0.2	0.0	0.2	0.1	0.0	0.0	0.2	0.1	0.0	0.3	0.2
Water non-Soluble protein	13.5	6.5	6.2	0.0	0.0	6.7	0.0	6.7	0.0	0.0	0.0	0.0	6.2	0.0
Water Soluble protein	5.0	5.0	0.5	4.3	0.0	0.0	0.0	0.0	0.0	3.4	0.0	3.4	0.5	4.3
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carotenoids	0.3	0.3	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.0	0.3
Carbohydrates	15.5	15.5	14.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	14.7	0.0
Nucleic Acids	6.8	6.8	0.0	6.8	0.0	0.0	0.0	0.0	0.0	5.4	0.0	5.4	0.0	6.8
Total mass	45.0	36.8	22.5	12.9	0.0	7.7	0.7	6.7	0.0	10.6	1.0	9.3	22.5	12.9

Products (details in appendix 6)

The final products of this conformation (Table 64) are streams 8 and 12, two streams of metal soap with EPA and DHA that can be used for nutraceuticals, stream 14 which contain protein hydrolysate and carotenoids that can be used as a plant bio-stimulant, and stream 4 which contains mostly carbohydrates.

Table 64 – Products for process option 1 + conformation 3.

Product	Stream	kg/kg of biomass	Applications
Carbohydrates	4	0.50	Animal Feed or paper industry
Metal Soap	8 and 12	0.04	Nutraceuticals
Protein hydrolysate stream	14	0.35	Bio-stimulant

4.2.2.3.5 Process option 2 + conformation 1

In this conformation (Figure 43), a different extraction method was used. In this process, a supercritical extraction was performed using supercritical CO_2 and 5% ethanol as co-solvent (Cardoso et al., 2012). However, the biomass first has to be dried, so a spray drier is used. The extraction will create a stream of oil containing ß-carotene and peridinin, while the remaining components will be retained in the spent biomass (Table 65).

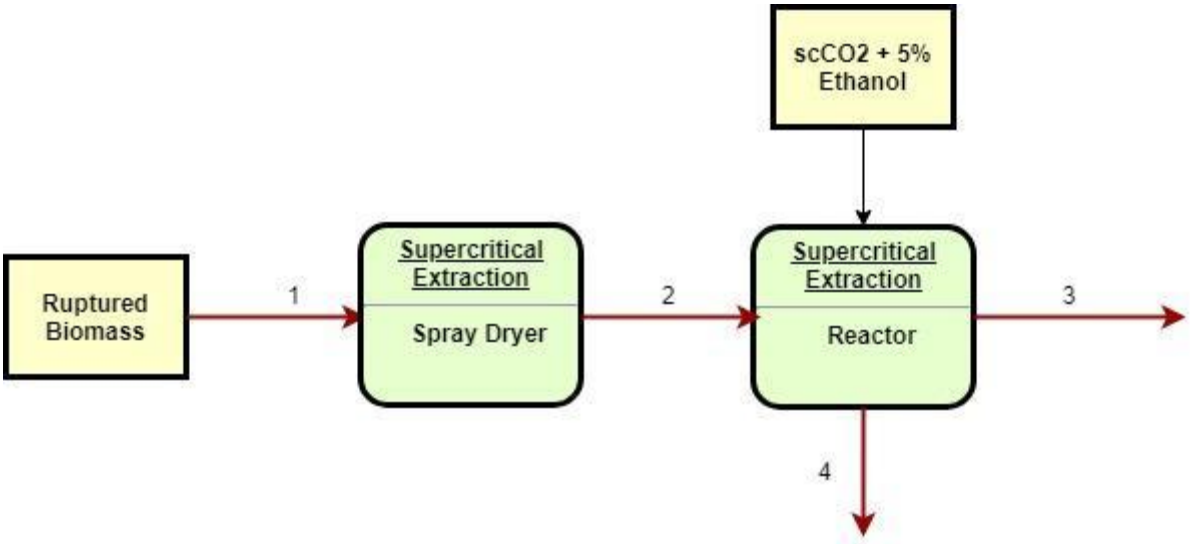

Figure 43 – *Process flowsheet for process option 2 + conformation 1.*

Table 65 – Process streams for process option 2 + conformation 1.

Component (kg/h) Stream	1	2	3	4
TAG	0.43	0.41	0.16	0.25
Phospholipids	3.05	2.90	2.11	0.78
Glycolipids	0.53	0.50	0.32	0.18
Water non-Soluble protein	13.49	12.15	12.15	0.00
Water Soluble protein	4.96	4.46	4.46	0.00
Amino acids	0.00	0.00	0.00	0.00
Carotenoids	0.34	0.34	0.00	0.31
Carbohydrates	15.45	13.14	13.14	0.00
Nucleic Acids	6.75	6.08	6.08	0.00
Total mass	45.00	39.96	38.41	1.52

Products (details in appendix 6)

In this conformation, two products will be produced (Table 66), stream 4 which is an oil, containing lipids and carotenoids, that can be used for nutraceuticals and stream 3 consisting in the remaining biomass, containing carbohydrates, proteins and some remaining lipids, that can be used as animal feed or also for the paper industry.

Table 66 - Products obtained in process option 2 + conformation 1.

Product	Stream	kg/kg of biomass	Applications
Spent Biomass rich in carbohydrates and proteins	3	0.85	Feed, Energy or paper industry
Lipid Oil with EPA+DHA + Carotenoids	4	0.03	Nutraceuticals

4.2.2.3.6 Process option 2 + conformation 2

In this conformation (Figure 44), in order to separate the carotenoids from the lipids, and obtain two purer streams, a purification process is used. This process will precipitate the lipids as a metal soap, and obtain a stream containing the carotenoids (Table 67).

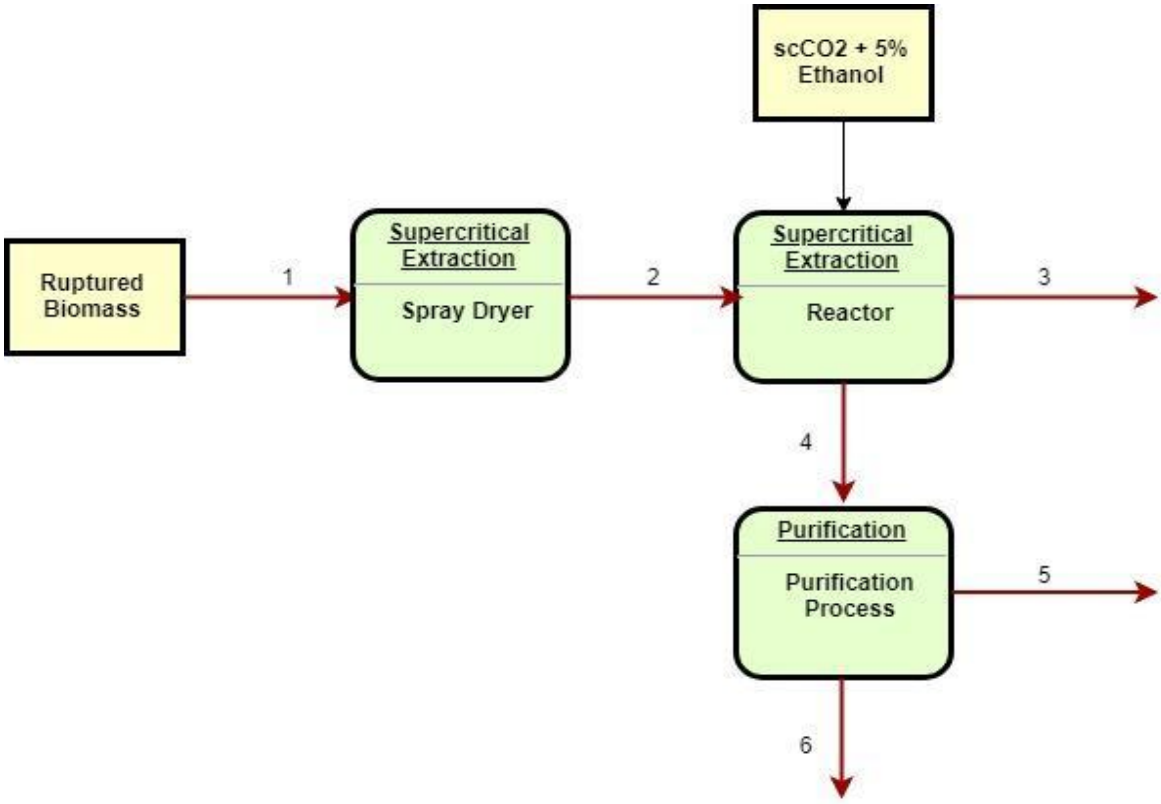

Figure 44 – Process flowsheet for process option 2 + conformation 2.

Table 67 – Process streams for process option 2 + conformation 2.

Component (kg/h) Stream	1	2	3	4	5	6
TAG	0.4	0.4	0.2	0.3	0.2	0.0
Phospholipids	3.1	2.9	2.1	0.8	0.5	0.1
Glycolipids	0.5	0.5	0.3	0.2	0.1	0.0
Water non-Soluble protein	13.5	12.2	12.2	0.0	0.0	0.0
Water Soluble protein	5.0	5.0	5.0	0.0	0.0	0.0
Amino acids	0.0	0.0	0.0	0.0	0.0	0.0
Carotenoids	0.3	0.3	0.0	0.3	0.0	0.3
Carbohydrates	15.5	13.1	13.1	0.0	0.0	0.0
Nucleic Acids	6.8	6.1	6.1	0.0	0.0	0.0
Total mass	45.0	40.00	38.4	1.5	0.8	0.4

Products (details in appendix 6)

Three product streams (Table 68) will be obtained in this setup: one is stream 3 with the spent biomass containing carbohydrates, proteins and some remaining lipids, that can be used as animal feed, stream 5 which is a metal soap rich in EPA and DHA, good for nutraceuticals and pharmaceutical applications, and stream 6, a dried stream of carotenoids (peridinin and ß-carotene) also good for nutraceuticals and pharmaceutical applications.

Table 68 - Products obtained in process option 2 + conformation 2.

Product	Stream	kg/kg of biomass	Applications
Spent Biomass rich in carbohydrates and proteins	3	0.85	Feed, Energy or paper industry
EPA+DHA	5	0.02	Nutraceuticals, Feed
Carotenoids	6	0.01	Nutraceuticals, Pharmaceuticals

4.2.2.3.7 Process option 2 + conformation 3

In this last conformation (Figure 45), a stirred tank is added to perform the hydrolysis of the proteins, contained in the biomass stream coming out of the supercritical extraction (stream 3). This step is again performed using an enzyme cocktail containing *Alcalase* and *Flavourzyme*. This stream then goes through a membrane system to purify the stream (Table 69).

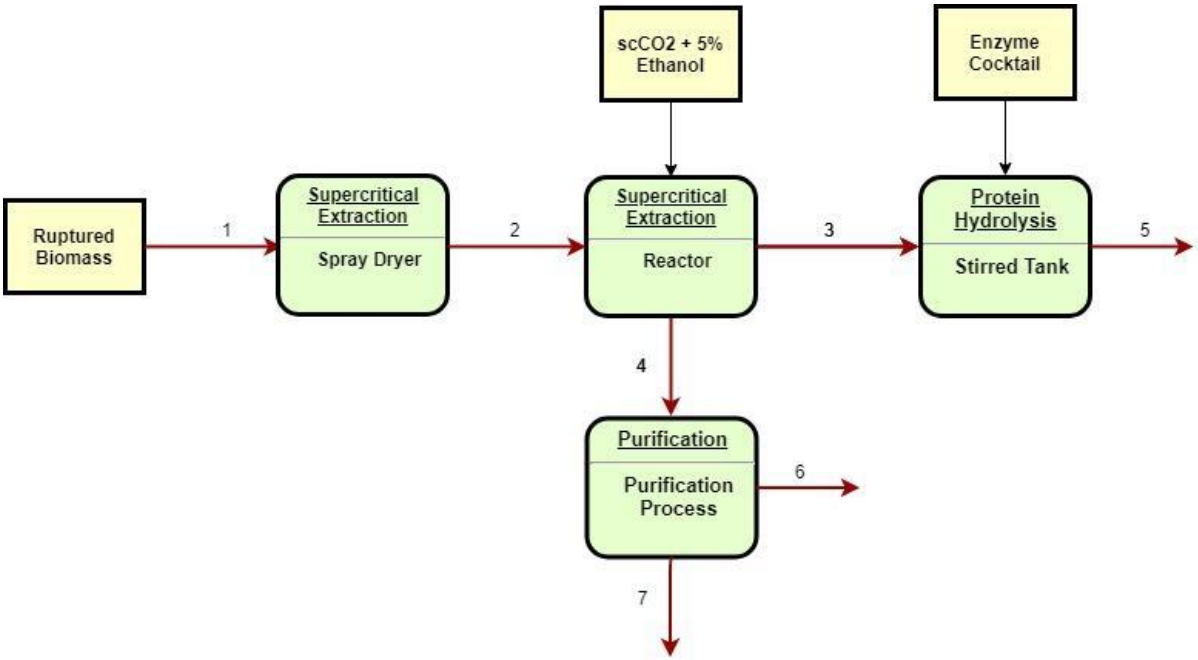

Figure 45 – Process flowsheet for process option 2 + conformation 3.

Table 69 - Process streams for process option 2 + conformation 3.

Component (kg/h) Stream	1	2	3	4	5	6	7
TAG	0.4	0.4	0.2	0.3	0.2	0.2	0.0
Phospholipids	3.1	2.9	2.1	0.8	2.0	0.5	0.1
Glycolipids	0.5	0.5	0.3	0.2	0.3	0.1	0.0
Water non-Soluble protein	13.5	12.2	12.2	0.00	5.0	0.0	0.0
Water Soluble protein	5.0	4.5	4.5	0.0	1.8	0.0	0.0
Amino acids	0.0	0.0	0.0	0.0	9.8	0.0	0.0
Carotenoids	0.3	0.3	0.0	0.3	0.0	0.0	0.3
Carbohydrates	15.5	13.1	13.1	0.0	13.1	0.0	0.0
Nucleic Acids	6.8	6.1	6.1	0.0	6.1	0.0	0.0
total mass	45.0	40.0	38.4	1.5	38.4	0.8	0.4

Products (details in appendix 6)

The final products of this process *(Table 70)* are stream 6, which can be used as a low-grade plant bio-stimulant containing carbohydrates, some lipids and amino acids (around 20%), and stream 7, which can also be used as a bio-stimulant but medium grade (with an amino acid content between 35 and 70%).

Table 70 - Products obtained in process option 2 + conformation 3.

Product	Stream	kg/kg of biomass	Applications
Protein Hydrolysate + Carbohydrates	5	0.85	Low grade Bio-stimulant
DHA+ EPA Soap	6	0.02	Nutraceuticals, Feed
Carotenoids	7	0.01	Nutraceuticals, Pharmaceuticals

4.2.3 Economic analysis

As stated before, to perform the economic analysis of these biorefinery scenarios, one must consider not only all the investments made to purchase equipment and land, but also the operational costs and the potential revenues resulting from selling the goods produced.

4.2.3.1 Assumptions

The assumptions and considerations used in the economic analysis are summarized as follows:

- ☐ Year zero is considered to be 2020
- ☐ Capex is 100% utilized in the zero year
- ☐ Capex value has a 10% security margin
- ☐ Production is assumed to be already 100% in the first year
- ☐ No Inflation on Opex is considered
- ☐ Opex value has a 5% security margin
- ☐ Land is considered to be of no value and non-arable and does not represent a cost
- ☐ No financing rate was considered
- ☐ Product prices are considered to be constant
- ☐ Discount rate – 5%
- ☐ Payback period is 8 years.

For each process sequence, the most important economic values are specified in Table 71 to Table 73. These are the values that will be inserted in the GAMS optimization software (https://www.gams.com/), which will calculate the economic parameters of all the possible biorefinery conformations in order to allow choosing the ones with the best economic performance.

4.2.3.2 Production, harvesting and biomass disruption economic values (all Capex and Opex values and explanation in appendix 7)

The economic results calculated for the different biorefinery processing scenarios are summarized in Table 71 to Table 72.

Table 71 – Economic results for Prorocentrum ruptured biomass production part 1.

Process Sequence	P.1.1	P.1.2	P.1.3	P.2.1	P.2.2	P.2.3	P.3.1
Capex	€ 14,788,116	€ 14,805,259	€ 14,709,084	€ 14,670,229	€ 14,655,259	€ 14,559,084	€ 14,495,797
Opex	€ 2,000,429	€ 2,013,351	€ 2,021,006	€ 2,003,783	€ 2,011,793	€ 2,019,447	€ 2,091,913

Table 72 – Economic results for Prorocentrum ruptured biomass production part 2.

Process Sequence	P.3.2	P.3.3	P.4.1	P.4.2	P.4.3	P.5.1	P.5.2
Capex	€ 14,578,569	€ 14,277,084	€ 14,561,185	€ 14,551,910	€ 14,299,084	€ 14,641,391	€ 14,863,845
Opex	€ 2,105,167	€ 2,078,734	€ 2,012,340	€ 2,017,907	€ 2,001,453	€ 1,989,103	€ 1,997,975

4.2.3.3 Extraction Values (all Capex and Opex values and explanation are found in appendix 7)

Since different concentrations and final biomass quantities are obtained, depending on the harvesting and biomass disruption methods selected, the extraction costs were calculated for different concentrations, and the final revenue was calculated per kg of biomass. The economic indicators are shown in Table 73, and the graphic representation of the Capex correlation with the biomass concentration is shown in Figure 46.

Table 73 – Capex and Opex values for different final ruptured biomass concentrations.

	Capex	Opex	Capex	Opex	Capex	Opex	Capex	Opex	Revenue (€/kg)
	Final biomass concentration g/L								
	50		100		150		200		
Process 1	€ 3,263,590	€ 2,983,010	€ 3,155,936	€ 2,327,259	€ 3,111,415	€ 2,108,071	€ 3,086,057	€ 1,998,260	€ 7.2
Process 2	€ 3,084,620	€ 2,616,083	€ 3,037,859	€ 2,579,702	€ 3,018,836	€ 2,567,334	€ 3,008,156	€ 2,561,069	€ 22.6
Process 3	€ 6,484,998	€ 2,858,714	€ 6,408,413	€ 2,718,301	€ 6,375,021	€ 2,671,286	€ 6,355,867	€ 2,647,719	€ 9.5
Process 4	€ 6,564,116	€ 3,316,141	€ 6,487,919	€ 3,175,703	€ 6,454,731	€ 3,128,675	€ 6,435,703	€ 3,105,100	€ 26.1
Process 5	€ 2,169,873	€ 2,616,712	€ 2,088,872	€ 1,971,357	€ 2,054,263	€ 1,755,706	€ 2,033,925	€ 1,647,668	€ 2.8
Process 6	€ 3,191,363	€ 2,817,124	€ 3,110,362	€ 2,171,769	€ 3,075,754	€ 1,956,118	€ 3,055,415	€ 1,848,080	€ 2.9
Process 7	€ 3,212,345	€ 3,747,778	€ 3,131,345	€ 3,102,423	€ 3,096,736	€ 2,886,772	€ 3,076,398	€ 2,778,734	€ 23.9

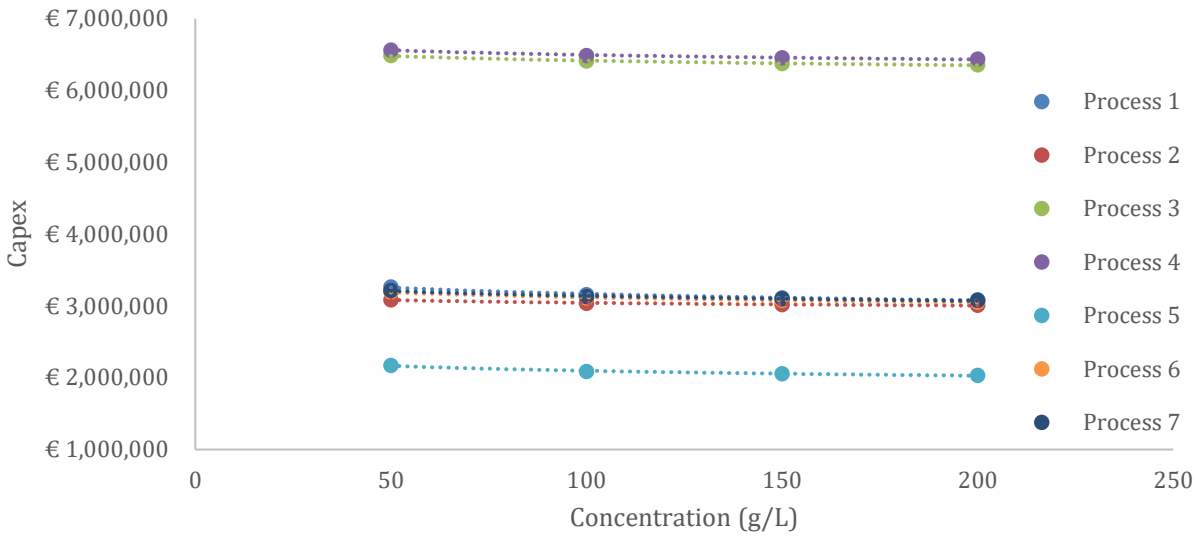

Figure 46 - Variation of the Capex of the extraction process with final ruptured biomass concentration.

From the previous graph (Figure 46), a logarithmic equation for the Capex was obtained, dependent on the final concentration that was obtained for each process, and is represented by eq. 22 to eq. 28.

$$Capex_{process\ 1} = -129{,}518 \times \ln(concentration) + 3{,}763{,}833 \quad (22)$$

$$Capex_{process\ 2} = -55{,}815 \times \ln(concentration) + 3{,}300{,}062 \quad (23)$$

$$Capex_{process\ 3} = -94{,}168 \times \ln(concentration) + 6{,}849{,}279 \quad (24)$$

$$Capex_{process\ 4} = -93{,}646 \times \ln(concentration) + 6{,}926{,}368 \quad (25)$$

$$Capex_{process\ 5} = -99{,}056 \times \ln(concentration) + 2{,}552{,}943 \quad (26)$$

$$Capex_{process\ 6} = -99{,}056 \times \ln(concentration) + 3{,}574{,}434 \quad (27)$$

$$Capex_{process\ 7} = -99{,}056 \times \ln(concentration) + 3{,}595{,}416 \quad (28)$$

A similar analysis of the effect of the biomass concentration on the Opex is represented in Figure 47.

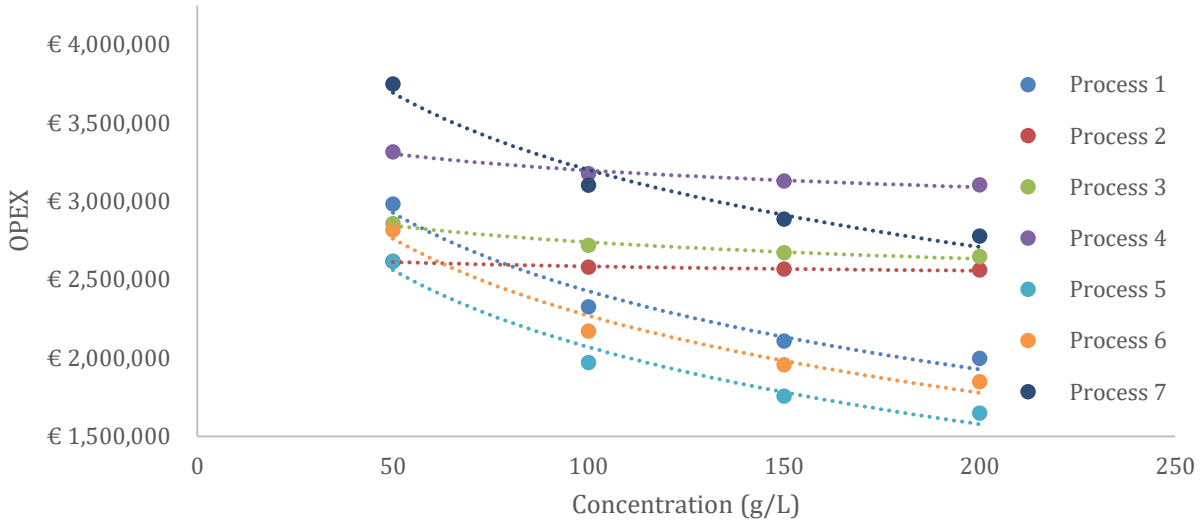

Figure 47 - Variation of the Opex of the extraction process with final ruptured biomass concentration.

From the previous graph (Figure 47) a logarithmic equation for the Opex was obtained, dependent on the final concentration that was obtained for each process, and is represented by eq. 29 to eq. 35

$$Opex_{process\ 1} = -721{,}590 \times ln\,(concentration) + 5{,}750{,}338 \quad (29)$$

$$Opex_{process\ 2} = -40{,}300 \times ln\,(concentration) + 2{,}770{,}719 \quad (30)$$

$$Opex_{process\ 3} = -154{,}608 \times ln\,(concentration) + 3{,}451{,}672 \quad (37)$$

$$Opex_{process\ 4} = -154{,}641 \times ln\,(concentration) + 3{,}909{,}228 \quad (32)$$

$$Opex_{process\ 5} = -710{,}081 \times ln\,(concentration) + 5{,}339{,}885 \quad (27)$$

$$Opex_{process\ 6} = -710{,}081 \times ln\,(concentration) + 5{,}540{,}297 \quad (34)$$

$$Opex_{process\ 7} = -710{,}081 \times ln\,(concentration) + 6{,}470{,}951 \quad (35)$$

The previously mentioned equations were introduced in the software GAMS and were calculated in order to obtain the different economic parameters necessary for the economic comparison. The code for the GAMS can be found in Appendix 10.

A first conclusion can be extracted from the analysis of the graphs Figure 46 and Figure 47, since the Capex of the scenarios with higher concentrations is lower than that of the scenarios with lower concentration. This is due to the lower volumes and therefore lower equipment capacity required to process the higher concentration strains, since the biomass input is the

same for all concentrations. The lower capacity also leads to a lower energy and water consumption and consequently to a lower Opex. However, to achieve higher concentration, more energy and more expensive equipment are required in the harvesting stage. Therefore, it is not always true to say that higher concentrations make the process economically more feasible.

4.2.3.4 Economic values for the different Scenario combinations

Capex

The Capex of the whole process was calculated based on eq. 17 and the values are presented in Table 74. The 5 lowest values are highlighted in green and the 5 highest are highlighted in red.

Opex

The Opex of the process were calculated based on eq. 18 and the values are found in Table 75. The 5 lowest values are highlighted in green and the 5 highest are highlighted in red

Revenues

The revenue was calculated based on eq. 19. As can be observed in Table 76, the scenario combinations with the largest revenue are the ones using membrane and the scenarios whose process exploits the biorefinery potential of the microalga. The 5 highest revenues are highlighted in green and the 5 lowest are highlighted in red.

Table 74 - Capex for all process combinations of Prorocentrum based biorefineries.

Extraction Scenarios							
	EP 1	**EP 2**	**EP 3**	**EP 4**	**EP 5**	**EP 6**	**EP 7**
P.1.1	€ 19,652,290	€ 19,571,700	€ 23,252,310	€ 23,340,150	€ 18,497,850	€ 19,621,490	€ 19,644,570
P.1.2	€ 19,671,150	€ 19,590,550	€ 23,271,170	€ 23,359,010	€ 18,516,710	€ 19,640,350	€ 19,663,430
P.1.3	€ 19,664,110	€ 19,527,320	€ 23,237,170	€ 23,324,620	€ 18,486,440	€ 19,610,080	€ 19,633,160
P.2.1	€ 19,563,600	€ 19,459,690	€ 23,152,430	€ 23,240,110	€ 18,399,520	€ 19,523,160	€ 19,546,240
P.2.2	€ 19,547,140	€ 19,443,220	€ 23,135,970	€ 23,223,640	€ 18,383,050	€ 19,506,690	€ 19,529,780
P.2.3	€ 19,499,110	€ 19,362,320	€ 23,072,170	€ 23,159,620	€ 18,321,440	€ 19,445,080	€ 19,468,160
P.3.1	€ 19,528,250	€ 19,335,260	€ 23,074,360	€ 23,161,400	€ 18,327,350	€ 19,450,990	€ 19,474,070
P.3.2	€ 19,619,300	€ 19,426,310	€ 23,165,410	€ 23,252,450	€ 18,418,400	€ 19,542,040	€ 19,565,120
P.3.3	€ 19,287,660	€ 19,094,680	€ 22,833,770	€ 22,920,820	€ 18,086,770	€ 19,210,410	€ 19,233,490
P.4.1	€ 19,574,200	€ 19,395,990	€ 23,127,400	€ 23,214,550	€ 18,379,410	€ 19,503,050	€ 19,526,140
P.4.2	€ 19,564,000	€ 19,385,790	€ 23,117,200	€ 23,204,340	€ 18,369,210	€ 19,492,850	€ 19,515,930
P.4.3	€ 19,285,890	€ 19,107,680	€ 22,839,090	€ 22,926,240	€ 18,091,100	€ 19,214,740	€ 19,237,820
P.5.1	€ 19,490,900	€ 19,410,300	€ 23,090,910	€ 23,178,750	€ 18,336,450	€ 19,460,090	€ 19,483,170
P.5.2	€ 19,735,600	€ 19,655,000	€ 23,335,610	€ 23,423,450	€ 18,581,150	€ 19,704,790	€ 19,727,870

Table 75 - Opex for all process combinations of Prorocentrum based biorefineries.

Extraction Scenarios							
	EP 1	**EP 2**	**EP 3**	**EP 4**	**EP 5**	**EP 6**	**EP 7**
P.1.1	€ 4,123,932	€ 4,785,507	€ 4,864,585	€ 5,344,837	€ 3,756,983	€ 3,967,416	€ 4,944,602
P.1.2	€ 4,137,500	€ 4,799,075	€ 4,878,154	€ 5,358,405	€ 3,770,552	€ 3,980,984	€ 4,958,171
P.1.3	€ 4,670,714	€ 4,836,443	€ 4,998,716	€ 5,478,991	€ 4,295,389	€ 4,505,822	€ 5,483,008
P.2.1	€ 4,345,421	€ 4,801,202	€ 4,914,809	€ 5,395,070	€ 3,974,996	€ 4,185,429	€ 5,162,615
P.2.2	€ 4,353,832	€ 4,809,612	€ 4,923,220	€ 5,403,481	€ 3,983,407	€ 4,193,840	€ 5,171,026
P.2.3	€ 4,669,078	€ 4,834,807	€ 4,997,079	€ 5,477,355	€ 4,293,753	€ 4,504,186	€ 5,481,372
P.3.1	€ 5,270,343	€ 4,940,226	€ 5,185,693	€ 5,665,992	€ 4,886,642	€ 5,097,074	€ 6,074,261
P.3.2	€ 5,284,259	€ 4,954,143	€ 5,199,609	€ 5,679,908	€ 4,900,558	€ 5,110,991	€ 6,088,178
P.3.3	€ 5,256,505	€ 4,926,389	€ 5,171,855	€ 5,652,154	€ 4,872,804	€ 5,083,237	€ 6,060,424
P.4.1	€ 5,048,652	€ 4,848,960	€ 5,072,543	€ 5,552,836	€ 4,667,154	€ 4,877,587	€ 5,854,773
P.4.2	€ 5,054,497	€ 4,854,805	€ 5,078,389	€ 5,558,682	€ 4,673,000	€ 4,883,432	€ 5,860,619
P.4.3	€ 5,037,220	€ 4,837,528	€ 5,061,112	€ 5,541,405	€ 4,655,722	€ 4,866,155	€ 5,843,342
P.5.1	€ 4,112,039	€ 4,773,615	€ 4,852,693	€ 5,332,944	€ 3,745,091	€ 3,955,524	€ 4,932,710
P.5.2	€ 4,121,355	€ 4,782,930	€ 4,862,009	€ 5,342,260	€ 3,754,406	€ 3,964,839	€ 4,942,026

Table 76 - *Revenue for all process combinations of Prorocentrum based biorefineries.*

Extraction Scenarios							
	EP 1	**EP 2**	**EP 3**	**EP 4**	**EP 5**	**EP 6**	**EP 7**
P.1.1	€ 2,487,575	€ 7,808,223	€ 3,282,217	€ 9,017,461	€ 967,390	€ 1,001,940	€ 8,257,368
P.1.2	€ 2,412,194	€ 7,571,610	€ 3,182,756	€ 8,744,204	€ 938,076	€ 971,578	€ 8,007,145
P.1.3	€ 2,512,702	€ 7,887,094	€ 3,315,371	€ 9,108,546	€ 977,162	€ 1,012,061	€ 8,340,776
P.2.1	€ 2,625,774	€ 8,242,013	€ 3,464,563	€ 9,518,431	€ 1,021,134	€ 1,057,603	€ 8,716,111
P.2.2	€ 2,546,205	€ 7,992,255	€ 3,359,576	€ 9,229,993	€ 990,191	€ 1,025,555	€ 8,451,986
P.2.3	€ 2,652,297	€ 8,325,265	€ 3,499,558	€ 9,614,576	€ 1,031,449	€ 1,068,286	€ 8,804,152
P.3.1	€ 2,487,575	€ 7,808,223	€ 3,282,217	€ 9,017,461	€ 967,390	€ 1,001,940	€ 8,257,368
P.3.2	€ 2,412,194	€ 7,571,610	€ 3,182,756	€ 8,744,204	€ 938,076	€ 971,578	€ 8,007,145
P.3.3	€ 2,512,702	€ 7,887,094	€ 3,315,371	€ 9,108,546	€ 977,162	€ 1,012,061	€ 8,340,776
P.4.1	€ 2,349,377	€ 7,374,432	€ 3,099,872	€ 8,516,491	€ 913,646	€ 946,277	€ 7,798,625
P.4.2	€ 2,278,183	€ 7,150,965	€ 3,005,937	€ 8,258,415	€ 885,960	€ 917,602	€ 7,562,303
P.4.3	€ 2,373,108	€ 7,448,922	€ 3,131,184	€ 8,602,516	€ 922,875	€ 955,835	€ 7,877,399
P.5.1	€ 2,363,197	€ 7,417,811	€ 3,118,107	€ 8,566,588	€ 919,021	€ 951,843	€ 7,844,500
P.5.2	€ 2,267,714	€ 7,118,102	€ 2,992,122	€ 8,220,463	€ 881,889	€ 913,385	€ 7,527,550

4.2.3.5 Economic indicators

To select the best scenarios, three indicators were used: Payback Period, Return on Investment and Net Present Value.

Highlighted in green in Table 77 are the scenario combinations with the shortest payback period. These values are over the desired 5 years. The common factor of all profitable scenarios is the fact that a biorefinery approach is applied to produce the highest number of products.

Table 77 - Payback period for all scenario combinations of *Prorocentrum* based biorefineries.

	Extraction Scenarios						
	EP 1	**EP 2**	**EP 3**	**EP 4**	**EP 5**	**EP 6**	**EP 7**
P.1.1	0	7	0	7	0	0	6
P.1.2	0	8	0	7	0	0	7
P.1.3	0	7	0	7	0	0	7
P.2.1	0	6	0	6	0	0	6
P.2.2	0	7	0	7	0	0	6
P.2.3	0	6	0	6	0	0	6
P.3.1	0	7	0	7	0	0	>10
P.3.2	0	8	0	8	0	0	>10
P.3.3	0	7	0	7	0	0	9
P.4.1	0	8	0	8	0	0	>10
P.4.2	0	9	0	9	0	0	>10
P.4.3	0	8	0	8	0	0	>10
P.5.1	0	8	0	8	0	0	7
P.5.2	0	9	0	9	0	0	8

The following indicator is the Return on investment.

The ROI was calculated for a period of 8 years (eq 20) and does not account for the depreciation of money value over time.

Highlighted in green in Table 78 are the 5 scenario combinations with the highest return on investment. As in the previous indicator, the best results correspond to the ones with a biorefinery approach, and from those scenarios, the ones with the highest ROI are the ones

with membrane harvesting. This is explained by the higher revenue obtained, since more biomass is harvested.

Table 78 - ROI values for all process combinations of Prorocentrum based biorefineries.

Extraction Scenarios							
	EP 1	**EP 2**	**EP 3**	**EP 4**	**EP 5**	**EP 6**	**EP 7**
P.1.1	<0%	0%	<0%	2%	<0%	<0%	9%
P.1.2	<0%	<0%	<0%	<0%	<0%	<0%	0%
P.1.3	<0%	1%	<0%	1%	<0%	<0%	<0%
P.2.1	<0%	14%	<0%	15%	<0%	<0%	18%
P.2.2	<0%	6%	<0%	7%	<0%	<0%	9%
P.2.3	<0%	17%	<0%	16%	<0%	<0%	10%
P.3.1	<0%	<0%	<0%	<0%	<0%	<0%	<0%
P.3.2	<0%	<0%	<0%	<0%	<0%	<0%	<0%
P.3.3	<0%	<0%	<0%	<0%	<0%	<0%	<0%
P.4.1	<0%	<0%	<0%	<0%	<0%	<0%	<0%
P.4.2	<0%	<0%	<0%	<0%	<0%	<0%	<0%
P.4.3	<0%	<0%	<0%	<0%	<0%	<0%	<0%
P.5.1	<0%	<0%	<0%	0%	<0%	<0%	<0%
P.5.2	<0%	<0%	<0%	<0%	<0%	<0%	<0%

Finally, the last indicator is the Net present value of the projects. It was calculated for the different process scenarios using eq. 21 and is shown in Table 79.

The 5 best scenarios are highlighted in green in Table 79. All the processes with positive NPV have a common denominator, which is the fact they are the ones where the highest number of products are produced, giving once more emphasis to the fact that biorefineries can have a positive impact on the economic feasibility of microalgae production. Furthermore, the scenarios with the highest NPV use a membrane for the harvesting step. Like the previous scenario, this is due to the highest efficiency of the membrane over all other harvesting methods.

As mentioned before, the scenarios with a negative NPV fail due to low or negative operating incomes, and because they cannot compensate for the high Capex values.

The low or negative operating income values are mostly due to the low value of the biomass obtained but also due to the high cost of certain extraction operations like the saponification

process (around 35% of the Opex in some cases). Also, the solvents for the solvent extraction can be as high as 35% of the extraction Opex. However, the biggest contribution to the total Opex are the operating costs of the production system (these values are shown in Appendix 7) as well as the manpower (up to 12.5% of the total Opex).

Table 79 - NPV for all process combinations.

Extraction Scenarios							
	EP 1	**EP 2**	**EP 3**	**EP 4**	**EP 5**	**EP 6**	**EP 7**
P.1.1	-€ 30,228,400	-€ 35,242	-€ 33,479,500	€ 396,801	-€ 36,527,600	-€ 38,788,000	€ 1,766,538
P.1.2	-€ 30,822,200	-€ 1,671,070	-€ 34,228,900	-€ 1,475,870	-€ 36,823,600	-€ 39,090,800	€ 42,738
P.1.3	-€ 33,611,800	€ 189,683	-€ 34,117,000	€ 133,971	-€ 39,932,900	-€ 42,191,000	-€ 1,162,800
P.2.1	-€ 30,678,000	€ 2,779,009	-€ 32,525,700	€ 3,410,049	-€ 37,491,000	-€ 39,739,000	€ 3,420,753
P.2.2	-€ 31,230,200	€ 1,126,877	-€ 33,242,100	€ 1,507,924	-€ 37,728,800	-€ 39,984,000	€ 1,675,767
P.2.3	-€ 32,534,000	€ 3,197,256	-€ 32,751,000	€ 3,580,128	-€ 39,406,400	-€ 41,652,000	€ 2,007,671
P.3.1	-€ 37,513,900	-€ 798,789	-€ 35,376,900	-€ 1,500,150	-€ 43,658,300	-€ 45,918,700	-€ 5,364,190
P.3.2	-€ 38,182,100	-€ 2,509,060	-€ 36,200,800	-€ 3,447,250	-€ 44,028,800	-€ 46,295,900	-€ 7,162,430
P.3.3	-€ 37,021,400	€ 40,989	-€ 34,832,600	-€ 581,422	-€ 43,265,100	-€ 45,523,300	-€ 4,495,090
P.4.1	-€ 37,020,200	-€ 3,073,330	-€ 35,877,200	-€ 4,059,820	-€ 42,639,100	-€ 44,911,900	-€ 6,962,610
P.4.2	-€ 37,507,900	-€ 4,545,220	-€ 36,511,900	-€ 5,755,390	-€ 42,845,700	-€ 45,124,900	-€ 8,517,580
P.4.3	-€ 36,504,600	-€ 2,229,690	-€ 35,312,600	-€ 3,141,620	-€ 42,217,300	-€ 44,488,000	-€ 6,091,280
P.5.1	-€ 30,794,000	-€ 2,320,290	-€ 34,301,900	-€ 2,279,030	-€ 36,601,900	-€ 38,873,500	-€ 663,660
P.5.2	-€ 31,716,100	-€ 4,562,290	-€ 35,421,100	-€ 4,821,010	-€ 37,146,800	-€ 39,427,000	-€ 3,017,080

4.2.3.6 Scenario choices

As could be observed in the previous section, the 5 scenario combinations with the best economic performances in Table 80, based on PBP, ROI and NPV, are 2.1 + EP 2, 2.1 + EP 4, 2.3 + EP 4, 2.3 + EP 2, 2.1 + EP 7. As in the previous scenario, as the objective is to design the most economically feasible and sustainable biorefinery, it is important to study the largest number of processes. Since three of the scenarios, from EP 4 have very similar equipment, in order to study the impact of other processes, it was decided to choose a similar alternative with different harvesting equipment. Therefore, 1.1 + EP 4 was chosen as an alternative to 2.1 + EP 4, and 2.2 + EP 7 was chosen as alternative to 2.3 + EP 2.

Table 80 - 3 scenarios with the best economic performance and the chosen scenarios for the LCA analysis for Prorocentrum based b*iorefinery.*

Combination	Best scenario	Chosen Scenario	Harvesting Step	Disruption Step	Products obtained
S2.1 + EP 2	**X**	**X**	Membrane	Bead mill	EPA+DHA Soap
					Protein concentrate
					Carbohydrate concentrate
S2.1+ EP 4	**X**		Membrane	Bead mill	EPA+DHA Soap
					Protein Hydrolysate
					Carbohydrate concentrate
S2.3 + EP 2	**X**		Membrane	Ultrasonicator	EPA+DHA Soap
					Protein Hydrolysate
					Carbohydrate concentrate
S 2.3 + EP 4	**X**	**X**	Membrane	Ultrasonicator	EPA+DHA Soap
					Protein Hydrolysate
					Carbohydrate concentrate
S2.1+ EP 7	**X**	**X**	Membrane	Bead mill	EPA+DHA Soap
					Protein Hydrolysate
					Carotenoids
S1.1 + EP 7		**X**	Centrifugation	Bead mill	EPA+DHA Soap
					Protein Hydrolysate
					Carotenoids
S2.4 + EP 4		**X**	Membrane	HPH	EPA+DHA Soap
					Protein Hydrolysate
					Carbohydrate concentrate

4.2.3.7 Sensitivity analysis

In order to study the robustness of the *Prorocentrum* based biorefinery, several what-if scenarios were proposed, aiming to perform a sensitivity analysis. All Values can be found in Appendix 11. As stated before, a number of different factors can change the performance of a process (Yilmaz Balaman, 2019); these are:

- Market demand and price
- Supply of biomass and other materials
- Processes and technology
- Transportation and logistics
- Governmental and regulatory policies

- Natural conditions.

In this part of this study, and similar to the case of *Synechocystis* based biorefinery process configurations, the effect of variations of the three first factors will be taken into consideration, as these are the ones that are more likely to have a serious impact on the final outcome of this project in the near future.

4.2.3.7.1 Market demand and price

As highlighted previously, the price of utilities, products and services fluctuates depending on the balance of demand and supply. Nevertheless, demand can change due to factors like availability of cheaper substitutes, consumer preferences, and the shifts in the price of complementary products. With this in mind, a study must be performed on the impact that variations of prices can have on the production process feasibility.

EPA + DHA Soap

The first product of this process is the DHA+EPA soap. It is a product common to all process configurations. According to Grand View Research (Grand View Research, 2018a), the global omega-3 market size is estimated to grow at a CAGR of 7.4% from 2019 to 2025. This increase is driven by the growing interest of the Active Pharmaceutical Ingredient (API) market in omega-3. However, this increase in demand can also bring in new competition, new technologies, and can cause a possible flood in the market, which can lead to a decrease in the value of the product. Therefore, it was analyzed the impact of 10 and 20% increase and decrease in EPA+DHA Soap prices, on the NPV of the process scenarios studied (Table 81 and Figure 48).

Table 81 - Impact of variation in EPA+DHA Soap prices on NPV.

Conformation	Change in EPA+DHA Price				
	-20%	-10%	0%	10%	20%
2.3 + EP 4	€ 2,389,694	€ 3,103,959	€ 3,580,128	€ 4,056,312	€ 4,770,577
2.1 +EP 7	€ 2,949,339	€ 3,185,046	€ 3,420,753	€ 3,656,461	€ 4,127,876
2.1 + EP 4	€ 2,231,518	€ 2,938,641	€ 3,410,049	€ 3,881,470	€ 4,588,593
2.3 + EP 2	€ 2,006,815	€ 2,959,168	€ 3,197,256	€ 3,673,433	€ 3,911,521
2.1 + EP 2	€ 1,600,472	€ 2,543,302	€ 2,779,009	€ 3,250,424	€ 3,486,132
2.3 + EP 7	€ 1,531,495	€ 1,769,583	€ 2,007,671	€ 2,245,760	€ 2,721,936
1.1 + EP 7	€ 1,319,934	€ 1,543,236	€ 1,766,538	€ 1,989,839	€ 2,436,443
2.2 + EP 7	€ 1,218,637	€ 1,447,202	€ 1,675,767	€ 1,904,332	€ 2,361,461
2.2 + EP 4	€ 365,107	€ 1,050,801	€ 1,507,924	€ 1,965,060	€ 2,650,755
2.2 + EP 2	-€ 15,947	€ 898,313	€ 1,126,877	€ 1,584,007	€ 1,812,572
No. profitable scenarios	9	10	15	16	17

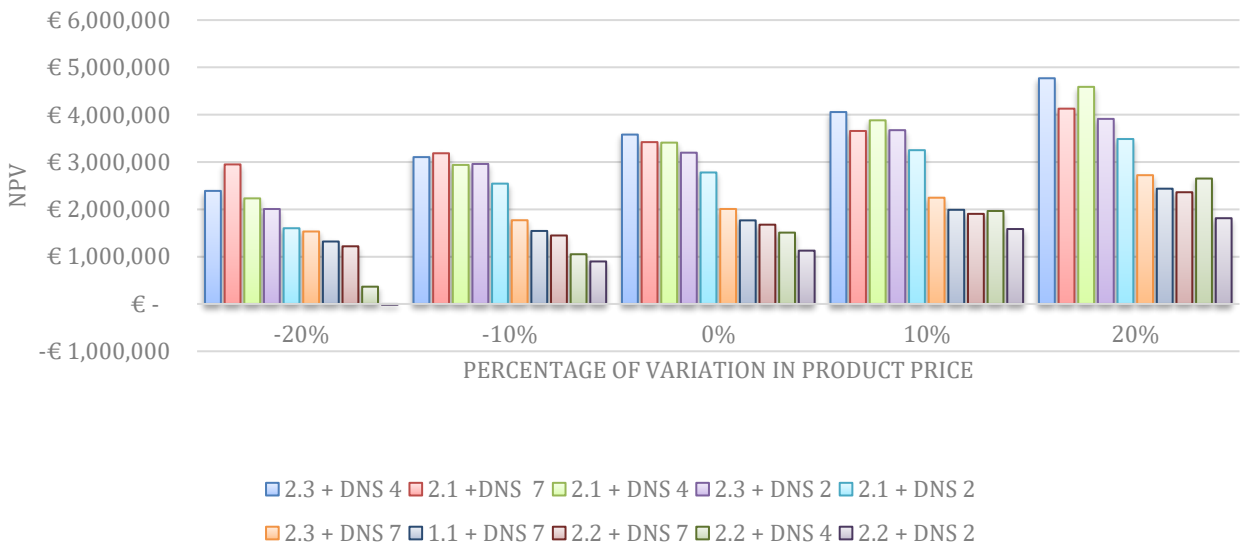

Figure 48 - NPV Variation with change in EPA+DHA Soap Prices (for more detail see Appendix 11).

As expected, as EPA + DHA Soap is a product common to all scenarios. Any change in the price will have impact on the economic values of all scenarios (Figure 48). However, as the production of EPA + DHA is not very high (around 1% of the biomass), it has a small impact

on the final economic performance, and so the impact of the change in EPA + DHA prices on NPV is also not very high. In fact, even if the prices decrease by 20%, none of the top 10 scenarios has a negative NPV. This observation also shows that the other products are more important in the economic feasibility of the process. It is also perceived that there are scenarios with more production of EPA+DHA Soap, due to the higher decrease in NPV due to the decrease in the revenue from that product. One example is the change of position of scenario 2.3 + EP 4 with 2.1 + EP 7. This shows that the extraction scenario 4 produces more EPA+DHA than scenario extraction 7 and therefore, it is more exposed to EPA + DHA Soap price changes. These results show that changes to the EPA+DHA market do not have a very large influence in the feasibility of the top 10 scenarios as the amount of EPA+DHA soap produced is not very high.

Protein and Bio-Stimulant

Besides the EPA + DHA soap, there are other components that can be obtained in the different conformations. This study will focus on the two products that have a higher impact on the top 10 scenarios: protein concentrate and bio-stimulant.

Currently the demand for proteins is increasing every year. The global protein ingredients market size is expected to witness a CAGR of 7.5% from 2019 to 2025. This is mainly due to the increase in demand from food products such as yogurt, milk sausages, bakery products, spicy sauces, among others (Grand View Research, 2018b). Likewise, the global bio-stimulant market is also expected to increase and reach 4.9 billion USD by 2025, with a CAGR of around 13% from 2015 to 2025. The main reasons for this increase will be the need to feed a larger population and the negative effects of climate change, and therefore, an increase in the productivity of agricultural crops in a sustainable manner is required. Thus, bio-stimulants will become an important solution in the plan to increase the yield level (Intelligence, 2017).

As shown in different reports, the demand for both products is increasing and therefore there is the possibility of product prices also increasing. However, this increase in demand can bring in more competition and better and cheaper products, and if the market is flooded with the same product or cheaper alternative products, the prices can decrease. As not all scenarios produce both products, in this study the increase and decrease of the price of both products were studied to understand what impact these changes would have in the ranking of the scenarios.

The first observation of Figure 49 is that the decrease in prices of the bio-stimulant has a high impact in all of the 10 scenarios with the highest NPV. This is due to the amount of bio-stimulant produced being quite high (from 35 – 85% of the biomass), though the product

price is low (around 4 €/L). Another evidence is the fact that if the price of bio-stimulant decreases up to 10%, none of the 10 scenarios will have a positive NPV, while decreasing the value of the protein concentrate to less than 10%, 9 of the top 10 scenarios are still profitable.

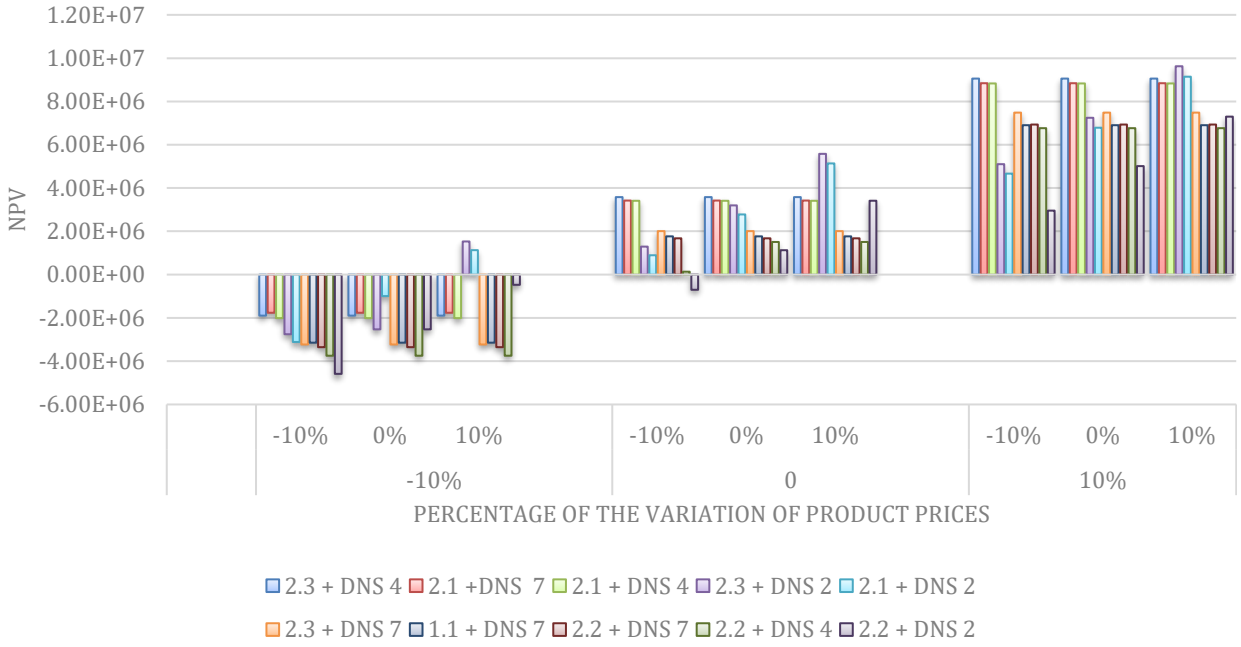

Figure 49 – NPV variation with variation of the prices of protein concentrate (top values) and bio-stimulant (bottom values) (for more detail see Appendix 11).

Likewise, it is possible to conclude that the bio-stimulant market has a higher importance in the economics of the processes than the EPA+DHA Market, since when decreasing the EPA+DHA soap price by 20% (Figure 48), only one of the top 10 scenarios has a negative NPV. Contrarily, when the price of the bio-stimulant is 10% lower, there are no profitable scenarios (Figure 49). Therefore, all of the top 10 scenarios depend on the stability of the biofertilizer market to be feasible. Furthermore, it is possible to see that the viability of the scenarios from extraction process 2 is very dependent on the changes in the Protein Concentrate Market as it accounts for around 20% of the revenue.

Energy prices

Similar to the *Synechocystis* based biorefinery, it is expected that a variation in energy prices affects the economic performance of the *Prorocentrum* based biorefinery. In order to mimic this event, two new scenarios were analyzed, one with an increase of 10% of the current energy price, and another with the increase of 25%.

When looking at the 10 best scenarios, the rank and NPV are shown in Table 82.

Table 82 - Variation of NPV of the top 10 scenarios with electricity cost increase.

NPV Rank	Energy cost increase					
	0%		10%		25%	
1	2.3 + EP 4	€ 3,580,128	2.3 + EP 4	€ 3,339,918	2.3 + EP 2	€ 2,527,871
2	2.1 +EP 7	€ 3,420,753	2.1 +EP 7	€ 3,196,447	2.3 + EP 4	€ 2,208,649
3	2.1 + EP 4	€ 3,410,049	2.1 + EP 4	€ 3,185,749	2.1 + EP 4	€ 2,178,734
4	2.3 + EP 2	€ 3,197,256	2.3 + EP 2	€ 2,957,039	2.1 + EP 2	€ 2,174,435
5	2.1 + EP 2	€ 2,779,009	2.1 + EP 2	€ 2,554,703	2.1 +EP 7	€ 2,079,164
6	2.3 + EP 7	€ 2,007,671	2.3 + EP 7	€ 1,767,454	1.1 + EP 7	€ 747,239
7	1.1 + EP 7	€ 1,766,538	1.1 + EP 7	€ 1,533,871	2.2 + EP 2	€ 506,935
8	2.2 + EP 7	€ 1,675,767	2.2 + EP 7	€ 1,445,313	2.2 + EP 7	€ 318,809
9	2.2 + EP 4	€ 1,507,924	2.2 + EP 4	€ 1,277,477	2.2 + EP 4	€ 261,241
10	2.2 + EP 2	€ 1,126,877	2.2 + EP 2	€ 896,423	2.3 + EP 7	€ 142,603
No. Profitable scenarios	15		11		10	

As can be seen in Figure 50, the energy price has different impact on the ranking of the 10 top scenarios. This is especially visible in the scenarios where the energy costs have a large impact on the operational costs like extraction scenario 4 and 7, where an evaporator is used and the energy costs are around 20% and 35% of the operational costs respectively. This difference can also be observed as Scenario 2.1 + EP 7 goes from rank 3 to 6 when the energy costs are increased by 15%. Furthermore, it is possible to see that the equipment used for harvesting plays an important role in the scenarios with evaporator, as the higher the concentration, the less water needs to be evaporated, which means lower energy consumption. This means that in scenarios where a centrifuge is used (scenario 1.1) as the concentration is higher, the impact of the increase of electric energy cost is smaller than in the scenarios where membrane separation is used.

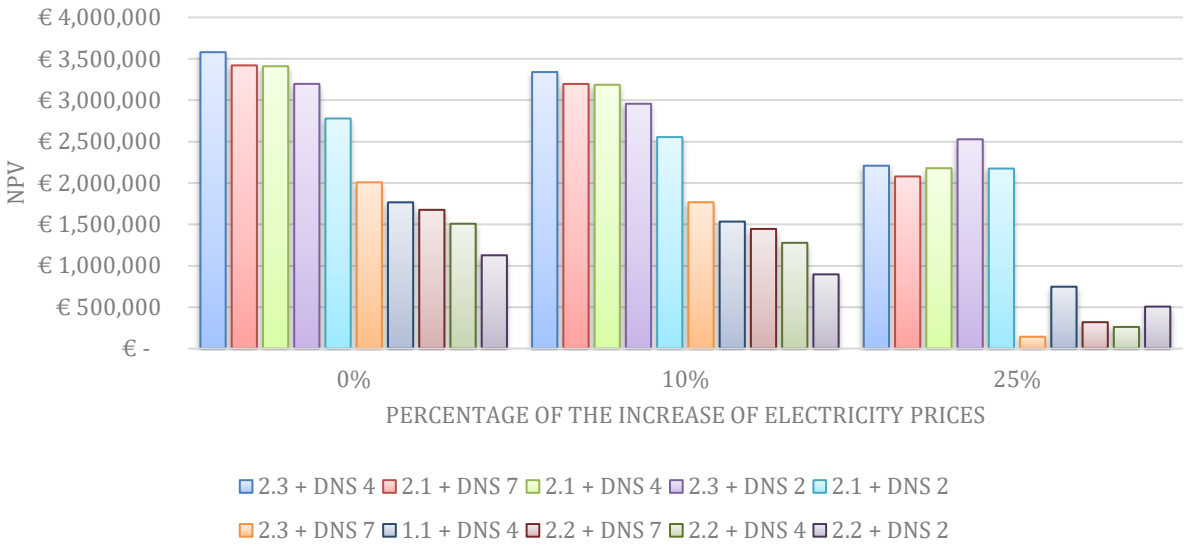

Figure 50 - Variation of NPV of the top 10 scenarios with electricity cost increase.

4.2.3.7.2 Technological improvement

Use of renewable energy

One possibility to improve the economic results of the biorefinery project is the implementation of renewable energy that will decrease the energy costs, but can increase the Capex due to the costs of setting up the equipment. As in the *Synechocystis* based biorefinery scenario, the price of setting up a Photovoltaic power plant is around 1700 €/kW.

As the biggest energy consumer is the production process, it was studied the impact of using 10%, 25%, 50% and 100% of renewable energy to power this step. The results are shown in Figure 51, Figure 52 and Table 83.

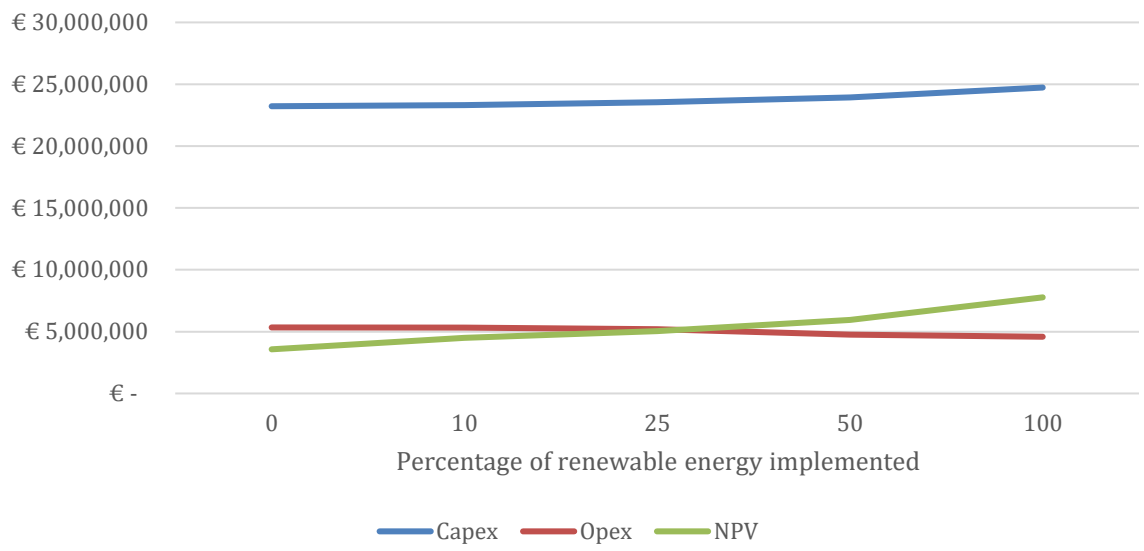

Figure 51 - Variation of the economic parameters with implementation of renewable energy.

Table 83 - Variation of the economic parameters with implementation of renewable energy.

	Share of renewable energy used in the microalgae production step (%)				
	0	**10**	**25**	**50**	**100**
Capex	€ 23,159,620	€ 23,318,840	€ 23,557,670	€ 23,955,730	€ 24,751,840
Opex	€ 5,477,355	€ 5,311,697	€ 5,186,451	€ 4,977,705	€ 4,560,223
NPV	€ 3,580,128	€ 4,491,593	€ 5,061,473	€ 6,013,358	€ 7,915,521
Capex change		1%	2%	3%	6%
Opex change		-3%	-6%	-10%	-20%
NPV change		20%	29%	40%	55%

As can be seen in Figure 52, the implementation of renewable energy has a positive impact in all scenarios, as the costs saved by the decrease of dependence in the electric grid are superior to the increase in the capital costs. The scenarios where the impact is higher are the scenarios with extraction scenario 4 and 7 where the consumption of energy has a higher impact on the operational costs. In the extraction scenario 4, the impact is higher as 16% of

the Opex is electricity costs due to the purification process, while in scenario 7 the impact is 12,5% due to the spray dryer.

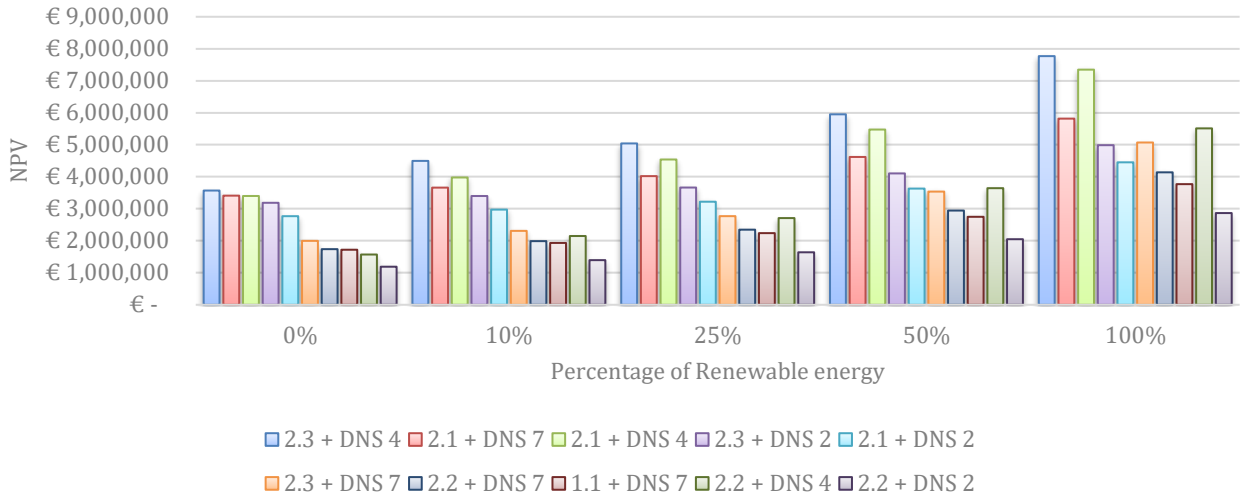

Figure 52 – NPV variation of the top 10 scenarios with implementation of renewable energy.

4.2.3.7.3 Supply of biomass and other materials

Biomass

As the biomass is the main source of raw material, the same factors as in the *Synechocystis* scenario decrease the amount of biomass produced, and any impact there is on the amount of biomass produced will have impact on the profitability and even on the economic sustainability of the process. Although the change of biomass can have some impact in the operational costs, as these were as low as 1%, they were not taken into account in the calculations.

As can be seen in Figure 53, if any of the previous negative factors would occur and the amount of biomass produced in 8 years would decrease below 10% of the initially proposed, then no scenario would be economically feasible. This is the result of the low value of biomass, as the highest value is only around 27 €/kg.

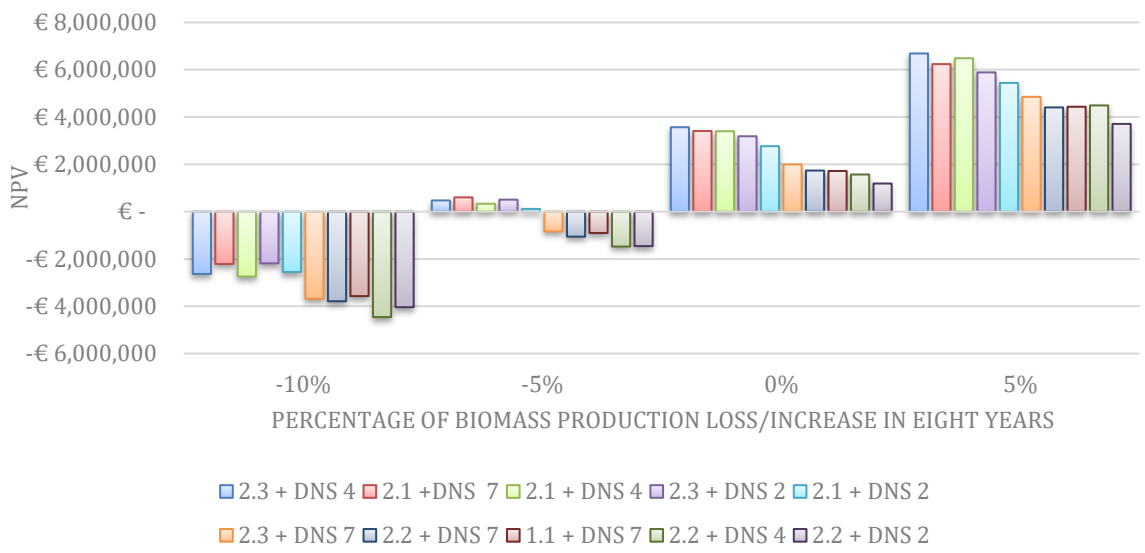

Figure 53 - NPV variation of the top 10 scenarios with variation of biomass produced (for more detail see Appendix 11).

4.3 Conclusion

From the economic analysis of both *Synechocystis* and *Prorocentrum* based biorefineries, it was possible to conclude that the biorefinery approach is an efficient way to obtain profit from these strains that without this approach would most likely fail. The main reason for the need of a biorefinery approach is the biomass value. In the *Synechocystis* based biorefinery, the biomass achieved values of almost 50 €/kg due to the possible products that could be obtained from that biomass which translated into 30 positive scenarios out of 136 scenarios. On the other hand, the *Prorocentrum* scenarios only achieved a price of around 25 €/kg, which is half of the value of the *Synechocystis* scenario, leading to only 15 profitable scenarios out of 98 scenarios. Another important aspect is the maximum concentration of biomass produced. This factor will influence the biomass production price per kg of biomass as well as the following harvesting step. Comparing both biorefineries, the *Prorocentrum* based biorefinery has a lower production capital cost/kg (around 2.2 €/kg) and a harvesting step with lower operational and capital costs, than the *Synechocystis* based biorefinery that has a production capital cost of around 5.0 €/kg and slightly higher harvesting costs. This happens because the concentration achieved during *Prorocentrum* production is higher (2.4 g/L) than the one of *Synechocystis* (0.52 g/L).

In both *Synechocystis* and *Prorocentrum* strains, the highest costs are associated to the biomass production process, and to the enzymatic hydrolysis or the saponification process. Another factor which increases the costs, is the high manpower costs, which are around 20% of the Opex.

The sensitivity analysis brought to light the most important factors that affect the profitability of the biorefineries, which are the products and the biomass production. In addition, it showed that the economic performance could be improved with the use of renewable energy and synergy with other industries by using waste streams.

Although a large number of process options were used, due to the large amount of possibilities some were not included, possibilities that could eventually be more profitable than the ones assumed. One of such possibilities would be to shift the focus of the *Prorocentrum* from the production of EPA+DHA to other products, as it was shown that the purification process suggested by IOI oleo (Friedl, 2017)is very expensive and the soap production does not compensate for the high costs. Another option could be to design a more efficient and less expensive process for the purification of the lipids.

5 Life Cycle Assessment

5.1 Introduction

Presently, and increasingly more in the future, the assessment of the viability of a new production process or changes to be implemented in existing systems will not be solely based on economic aspects, as the environmental and social dimensions of sustainability must also be accounted for. This is the result of the increased awareness and urgency of the various stakeholders of the impacts that activities have on a more sustainable development, that leads to the development and implementation of specific tools and strategies to support it (Hauschild et al., 2017).

Therefore, in order to create a sustainable microalgae biorefinery, one must look not only at the economic viability but also to its environmental and social impacts. The economic viability was first discussed in detail in the previous chapter, and in this section, focus is given to the study of the environmental impacts.

In this thesis, the life cycle analysis (LCA) was selected to study the environmental impact of the microalgae cultivation and processing, in order to assess which of the previously chosen scenarios is the most sustainable. The aim of the LCA study was to evaluate the impact of the production of different products on the environment, and to identify hotspots in a process to prioritise enhancements in order to improve the product and process environmental performance. In the following subsections the main assumptions, data sources and calculations performed, environmental categories and corresponding environmental indicators calculations, and main results are presented and discussed in detail.

5.1.1 Goal and Scope

5.1.1.1 Goal

The main goal of an LCA study may vary, but normally it is either the evaluation of the environmental impacts of a product or process system, or the comparison between products/processes with similar functions, that may involve the comparison with a benchmark system. In this project, the purpose of this LCA study is:

> *To compare the environmental impact of 5 different microalgae biorefineries to obtain 1 kg of a defined product and choose the one with the lowest environmental impact.*

The 5 biorefineries layouts were defined taking into account the analysis performed in the previous chapter (chapter 4 – preliminary economic analysis).This study was performed to assist in the development process of a sustainable microalgae cultivation and processing facility, and will be used to compare different technologies, and help in the choice of the ones that are more sustainable, not only economically but also environmentally, in order to define which one is the most adequate based on the assumptions made and data available. This study will also allow the identification of the processes hotspots, that should be improved first in a cost-benefit perspective to improve the systems overall environmental performance.

5.1.1.2 Scope

The product system studied is a microalgae biorefinery facility, where the function of the system is to produce different products from microalgae. The analysis was only performed "cradle-to-gate", from the cultivation of microalgae to the final purification of the final product and did not take into account the applications of the products. This decision was taken because the products considered were business to business and the products can have more than one final application, rendering impossible the definition of proper end-of-life scenarios.

5.1.1.3 Functional Unit

As was mentioned previously, the objective was to compare 5 different microalgae biorefinery configurations. Thus, a simpler form to perform the comparison is to define a common product found in all scenarios, in particular phycocyanin for scenario 1 and EPA+DHA soap for scenario 2. Since the objective was to compare the different refineries, the functional unit chosen was "*1 kg of phycocyanin*" for scenario 1 and "*1 kg of EPA+DHA soap*" for scenario 2. As 5 different biorefineries layouts, for each microalga, are analyzed in this work, this represents a total of 5 variants studied and compared with each other.

5.1.2 Study Approach

Two approaches can be followed in an LCA study: attributional or consequential (Ekvall et al., 2016).

The first studies the environmental impacts of an entire product or process system. It uses average data to study the direct environmental impact of a product. It answers the question: "What are the total emissions from the processes and material flows directly used in the life cycle of a product?" The second aims to evaluate the changes in environment impact on an existing product or process system. It uses marginal data to study how the global

environmental burdens are affected by the production and use of the product. It answers the question: "What is the change in total emissions as a result of a marginal change in the production of a product?" (Brander et al., 2008; Ekvall, 2020)

As the main study goal is the comparison between the total impacts of different microalgae biorefinery processes, an attributional approach was considered, as the full system needs to be analysed.

5.1.2.1 Geographic, technological and temporal coverage

Regional conditions can play a crucial role in the economic and environmental impact of a product or process system. The location will define the microalgae productivity (through the solar radiation, temperature, etc.), the transportation costs and environmental impacts, as well as the electricity generation environmental impacts and prices. Therefore, it is important to define the location of the process and, in this study, the location selected was Lisbon, Portugal.

Regarding the technological and temporal coverages, it was considered that the best available technologies are used, and the calculations/results are valid for a 10-year period starting in the present.

It was assumed that the facility and all process equipment have an operational lifetime of 20 years from commissioning. Only the membrane filter modules were considered to have a lifetime of 2 years.

5.1.2.2 Data Considerations

Where possible, primary data was used in order to make the project as similar to real life as possible. Most of the primary data was based on information obtained from the *PUFAChain* and *DEMA* European projects (Friedl, 2017; University of Limerick, 2018). Other data was supplied by the company *A4F* – Algae for Future.

In some cases, even in the previously mentioned projects, primary information on certain processes was not available due to intellectual property protection. However, some information on those processes (energy, water and chemical consumption) was still available, so if any of these processes was utilised, it was considered as a black box.

In some cases, high quality data was not available. In this case, in the first instance, the *Ecoinvent* 3.5 life cycle inventory database was utilized, as this (or previous versions) are regularly used in microalgae biofuel studies and other LCA studies.

If data was not available via primary sources or the previously mentioned databases, then other sources, such as literature data, were used. For all unit-processes used, a shared database between the projects was developed to show all data sources (primary, *Ecoinvent*, etc.). When no primary information on equipment materials was available, information about these was taken from equipment suppliers.

5.1.2.3 Cut-off

According to the ILCD handbook (European Commission Joint Research Centre Institute for Environment and Sustainability, 2010), the cut-off for each of the impact categories should be at 1%. Nevertheless, as recommended by the handbook, the flows not considered are still identified and will stay in the inventory, but without stating an amount and being marked as "missing relevant" or "missing irrelevant", as applicable (European Commission Joint Research Centre Institute for Environment and Sustainability, 2010).

5.2 System Description

The system description involves two parts: the definition of system boundaries through which there is exchange of materials and energy with the exterior, and the definition of system structure and how they interrelate with each other.

Based on the analysis performed in the previous chapter (economic analysis) 5 different process systems were chosen for the LCA. Each production system scenario can be divided in three main parts, which are done sequentially:

- Microalgae biomass production, using the photobioreactors where the microalgae are cultivated;
- Microalgae cell harvesting and rupture, divided in two parts, one for harvesting and other for cell wall rupture;
- Product extraction and purification, that consist in the combination of unit processes needed to obtain the final product ready to be sold.

Associated with each part of the process, there are some support processes, for example to power up the system, to produce the reagents, etc. In Figure 54, a general process system is presented where one can identify what are the main and auxiliary system processes, represented in black and grey, respectively.

Depending on the system variant under study, different unit operations were considered for the main processes after biomass cultivation, which was similar for all scenarios, and they represent the main differences between them. In particular, for each main process:

- Harvesting: membrane system, flocculation tank or a centrifuge;

- Cell rupture: bead mill, ultrasonication, or High Pressure Homogenizer (HPH);
- Extraction processing: protein hydrolysis (common in all scenarios), diafiltration or conventional solvent extraction;
- Ethanol recovery: pervaporation membrane alone or combined with a distillation column.

For the *Synechocystis* based biorefinery and the *Prorocentrum* based biorefinery the various scenarios considered are presented in Table 84 and Table 85, repectively. Each unit process/operation is described in detail below, and detailled diagrams of each scenario can be found in section 5.3.2.

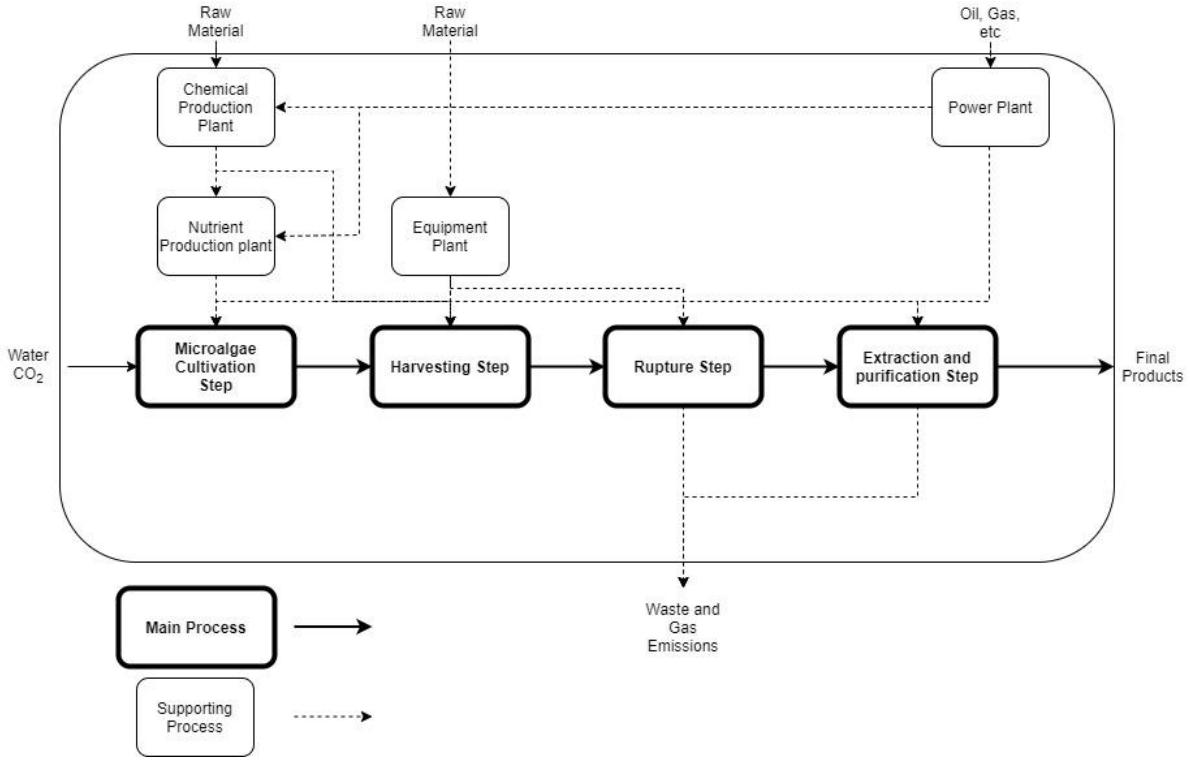

Figure 54 - System boundary and process components of the system.

Table 84 – Scenarios description for Synechocystis based biorefinery.

Scenario	Harvesting	Rupture	Extraction	Ethanol Recovery
Scenario S1	Membrane System	Bead mill	Diafiltration Protein Hydrolysis	Pervaporation
Scenario S2	Membrane System	Ultrasonication	Diafiltration Conventional Solvent Extraction Protein Hydrolysis	Pervaporation
Scenario S3	Flocculation Tank	Ultrasonication	Diafiltration Protein Hydrolysis	Distillation Column + Pervaporation
Scenario S4	Centrifuge	Bead mill	Diafiltration Protein Hydrolysis	Distillation Column + Pervaporation
Scenario S5	Membrane System	Bead mill	Diafiltration Protein Hydrolysis	Distillation Column + Pervaporation

Table 85 – Scenarios description for Prorocentrum based biorefinery.

Scenario	Harvesting Step	Disruption Step	Products obtained
Scenario P1	Centrifugation	Bead mill	EPA+DHA Soap Protein Hydrolysate Carotenoids
Scenario P2	Membrane	Bead mill	EPA+DHA Soap Protein Hydrolysate Carotenoids
Scenario P3	Membrane	HPH	EPA+DHA Soap Protein Hydrolysate Carbohydrate concentrate
Scenario P4	Membrane	Bead mill	EPA+DHA Soap Protein concentrate Carbohydrate concentrate
Scenario P5	Membrane	Ultrasonication	EPA+DHA Soap Protein Hydrolysate Carbohydrate concentrate

5.2.1 Biomass Production

In both scenarios, the microalgae biomass production occurs in UHT-PBRs designed by A4F, similar to those shown in Figure 55. The production process lasts 4 months, and afterwards the reactor is cleaned using a sodium hypochlorite solution that later is neutralized using sodium thiosulfate. Water is used for medium manufacture and to cool down the system when the temperature is over a certain threshold. Sodium Nitrate and Potassium Phosphate are considered as the macronutrients used for the microalgae, and CO_2 (from industrial flue gas in the *Synechocystis* scenario and from gas cylinders in the *Prorocentrum* scenario) is injected in the system. Electricity is mostly used to power the circulation pump.

Figure 55 - UHT-PBR considered in this work.

5.2.2 Harvesting Step

This step follows the cultivation stage and starts with the concentration of the microalgae biomass. Usually, after the production stage, the final concentration of the biomass is between 0.1-10 g/L. The objective of this step is to increase the concentration 50 to 200 fold, to simplify extraction processing. Afterwards, in order to facilitate the separation of the various biomass fractions and the components of interest present inside the microalgae, the microalgae cell wall must be ruptured.

In scenarios S1, S2 and S5, the microalgae biomass is considered to be harvested and concentrated to 100 g/L using a membrane system. In scenarios P2, P3, P4 and P5, the microalgae biomass is considered to be harvested and concentrated to 150 g/L, using a membrane system. The membrane system equipment (tubing, pumps, support structure, membrane casings) is considered to be made out mostly of stainless steel 316L. The membranes used are considered to be made out of Polyethersulfone (PES) and have a durability of 2 years.

In scenario S3, the microalgae biomass is considered to be harvested and concentrated to 50 g/L using a flocculation tank. The flocculation tank is considered to be made with glass fiber reinforced polyester, and a structure made of carbon steel. To flocculate the biomass and improve the settling time, chitosan is considered to be used as a flocculant agent.

In scenario S4, the microalgae biomass is considered to be harvested and concentrated to 200 g/L using 3 centrifuges. In scenario P1, the microalgae biomass is considered to be harvested and concentrated to 200 g/L using 2 centrifuges. Each Centrifuge was considered to have a cast iron structure and the bowl body made of stainless steel 316L.

5.2.3 Cell Rupture Step

After harvesting, the microalgae biomass is ruptured. In scenarios S1, S4 and S5, and in scenarios P1, P2 and P3 the microalgae biomass is considered to be ruptured by a bead mill. The bead mill was considered to have stainless steel structure (cylinder and supporting structure). To rupture the cells, half the barrel volume of stainless steel spheres is used.

For scenarios S2 and S3 and scenario P5 the rupture method considered was an ultrasonication system. The ultrasonication system is constituted by a stainless-steel structure, and a titanium alloy ultrasonic processer.

In the case of scenario P4, the rupture is considered to be performed by an high-pressure homogenizer. The body of the homogenizer is considered to be made of stainless steel 316L.

5.2.4 Extraction Process

5.2.4.1 *Synechocystis* based biorefinery scenarios

For all scenarios, the first extraction step consists in the extraction of phycocyanin and soluble proteins from the remaining components. This step is performed using a Membrane System where a diafiltration is performed. In this membrane system most components (tubing, pumps, support structure, membrane casings) are considered to be made of stainless steel 316L and Polyethersulfone Membranes (PES).

The phycocyanin stream still requires further purification. Therefore, chitosan is added to the mixing/settler tank to purify the stream and create food grade phycocyanin. The tank (stirrer, support structure) is made of stainless steel 316L. The stream then goes through a membrane system. In this membrane system most components (tubing, pumps, support structure, membrane casings) are also considered to be made of stainless steel 316L and Polyethersulfone Membranes (PES).

In scenario S2 the remaining biomass, composed of the non-soluble components, goes into a stirred tank where ethanol is added to separate polar lipids and other components from the non-polar components. The main components (tank and stirrer) are considered to be made of stainless steel. The ethanol fraction goes into a series of purification steps (designed by IOI Oleo) whose components are considered to be made of stainless steel 316L and stainless steel 304. For the previous purification steps, NaOH, $Mg(OH)_2$ and heptane are added during the process along with water.

The remaining biomass of scenario S1, S3, S4 and S5, along with the stream without ethanol from scenario S2, composed of the non-soluble compounds of the biomass (lipids, carbohydrates, non-soluble proteins) is then fed to another stirred tank. In this tank, an enzyme cocktail (containing Flavourzyme and Alcazyme) is added in order to hydrolyze the remaining proteins. The main components (tank and stirrer) are considered to be made out of stainless steel 316L as well.

5.2.4.2 *Prorocentrum* based biorefinery scenarios

In scenario P1 and P2 the biomass is first considered to be dried in a Spray dryer made of stainless steel 316L. The lipids, along with part of the carotenoids, from the biomass, are considered to be extracted from the remaining biomass in a High-Pressure reactor with supercritical carbon dioxide and 5% V/V ethanol. The reactor is considered to be made mostly of titanium alloy.

The remaining biomass containing proteins and carbohydrates, is then fed to another stirred tank. In this tank, an enzyme cocktail (containing Flavourzyme and Alcazyme) is added in order to hydrolyze the remaining proteins. The tank´s main components (tank and stirrer) are considered to be made of stainless steel.

The soluble components (soluble proteins and amino acids) are then considered to be separated in a membrane system where most components (tubing, pumps, support structure, membrane casings) are made of Stainless Steel 316L and use Polyethersulfone Membranes (PES).

The fraction that is extracted with the supercritical CO_2 is considered to go into a series of purification steps with components made of stainless steel 316L and stainless steel 304.

In scenario P3 and P5, the first extraction step is considered to be performed in a stainless steel 316L stirred tank where heptane is added to separate the non-polar lipids from the remaining biomass. To separate heptane from water, a decanter centrifuge is used. The decanter centrifuge body is considered to be made of stainless steel 316L.

To extract the polar lipids, ethanol is added to another stirred tank. The tank system (tank, stirrer, support structure) is considered to be made of stainless steel 316L. The stream is then considered to go through a membrane system to separate ethanol and its soluble components from the non-soluble components. The membrane system components (tubing, pumps, support structure, membrane casings) are considered to be made of stainless steel 316L and the membranes from Polyethersulfone (PES).

After extraction, it is considered that both lipid streams go through a series of purification steps where the components are made of stainless steel 316L and stainless steel 304. For the previous purification steps, NaOH, $Mg(OH)_2$ and heptane are added during the process along with water.

The remaining components are then considered to be fed to another stirred tank. In this tank, an enzyme cocktail (containing Flavourzyme and Alcazyme) is added, in order to hydrolyze the remaining proteins. The main components (tank and stirrer) are considered to be made of stainless steel.

In scenario P4, the first extraction step is considered to be processed in a stainless steel 316L stirred tank where heptane is added to separate the non-polar lipids from the remaining biomass. To separate heptane from water, a decanter centrifuge is used. The decanter centrifuge body is considered to be made of stainless steel 316L.

After extraction, the lipid stream is considered to go through a series of purification steps, with components made of stainless steel 316L and stainless steel 304. For the previous purification steps, NaOH, $Mg(OH)_2$ and heptane are added during the process along with water.

The remaining components are then considered to be fed to another stirred tank. In this tank, an enzyme cocktail (containing Flavourzyme and Alcazyme) is added, in order to hydrolyze the remaining proteins. The main components (tank and stirrer) are considered to be made of stainless steel 316L.

5.2.4.3 Ethanol recovery

In scenarios S1 and S2, ethanol was considered to be recovered by means of a pervaporation membrane. The pervaporation membrane system was considered to be mainly constituted by stainless steel structure (tubing, pumps, structure, membrane casings of stainless steel 316L, and Polydimethylsiloxane (PDMS) membranes.

In scenarios S3, S4 and S5, ethanol was considered to be recovered by combining a distillation column (concentrate up to 80% of ethanol), and a pervaporation membrane (concentrate to 99.5%). The distillation column is considered to be manufactured in stainless steel 316L. The distillation uses high-pressure steam to supply energy to perform the separation. The pervaporation membrane system was considered to be mainly constituted by stainless steel structure (tubing, pumps, structure, membrane casings of stainless steel 316L, and Polydimethylsiloxane (PDMS) membranes.

5.2.5 Supporting Processes

The main advantage of an LCA study is that it focuses not only on the processes that produces the desired product, but it also looks into the processes essential to produce all the equipment, consumables and energy that the main production line requires.

5.2.5.1 Compounds and consumables

In each process, different inputs (reagents, cleaning products, equipment consumables) and outputs (products and wastes) are used and produced. The production of the consumed compounds and the disposal of the waste can also have significant impacts on the environment and, therefore, must be included in the process. The compounds and wastes considered are:

- Water
- CO_2
- Nutrients ($NaNO_3$ and NaH_2PO_4)
- Solvents (ethanol, heptane)
- Chemicals ($Mg(OH)_2$, NaOH)
- Cleaning Products (Cleaning Chemical, Sodium Hypochlorite)
- Chitosan
- Enzymes
- Membranes
- Wastewater
- Storage Vessels (polypropylene 25 kg bags for dry products, polyethylene 200 L Drum for liquid products).

5.2.5.2 Facility and equipment Construction

The models include the major capital assets used in the cultivation, harvesting and cell rupture of the microalgae, and in the extraction of the desired components from the ruptured microalgae. More specifically, they include:

- Photobioreactors
- Harvesting and Rupture equipment
- Extraction and purification Equipment
- Ethanol extraction Equipment.

The building facilities where the biorefinery are installed were considered to be the same size in all scenarios and therefore the impact is similar in all scenarios. For these reasons, the building construction impacts were not included in the LCA.

The land occupied by the biorefinery was considered to be the same size for all scenarios, and therefore the environmental impacts of its utilization have not been accounted for in the comparison study. However, when comparing the Land Use with other processes the land was considered to be non-arable and with low biodiversity.

All equipment chosen is commercially available with the same or similar working capacities of the process units considered in this work and used in the context of microalgae cultivation and processing or similar processes. Moreover, technical information is available allowing the calculation of the material weight. In cases that the equipment information was not available, the values were downsized according to existing equipment.

5.2.5.3 Distribution and Transportation

The distribution and transportation of the products was not included in the LCA since the analysis is “cradle-to-gate”. Further, it was considered that the impact would have the same weight for all the scenarios and therefore would not significantly influence the LCA study results. Furthermore, it was considered that the production of biomass and further treatment will occur in the same industrial facility and therefore no transportation is required. As not much detail on transportation is available, it was assumed that the transportation is one way trip only (Bilec et al., 2010).

The transportation of the remaining components was divided into 2 main groups; equipment and consumables. The first is composed of the transportation of the glass tubes from Germany to Lisbon, Portugal. This transportation is performed by a 32-ton truck for 2500 km. The remaining production equipment (aside of the glass tubes) is manufactured by a local company, transported in a 32-ton truck at a distance of 100 km. It was considered that all harvesting equipment and ethanol recovery equipment (except the pervaporation membranes) are shipped from Italy to Lisbon Portugal. The transportation is performed by

a 32-ton truck for 2200 km. The pervaporation membranes are considered to be transported from the Netherlands, and their transportation is performed by a 32-ton truck for 2500 km.

The second group contains the transportation of the chemicals used. The transportation of the bottled CO_2 for the biomass cultivation, from Estarreja, Portugal, to Lisbon, Portugal, is done by a 32-ton truck for 275 km. The remaining chemicals are considered to be supplied by distribution truck from a local supplier (around 100 km) that imports the chemicals in larger quantities, not being considered in the LCA study in this work, as no information is available.

5.2.5.4 Electricity

All the process, from the production line to the auxiliary processes, require electricity to function. The source of that electricity can be very vast, with each source having different environmental impacts. As the location considered was Portugal, the electricity mix used was related to that of Portugal.

5.3 Life Cycle Inventory

The data used in the LCA study was obtained from primary sources, in particular from the *DEMA* project and *PUFAChain* projects (Friedl, 2017; University of Limerick, 2018), and was provided by A4F. When primary data was not available, life cycle inventory database, published data and estimates were used. All equipment material, energy and equipment maintenance, were obtained from equipment supplier datasheets. The data for the electricity mix was provided by the *Ecoinvent* 3.5 database regarding Portugal. The cleaning agent main components were considered to be 15% of NaOH, 5% of KOH and 5% of EDTA (tetrasodium ethylenediaminetetraacetate) (Diversey, 2017).

The inventory is presented in the following subsections and is divided by type of information, in particular:

- products obtained in each scenario
- global inventories.

The values found in the global inventories were related to the functional unit, phycocyanin in the *Synechocystis* scenarios, and EPA+DHA Soap in the *Prorocentrum* scenarios. This was done in order to allow the aggregation of results and permit a correct and coherent comparison between the scenarios.

The following assumptions were made, concerning the process operation:

- Microalgae production works 24 hours/day for 350 days/year
    - Productivity information for both strains can be found in the Economic Analysis Chapter

- Harvesting, rupture and extraction equipment work for 350 days a year, for 22 hours/day with the remaining 2 hours/day used for cleaning and maintenance
- All extraction solvents are recovered with a recovery rate of 90%
- For the membranes, centrifuges and ultrasonication system, a solution of 4% Volume of cleaning agent is used for cleaning. The total volume of cleaning liquid used for this is the full volume of the equipment
- For the remaining equipment, the volume of the cleaning agent used is 10% of the equipment volume (Brandt, 2013)
- The equipment materials inventory was calculated dividing the weight of the material by the lifetime of the equipment and relating it to the functional unit (facility and all process equipment have an operational lifetime of 20 years; the membrane filter modules a lifetime of 2 years).

5.3.1 CO_2 Capture

As microalgae consume CO_2, it was considered that they have a positive impact on the global warming potential ($kgCO_2$ eq.), and therefore the consumed CO_2 was subtracted to the total global warming potential value (Bhola et al., 2014).

The CO_2 fixation was calculated based on the following equation (36) (Adamczyk et al., 2016);

$$\mathrm{CO_2biofixation} = C \times P \times \left(\frac{MCO_2}{MC}\right) \quad (36)$$

Where *C* is the carbon content in the microalgae biomass, *P is* productivity, MCO_2 is the molar mass of carbon dioxide, and *MC* is the molar mass of carbon.

In the case of *Synechocystis*, due to the production of Ethanol it was assumed that the CO_2 was double that of the one obtained in eq. 36.

5.3.2 Global Inventories (See appendix 12 for full inventory)

5.3.2.1 Scenario S1

In this scenario (Figure 56 and Table 86), 180.5 tonnes of microalgae biomass are produced per year. This biomass is harvested and afterwards ruptured. The amount of ruptured biomass is 161.3 tonnes per year. About 368.5 m^3 of ethanol are recovered by a pervaporation membrane. The extraction of components from the ruptured biomass produces two products: 15.9 tonnes per year of phycocyanin and 705.6 m^3 per year of a protein hydrolysate.

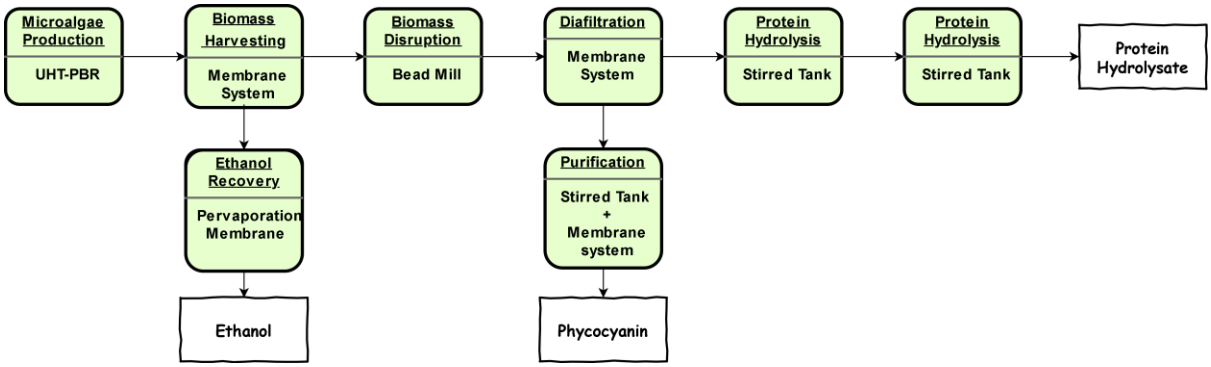

Figure 56 - Scenario S1 process diagram.

Table 86 - Scenario S1 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
Phycocyanin	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	2.20	No environmental burdens were considered since the values are under the legal limit to disposed to sewage
Amino acid Hydrolysate	m^3	44.42	Final Product
Ethanol	m^3	23.9	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.30	For 20 years
Borosilicate Glass	kg	2.06	For 20 years
Wood (any type)	kg	0.21	For 20 years
Nitrile Rubber	kg	0.02	For 20 years
Polypropylene	kg	0.03	For 20 years
Polyethersulfone (PES)	kg	0.01	For 2 years
Polydimethylsiloxane (PDMS)	kg	v.t.s.	For 2 years
Polypropylene	kg	v.t.s.	Used in the Storage Bags
Polyethylene	kg	3.37	Used in the Storage Barrels
Chemicals			
Water	m^3	15.03	Used for production, cooling and extraction
Sodium thiosulfate	kg	0.03	-
$NaNO_3$	kg	7.39	-
NaH_2PO_4	kg	0.62	-
CO_2	t	0.07	-

Exchanges	Unit	Amount	Comments
Cleaning Agent	m^3	0.03	-
Enzymes	m^3	1.12	Alcalase and Flavourzyme mixture
Chitosan	kg	0.07	-
Electricity			
Electricity	kWh	255.3	Includes all equipment
Transport			
Transport	tkm	3.70	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. - value too small

5.3.2.2 Scenario S2

In this scenario (Figure 57 and Table 87), 180.5 tonnes of microalgae biomass are produced per year. This biomass is harvested and ruptured, and the amount of ruptured biomass is 162.9 tonnes per year. Around 368.5 m^3 of ethanol are recovered. The biomass goes through a extraction process producing not only 16.0 tonnes per year of phycocyanin, but also 5.7 tonnes per year of a magnesium soap, 2.4 tonnes per year of carotenoids and 672.0 m^3 per year of a protein hydrolysate stream.

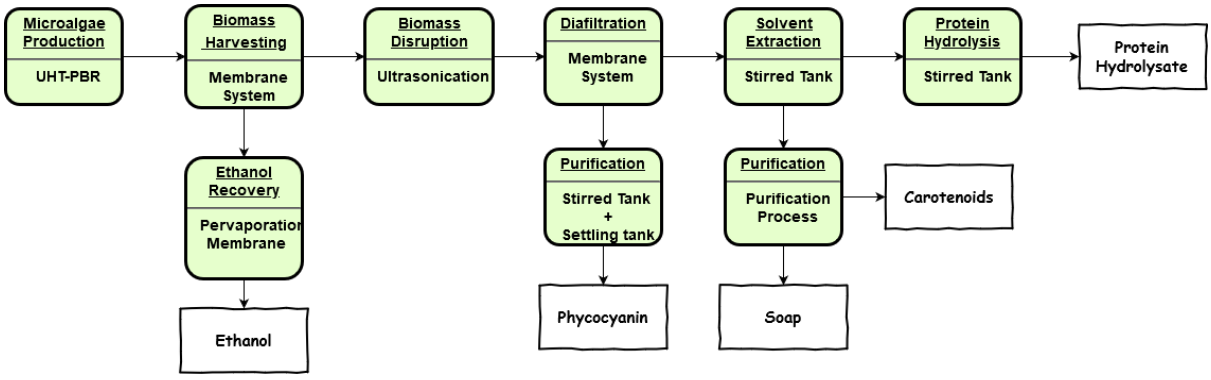

Figure 57 – Scenario S2 process diagram.

Table 87 - Scenario S2 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
Phycocyanin	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	2.15	No environmental burdens were considered since the values are under the legal limit to disposed to sewage
Amino acid Hydrolysate	m^3	41.88	Final Product
Zeaxanthin	kg	0.15	Final Product

Exchanges	Unit	Amount	Comments
Soap	kg	0.36	Final Product
Ethanol	m^3	22.96	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.34	For 20 years
Titanium Alloy	kg	0.00	For 20 years
Borosilicate Glass	kg	2.04	For 20 years
Wood (any type)	kg	0.21	For 20 years
Nitrile Rubber	kg	0.02	For 20 years
Polypropylene	kg	0.03	For 20 years
Polyethersulfone (PES)	kg	0.01	For 2 years
Polydimethylsiloxane (PDMS)	kg	v.t.s.	For 2 years
Polypropylene	kg	v.t.s.	Used in the Storage Bags
Polyethylene	kg	3.24	Used in the Storage Barrels
Chemicals			
Water	m^3	14.89	Used for production, cooling and extraction
Sodium thiosulfate	kg	0.03	-
$NaNO_3$	kg	7.31	-
NaH_2PO_4	kg	0.62	-
CO_2	t	0.07	-
Heptane	m^3	v.t.s.	-
Ethanol	m^3	12.33	-
NaOH	kg	v.t.s.	Used in the purification process
$Mg(OH)_2$	kg	v.t.s.	Used in the purification process
Cleaning agent	m^3	0.03	-
Enzymes	kg	0.94	Alcalase and Flavourzyme
Chitosan	kg	0.07	-
Electricity			
Electricity	kWh	274.35	Includes all equipment
Transport			
Transport, road	tkm	4.93	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. – value too small

5.3.2.3 Scenario S3

In this scenario (Figure 58 and Table 88), 180.5 tonnes per year of microalgae biomass are produced. The biomass is harvested and ruptured, and the ethanol recovered from the

medium. The amount of ruptured biomass is 154.3 tonnes per year and around 368.5 m^3 per year of ethanol are recovered. The extraction process performed on the ruptured biomass produced 15.2 tonnes per year of phycocyanin along with 675.2 m^3 per year of a protein hydrolysate.

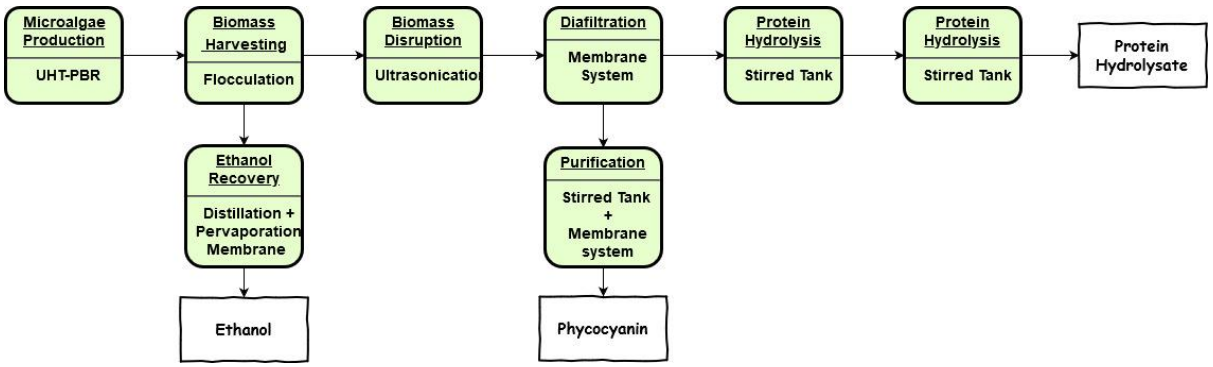

Figure 58 - Scenario S3 process diagram.

Table 88 - Scenario S3 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
Phycocyanin	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	2.15	No environmental burdens were considered since the values are under the legal limit to disposed to sewage
Amino acid Hydrolysate	m^3	44.4	Final Product
Ethanol	m^3	24.15	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.28	For 20 years
Titanium Alloy	kg	0.01	For 20 years
Glass fibre reinforced Polyester	kg	0.02	For 20 years
Carbon Steel	kg	0.01	For 20 years
Borosilicate Glass	kg	2.04	For 20 years
Wood (any type)	kg	0.21	For 20 years
Nitrile Rubber	kg	0.02	For 20 years
Polypropylene	kg	0.03	For 20 years
Polyethersulfone (PES)	kg	v.t.s.	For 2 years
Polydimethylsiloxane (PDMS)	kg	v.t.s.	For 2 years
Polypropylene	kg	v.t.s.	Used in the Storage Bags
Polyethylene	kg	3.24	Used in the Storage Barrels
Chemicals			
Water	m^3	14.33	Used for production, cooling and extraction

Exchanges	Unit	Amount	Comments
Sodium thiosulfate	t	0.03	-
$NaNO_3$	kg	7.31	-
NaH_2PO_4	kg	0.62	-
CO_2	t	0.07	-
Cleaning Agent	m^3	0.01	-
Enzymes	kg	1.06	Alcalase and Flavourzyme mixture
Chitosan	kg	0.29	Includes harvesting and extraction processes
Electricity and Utilities			
Electricity	kWh	225.45	Includes all equipment
Steam	t	0.23	For the distillation Column
Transport			
Transport, road	tkm	1.46	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. – value too small

5.3.2.4 Scenario S4

In this scenario (Figure 59 and Table 89, 180.5 tonnes per year of microalgae biomass are produced, harvested and ruptured. The process produces 152.8 tonnes per year of ruptured biomass. Around 369.4 m^3 per year of ethanol are also recovered from the culture medium. The extraction process of the ruptured biomass produces 15.0 tonnes per year of phycocyanin, producing 668.4 m^3 per year of a protein hydrolysate.

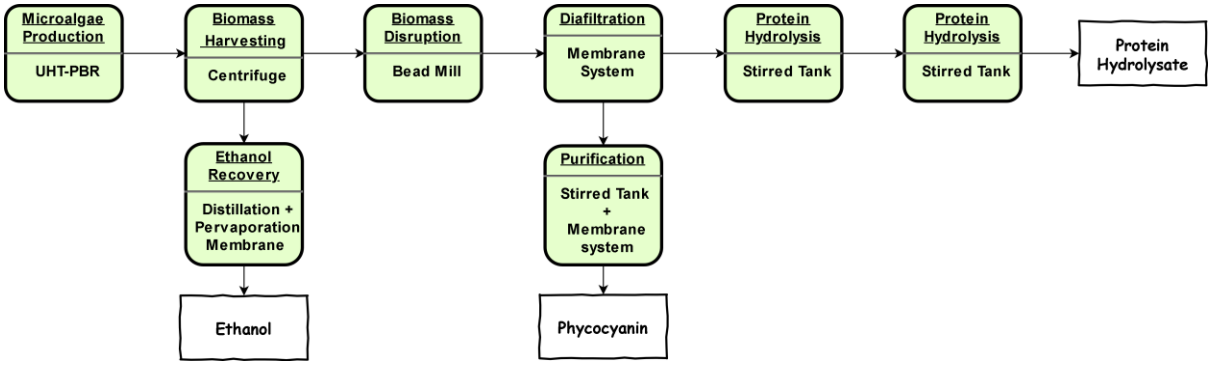

Figure 59 – Scenario S4 process diagram.

Table 89 - Scenario S4 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
Phycocyanin	kg	1	Reference Flow
Output of by-products:			

Exchanges	Unit	Amount	Comments
Wastewater	m^3	2.16	No environmental burdens considered since values under limit. Disposed to sewage
Amino acid Hydrolysate	m^3	44.4	Final Product
Ethanol	m^3	24.55	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.31	For 20 years
Cast Iron	kg	0.01	For 20 years
Borosilicate Glass	kg	2.04	For 20 years
Wood (any type)	kg	0.21	For 20 years
Nitrile Rubber	kg	0.02	For 20 years
Polypropylene	kg	0.03	For 20 years
Polyethersulfone PES)	kg	v.t.s.	For 2 years
Polydimethylsiloxane (PDMS)	kg	v.t.s.	For 2 years
Polypropylene	kg	v.t.s.	Used in the Storage Bags
Polyethylene	kg	3.23	Used in the Storage Barrels
Chemicals			
Water	m^3	14.57	Used for production, cooling and extraction
Sodium thiosulfate	t	0.03	-
$NaNO_3$	kg	7.31	-
NaH_2PO_4	kg	0.62	-
CO_2	t	0.07	-
Cleaning Agent	m^3	0.02	-
Enzymes	kg	1.05	Alcalase and Flavourzyme mixture
Chitosan	kg	0.07	Includes harvesting and extraction processes
Electricity and Utilities			
Electricity	kWh	221.76	Includes all equipment
Steam	t	0.23	For the distillation Column
Transport			
Transport, road	tkm	2.35	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. – value too small

5.3.2.5 Scenario S5

In this scenario (Figure 60 and Table 90), 180.5 tonnes per year of microalgae biomass are produced. This biomass is harvested and afterwards ruptured producing 162.9 tonnes per year of ruptured biomass. About 368.5 m^3 per year of ethanol are also recovered. The extraction of components from the ruptured biomass produces 16.0 tonnes per year of phycocyanin, and 712.7 m^3 per year of a protein hydrolysate.

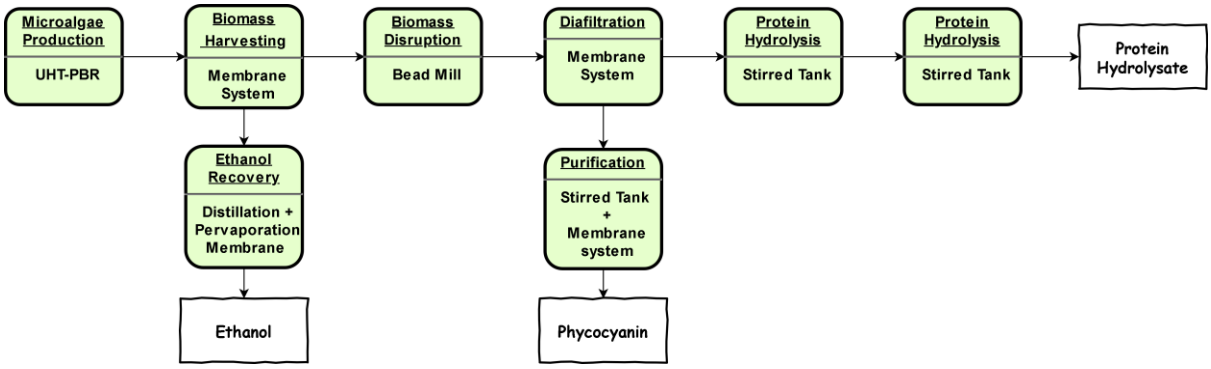

Figure 60 – Scenario S5 process diagram.

Table 90 - Scenario S5 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
Phycocyanin	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	0.71	No environmental burdens considered since values under limit. Disposed to sewage
Amino acid Hydrolysate	m^3	44.42	Final Product
Ethanol	m^3	22.96	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.33	For 20 years
Borosilicate Glass	kg	2.17	For 20 years
Wood (any type)	kg	0.22	For 20 years
Nitrile Rubber	kg	0.02	For 20 years
Polypropylene	kg	0.03	For 20 years
Polyethersulfone (PES)	kg	0.01	For 2 years
Polydimethylsiloxane (PDMS)	kg	v.t.s.	For 2 years

Exchanges	Unit	Amount	Comments
Polypropylene	kg	v.t.s.	Used in the Storage Bags
Polyethylene	kg	3.58	Used in the Storage Barrels
Chemicals			
Water	m^3	15.87	Used for production, cooling and extraction
Sodium thiosulfate	t	0.03	-
$NaNO_3$	kg	7.80	-
NaH_2PO_4	kg	0.66	-
CO_2	ton	0.07	-
Cleaning Agent	m^3	0.03	-
Enzymes	kg	1.19	Alcalase and Flavourzyme mixture
Chitosan	kg	0.08	-
Electricity and Utilities			
Electricity	kWh	238.79	Includes all equipment
Steam	t	0.25	For the distillation Column
Transport			
Transport, road	tkm	3.93	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. - value too small

5.3.2.6 Scenario P1

In this scenario (Figure 61 and Table 91), 408.2 tonnes per year of microalgae biomass are produced. This biomass is harvested and afterwards ruptured. The amount of ruptured biomass is 345.5 tonnes per year. The extraction of the ruptured biomass produces three products: 6.4 tonnes per year of EPA+DHA soap, 1881.0 m^3 per year of a protein hydrolysate and 3.4 tonnes per year of carotenoid mixture.

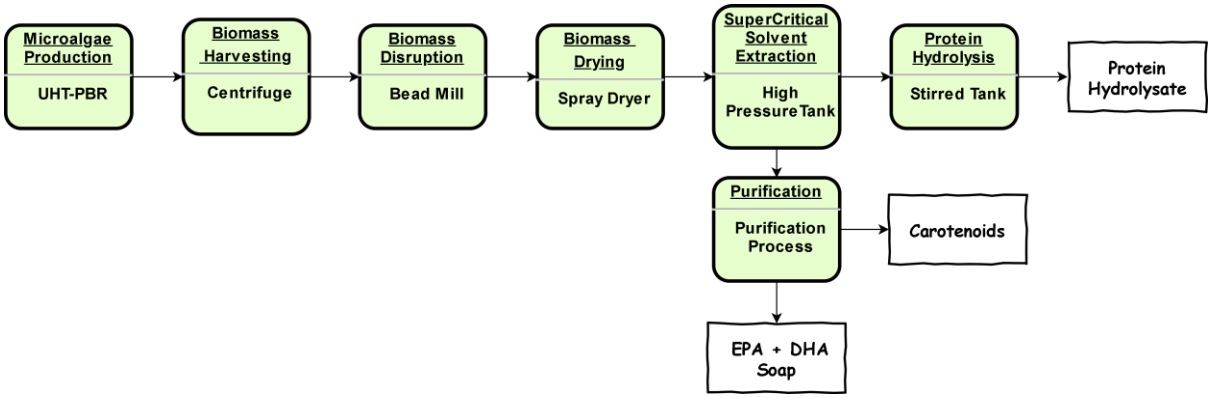

Figure 61 - Scenario P1 process diagram.

Table 91 - Scenario P1 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
EPA+DHA Soap	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	5.26	No environmental burdens considered since values under limit. Disposed to sewage
Amino acid Hydrolysate	m^3	291.67	Final Product
Carotenoid Mixture	kg	0.52	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.78	For 20 years
Cast Iron	kg	v.t.s.	For 20 years
Borosilicate Glass	kg	5.07	For 20 years
Wood (any type)	kg	0.51	For 20 years
Nitrile Rubber	kg	0.04	For 20 years
Polypropylene	kg	0.07	For 20 years
Polypropylene	kg	v.t.s.	Used in the Storage Bags
Polyethylene	kg	14.55	Used in the Storage Barrels
Chemicals			
Water	m^3	35.93	Used for production, cooling and extraction
Sodium thiosulfate	kg	0.23	-
$NaNO_3$	kg	34.01	-
NaH_2PO_4	kg	2.88	-
CO_2	t	0.18	-
NaCl	kg	75.07	
Heptane (m3)	m^3	v.t.s.	-
Ethanol (m3)	m^3	v.t.s.	-
NaOH	kg	0.15	Used in the purification process
Mg(OH)2	kg	0.11	Used in the purification process
Cleaning agent	m^3	0.01	-
Enzymes	kg	12.10	Alcalase and Flavourzyme
Electricity			
Electricity	kWh	753.86	Includes all equipment
Transport			
Transport, road	tkm	57.34	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. – value too small

5.3.2.7 Scenario P2

In this scenario (Figure 62 and Table 92), 408.2 tonnes per year of microalgae biomass are produced. This biomass is harvested and ruptured, and the amount of ruptured biomass is 364.7 tonnes per year. The biomass goes through an extraction process producing 6.8 tonnes per year of EPA+DHA Soap but also producing 3.7 tonnes per year of carotenoid and 1985.5 m^3 per year of a protein hydrolysate stream.

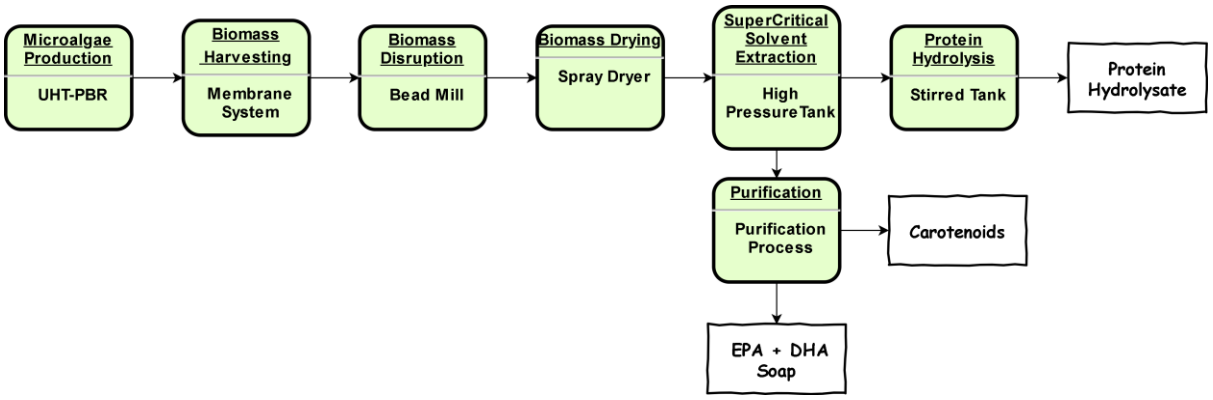

Figure 62 – Scenario P2 process diagram.

Table 92 - Scenario P2 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
EPA+DHA soap	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	5.11	No environmental burdens considered since values under limit. Disposed to sewage
Amino acid Hydrolysate	m^3	291.67	Final Product
Carotenoid mixture	kg	0.52	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.66	For 20 years
Titanium Alloy	kg	v.t.s.	For 20 years
Borosilicate Glass	kg	4.80	For 20 years
Wood (any type)	kg	0.49	For 20 years
Nitrile Rubber	kg	0.04	For 20 years
Polypropylene	kg	0.06	For 20 years

Exchanges	Unit	Amount	Comments
Polyethersulfone (PES)	kg	0.01	For 2 years
Polypropylene	kg	v.t.s.	Used in the Storage Bags
Polyethylene	kg	14.55	Used in the Storage Barrels
Chemicals			
Water	m^3	34.88	Used for production, cooling and extraction
Sodium thiosulfate	kg	0.22	-
$NaNO_3$	kg	32.22	-
NaH_2PO_4	kg	2.73	-
CO_2	ton	0.18	-
NaCl	kg	71.11	-
Heptane (m3)	m^3	v.t.s.	-
Ethanol (m3)	m^3	v.t.s	-
NaOH	kg	0.15	-
$Mg(OH)_2$	kg	0.11	-
Cleaning agent	m^3	0.01	-
Enzymes	kg	12.10	Alcalase and Flavourzyme
Electricity			
Electricity	kWh	821.42	Includes all equipment
Transport			
Transport, road	tkm	56.81	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. – value too small

5.3.2.8 Scenario P3

In this scenario (Figure 63 and Table 93) 408.2 tonnes per year of microalgae biomass are produced. This biomass is harvested and ruptured. The amount of ruptured biomass is 139.1 tonnes per year. The extraction process performed on the ruptured biomass produces 13.9 tonnes per year of EPA + DHA soap along with 1145.4 m^3 per year of a protein hydrolysate and 176.3 ton per year of carbohydrate feed.

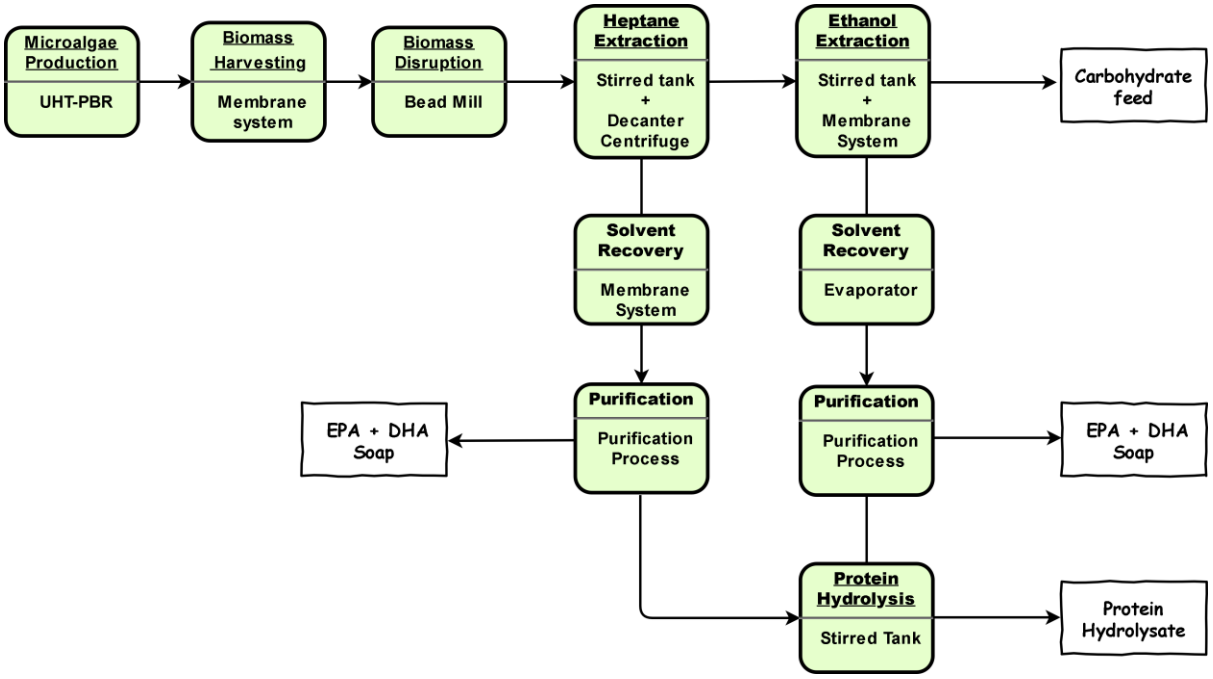

Figure 63 - Scenario P3 process diagram.

Table 93 - Scenario P3 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
EPA+DHA Soap	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	3.87	No environmental burdens considered since values under limit. Disposed to sewage
Amino acid Hydrolysate	m^3	82.34	Final Product
Carbohydrate Feed	kg	12.68	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.41	For 20 years
Borosilicate Glass	kg	2.35	For 20 years
Wood (any type)	kg	0.24	For 20 years
Nitrile Rubber	kg	0.02	For 20 years
Polypropylene	kg	0.03	For 20 years
Polyethersulfone (PES)	kg	0.01	For 2 years
Polyethylene	kg	0.02	Used in storage bags
Polypropylene	kg	4.11	Used in the Storage Barrels
Chemicals			
Water	m^3	17.01	Used for production, cooling and extraction

Exchanges	Unit	Amount	Comments
Sodium thiosulfate	kg	0.11	-
$NaNO_3$	kg	15.77	-
NaH_2PO_4	kg	1.33	-
CO_2	t	0.09	-
NaCl	kg	34.80	-
Heptane (m3)	m^3	0.01	-
Ethanol (m3)	m^3	0.04	-
NaOH	kg	0.15	Used in the purification process
$Mg(OH)_2$	kg	0.11	Used in the purification process
Cleaning agent	m^3	0.00	-
Enzymes	kg	2.51	Alcalase and Flavourzyme
Electricity			
Electricity	kWh	463.14	Includes all equipment
Transport			
Transport, road	tkm	31.36	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

5.3.2.9 Scenario P4

In this scenario (Figure 64 and Table 94), 408.2 tonnes per year of microalgae biomass are produced and the biomass is then harvested and ruptured, producing 345.5 tonnes per year of ruptured biomass. The extraction of components from the ruptured biomass produces 8.1 tonnes per year of EPA+DHA Soap, also producing 1295.6 m^3 per year of a protein hydrolysate and 55.1 tonnes per year of protein concentrate.

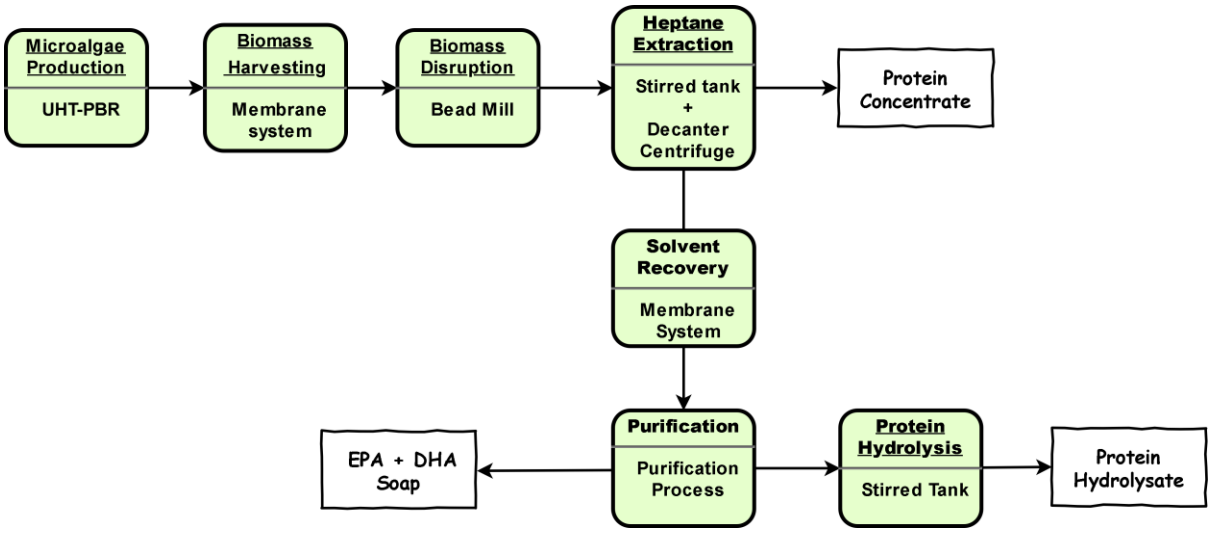

Figure 64 – Scenario P4 process diagram.

Table 94 - Scenario P4 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
EPA+DHA Soap	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	6.51	No environmental burdens considered since values under limit. Disposed to sewage
Amino acid Hydrolysate	m^3	159.2	Final Product
Protein Concentrate	kg	6.77	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.63	For 20 years
Borosilicate Glass	kg	4.02	For 20 years
Wood (any type)	kg	0.41	For 20 years
Nitrile Rubber	kg	0.04	For 20 years
Polypropylene	kg	0.05	For 20 years
Polyethersulfone (PES)	kg	0.01	For 2 years
Polypropylene	kg	v.t.s.	Used in Storage bags
Polyethylene	kg	7.94	Used in the Storage Barrels
Chemicals			
Water	m^3	28.79	Used for production, cooling and extraction
Sodium thiosulfate	kg	0.19	-
$NaNO_3$	kg	26.95	-
NaH_2PO_4	kg	2.28	-
CO_2	t	0.15	-
NaCl	kg	59.49	
Heptane (m3)	m^3	0.01	-
NaOH	kg	0.24	Used in the purification process
$Mg(OH)_2$	kg	0.18	Used in the purification process
Cleaning agent	m^3	v.t.s.	-
Enzymes	kg	9.11	Alcalase and Flavourzyme
Electricity			
Electricity	kWh	418.91	Includes all equipment
Transport			
Transport, road	tkm	45.50	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. - value too small

5.3.2.10 Scenario P5

In this scenario (Figure 65 and Table 95), 408.2 tonnes per year of microalgae biomass are produced. This biomass is harvested and afterwards ruptured producing 368.4 tonnes per year of ruptured biomass. The extraction process performed on the ruptured biomass produces 14.5 tonnes per year of EPA+DHA Soap, along with 1193.1 m^3 per year of a protein hydrolysate and 183.7 tonnes per year of Carbohydrate feed.

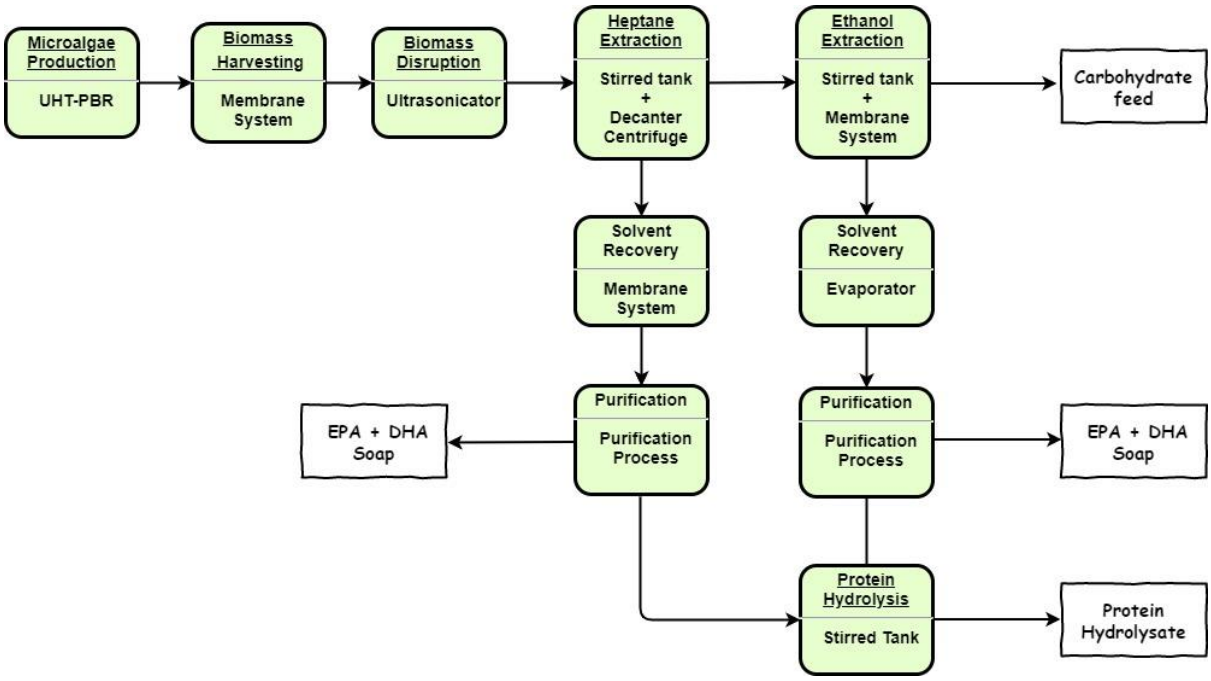

Figure 65 – Scenario P5 process diagram.

Table 95 - Scenario P5 LCA Global Inventory.

Exchanges	Unit	Amount	Comments
Output of products/services:			
EPA+DHA Soap	kg	1	Reference Flow
Output of by-products:			
Wastewater	m^3	3.81	No environmental burdens considered since values under limit. Disposed to sewage
Amino acid Hydrolysate	m^3	82.28	Final Product
Carbohydrate Feed	kg	12.69	Final Product
Input of products/services:			
Equipment Material			
Stainless steel 316L	kg	0.39	For 20 years
Titanium Alloy	kg	v.t.s.	For 20 years

Exchanges	Unit	Amount	Comments
Borosilicate Glass	kg	2.26	For 20 years
Wood (any type)	kg	0.23	For 20 years
Nitrile Rubber	kg	0.02	For 20 years
Polypropylene	kg	0.03	For 20 years
Polyethersulfone (PES)	kg	v.t.s.	For 2 years
Polypropylene	kg	0.02	Used in Storage Bags
Polyethylene	kg	4.11	Used in the Storage Barrels
Chemicals			
Water	m^3	16.34	Used for production, cooling and extraction
Sodium thiosulfate	kg	0.10	-
$NaNO_3$	kg	15.14	-
NaH_2PO_4	kg	1.28	-
CO_2	t	0.08	-
NaCl		33.41	
Heptane (m3)	m^3	0.01	-
Ethanol (m3)	m^3	0.04	-
NaOH	kg	0.15	Used in the purification process
$Mg(OH)_2$	kg	0.11	Used in the purification process
Cleaning agent	m^3	0.01	-
Enzymes	kg	2.51	Alcalase and Flavourzyme
Electricity			
Electricity	kWh	457.37	Includes all equipment
Transport			
Transport, road	tkm	30.29	Ecoinvent dataset: Transport, freight, lorry 16-32 metric ton

*v.t.s. – value too small

5.4 Life Cycle impact assessment

Based on the data gathered in the life cycle inventory phase, the values of the environmental impacts of the process system were quantified. The calculated values can serve various purposes as, for example, the identification of the process environmental hotspots, support decision making concerning process adjustments and/or improvements, among other. This stage always involves two parts, done sequentially: the definition of the environmental impact categories deemed relevant for the product/process system under study, and the calculation of respective indicators using an adequate environmental impact assessment methodology. As there are no consensual or universal applicable environmental

categories and impact assessment methodologies, depending on the study goals and objectives, both parts are usually considered simultaneously. In the following sub sections both steps are considered separately.

5.4.1 Environmental Impact Categories

Several issues are relevant when selecting the environmental impact categories adequate for a given process system. In particular, they should be representative of the expected main environmental impacts, which are dependent on the system material and energy consumptions and emissions. Further, the environmental indicators representing the impact categories should be consensual as much as possible, and calculable using proper and science based environmental impacts assessment methodologies. Moreover, if the results of the study are compared with other studies available in the literature, the same environmental impact categories, indicators, and impact evaluation methodologies should be used, to ensure an objective comparison between studies.

The starting point to select the relevant environmental impact categories is a qualitative analysis of the process operational conditions and of the inventory data, in particular what are main energy and material consumptions and flows between the system and the exterior, coupled with and understanding of the currently seen as the most important environmental issues. Following the system description made before, the main system inputs and outputs are:

- Energy consumption, in particular electricity to power the pumps that make the fluids move inside the photobioreactors or operate the harvesting, cell rupture and extraction processes;
- Water, either for microalgae cultivation or wastewater;
- Nutrients for microalgae cultivation.

As currently the burning of fossil resources (oil, coal and natural gas) are the dominant sources of energy, environmental impact categories directly related to energy production and generation using fossil fuels should be considered. Thus, Climate Change (CC) is a consensual indicator, as it is a direct measure of the process energy efficiency and greenhouse emissions. Likewise, as fossil fuels are produced from non-renewable resources, they will contribute to resource depletion. Furthermore, the production of the reactants and other materials needed to operate the process system also uses other non-renewable resources, in particular minerals, thus supporting the inclusion of an environmental impact category directly related to mineral and fossil resources consumption and depletion.

Additionally, the combustion of fossil fuels generates pollutants, in particular NOx, SOx, unburned fuel, and particulate matter that have not only potential environmental impacts but also potential negative effects in human health. To take into account the last aspect, the Human Toxicity environmental impact category was chosen. NOx and SOx are acid gases that in contact with water form strong acids, that lowers the rain and water bodies' pH, with potential negative impacts, in ecosystems, buildings, agriculture, among others. This justifies the inclusion of an acidification potential environmental impact category. When they interact with particulate and unburned fuel in the atmosphere in the presence of ultraviolet (UV) radiation, they can generate other organic compounds that can contribute to smog generating and/or negatively impacting human health and the environment, supporting the inclusion of a human and ecotoxicity impact categories. NOx represents also a nitrogen source that when reaches the soil and bodies of water, in particular lakes, will contribute to biomass growth, contributing to their eutrophication, validating the need for a specific indicator for eutrophication potential, either water or terrestrial. NOx in the troposphere also reacts with ozone in the presence of UV radiation and contributing to its depletion, thus sanctioning the inclusion of an ozone depletion environmental impact category.

Nutrients are also produced using energy that comes mainly from fossils resources, so their production will also have impacts in the categories considered above. In addition, the generation of wastewater with a high organic load, typical of a bio-based process, also contributes to eutrophication. More specific to the process system under study, water consumption is also a relevant environmental indicator, as microalgae cultivation normally requires large quantities of fresh water.

The environmental impact categories selected in this work were a combination of categories from the ReCiPe methodology (Huijbregts et al., 2016), and CML methodology (Frischknecht et al., 2007) since they are the most used methods in other LCA studies on microalgae processing (Collet et al., 2013; Collotta et al., 2017). This allows a more objective comparison between the results of this work with other studies, as ensures that a more objective comparison can be made. Table 96 presents the environmental categories of both methodologies, obtained from the ILCD handbook (European Commission Joint Research Centre Institute for Environment and Sustainability, 2010) and the work by Acero et al. (Acero et al., 2014). Highlighted in green are the categories shared by both methods, and those were the environmental categories considered in this work.

Table 96 - CML and ReCiPe Impact factors (European Commission Joint Research Centre Institute for Environment and Sustainability, 2010).

Impact Category	CML	RECIPE
Climate change	X	X
Ozone depletion	X	X
Respiratory inorganics		X
Human toxicity	X	X
Ionising radiation		X
Ecotoxicity	X	X
Ozone formation	X	X
Acidification	X	X
Terrestrial Eutrophication	X	X
Land Use		X
Aquatic Eutrophication.	X	X
Resource Consumption	X	X

As the ReCiPe methodology is more recent than the CML methodology, it is expected that it yields more truthful estimates of the environmental impacts. Thus, it was the methodology considered to evaluate the environmental indicators. Further, the ReCiPe also evaluates the water consumption, which is an important aspect of the process system under analysis, as it is based on the cultivation in aqueous media of microalgae. Of the three variants of the ReCiPe methodology, the egalitarian approach was used, as it is the most precautionary perspective, thus being more conservative. Also, it considers the longest time frame of the three perspectives: Individualist, Hierarchist and Egalitarian; and impact types that are not yet fully established but for which some indication is available (Huijbregts et al., 2017).

In Table 97 the environmental impact categories selected in this work are presented, together with the measuring units. Only midpoints were considered in this work. Although the ReCiPe method also considers endpoints, they were not calculated in this work. Besides reducing the number of environmental indicators, with the consequent loss of information, their determination involves the definition of weights in their evaluations, a process that is never completely objective and that is dependent on external information. Thus, a better analysis of results is performed with the midpoints, and even a comparison with results published in the literature, as the midpoint are the values normally reported in the literature or in LCA studies.

An additional environmental impact category, the Primary energy consumption [kWh], was also considered. It represents a measure of the energy consumption and efficiency in the

process system. Although related to other environmental categories, in particular the Global Warming indicator, as renewable energy may be used in the process, is relevant to also calculate it, as it represents a better measure of the system performance in terms of energy consumption.

Table 97 – Mid Point Impact Categories in Recipe Methodology.

Impact Category	Abbreviation	Unit
Global warming	GW	kg CO_2 equivalent
Stratospheric ozone depletion	SOD	kg CFC11 equivalent
Ozone formation, Human health	OF,H	kg NOx equivalent
Terrestrial acidification	TA	kg SO_2 equivalent
Freshwater eutrophication	FE	kg P equivalent
Marine eutrophication	ME	kg N equivalent
Freshwater ecotoxicity	FET	kg 1,4-DCB
Marine ecotoxicity	MET	kg 1,4-DCB
Human carcinogenic toxicity	HCT	kg 1,4-DCB
Mineral resource scarcity	MRS	kg Cu equivalent
Fossil resource scarcity	FRS	kg oil equivalent
Water consumption	WC	m^3

Concerning the water consumption indicator, it corresponds to the blue water consumption (ground water + lake water + river water + fossil ground water but excluding rainwater) according to the water footprint methodology of Hoekstra et al. (2009). This blue water consumption considers freshwater lost to the watershed due to water vaporization to air, evapotranspiration, water incorporation into products, and water release to sea. Therefore, it can be calculated as input of ground water, lake water, river water, and fossil ground water minus total blue water release from Technosphere into rivers or lakes (water outputs).

5.4.2 Calculation of the Environmental Indicators Values

The evaluation of the environmental impact indicators usually requires using specific software, as the calculations are too complex to be done by hand. In this study, all the necessary calculations were done using the LCA software Simapro V8.5.2 PhD version. Due to the linear and simple structure of the process systems of the various scenarios, as presented in section 5.3, that does not include recycling or end of life modelling, instead of modelling the process entirely in the software, a simpler approach was considered in this work.

Since the Simapro software also includes a Life Cycle Inventory (LCI) database, in particular the *EcoInvent* V3.5 database, the environmental impacts were evaluated using impact factors, also known as characterization factors, expressed per unit mass, energy, or other relevant unit. For example, the climate change is expressed in terms of kilograms of CO_2 equivalent per unit mass of a given compound and quantifies the emission of a substance relative to that induced by 1 kg of CO_2.

They can be calculated using LCI data and using the impact assessment methodology implemented in the software. Using the data gathered in the inventory phase, the calculation of the environmental impacts values is done by multiplying the impact factor by the amount of material used, thus calculating the emissions related to the production of the material considered. For transportation, special care must be placed in the calculations, as the impact factors are expressed per tonnes kilometer, tkm. Thus, it is necessary to convert the quantity used into tonnes and multiply the emission factor by the distance travelled and the quantity in tonnes, to calculate the emissions related to transport. For electricity/energy, it is sufficient to multiply the impact factor by the amount of energy used. It was assumed that only electricity is used, otherwise as many impact factors as there are forms of energy used should have been used, considering the specific energy consumption for each form of energy.

Using the information obtained in the Life Cycle Inventory, an assessment was performed in order to quantify the impact each scenario had on the environment. Each scenario was analyzed individually, to identify the steps with the largest contribution to the environmental impacts, followed by a comparison of the different scenarios to identify the one with the lowest impact, and therefore, the most sustainable one. All values can be found in Appendix 13.

5.5 *Synechocystis* based biorefinery scenarios

5.5.1 Production stage

The impact distribution of the production stage is the same for all scenarios, as the production process and equipment are the same for all scenarios. They only differ slightly when comparing the impacts generated by the production of 1 kg of phycocyanin. In this situation, the scenarios with the smallest impact are the S2 and S5 scenario, as these processes produce the biggest amount of phycocyanin and therefore the impact is smaller for each kg of phycocyanin produced.

From *Figure 66*, it can be observed that the biggest contributors to the environmental impacts are the nutrients used ($NaNO_3$ and NaH_2PO_4), water and electricity used to power

the pumps and other auxiliary systems. These observations corroborate the information obtained in previous LCA studies performed on the topic (Schneider et al., 2018; Taelman and Sfez, 2015). In most categories, the largest contributor are the nutrients used. Most of the nutrients used in microalgae production are produced in a way similar to that of fertilizers. And as well known, fertilizer production has severe impact on environment, especially as it comes from mineral sources (Basosi et al., 2014; Lenka et al., 2016). Microalgae production also requires large amount of water for cultivation. As the water is treated and the chemicals used in the treatments also have impact on the environment (Zijp and van der Laan, 2015), water represents an important environmental impact. Finally, yet importantly, the process also requires large amount of electricity. In this situation, the impact depends on the electricity mix. In Portugal the mix is almost half renewable energies, half fossil fuels (EDP, 2019). The transportation of equipment and equipment production do not have a large impact, as the equipment is considered to have a 20 years life span and therefore the impacts are distributed throughout those 20 years. As their impact is lower than 1%, they have not been considered. Unlike all other components, as the CO_2 used is a waste from another industry, it has a positive effect in the Global Warming impact.

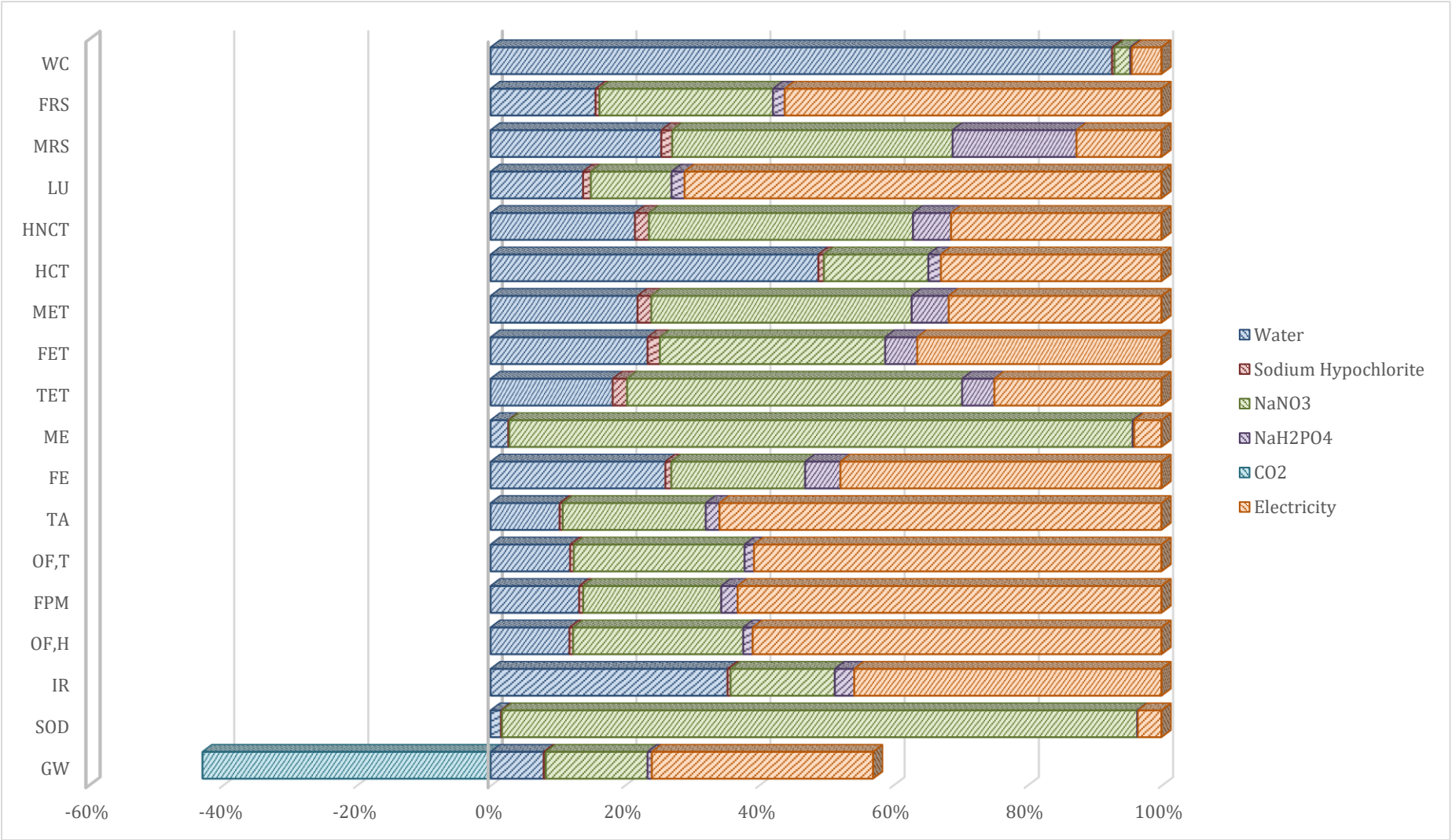

Figure 66 – Eco-Profile of all Scenarios Production stage at midpoint categories.

5.5.2 Harvesting and rupture stage

In the harvesting and rupture stage of all scenarios (Figure 67), except scenario S3 (Figure 68), the distribution of the impacts is similar. One of the biggest contributors to all impacts is the cleaning agent, through the components NaOH, EDTA and KOH. Of these 3 components, the highest impact comes from NaOH, where electricity and raw salt production account for over 90% of the overall environmental burden (Hong et al., 2014; Thannimalay et al., 2013). On the other hand, although the treated wastewater has a negative impact on most categories, due to the chemicals and energy required to treat the water (Raghuvanshi et al., 2017), it has a positive impact on the water consumption, as it returns treated water to the environment. In the case of global warming, the biggest impact comes from the recovery of the ethanol. In scenarios S1 and S2, this impact comes from the electricity required by the pervaporation membrane and in scenario S4 and S5 from the steam required by the distillation column and electricity consumed by the pervaporation membrane. While the electricity impact is explained by the use of fossil fuels, steam has high impact due to the release of CO_2 into the atmosphere during production, due to the combustion of natural gas, as well as a large energy consumption to produce the high temperatures required in the process (Amran et al., 2017; Usubharatana and Phungrassami, 2018).

When looking at the impact of each scenario per kg of phycocyanin produced, the 4th scenario has a slightly smaller impact, due to the size of the harvesting equipment (centrifuge), which requires less cleaning agent.

In the harvesting and rupture stage of scenario S3 (*Figure 68*), the three biggest contributors to all impacts (except water consumption and global warming) are chitosan, used in the flocculation step, cleaning agent, through the components NaOH, EDTA and KOH, and the wastewater treatment. In the case of chitosan, most of the negative effects come from the production process, especially due to energy consumption and the use of NaOH and HCl in the process. However, the utilization of chitosan also has a positive effect in the acidification impact category. This effect comes from avoiding the ammonia emissions associated to composting of the crab shells (Muñoz et al., 2018). Again, the wastewater negative effects come from the chemicals and energy required for the treatment. The treated wastewater has a positive impact on the water consumption, as it returns treated water to the environment. The global warming impact and fossil resources depletion factors main contributor is again the steam used in the distillation column.

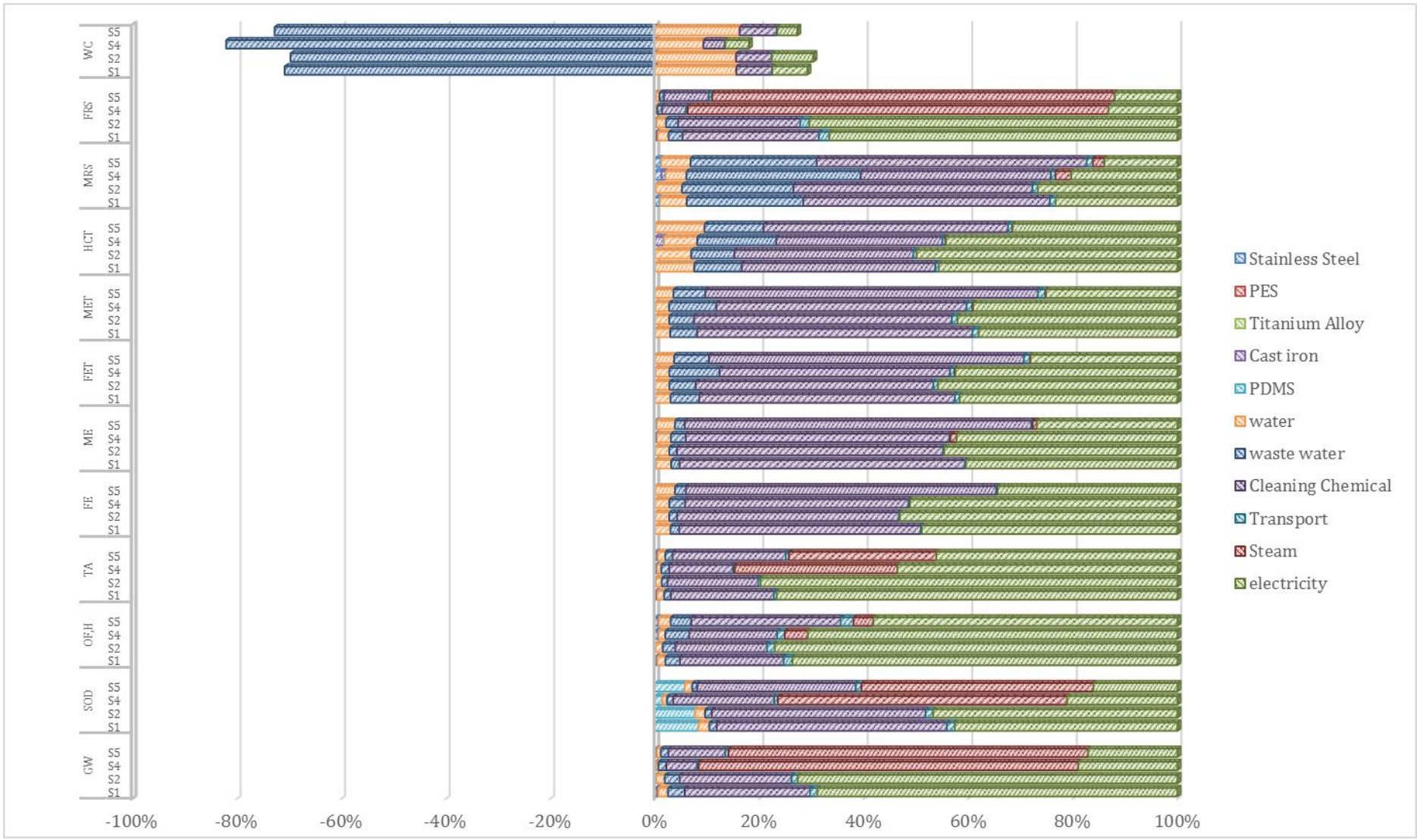

Figure 67 – Eco-Profile of Scenario S1, S2, S4 and S5 harvesting and rupture stage at midpoint categories.

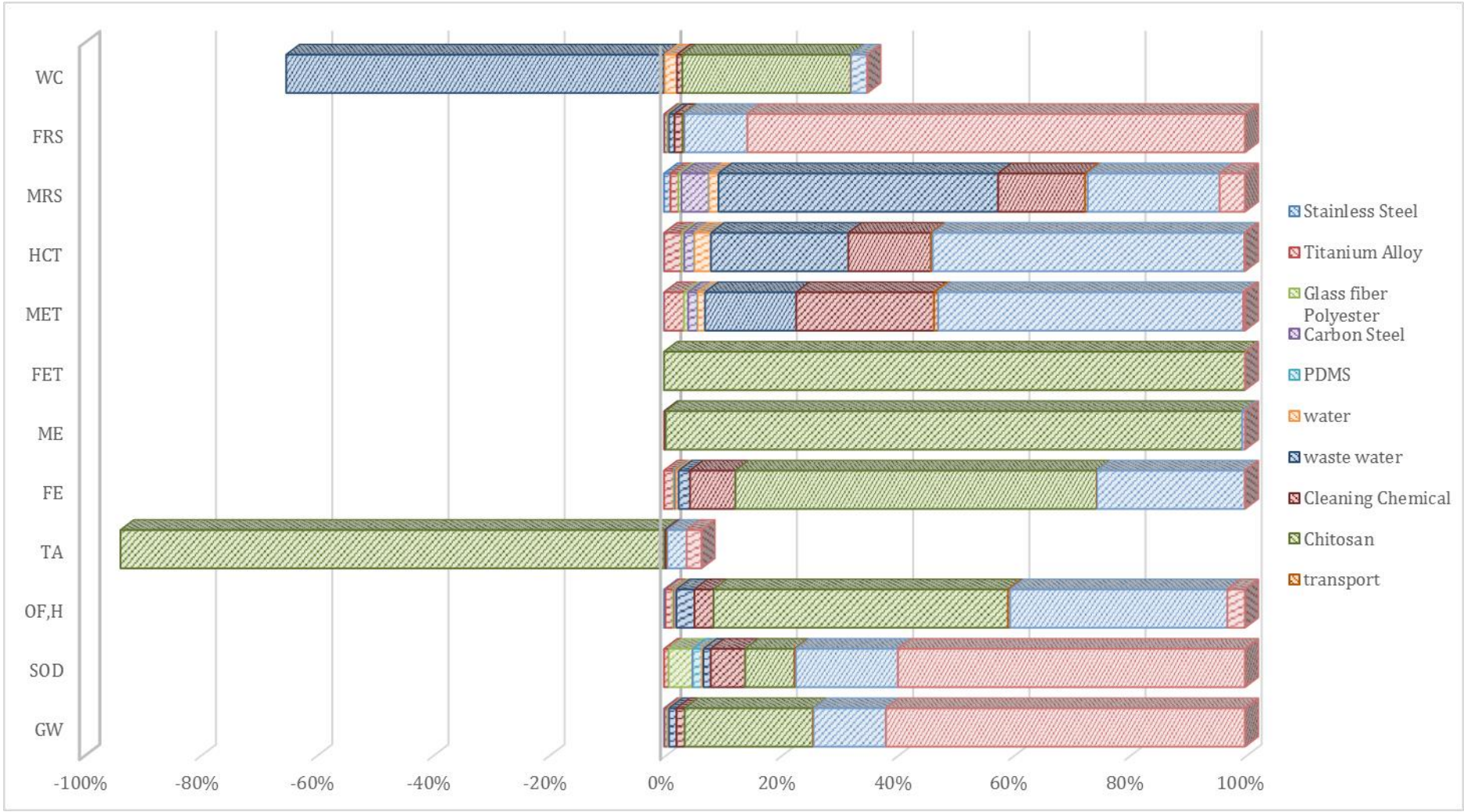

Figure 68 - Eco-Profile of Scenario S3 harvesting and rupture stage at midpoint categories.

5.5.3 Extraction Stage.

In the extraction stage of scenarios S1, S3, S4 and S5 (Figure 69), the environmental impact main contributors are the same, since the extraction process of these scenarios is identical. The highest contributors are enzymes, chitosan, polyethylene storage containers and electric energy. In the first case (enzymes), most of the negative impact comes not only from the production and fermentation processes (Nielsen et al., 2007), but also from the cleaning processes, as the equipment has to be maintained aseptic and sterile (Feijoo et al., 2017). In the case of chitosan, most of the negative impact comes from the production process, especially from the energy consumption and the use of NaOH and HCl in the process. The positive effect caused by chitosan in the acidification impacts comes from avoiding the ammonia emissions associated to composting of the crab shells (Muñoz et al., 2018). The energy high impact is mostly due to the high energy consumption of the spray dryer used to produce the phycocyanin powder. With a smaller influence, the water used in the extraction of phycocyanin also has some impact, especially in the water consumption impact category (Zijp and van der Laan, 2015). Polyethylene, used for the storage bags, also has a small negative influence on the impact factors, especially in the fossil resources scarcity, as currently most is produced from crude oil and requires large amounts of energy (Liptow and Tillman, 2012). The main difference between the scenarios is due to the harvesting step. As in scenario S4 harvesting step is done by centrifugation, the final concentration is higher than that achieved by all the other harvesting methods. Therefore the equipment required for extraction processing is smaller, leading to smaller consumption of energy, chemicals and consumables.

In the extraction stage of scenario S2 (Figure 70), the highest contributor (negative and positive) to the impact factors is the ethanol, used as solvent in the lipid extraction step. Although ethanol used in this process is made from rye, it still carries a high environmental impact. The largest impact of ethanol production is due to the fermentation stage, owing to production impacts of the enzymes used (discussed further down this paragraph), and high energy consumption (Aroca et al., 2013; Borrion et al., 2012). Another contributor, but in smaller scale, is the use of yeasts. In this case, most of the impact comes from the production and fermentation processes (Nielsen et al., 2007), but also from the cleaning processes, as the equipment has to be maintained aseptic and sterile (Feijoo et al., 2017).

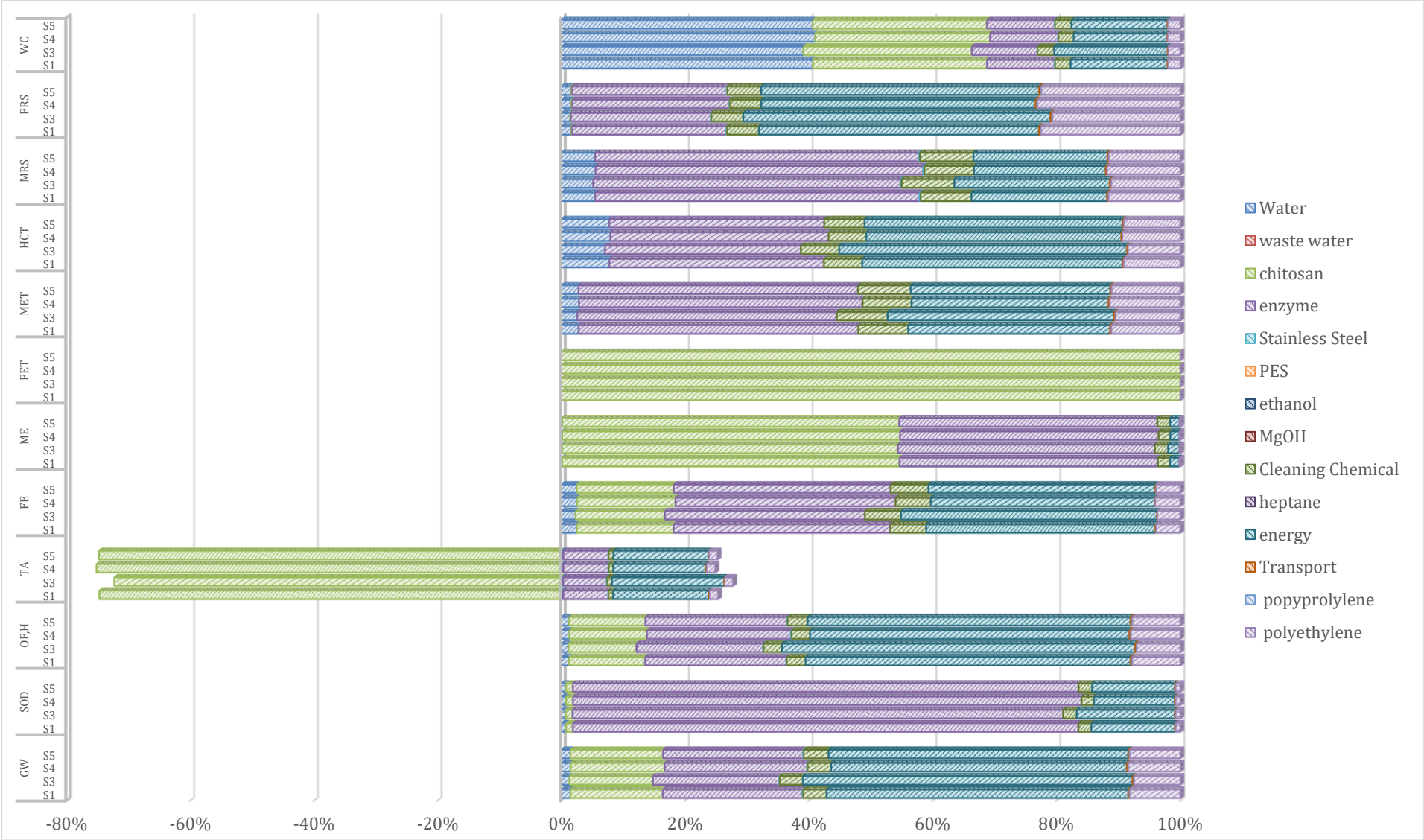

Figure 69 - Eco-Profile of Scenario S1, S3, S4 and S5 extraction stage at midpoint categories.

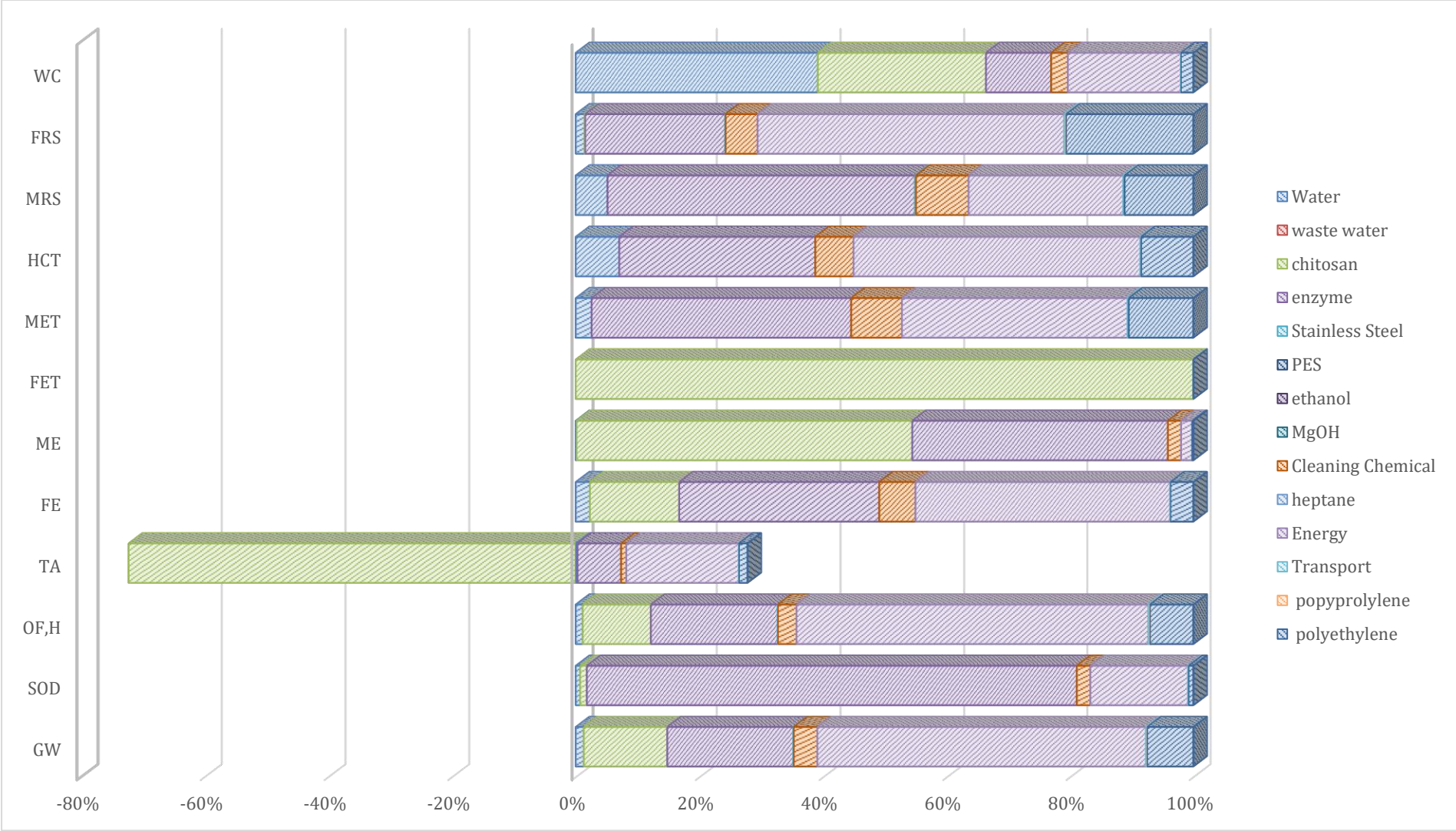

Figure 70 - Eco-Profile of Scenario S2 extraction stage at midpoint categories .

In some impact factors, chitosan also plays a small role. With chitosan, most of the negative impact comes from the production process, especially from energy consumption and the use of NaOH and HCl. However, chitosan has a positive effect in the acidification impact category, due to avoiding the ammonia emissions associated to composting of the crab shells. (Muñoz et al., 2018). Finally, yet still important, the energy consumed, especially during the purification phase. The polyethylene storage containers and the cleaning chemicals also play an important role in this scenario.

5.5.4 Stage comparison

5.5.4.1 Scenarios S1 and S5

Analyzing Figure 71, what can be observed is that the production stage has the largest contribution to all impact factors except land use, terrestrial ecotoxicity and terrestrial acidification. These results are comparable to those obtained in previous LCA studies (Sun et al., 2019; Togarcheti et al., 2017).

Looking into the highest contributors for the environmental impacts with more detail, it can be observed that water, nutrients and electricity used for the production of microalgae have the biggest impact on most factors. These results validate the reason why the production stage is the one with the highest impacts. However, the effect due to the use of chitosan in the extraction stage, on impact categories such as fresh water ecotoxicity and land use is very high. Other contributors to all impacts, but with smaller roles, are the enzymes used in the extraction stage, water and electricity (used in the remaining stages), and finally the cleaning agents also have some impact, with the cleaning agent used in the harvesting stage being the highest contributor for this stage. In the case of Scenario S5, steam also plays a small role in the global warming and fossil resources scarcity.

However, the terrestrial acidification has a negative value and therefore a positive impact on the environment, as the use of crab shells to produce chitosan avoids the ammonia emissions associated to composting of the crab shells. Furthermore, the consumption of CO_2 by microalgae and the treatment of wastewater have a positive impact on the environmental impact factors.

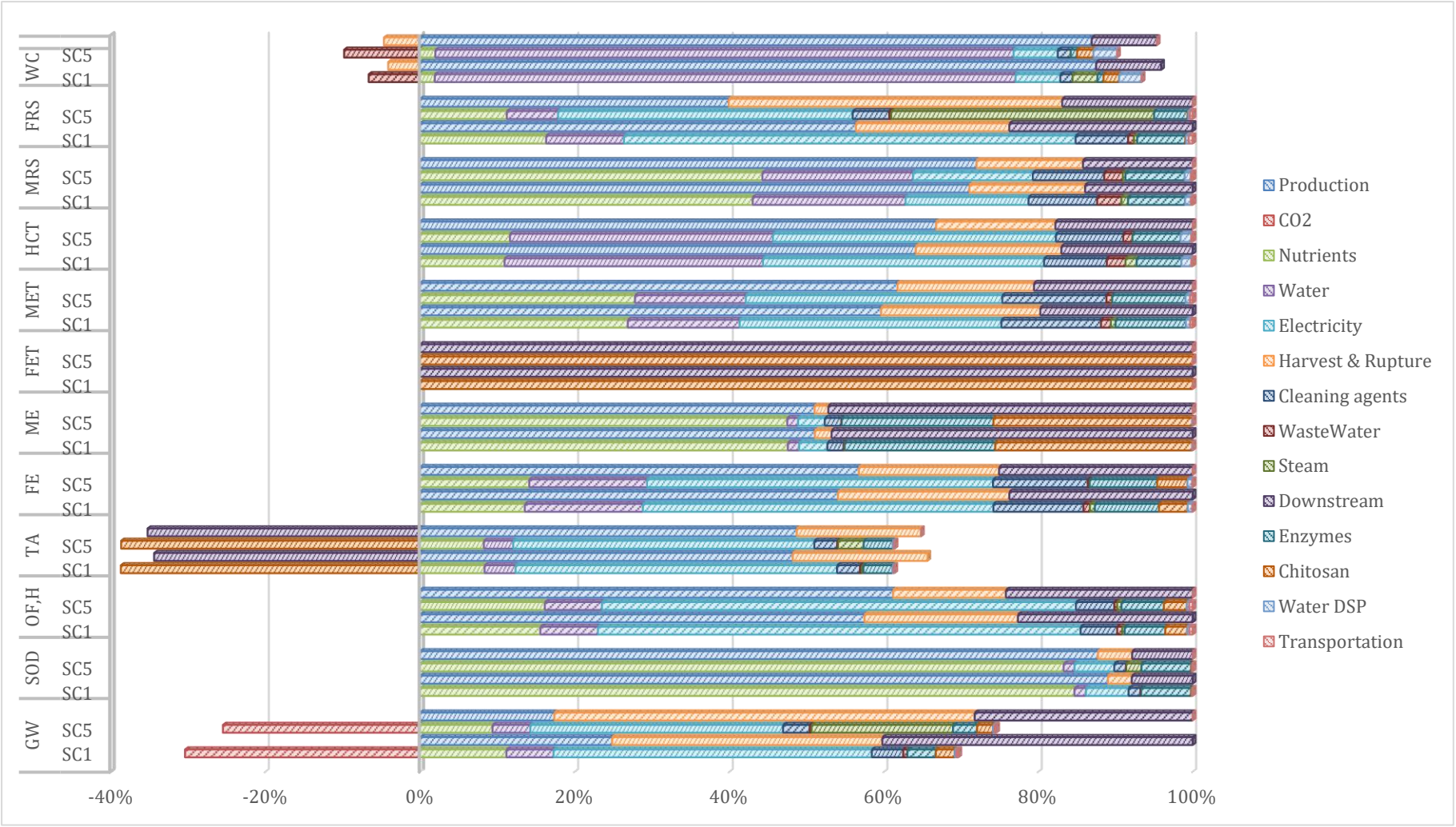

Figure 71 - Eco-Profile of the highest contributors in Scenario S1 and S5 at midpoint categories with the impact of each stage.

5.5.4.2 Scenario S2

As shown in Figure 72, the stage with the highest impact on most categories is again the production stage. However, in this scenario the extraction processing has a higher impact than in the previous scenarios.

Looking closer at the highest contributors, it can be observed that the production stage has a higher impact due to the high contributions of Nutrients, electricity and water used for the production of microalgae. In the extraction stage, the main contributors are the ethanol, along with enzymes, except in the freshwater ecotoxicity and land use, where chitosan plays a major role. It is the use of ethanol that plays an important role in the negative impact of the extraction stage, in this scenario. On the other hand, the cleaning agents are the largest contributor of the harvesting and rupture stage but have a mild impact on the different categories. Once again, some of the global warming effects are mitigated by CO_2 consumption by microalgae (lower global warming), the wastewater treatment returning clean water (lower water consumption) and use of crab shells for chitosan production (lower terrestrial acidification).

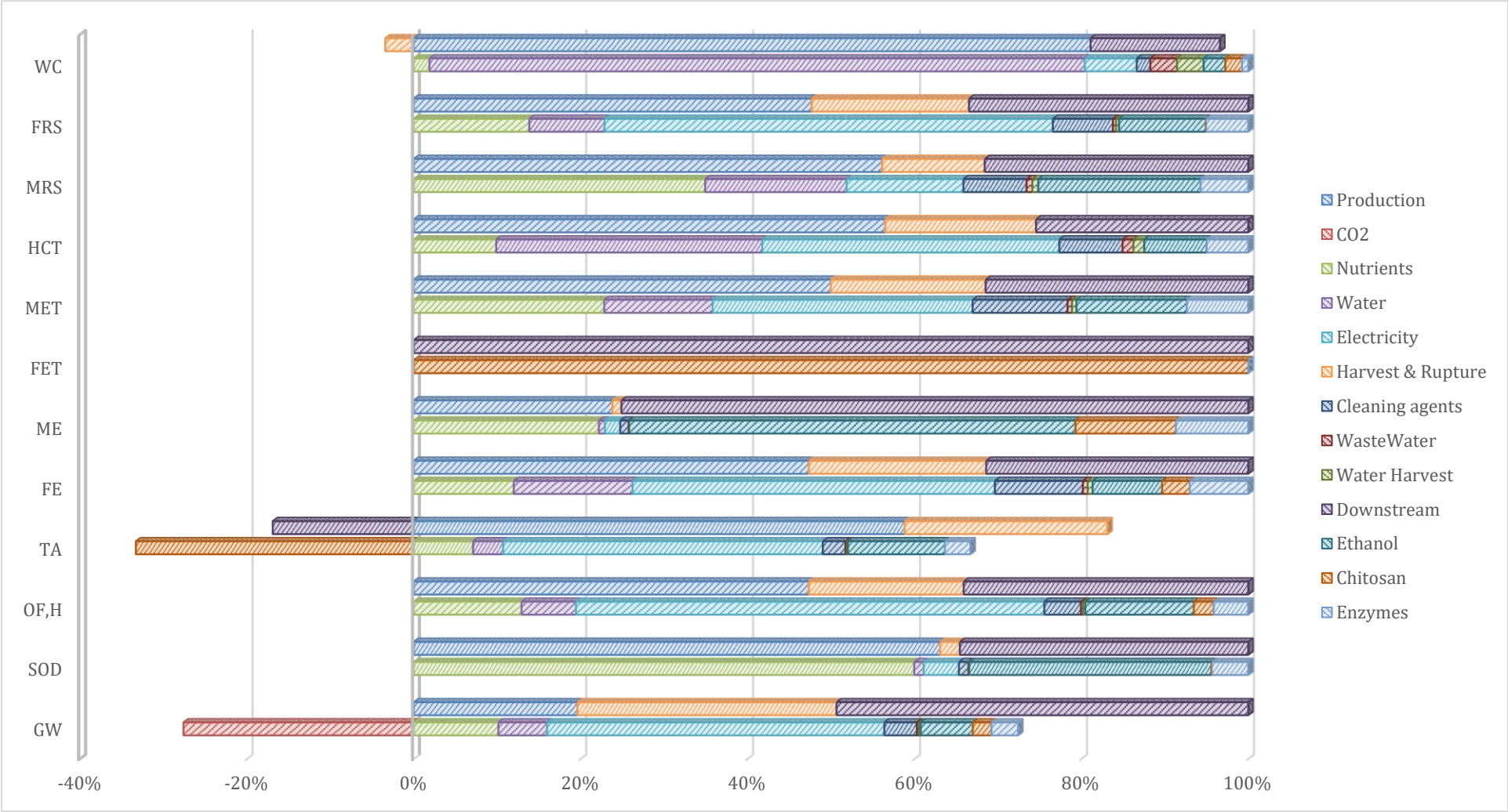

Figure 72 - Eco-Profile of Scenario S2 at midpoint categories with the impact of each stage.

5.5.4.3 Scenario S3

As can be observed in Figure 73 the stage with the highest impacts, as expected, is the production stage. Looking with more detail into the major contributors in this scenario, one can understand that the production impact has the biggest role since the biggest contributors are nutrients, water and electricity. Furthermore, chitosan has also a very large effect on some impact factors. In this case, most of the impact comes from the chitosan used in biomass harvesting. Like in the previous scenarios, chitosan has a positive impact on the terrestrial acidification, even more significant in this scenario as higher amount of chitosan was used. The CO_2 consumption by microalgae mitigates the global warming effect, while wastewater also has a positive impact on the water consumption, as it returns treated water into the environment. Further, the steam used for distillation has a negative impact on the global warming.

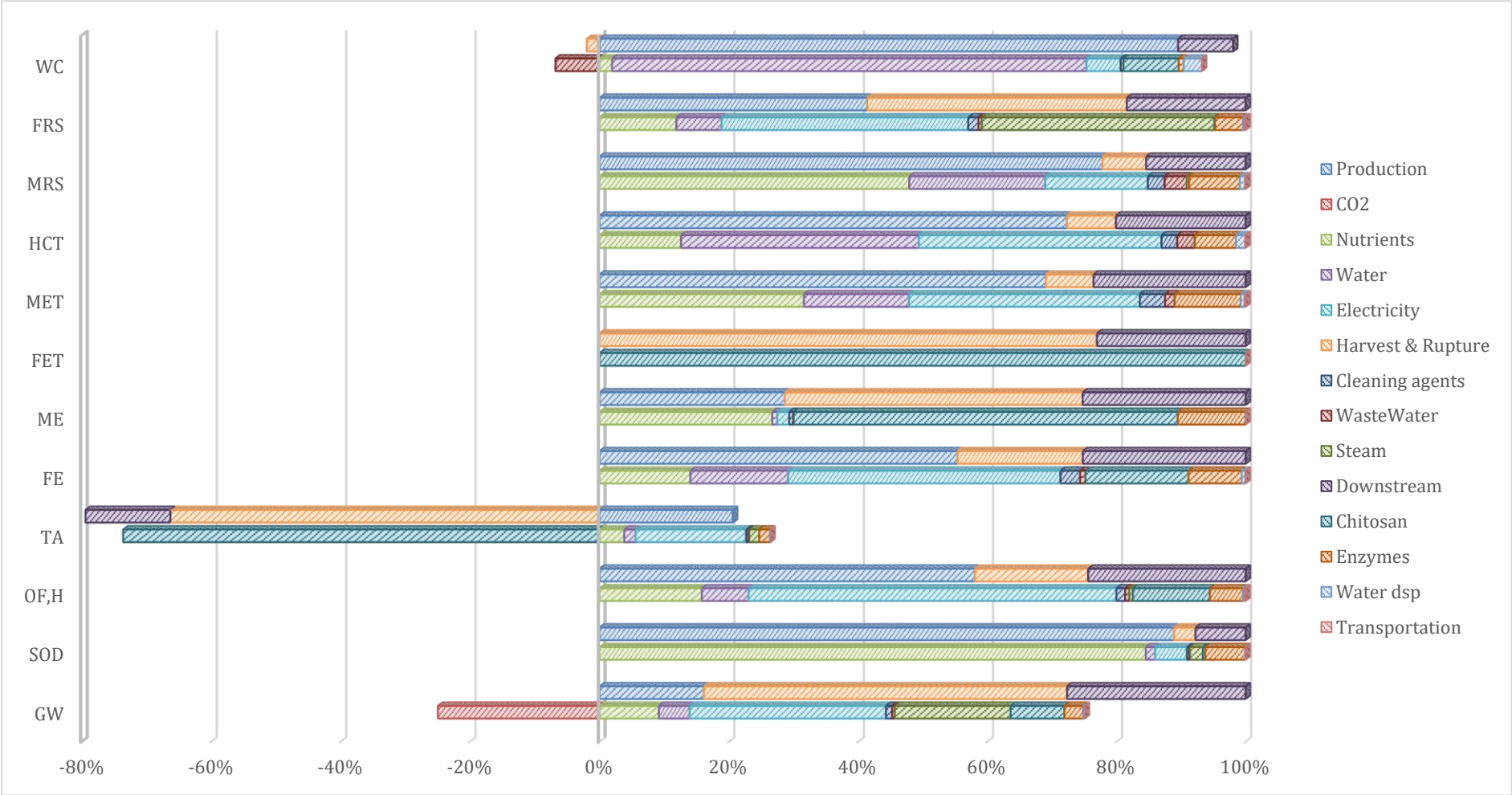

Figure 73 - Eco-Profile of Scenario S3 at midpoint categories with the impact of each stage.

5.5.4.4 Scenario S4

As can be observed in *Figure 74*, the stage with the highest impacts, as expected, is the production stage. When looking with more detail into the major contributors, it is clear why the production step has the biggest impact, as the biggest contributors are nutrients, water and electricity (which is mostly used in the microalgae biomass production stage). Further, it can be seen that chitosan has also a very large impact on some impact factors. In this case, most of the impacts come from the chitosan used in biomass harvesting. Like in the previous scenarios, chitosan has a positive impact on the terrestrial acidification, and in this stage even more as higher amount of chitosan was used. The CO_2 consumption by microalgae mitigates the global warming effects while wastewater also has a positive impact on the water consumption as it returns treated water into the environment.

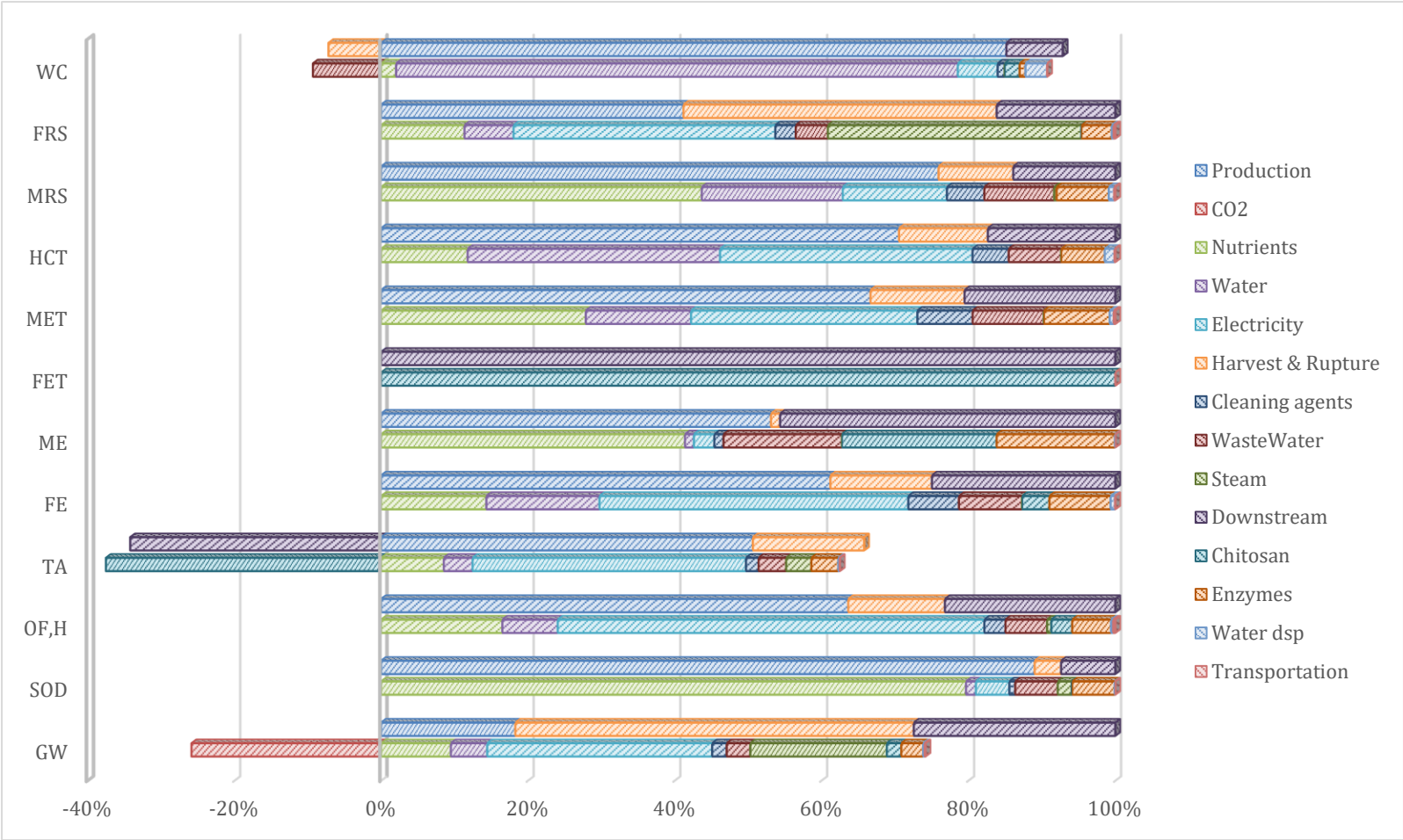

Figure 74 - Eco-Profile of Scenario S4 at midpoint categories with the impact of each stage.

5.5.5 Scenario comparison

After each scenario has been scrutinized, it is necessary to compare the different scenarios to choose the most sustainable. In order to compare the different scenarios and choose the one with the lowest impact, the values were normalized and scenario S1 was considered to be the standard, and all the other scenarios were compared against it (Figure 75). The absolute values of environmental impacts can be found in the Appendix 13.

It is possible to conclude that scenarios S2 and S3 have the highest impacts of all scenarios. In the case of scenario S2, the main contributor to this negative performance is lipid extraction and consequent treatment. The reason for this is that the main contributors are not only the ethanol used in the lipid extraction, but also all the chemicals and energy required to purify the lipids.

In the case of scenario S3 this is mainly due to the use of flocculation as a harvesting method. The flocculation method uses chitosan, which has a high negative impact on most factors, but also this method produces a low concentration stream, which leads to an increase in equipment size, increase in consumables and energy consumption, therefore leading to higher negative impacts.

As the remaining scenarios are very similar, a more in-depth analysis was performed and each stage was separated in order to access which stage has the best performance.

In Figure 76, the impact of each different stage of scenarios S1, S4 and S5 can be observed. The first conclusion is that the production stage has the largest contribution on most scenarios. However, when looking more closely at the production column, it is evident that stage S5 has the lowest impacts. This is due to the higher amount of phycocyanin produced in that scenario, as a membrane is being used for microalgae harvesting and an ultrasonicator was used to rupture the biomass. The difference to scenario S1 is due to the use of a bead mill in this scenario that has slightly lower efficiency in the cell rupture. Scenario S4 has shown the worst performance as it uses a centrifuge, which has a lower harvesting efficiency.

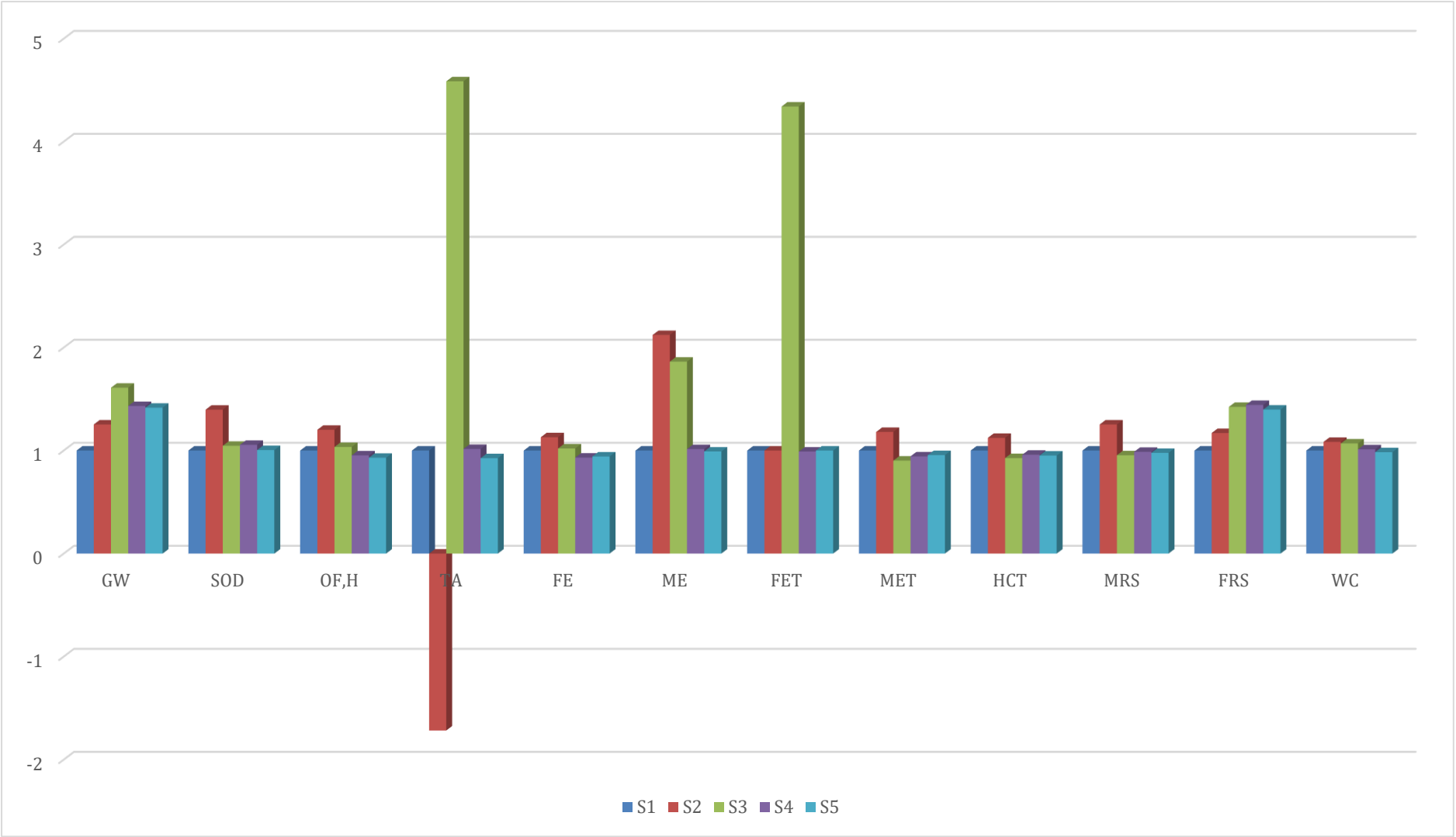

Figure 75 – Normalized Eco-Profile of all scenarios at midpoint categories.

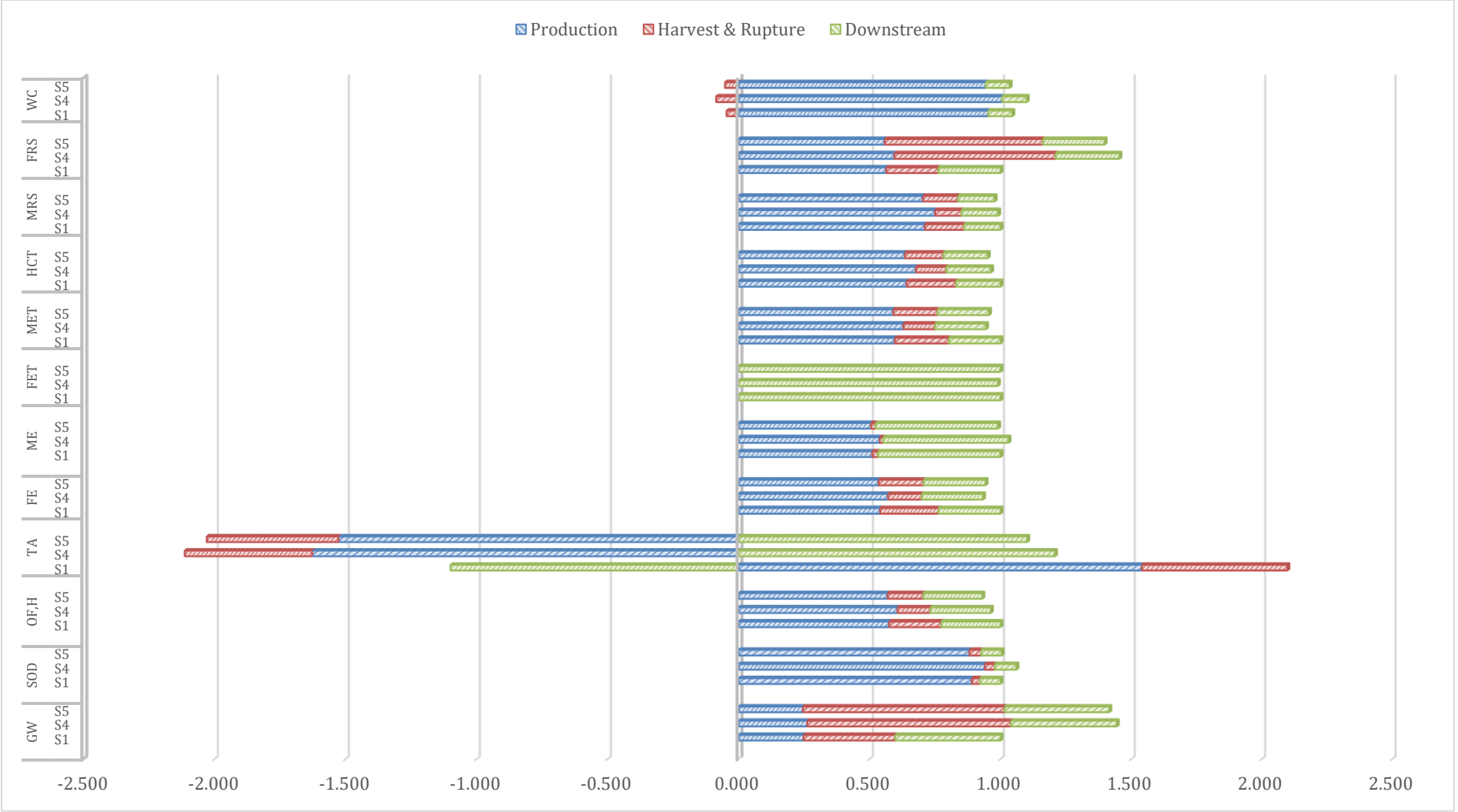

Figure 76 - Normalized Eco-Profile of scenario S1, S4 and S5 stages at midpoint categories.

However, when looking at the harvesting stage, scenario S4 seems to have the best performance, except in the global warming and fossil resources scarcity, due to the use of a centrifuge which requires less cleaning chemicals, but also due to the higher concentration achieved, smaller equipment is used and consequently less consumables. The reason why it has high impacts in the other two factors is due to the use of steam in the distillation process. When comparing scenarios S1 against S5, the main difference is the use of a pervaporation membrane in scenario S1 and a combination of pervaporation and distillation column in scenario S5. Here the difference is due to the energy consumption in scenario S1, which is higher due to the pervaporation membrane, except on the global warming and fossil fuel resources impact, where the steam used in scenario S5 has a much larger weight.

Finally, when looking at the extraction stage, the differences are once again due to the harvesting method. Here, the scenario S4 has a lower impact than the other two scenarios, due to the higher concentration achieved by the centrifuge which in turn leads to lower consumption of consumables like cleaning chemicals, and also the use of smaller equipment and less energy. For the two other scenarios, the main difference is the amount of phycocyanin produced, and the scenario S5 is the winner, as it produces more phycocyanin, so the impact is slightly lower than that of scenario S1.

Thus, when looking at the impact categories of all scenarios, scenario S4 has 2 categories with the lowest impact (IR and FET), scenario S1 also has two categories with the best performance (GW and FRS) and, scenario S5 has 6 categories with the best performance. If one is interested in decreasing the Global Warming and the Fossil resource consumption, then scenario S1 should be the chosen option; however, if one is analyzing the scenarios at an overall perspective, and if all impact categories are weighed equally, then scenario S5 is the best option.

5.5.6 Energy consumption

As shown in Figure 77 and Table 98, and for the previous observations, it is obvious that in all scenarios the stage with the highest energy consumption is the production stage. This is due to the energy required for the pumps, as well as heating and cooling systems. When comparing the scenarios, those with the lowest energy consumption per kg of phycocyanin in the production stage are scenarios S1, S2 and S5, because these are the ones that produce the highest amount of phycocyanin. In the harvesting and rupture stage, scenarios S1 and S2 have the largest energy consumption, due to the energy used by the membrane system in the harvesting of biomass and only the pervaporation membrane as scenarios S3, S4 and S5 use a distillation column that uses steam that is bought directly, so no energy

consumption is required. Furthermore, since scenario S3 uses flocculation, which is a very low energy-consuming step, it has very low energy consumption in the harvesting stage.

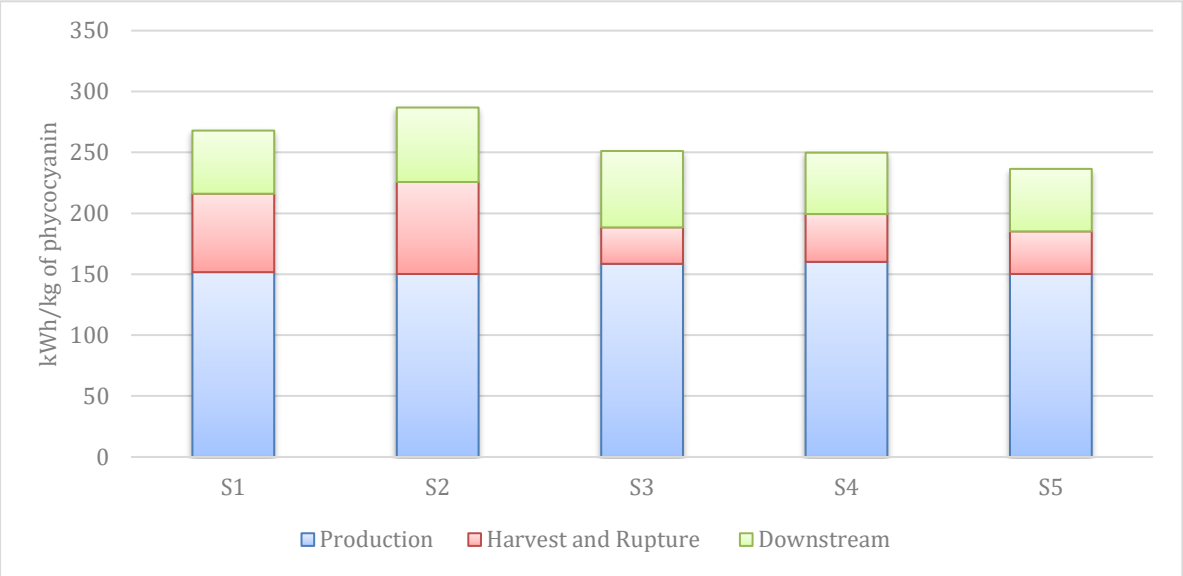

Figure 77 – Total energy consumption per kg of phycocyanin for each scenario.

In the extraction stage, energy consumption occurs mostly during the purification step in scenario S2 and by the spray dryers used in all other scenarios. In the scenarios that use spray dryers, S3 stands out as the largest consumer, which is mostly due to the harvesting step, which although has a lower energy consumption, also has a lower concentration factor, leading to larger equipment and energy consumption.

When comparing all scenarios, it can be concluded (Table 98) that scenario S4 has the lowest energy consumption. This is due to the fact that scenario S4 uses a centrifuge for harvesting, and therefore produces a higher concentration stream, leading to smaller extraction equipment and energy consumption. However, looking at the energy consumption per kg of phycocyanin, Table 99, scenario S5 is the one with the lowest specific consumption, as it produces more phycocyanin than scenario S4. On the other hand, scenarios S1 and S2 have the largest consumption of energy. Energy consumption in scenario S1 is due to the energy spent by the pervaporation system and spray dryer in the extraction processing, while in scenario S2 extra energy is consumed in the extraction and purification of lipids.

Table 98 - Energy consumption (kWh/year) in Synechocystis based biorefinery.

Scenario	S1	S2	S3	S4	S5	unit
Production	2410560	2410560	2410560	2410560	2410560	kWh/year
Harvesting	1023087	1211737	454910	590969	561178	kWh/year
Extraction Proc.	822865	980899	952995	757723	822865	kWh/year
Total	4256512	4603196	3818465	3759252	3794603	kWh/year

Table 99 - Energy consumption per functional unit (kWh/kg FU) in Synechocystis based biorefinery.

Scenario	S1	S2	S3	S4	S5	unit
Production	47	46	49	49	46	kWh/kg PC
Harvesting and Rupture	20	23	9	12	11	kWh/kg PC
Extraction Proc.	16	19	19	15	16	kWh/kg PC
Total	82	88	77	77	73	kWh/kg PC

5.5.7 Blue Water Footprint

For microalgae-based biofuels, the blue water footprint is the sum of the water directly used to supply the cultivation and process needs. In this process, water is required for microalgae cultivation, reactor refrigeration, for cleaning the equipment, for the extraction of phycocyanin and, in scenario S2, for the purification process. Not included are water required for the production of the equipment needed, chemicals and utilities used in the process (indirect water use).

From Figure 78, it is concluded that, except for scenario S2, the production stage in all scenarios has the highest blue water consumption, mostly due to the water required for cooling. The difference in this stage is due to the phycocyanin production. When looking at the Harvesting and Rupture stage, the largest water consumption is due to the water used for cleaning. Here the difference is the size and type of equipment. The flocculation tank requires less cleaning agent, followed by the centrifuge and last by the membrane. In the case of the extraction process, the process that uses higher amount of water is phycocyanin extraction. When comparing the 5 scenarios, one can conclude that all scenarios are very similar in terms of water consumption per kg of phycocyanin. However scenario S5 is the one with the lowest blue water consumption, mostly due to the fact that it is the scenario where the highest amount of phycocyanin is produced.

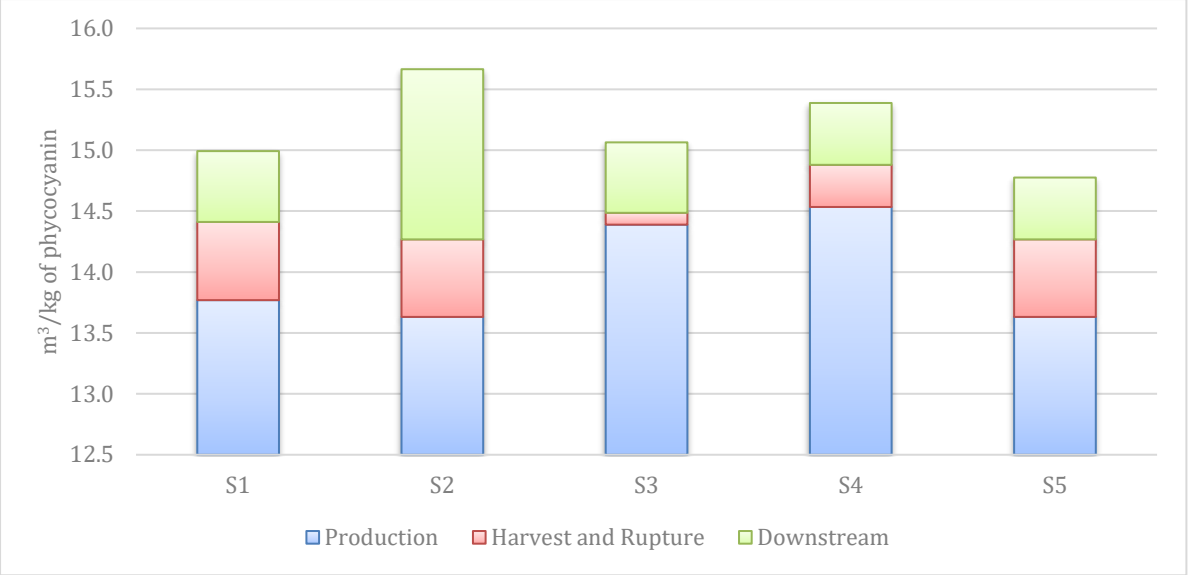

Figure 78 - Blue Water consumption per kg of phycocyanin produced.

Therefore, when looking at both energy and water consumption, it is concluded that they are very similar in all scenarios, and the difference is mostly due to the harvesting method, which not only dictates the amount of phycocyanin produced, but also the size of the following steps and consequent energy and water consumption. Thus, one can also conclude that the harvesting step is the bottleneck of the whole process.

5.6 Discussion on *Synechocystis* based biorefinery scenarios environmental performance

From the previously mentioned factors, ReCiPe midpoints and blue water and energy consumption, one can observe that scenarios S1 and S5 show very similar impact values. However, scenario S5 has slightly higher number of lower impact categories. As the objective was to choose the scenario with the highest number of lower impact categories and as scenario S5 also has a lower energy and water consumption per kg of phycocyanin, the final choice of the most sustainable scenario falls on scenario S5.

Although there have been other studies performed on the topic of phycocyanin extraction from microalgae, their approach can be very simplistic, and so the results cannot be quantitatively compared. However, they help to understand if the study is on the right track.

A study performed by Papadaki et al. (2017), compared different options for phycocyanin extraction, one with wet biomass and 3 solvents (water, ethanol and phosphate buffer) and a second with dried biomass and the same 3 solvents. The results of the extraction using water as solvent, were compared with the ones obtained by the chosen scenario only producing phycocyanin, as it was the same solvent used in this study. It can be concluded

that the results from this study are similar to those of dried biomass (Figure 79). Besides the use of different equipment and processes, the difference in the impacts are very likely to be due to the amount of phycocyanin obtained, as all results are presented per kg of phycocyanin. While in the work by Papadaki et al. (2017) the maximum amount of phycocyanin obtained was 3% of the biomass content, in this study it was assumed a 9% of phycocyanin in the biomass. The reason why the results with dry *Arthrospira* are so close could be due to the fact that Papadaki et al. only considered the impact of water, CO_2, nutrients and energy consumption.

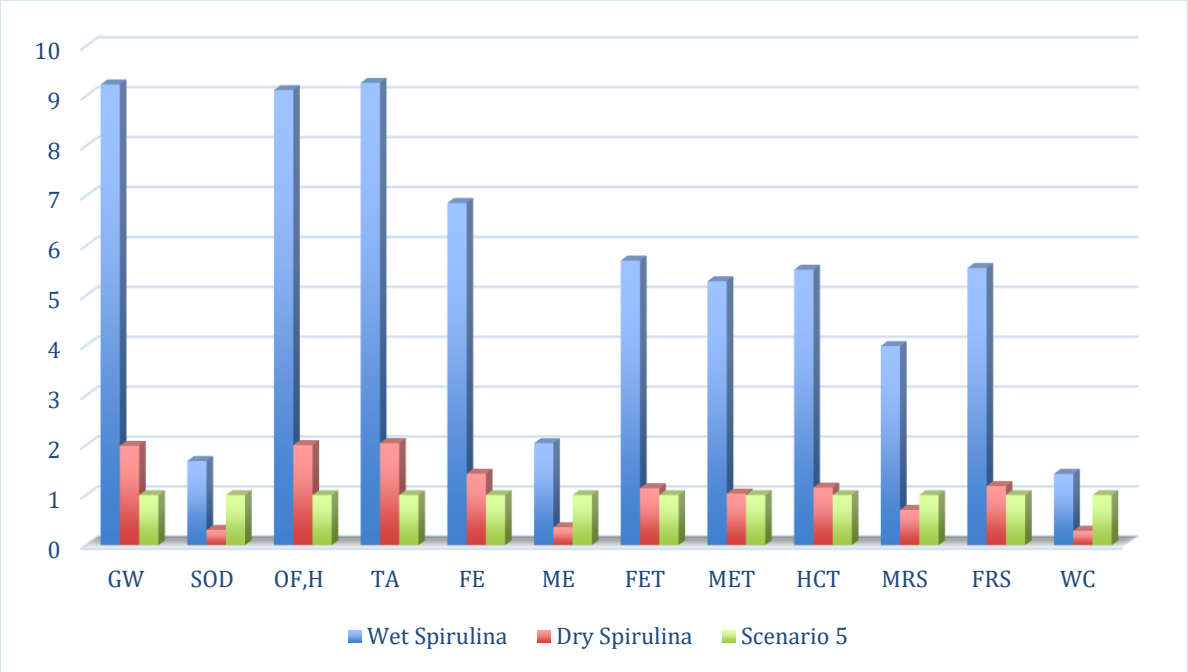

Figure 79 - Comparison of the normalized results by Papadaki et al. (2017) *(for wet and dry Arthrospira) and those obtained in this work for scenario S5 (only producing phycocyanin).*

5.6.1 Possible improvements on the performance of the proposed scenarios

As was mentioned in the introduction chapter, in the LCA section, there are different possibilities to improve the environmental performance of microalgae biorefineries. Two of such improvements are the use of wastewater, providing the nutrients required by microalgae, and the use of renewable energies to power the processing unit. Considering these two improvements, an analysis on all the scenarios was performed and compared the best results previously obtained.

5.6.1.1 Use of wastewater to replace the use of nutrients

It is well known and has been discussed in several studies (Gupta et al., 2019; Schneider et al., 2018) that wastewater can be used to supply nutrients to a microalgae production process. Using wastewater would not only provide nutrients but also water, decreasing water consumption. Taking this into account, a wastewater with the composition shown in Table 100 was used to supply part of the nutrients required for microalgae production. This composition was only able to supply part of the nutrients, as the nutrient contents was low; however, waste streams with higher nutrient content are also available.

Table 100 - Wastewater composition.

Nutrients present	kg/m^3	Nutrient required (kg)	Nutrients supplied (kg)	Water Supplied
N	0.061	19324	2101	34456
P	0.008	2260	289	

Considering the previously presented wastewater composition, it would be possible to supply about 10% of the nutrient requirements by adding wastewater. Also 100% of fresh water required for cultivation would be saved, as all the water for production would be provided by the wastewater.

This is shown in Figure 80 and in Table 101, where it can be observed that in all impact categories, the production impact decreases. Most positive impact is on the water consumption impact, as all the water is supplied by the wastewater instead of fresh water, but also in the human carcinogenic toxicity and fossil resource scarcity, due to the lower mineral consumption, and consequent extraction. Therefore, any decrease in nutrient use causes lower impacts in all impact categories.

Table 101 – Total impacts of selected categories for scenario S5 with and without wastewater usage.

	GW	MET	HCT	MRS	FRS	WC
Scenario S5 no improvements						
Production	18.4	12801.6	135.3	0.2	9.9	4.8
Harvesting & Rupture	21.6	2063.9	23.1	0.0	7.3	-0.3
Extraction	61.0	2134.2	23.5	0.0	2.4	4.2
Total	100.9	16999.7	181.9	0.2	19.6	8.8
Scenario S5 with wastewater						
Production	16.9	12198.6	125.7	0.1	9.6	4.1
Harvesting & Rupture	21.6	2063.9	23.1	0.0	7.3	-0.3
Extraction	61.0	2134.2	23.5	0.0	2.4	4.2
Total	99.5	16396.7	172.3	0.2	19.2	8.1

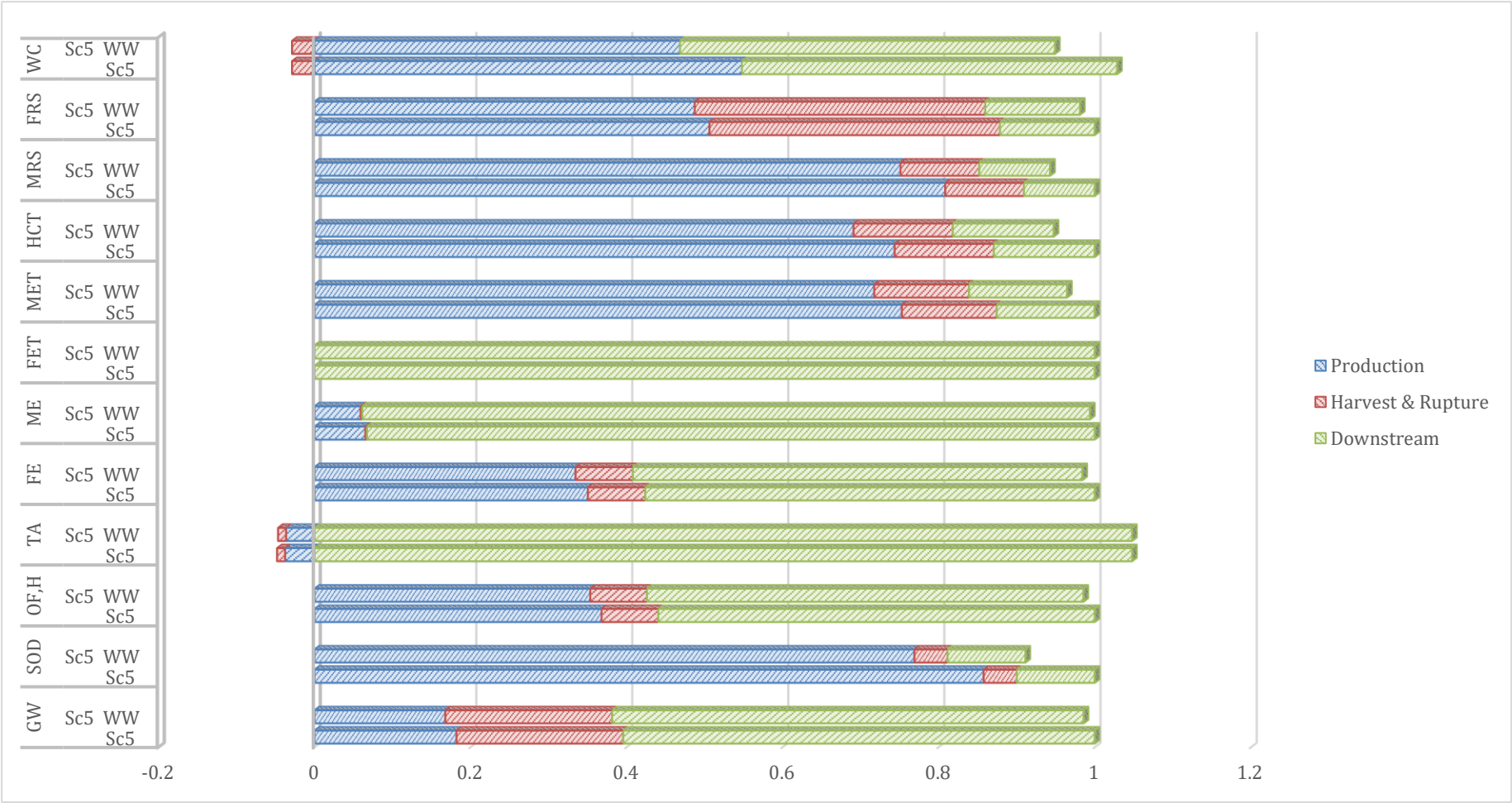

Figure 80 - Comparison of the performance of the Synechocystis scenario S5 with (Sc5 WW) and without (Sc5) the use of wastewater using normalised impact factors.

5.6.1.2 Use of renewable energy

As mentioned in the LCA chapter, in the introduction section, the use of renewable energies, like solar panels, wind power and others, can decrease the use of electric power from the main grid, and with it the global warming effects and the fossil resources depletion. With this in mind, in this study the energy used in all the scenarios was replaced by photovoltaic energy to evaluate the impact it would have on our proposed scenarios. The photovoltaic energy was chosen since it is the most used renewable energy source in industrial units.

To first understand the distribution, the results of our chosen scenario, scenario S5, were compared with and without using photovoltaic energy. The results are shown in Figure 81 and in Table 102. As expected, in the two previously mentioned categories, and many others related to the production of electricity from fossil fuels, like ozone formation, there is a large decrease in the negative impact, especially in the stages where highest amount of energy is consumed, which is the case of the production stage. What was also observed is that the mineral resources depletion and marine environment toxicity have an increase. This is due to the use of minerals in the production of photovoltaic panels. The mining of those minerals also has a large impact on other categories that the electricity supplied from fossil fuels does not have. However, the use of photovoltaic modules has more positive performance in most categories than grid energy. However, if a different material was used, or the material of photovoltaic panels was recycled, then the impact factors could be better.

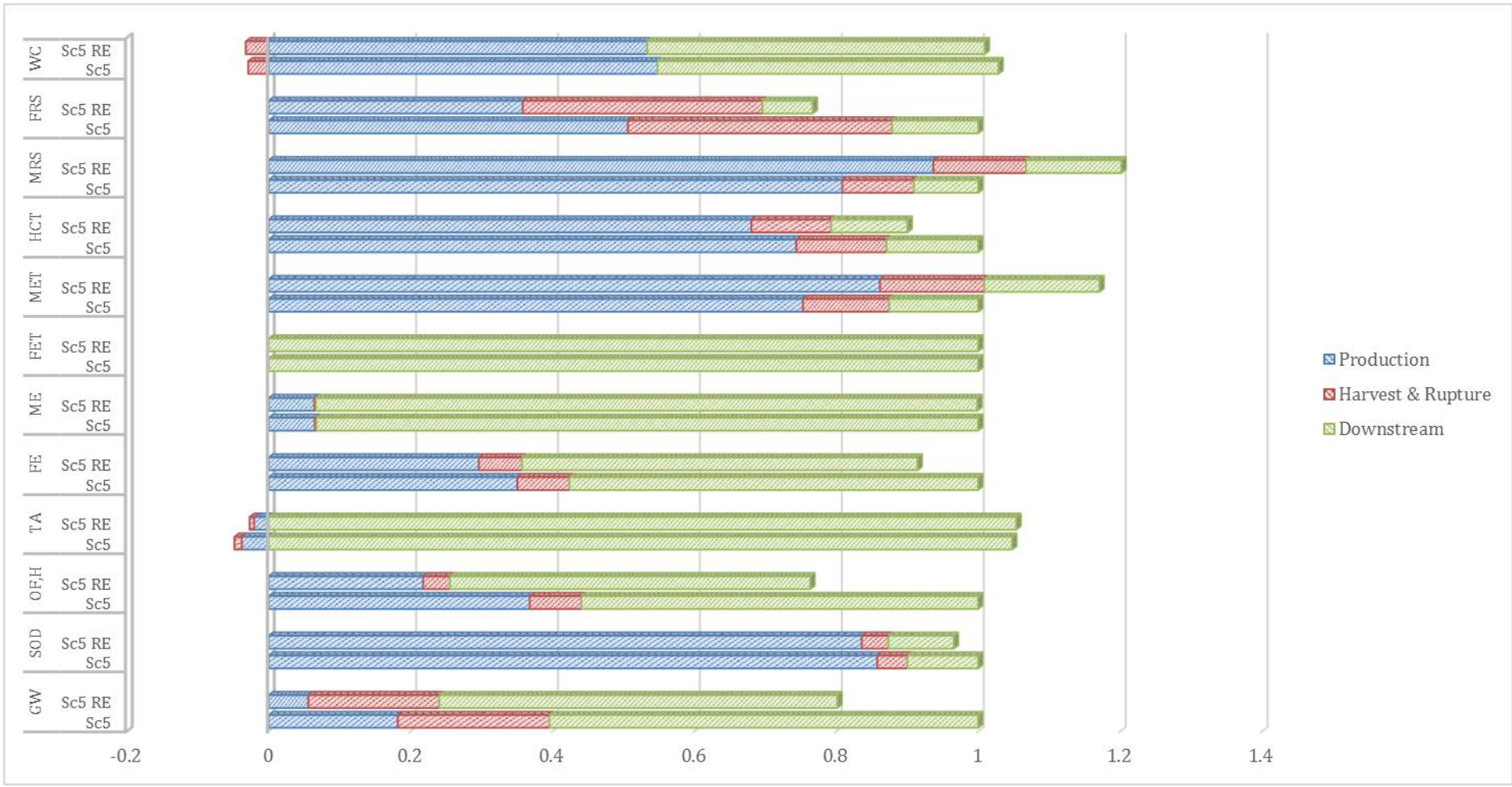

Figure 81 – Comparison of the performance of the Synechocystis scenario S5 with (Sc5 RE) and without (Sc5) use of renewable energy, using normalised impact factors.

Now, when looking at the changes in all scenarios (the impact categories where the difference was very small were discarded), it can be concluded that the changes in the impact categories are different from scenario to scenario. One of the first observations is that scenario S2 has the highest changes in the impact categories values, as it is the scenario which consumes more energy (see Table 98 and Table 102). On the other hand, as scenario S4 consumes the lowest amount of energy, it is the scenario with the smallest value changes. If the number of categories that have a better performance is taken into account, one can say that the photovoltaic energy has a better performance than the energy from the national grid. Moreover, as mentioned before, the main difference between the two best scenarios is that one uses a pervaporation membrane (scenario S1) and the other distillation (scenario S5), to recover ethanol. Therefore, if one was interested in setting up photovoltaic panels, maybe then scenario S1 would be more interesting, as more energy related impacts would be mitigated.

Table 102 - Total impacts of selected categories for all scenarios with and without renewable energy usage.

	GW	OF	HCT	MET	MRS	FRS
Scenarios without renewable energy						
S1	89.42	0.25	190.76	17622.03	0.20	14.85
S2	106.81	0.30	209.11	19693.40	0.23	17.03
S3	107.03	0.25	180.89	16682.07	0.19	20.11
S4	101.69	0.24	186.36	17248.81	0.20	20.39
S5	100.91	0.24	181.86	16999.71	0.19	19.62
Scenarios with renewable energy						
S1 RE	66.73	0.18	170.22	20915.96	0.24	9.67
S2 RE	82.52	0.23	187.11	23219.98	0.28	11.48
S3 RE	85.76	0.19	161.63	19769.98	0.23	15.25
S4 RE	80.58	0.18	167.24	20313.36	0.24	15.57
S5 RE	80.88	0.18	163.73	19906.82	0.23	15.05

The comparison of the environmental performance of the 5 *Synechocystis* based biorefinery scenarios without and with renewable (photovoltaic) energy replacement in all the impact categories evaluated is shown in Figure 82 and Figure 83.

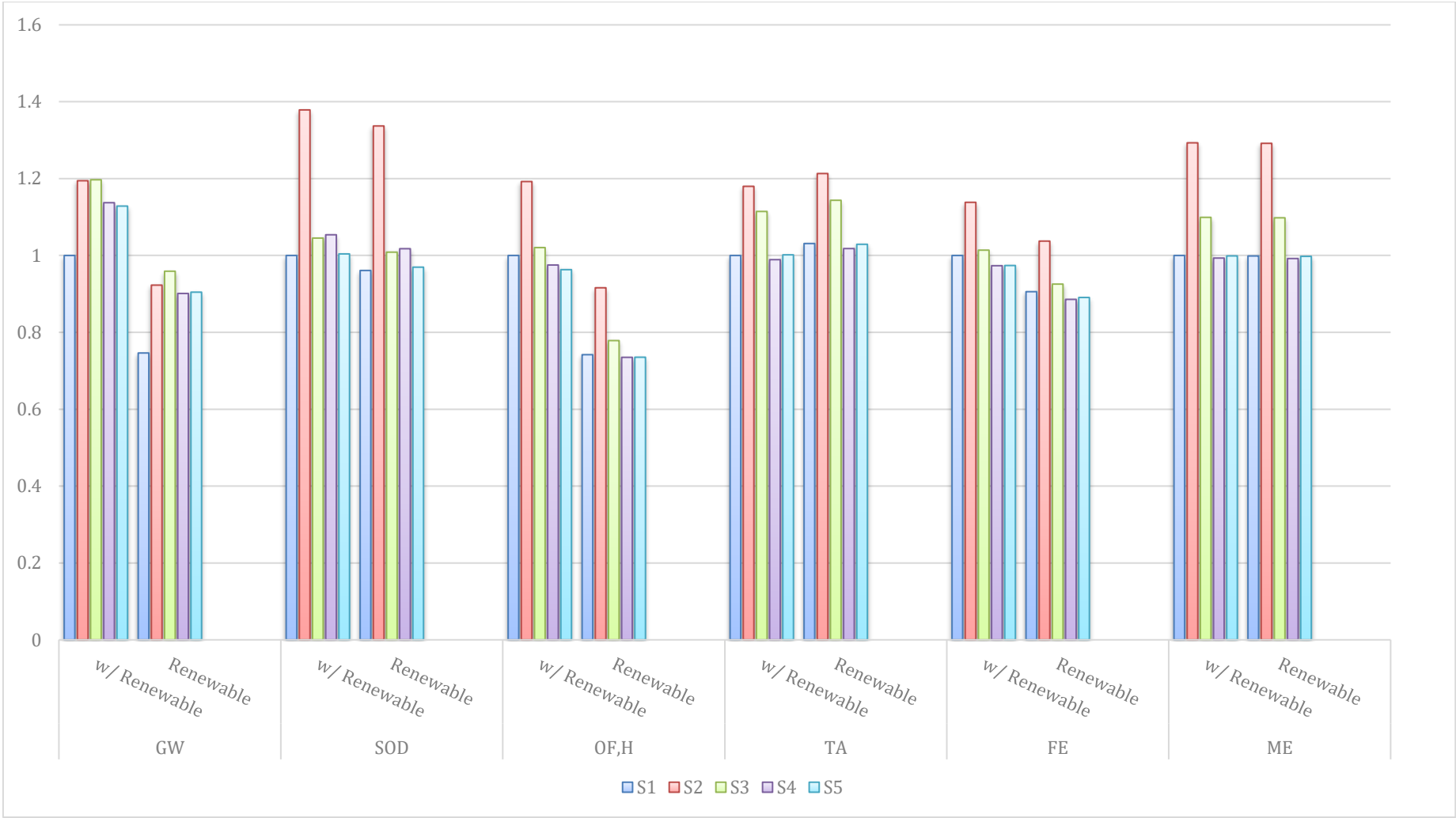

Figure 82 – Comparison of the performance of the 5 Synechocystis based biorefinery scenarios with and w/ renewable energy, using normalised impact factors (part 1).

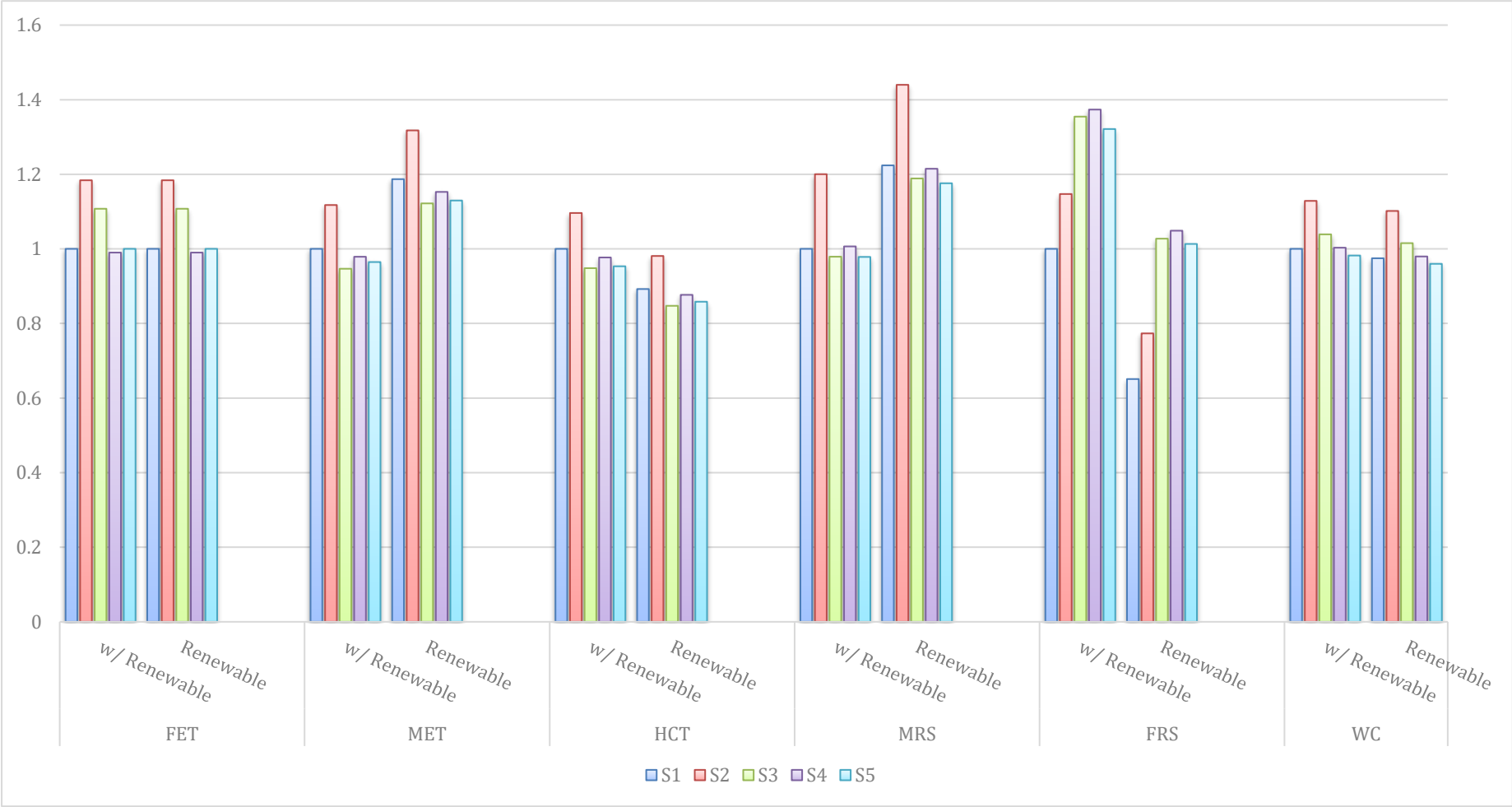

Figure 83 – Comparison of the performance of the 5 Synechocystis based biorefinery scenarios with and w/renewable energy, using normalised impact factors (part 2).

5.7 *Prorocentrum* based biorefinery scenario

5.7.1 Production stage

The impact distribution of the production stage is the same for all scenarios, as the production process is the same for all scenarios. They only differ slightly when the impacts generated by the production of 1 kg of EPA+DHA Soap are compared. In this situation, the scenario with the smallest impact is the P5 scenario as this is the process where the production of EPA+DHA is the largest, and therefore, the impact is smaller for each 1 kg of EPA+DHA produced.

From Figure 84, it can be concluded that the biggest contributors to the impact categories are CO_2, the nutrients used ($NaNO_3$ and NaH_2PO_4), water, NaCl and electricity. These observations corroborate the information obtained in previous LCA studies performed on the topic (Schneider et al., 2018; Taelman and Sfez, 2015)). The first contributor is CO_2. Although it can be observed that it has a positive effect on the global warming category, because it is consumed by microalgae, one must remember that the CO_2 used is bottled CO_2, whose production incurs on some impacts to the environment. Another contributor are the nutrients used. Most of the nutrients used in microalgae production are very similar to fertilizer. And it is well known that fertilizer production has a certain impact on the environment (Basosi et al., 2014; Lenka et al., 2016). Therefore, due to the high amounts of nutrients required, especially $NaNO_3$, this has a high impact on the impact categories. Microalgae production also required large amount of water for cultivation. As the water used is treated, these treatments also have impact on the environment. Furthermore, as *Prorocentrum* is a saltwater microalga, it requires salt in the form of NaCl, if the cultivation medium does not resort to seawater. NaCl is produced from the mining of salt deposits, which incurs in a high impact on the environment not only from the decrease in mineral resources but also due to the energy consumed for mining (Michael Fitch et al., 2012). Finally, the process also requires large amount of electricity for the pumps, heating and cooling systems, among others. In this situation, the impact depends on the electricity mix. In Portugal the mix is almost half renewable energies, half fossil fuel (EDP, 2019).

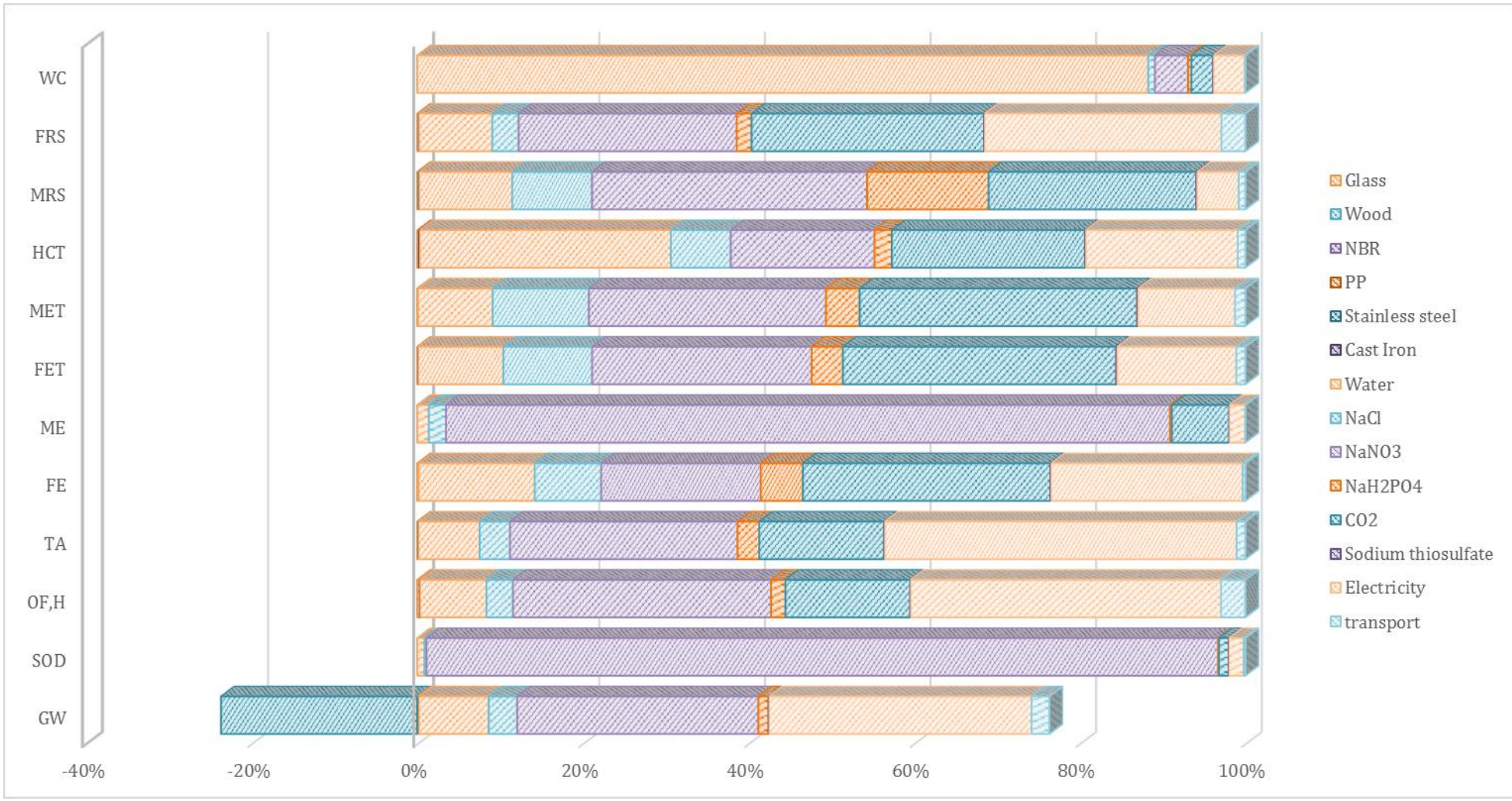

Figure 84 – Eco-Profile of all Scenario Production stages of Prorocentrum based biorefinery at midpoint categories.

5.7.2 Harvesting and rupture stage

Although some of the processes in this stage are different, leading to a slight difference in distribution of the impact factors, the main contributors in the impact categories are the same for all scenarios. In the harvesting and rupture stage, the biggest contributors to all impacts (except water consumption) are the cleaning agent (in some categories over 90% of the impact) and the energy consumption (Figure 85). The impact of the cleaning agent is mostly due to the components NaOH (Thannimalay et al., 2013), EDTA and KOH. From these three, the highest impact comes from NaOH, where electricity and raw salt production account for >90% of the overall environmental burden (Hong et al., 2014). The electricity impacts are due to the consumption of fossil fuel, as 50% of the electricity produced in Portugal is still produced from fossil fuels.

On the other hand, the treated wastewater has a positive impact on the water consumption, as it returns treated water to the environment, although the process for wastewater treatment is the third highest negative contributor, due to the chemicals and energy required to treat the wastewater.

The main differences between the scenarios are that scenario P1 uses a centrifuge, which requires less cleaning water and chemicals. Therefore, the impact of the cleaning chemical will have a smaller contribution with energy having a larger impact, as the energy consumption of the centrifuge is higher than that of the membranes, which are used in all other scenarios. On the other hand, scenario P5 has a larger impact caused by the cleaning agent, as the ultrasonicator used requires more cleaning agent than the other rupture methods.

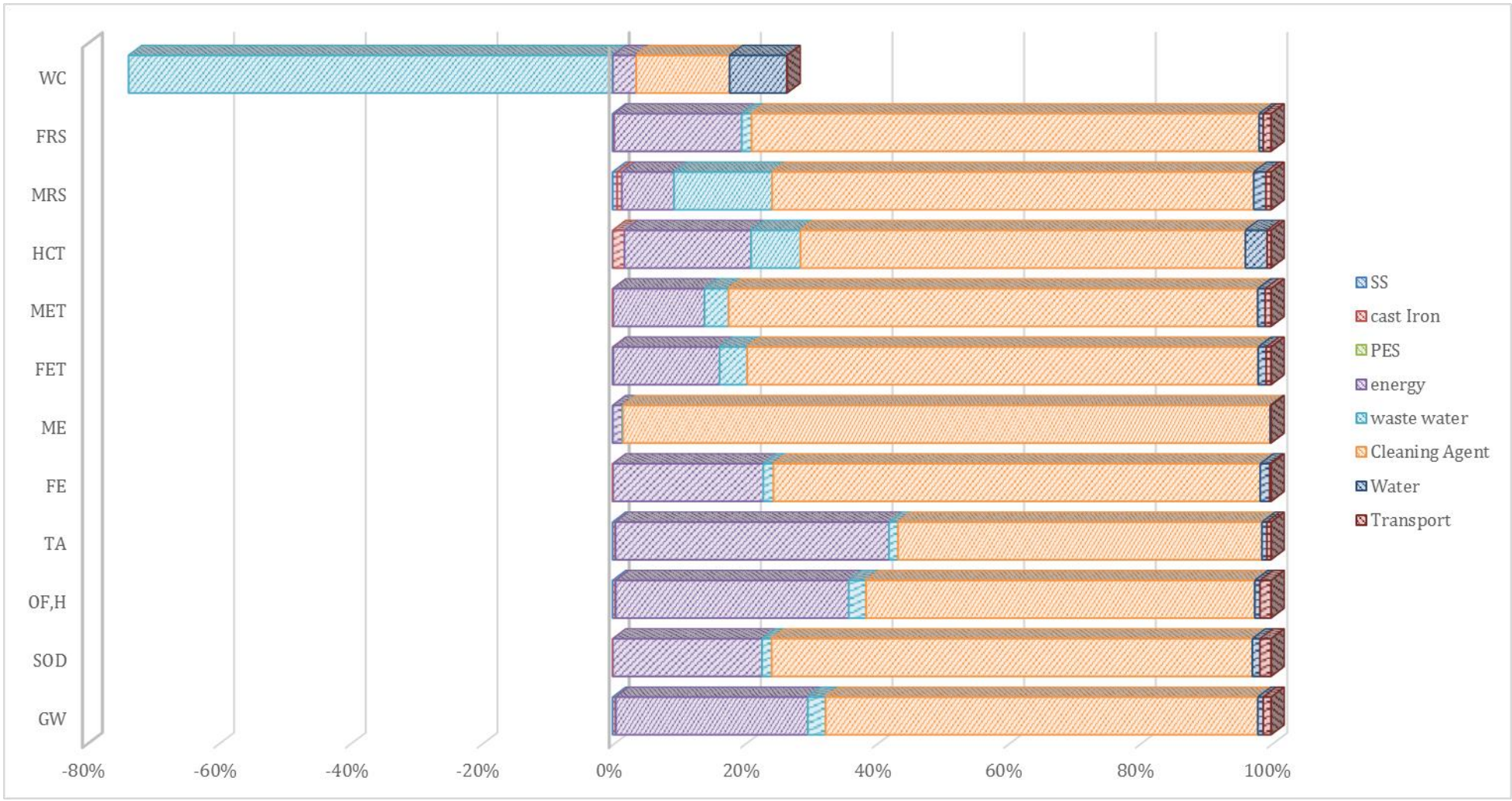

Figure 85 - Eco-Profile of the harvesting and rupture stage of Prorocentrum based biorefinery at midpoint categories.

5.7.3 Extraction Stage

In the extraction stage, the highest contributors (negative and positive) to the impact factors are the enzymes, used for the production of the amino acid hydrolysate, the ethanol, used in the lipid extraction, the CO_2 used in the supercritical extraction, the cleaning agent and the electricity used, especially due to the spray dryer.

In the first case (enzymes) most of the impact comes from the production and fermentation processes (Nielsen et al., 2007), but also from the cleaning processes, as the equipment has to be maintained aseptic and sterile (Feijoo et al., 2017).

Ethanol also has significant negative impacts due to the use of enzymes in the saccharification process (it was assumed the ethanol used is produced from rye), and the high energy consumption of the process (Aroca et al., 2013; Borrion et al., 2012).

In the case of CO_2, most of the negative impact comes from the production process, as CO_2 can be a co-product of a Power plant (Hertwich et al., 2008; Volkart et al., 2013) or from mineral production (Khoo et al., 2011). Adding to these negative impacts, is also the impact of CO_2 capture that consumes energy and therefore, has some negative impacts on the environment (Henkel, 2006). The impact of the cleaning agent is mostly due to the components NaOH (Thannimalay et al., 2013), EDTA and KOH as was explained before.

In the extraction process of scenarios P1 and P2, the highest contributors (negative and positive) to the impact categories are the enzymes and electricity consumption (Figure 86). The large electricity consumption is mostly due to the energy required for the spray dryer. In addition, with some significant impacts are the CO_2 and the ethanol used in the supercritical extraction. Scenario P1 has slightly lower impact as the centrifuge produces a more concentrated biomass, leading to a lower consumption of energy and therefore a lower impact of energy. However, as these impact factors are distributed by contribution to the total impact, this means that all other contributors have a higher contribution. Another contributor to the negative impacts, especially in fossil resources scarcity factor, is the polyethylene storage containers, because they are produced from fossil fuels and require a large energy consumption (Liptow and Tillman, 2012).

In the case of scenario P3 and P5, the extraction process is the same, with the highest contribution coming from enzymes, electricity used in the evaporator and spray dryer, and ethanol used in the extraction process (Figure 87). The impact distribution for these scenarios is the same with the difference of distribution being due to the amount of EPA+DHA produced, as the values shown are per kg of EPA+DHA Soap. As less soap is

produced in scenario P3, the ethanol impact on that scenario is higher. Also playing a small role in the negative impacts are the polyethylene storage containers.

In the case of scenario P4, the highest contributors are enzymes as well as energy consumption and heptane (Figure 88). Unlike the previous scenarios, energy consumption has a lower impact, because in this scenario it is not required to dry the biomass in any step of the process and, therefore, the energy consumption is much lower (around 10 fold lower). With this in mind, some of the components that did not have a large impact on the previous scenarios now play a bigger role in the impact categories. This is the case of heptane; the negative impact of heptane comes from the refining of fossil fuels and therefore has a large impact in areas as global warming, fossil resources depletion and ozone formation. As in scenario P1 and P2, the storage containers also have some influence on the negative impacts, especially on the fossil resources scarcity.

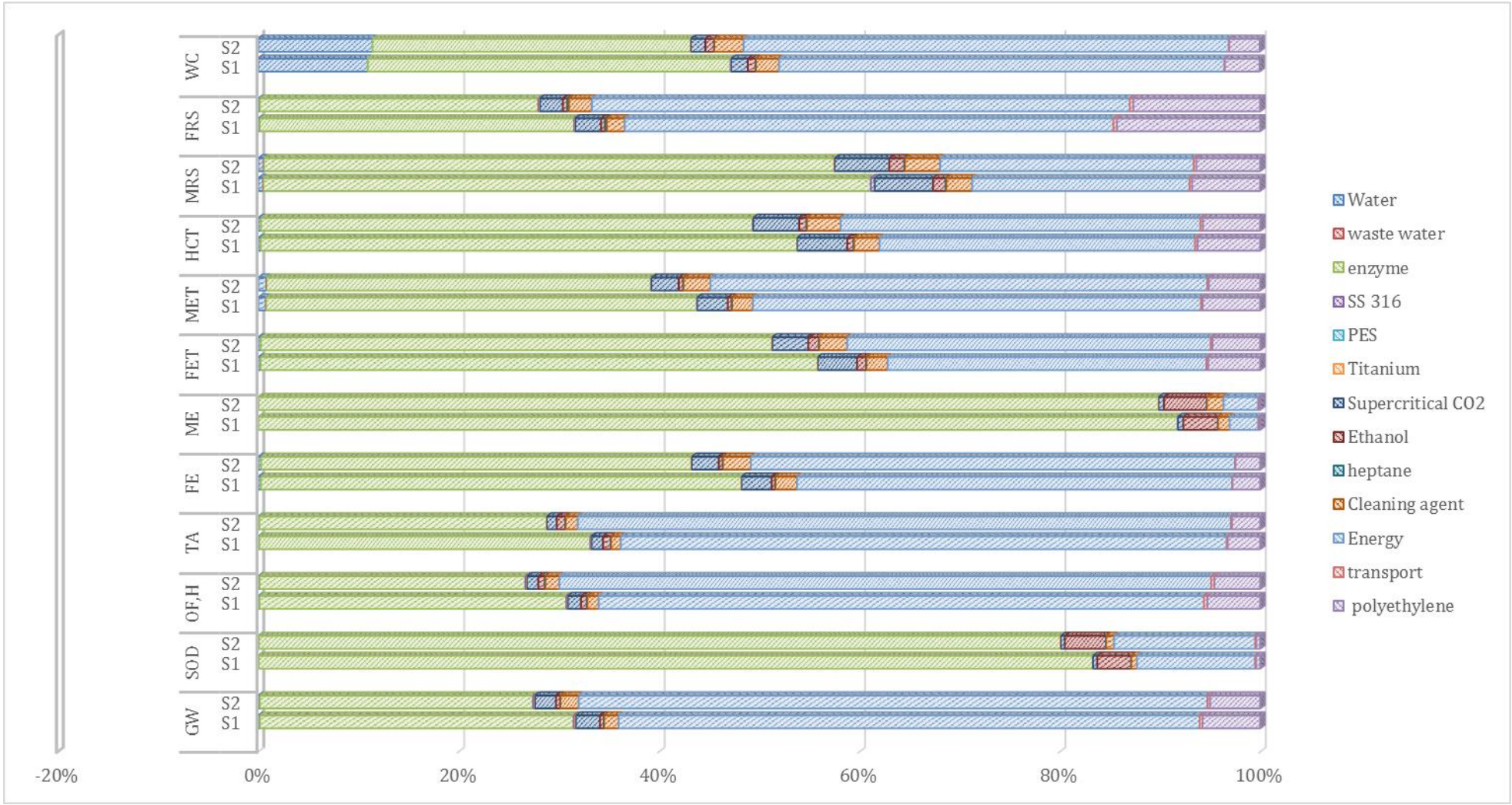

Figure 86 - Eco-Profile of Prorocentrum based biorefinery Scenarios P1 and P2 extraction stage at midpoint categories.

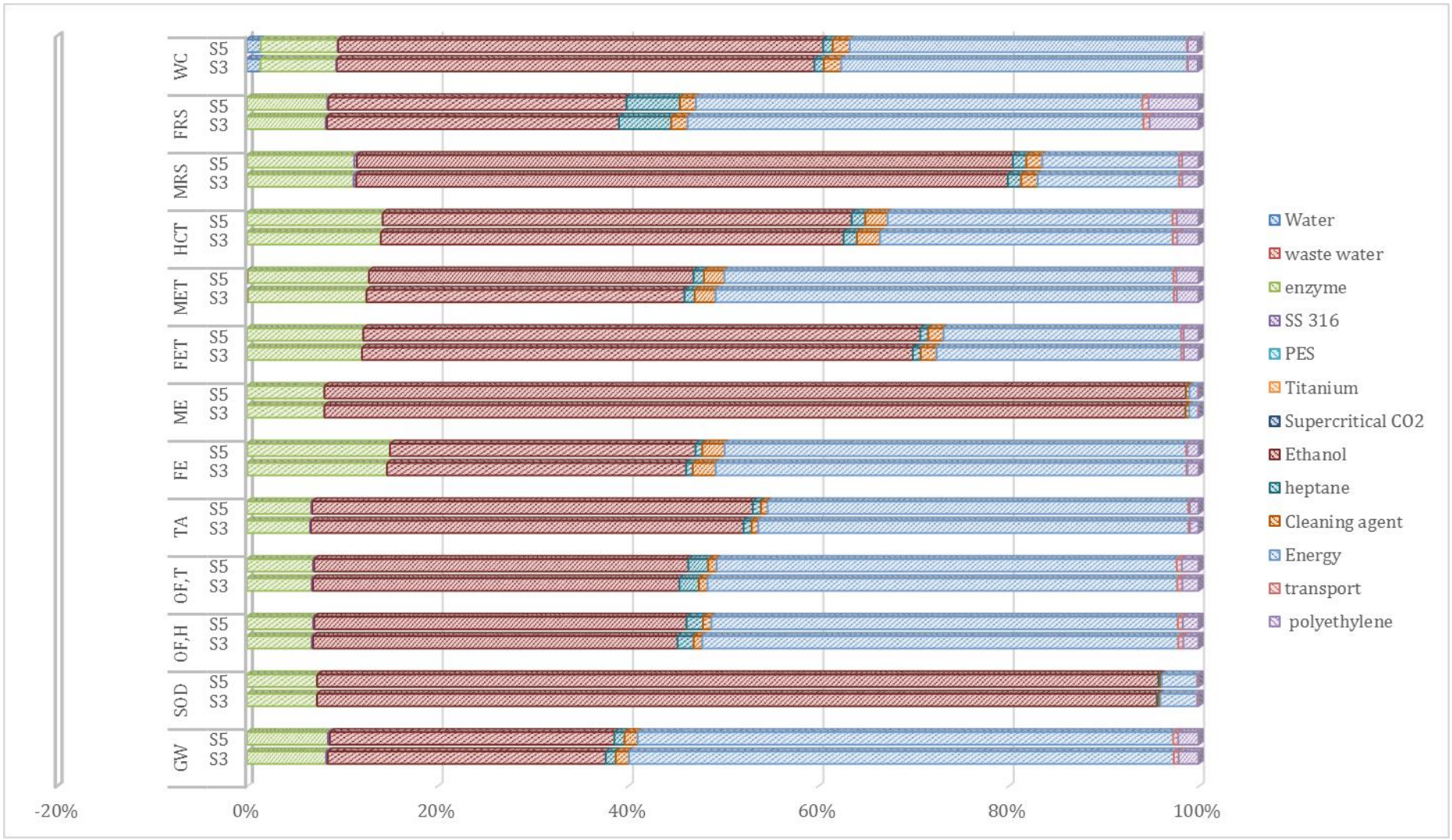

Figure 87 - Eco-Profile of Prorocentrum based biorefinery Scenario P3 and P5 extraction stage at midpoint categories.

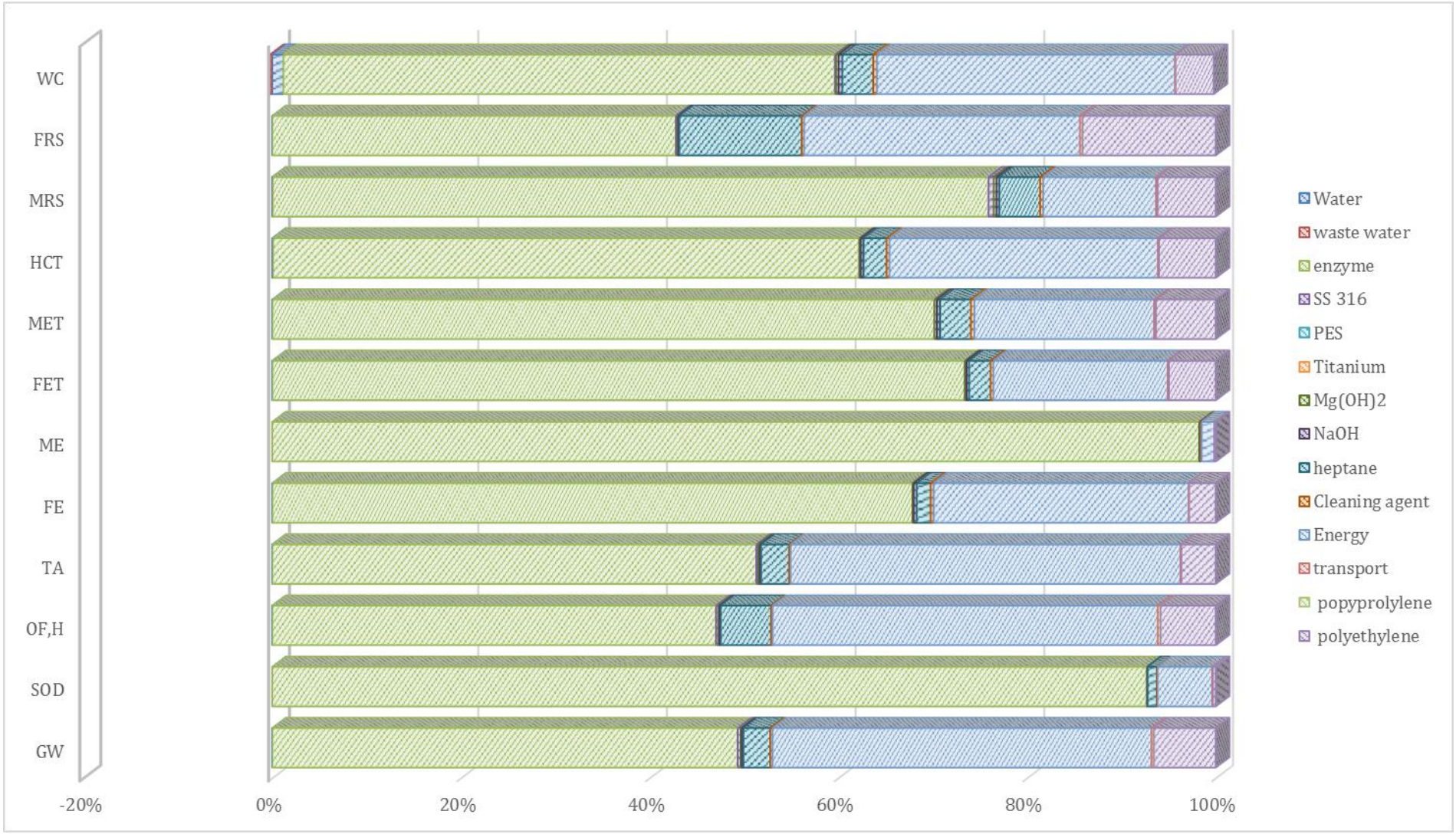

Figure 88 - Eco-Profile of Prorocentrum based biorefinery Scenario P4 extraction stage at midpoint categories.

5.7.4 Stage comparison

5.7.4.1 Scenario P1 and P2

As in the *Synechocystis* scenario the first conclusion that can be taken from Figure 89 is that the production stage has the largest contribution to all impact factors except global warming and Marine Eutrophication, where the extraction takes the lead on the negative impacts. Again, this scenario is similar to what was reported in previously published research (Schneider et al., 2018; Sun et al., 2019; Togarcheti et al., 2017), where the highest contributors are CO_2, the nutrients and electricity consumption, explaining why the production stage has the highest contribution to the impact factors. On the other side, CO_2 has a positive impact on the global warming (GW), as it is consumed by microalgae. The highest contributors in the extraction stage are energy consumption and the use of supercritical CO_2 and enzymes, as well as the storage containers made of polyethylene. The harvesting and rupture stage have very little impact, when compared to the other two stages, and the highest contributor is the cleaning agent; however, it has a positive impact on the water consumption due to the treatment of wastewater. It is in this stage that the biggest difference between both scenarios is found. As scenario P1 uses centrifuges, the amount of cleaning chemicals, for this stage, is smaller and therefore, the negative impact is smaller. Furthermore, the choice of harvesting method has consequences on the extraction stage, because with a more concentrated stream, smaller equipment is required in the extraction stage, leading to smaller equipment and lower energy consumption.

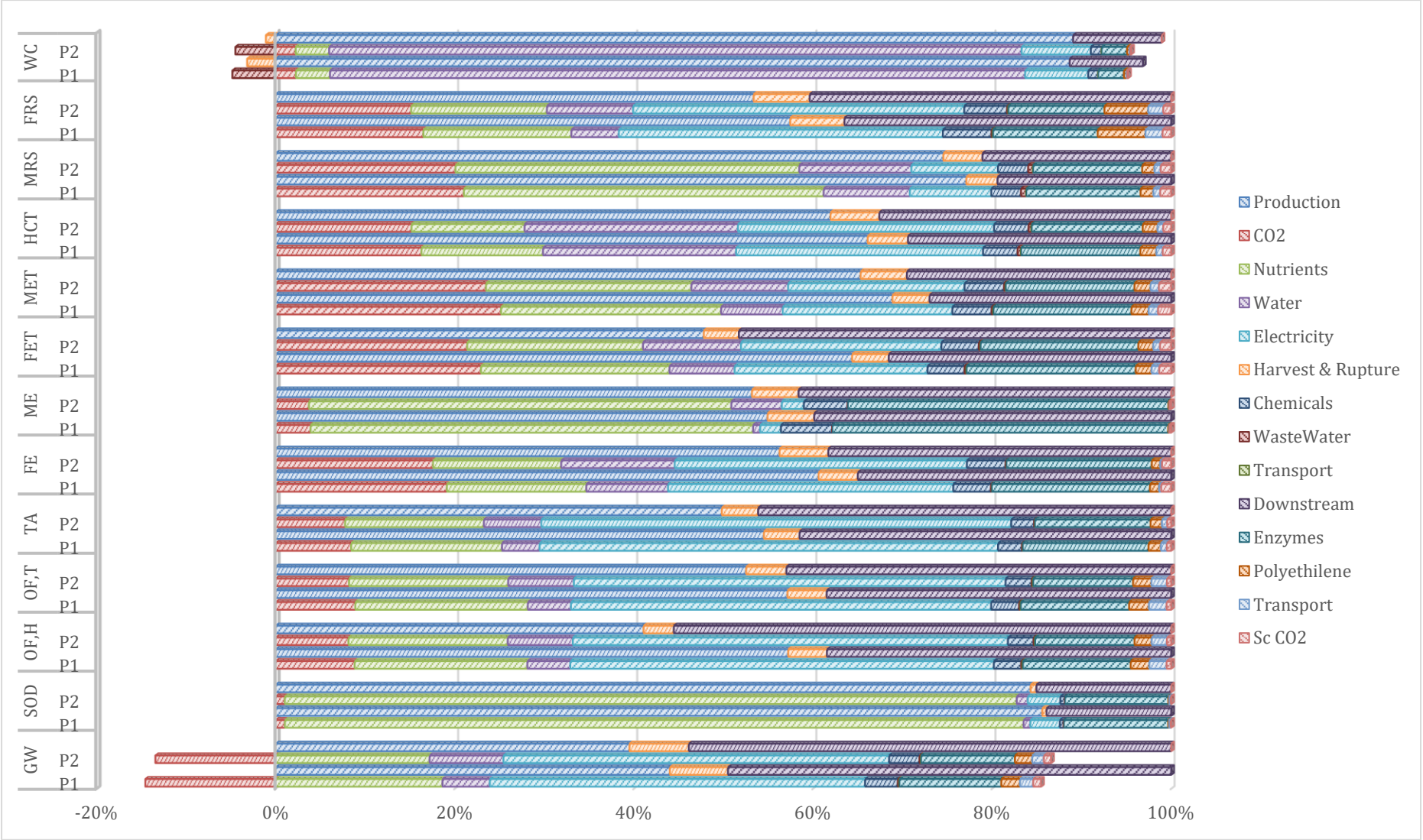

Figure 89- Eco-Profile of the highest contributors in Prorocentrum based biorefinery Scenario P1 and P2 at midpoint categories.

5.7.4.2 Scenario P3 and P5

In Figure 90, it can be observed that the production stage has the largest contribution to all impact categories, except global warming, ozone formation, marine eutrophication and terrestrial acidification, where the extraction stage is responsible for the bulk of the negative impacts. When looking at the contributors more closely, it is observed, in line with information from previously published results (Schneider et al., 2018; Sun et al., 2019; Togarcheti et al., 2017), that the highest contributors are CO_2, the nutrients and electricity consumption. This explains why the production stage has the highest contribution to the impact factors, although CO_2 has a positive impact on the global warming category. In the case of the extraction stage, the highest contributors are energy consumption and the use of ethanol and enzymes. The harvesting and rupture stage have very little impact, when compared to the other two stages, and the highest contributor is the cleaning agent. However, it is the only stage with a positive impact, as the wastewater treatment has a positive impact on the water consumption. When comparing both scenarios, it is observed that the differences are very small. However, as scenario P5 produces slightly more EPA+DHA soap, it has a smaller impact per kg of soap.

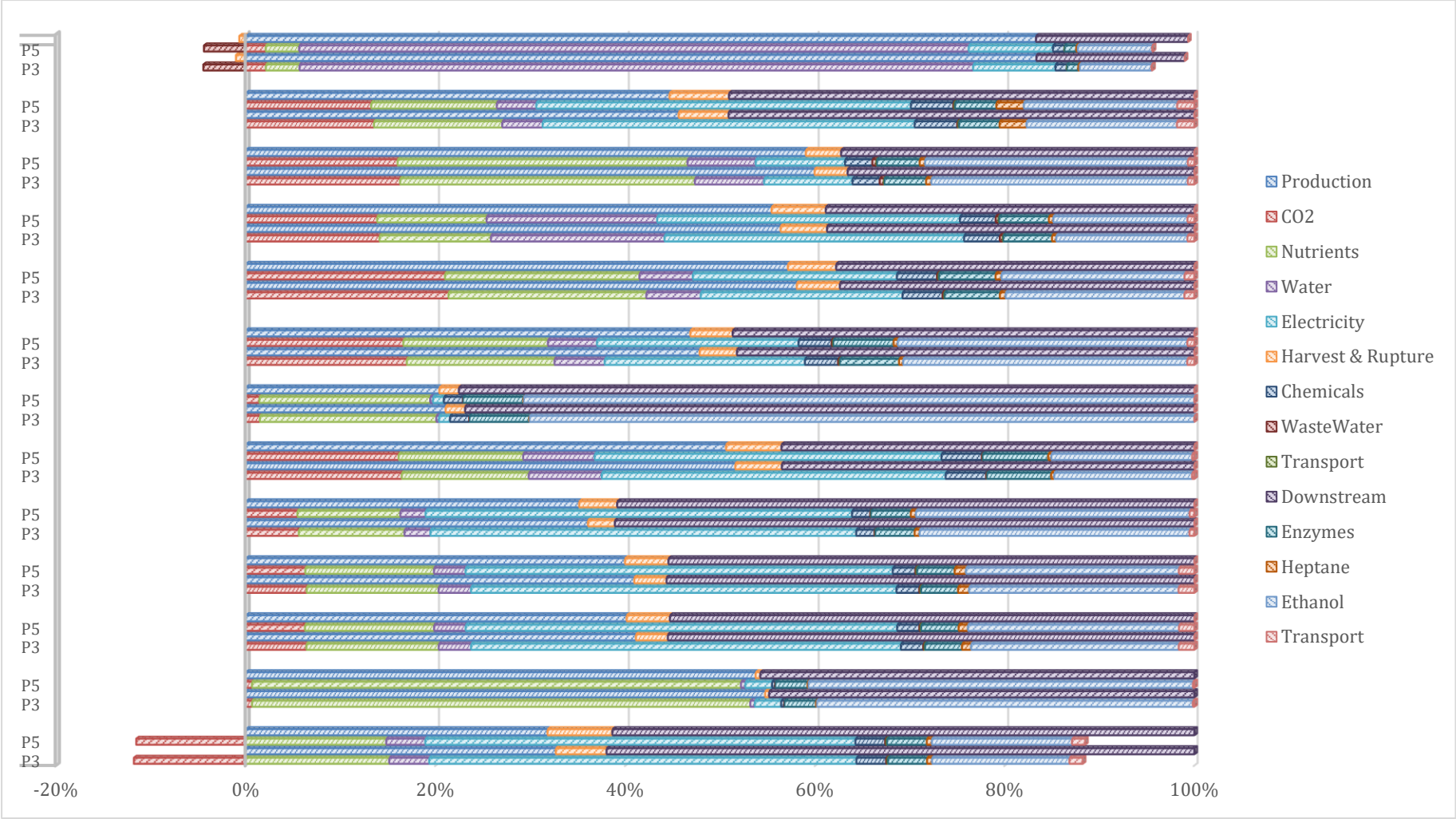

Figure 90 - Eco-Profile of the highest contributors in Prorocentrum based biorefinery Scenario P3 and P5 at midpoint categories.

5.7.4.3 Scenario P4

In Figure 91, once again, the largest contributor to the impact categories is the production scenario, with CO_2, the nutrients and electricity being the largest contributors.

In this scenario, the extraction process has a smaller impact than in the other scenarios, due mostly to the lower energy consumption, as no liquid evaporation is required. Therefore, enzymes, polyethylene from the storage containers and heptane, have larger impacts. This phenomenon also explains why the electricity impact is lower in this scenario than in the previous ones. Again, the harvesting and rupture stage has a smaller impact than the other two and, in this stage, the cleaning agent plays a major role. Also due to the positive impact of the treatment of wastewater in the water consumption, it is the only stage with a positive impact.

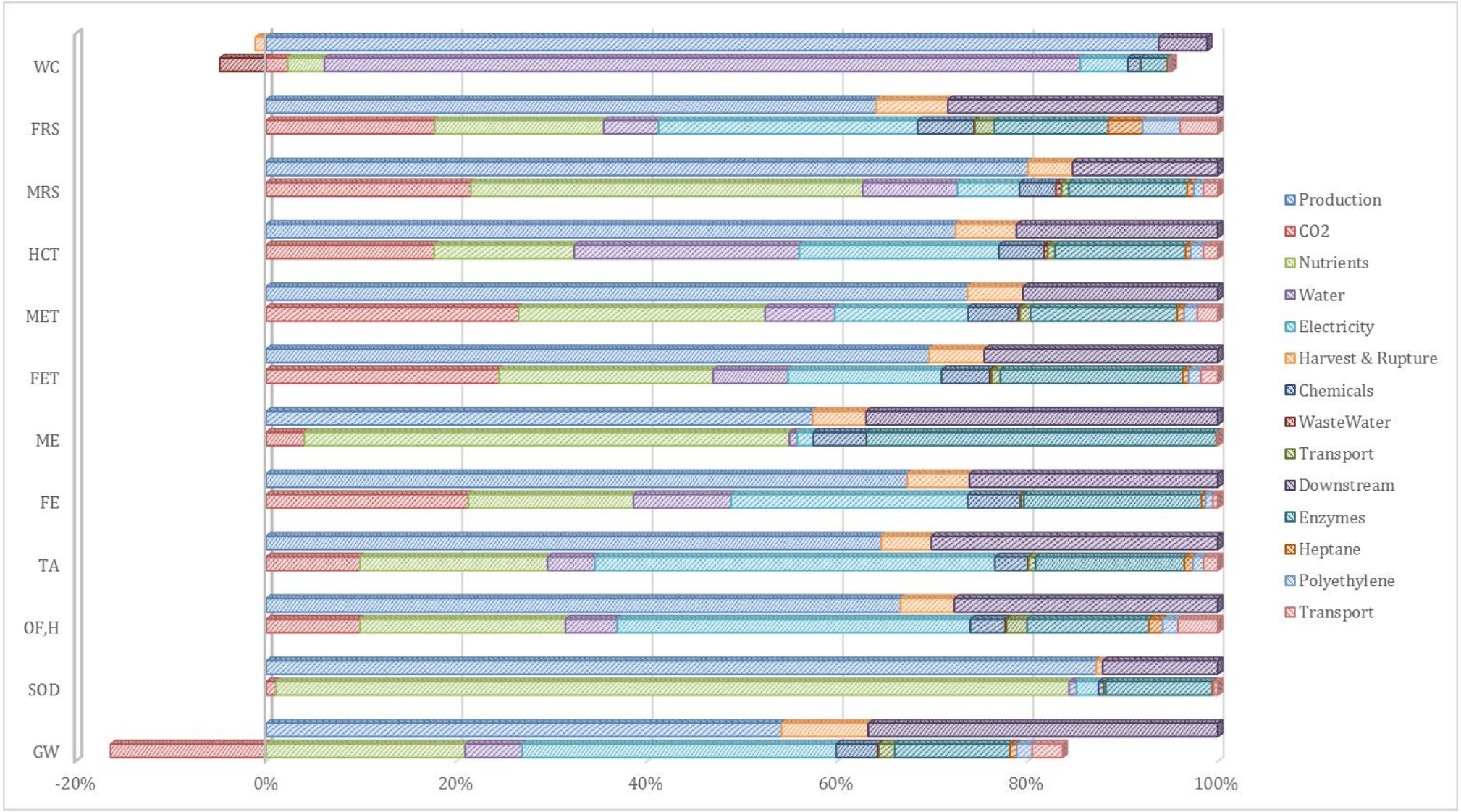

Figure 91 - Eco-Profile of the highest contributors in Prorocentrum based biorefinery Scenario P4 at midpoint categories.

5.7.5 Scenarios comparison

In order to compare the different scenarios, and choose the one with the lowest impact, scenario P1 was considered the standard, and the other scenarios were compared against it, by normalizing their values of the environmental indicators with respect to scenario P1 (Figure 92). The absolute values of the environmental impacts are tabulated in Appendix 13.

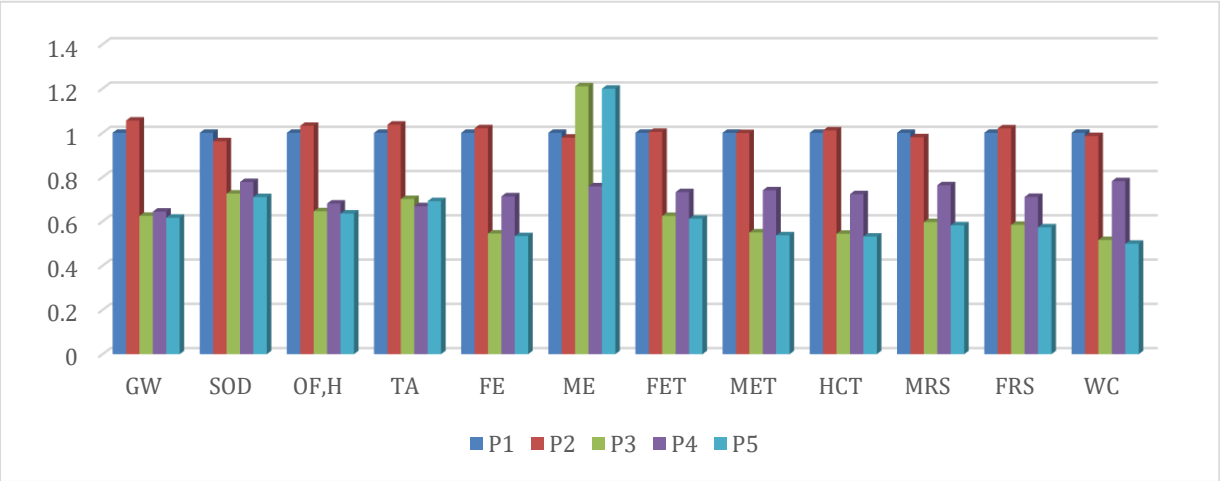

Figure 92 - Normalized Eco-Profile of all Prorocentrum based biorefinery scenarios at midpoint categories.

From Figure 92, it is possible to observe that the scenarios with the highest impact are P1, P2 and P4. The main reason behind this result is the low amount of EPA+DHA soap produced by these scenarios (The amount of EPA+DHA soap produced is 40% less than that of the other scenarios). This has a very large impact on the final results.

As the two remaining scenarios are very similar a more in-depth analysis was performed, and each stage was separated in order to access which stage has the best performance and why.

In Figure 93 the impact of each different stage can be observed. When looking at each individual stage, it is possible to conclude that the production stage and the extraction processing stage share the weight of the impact. In the production stage, although the process is the same for all scenarios, scenario P5 has the lowest impact, because it produces more EPA+DHA soap than the other scenarios.

When looking at the scenario with the best performance, it can be stated that it is scenario P5, mostly because it is the scenario that produces more EPA+DHA soap.

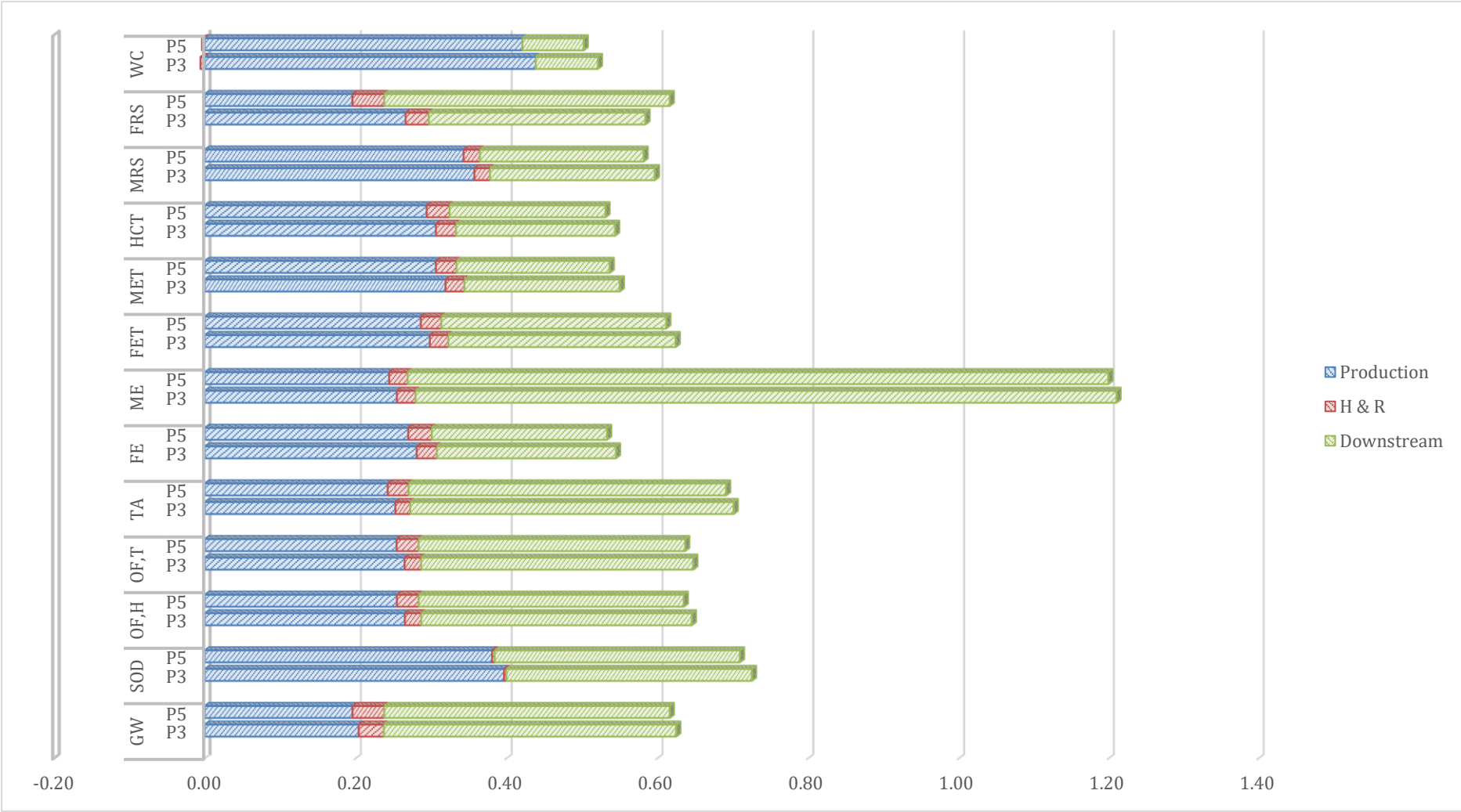

Figure 93 - Normalized Eco-Profile of all stages in Prorocentrum based biorefinery scenarios P3 and P5 at midpoint categories.

5.7.6 Energy consumption

As can be seen in Figure 94 and Table 103, and in previous observations, in most scenarios (except P4), the highest energy consumption comes from the extraction process stage. This is due to the energy required for the drying steps of the extraction process. As was mentioned in several studies on the Microalgae LCA topic (Taelman et al., 2013; Zaimes and Khanna, 2013), the drying of biomass and products is one of the largest contributors to the negative impacts of a process. In the 4th scenario, as no drying is required, the largest contributor to the energy consumption is the production process. Also, it is possible to observe in Table 103 that scenario P3 and P5 have the highest energy consumption, due to the purification process, however in Table 104 they have a lower energy consumption per kg of EPA+DHA soap than scenario P2 and very close to scenario P1. This observation is due to the fact that these are the scenarios which produce the most EPA+DHA Soap, and therefore use less energy per kg of EPA+DHA soap. The fact they produce the most EPA+DHA Soap also explains the higher amount of energy spent in the purification process. Also, as can be seen in both tables, scenario P4 has the lower energy consumption.

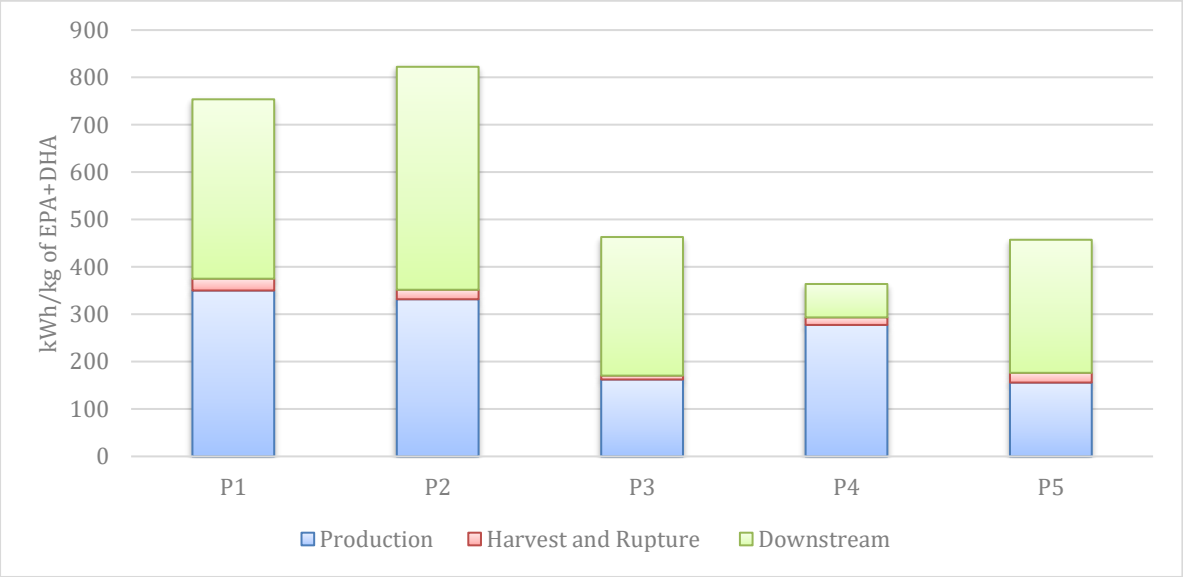

Figure 94 – Total energy consumption per kg of EPA+DHA soap for each scenario.

Table 103 – Total energy consumption (kWh/year) in the Prorocentrum based biorefinery.

Scenario	P1	P2	P3	P4	P5	units
Production	2258519	2258519	2258519	2258519	2258519	kWh/year
Harvesting & Rupture	161000	129500	114100	129500	298900	kWh/year
Extraction process	3101181	3203857	4069537	1021256	4069537	kWh/year
Total	5520701	5591876	6442156	3409275	6626956	kWh/year

Table 104 - Energy consumption per functional unit (kWh/FU) in the Prorocentrum based biorefinery.

Scenario	P1	P2	P3	P4	P5	units
Production	166	332	162	278	156	kWh/kg prod
Harvesting & Rupture	12	10	8	16	21	kWh/kg prod
Extraction process	228	471	293	70	281	kWh/kg prod
Total	406	821	463	235	457	kWh/kg prod

5.7.7 Blue Water Footprint

For microalgae-based biofuels, the blue water footprint is the sum of the water directly used to supply the cultivation and process needs. In this process, water is required for microalgae cultivation and reactor cooling, for cleaning the equipment, and for the purification process. Also included is the water required for the production of the equipment, chemicals and utilities used on the process.

From Figure 95, it is observed that the production stage in all scenarios has the highest blue water consumption, mostly due to the water required for cooling.

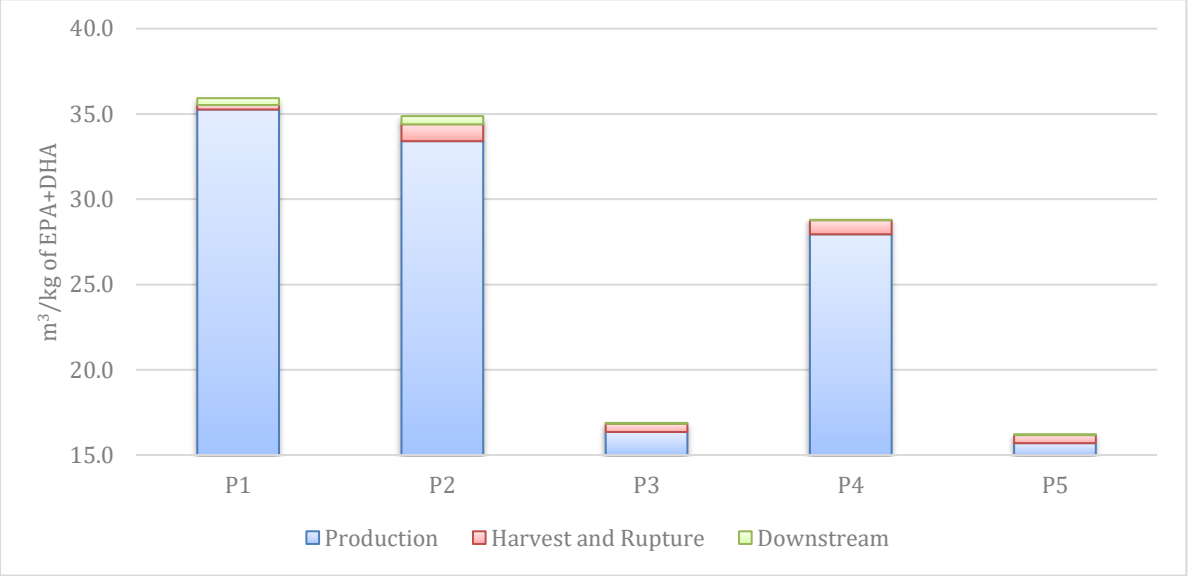

Figure 95 - Blue Water consumption in the Prorocentrum based biorefinery per kg of EPA + DHA Soap.

When looking at the Harvesting and Rupture stage, the largest water consumption is due to the water used for cleaning. In the extraction stage, the use of water is very small and is mostly for equipment cleaning and for the purification stage. When comparing the 5 scenarios, one can observe that scenarios P3 and P5 are very similar in terms of water consumption per kg of EPA+DHA. However, scenario P5 is the one with the lowest blue water

consumption, mostly due to the fact that it is the scenario where the highest amount of EPA+DHA Soap is produced.

However, as in the energy analysis, when looking at the total water consumed shown in Table 105, the total consumption is very similar, being that scenario P1 actually has the lowest consumption.

Table 105 - Total water consumption.

Stage Scenario	P1	P2	P3	P4	P5	Units
Production	2.27E+05	2.27E+05	2.27E+05	2.27E+05	2.27E+05	m^3/year
Harvesting & Rupture	1.70E+03	6.74E+03	6.73E+03	6.74E+03	6.85E+03	m^3/year
Extraction	7.79E+02	1.67E+03	7.93E+02	1.18E+03	8.18E+02	m^3/year
Total	2.30E+05	2.36E+05	2.35E+05	2.35E+05	2.35E+05	m^3/year

Therefore, when looking at both energy and water consumption, it is concluded that they are very similar in all scenarios, and the difference is mostly due to the efficiency of the production of EPA+DHA Soap. Moreover, one can conclude that the production stage has the largest impact in all scenarios.

5.8 Discussion on *Prorocentrum* based biorefinery scenarios environmental performance

From the previously mentioned factors, midpoints and blue water and energy consumption, one can observe that scenarios P3 and P5 have very similar values. However, scenario P5 has slightly lower impact categories, as well as lower energy and water consumptions and therefore, is considered the most sustainable scenario.

Comparing the chosen scenario with another study by Perez-Lopez et al. (2014) where PUFAs are produced from *Phaeodactylum tricornutum*, using indoor bubble columns, and recovered using hexane, it is possible to observe in Figure 96 that the values are similar (the scenario P5 used for comparison does not include the enzymatic hydrolysis since the study by Perez-Lopez et al. only contemplates the production of PUFAs). In most cases, the study by Perez-Lopez et al. has slightly better performance. The reason behind this difference can be the fact that *Prorocentrum biomass* only accumulates around 5.8% of TFAs while *P. tricornutum* biomass can accumulate a slightly higher value of 6-7% (Fajardo et al., 2007). This means that the impacts per kg of PUFAs can also be smaller in the study by Perez-Lopez

et al., as more PUFAs are obtained using the same amount of biomass. Further, as the biomass production is usually the biggest responsible for the negative impacts, the less biomass required to produced 1 kg of PUFAs, the lower the impacts. The difference in the global warming can be due to the fact that more energy is consumed in the study by Perez-Lopez et al. The confirmation for this result is that for 1 kg of PUFA produced, the study by Perez-Lopez et al. used 488 kWh and in the present study the amount used was 455 kWh. This difference can be due to the use of different reactors, (bubble columns vs UHT-PBR, respectively) different harvesting methods (centrifuge vs membrane, respectively) and due to the fact that Perez-Lopez et al. used a lighting system as the microalga production was done indoor. The largest difference is in the Marine Ecotoxicity category, and this is due to the use of ethanol in the extraction process of scenario P5, which has a large negative impact on this category. However, the use of ethanol is necessary to obtain the maximum amount of PUFAs, as their concentration is so low. These results help us conclude that for a better environmental performance using this process, it would be more favorable to use a microalga with higher percentage of PUFA content in the biomass and maybe skip the ethanol extraction step.

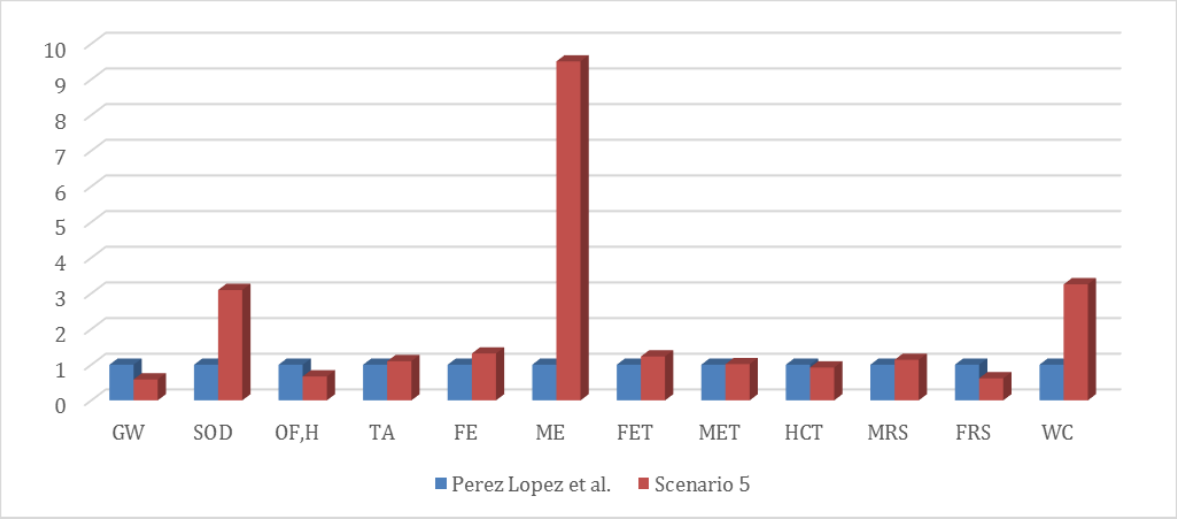

Figure 96 – Comparison of the normalized values between Perez-Lopez et al. (2014) *and scenario P5.*

Unfortunately, due to the lack of data, it was not possible to compare the production of PUFA with other industries, however some conclusions can be taken from other studies. In the study by IFEU "Environmental assessment of algae-based PUFA production" (Keller et al., 2017), it was explained that in categories like land use (that was not used as a comparison category, but is an important category in the debate between microalgae and food crops), the impact of microalgae is smaller, as non-fertile land can be used for the

production of microalga, while for soy and other plants, fertile land is required, decreasing the availability for food crops, and increasing soil degradation. Also, when comparing the impact of consuming omega 3 fatty acids from microalgae with the consumption of commonly consumed sources like meat and dairy products (Coelho et al., 2016) it is possible to observe that to consume the same amount of DHA+EPA the global warming potential is much lower using microalgae, as is the land use (Table 106). These results are mostly due to the smaller concentration of EPA+DHA in the other products.

Table 106 – Value of Global warming potential and Land Competition for different sources of DHA and EPA (adapted from (Coelho et al., 2016)).

			Global warming potential (GWP100a)		Land competition	
Animal food	**EPA (mg/100 g)**	**DHA (mg/100 g)**	**kg CO2 eq/kg of food**	**kg CO2 eq/kg of DHA+EPA**	**m^2a/kg of food**	**m^2a/kg of EPA + DHA**
Beef flank steak, cooked	7.97	1.27	38.75	419266.63	72.81	787780.47
Roast beef, cooked	8.91	2.23	39.08	350843.70	72.82	653727.84
Chicken leg, cooked	2.50	5.20	7.11	92384.84	8.85	114886.39
Pork, ham cooked	5.46	1.64	9.24	130211.21	12.07	170047.34
Pork, dried sausage	7.79	15.80	9.57	40579.75	13.04	55293.42
Camembert	8.55	0.00	6.19	72431.47	8.46	98933.95
Emmental	12.83	0.00	7.67	59781.75	11.15	86910.15
Goat cheese	11.29	0.00	5.98	52973.37	10.24	90697.58
Roquefort	13.73	0.00	13.05	95079.66	35.74	260387.23
Crème fraîche	13.26	0.00	4.24	32004.72	5.92	44673.18
UHT milk, semi-skimmed	0.65	0.00	1.21	185011.28	1.59	242926.92
microalgae	2500.00		1.12	281.03	0.77	192.02

Also, in the same table (Table 106) it is possible to see that for 1 kg of microalgae, the amount of CO_2 produced is smaller than all other sources of DHA+EPA.

5.8.1 Possible improvements on the proposed scenarios of the *Prorocentrum* based biorefinery

As mentioned in the introduction chapter, in the LCA section, there are different possibilities to improve the environmental performance of microalgae biorefineries. Two of such improvements are the use of wastewater with the nutrients required by the microalgae and the use of renewable energies to power the processing unit. Taking these two

improvements into account, an analysis was performed using these scenarios and the scenario with the best results previously obtained was compared to the obtained results. Another improvement possible would be the use of flue gas from other industries to supply the CO_2. Looking at the *Synechocystis* scenarios, where the CO_2 used is supplied via flue gas, there is only the positive impact of consuming the CO_2. This step would reduce the negative impact on the production stage, as this is one of the largest contributors. Another possible improvement would be to use steam from a nearby industry for the distillation step.

5.8.1.1 Use of wastewater in the *Prorocentrum* based biorefinery

It is well known and has been discussed in several studies that wastewater can be used to supply nutrients to a microalgae production process. Using wastewater would not only provide nutrients but also water, decreasing water consumption. Taking this into account, a wastewater with the composition found in Table 107 was considered to be used to supply the nutrients to microalgae production.

Table 107 - Wastewater composition.

Nutrients present	kg/m^3	Nutrient required (kg)	Nutrients supplied (Kg)	Water Supplied
N	0.061	36125	835	13692
P	0.008	5137	115	

Taking into account the presented wastewater composition, it would be possible to supply only about 3% of the nutrient requirements by adding wastewater. Further, all the water required for production would be saved.

However, as the amount of nutrients and water saved are very low, using wastewater with this composition, would not have a significant impact on the environmental performance. This is shown in Figure 97, in all impact categories, and in Table 108 where some selected categories are shown. The reason is that as 90% of the water used for production is recycled the largest impact comes from the water used for cooling the reactors. If a more nutrient rich wastewater would be used, then the impact would be larger.

Table 108 - Total impacts of selected categories for scenarios P5 with and without wastewater usage.

	GW	MET	HCT	FRS	WC
Scenario P5 without wastewater					
Production	89.25	65720.60	599.46	44.57	18.44
Harvesting & Rupture	19.18	5855.73	61.76	6.25	-0.15
Extraction	169.19	42591.49	412.69	46.79	3.52
Total	277.62	114167.82	1073.92	97.60	21.82
Scenario P5 with wastewater					
Production	89.00	65601.75	598.83	44.50	18.44
Harvesting & Rupture	19.18	5855.73	61.76	6.25	-0.15
Downstream	169.19	42591.49	412.69	46.79	3.52
Total	277.37	114048.97	1073.29	97.54	21.81

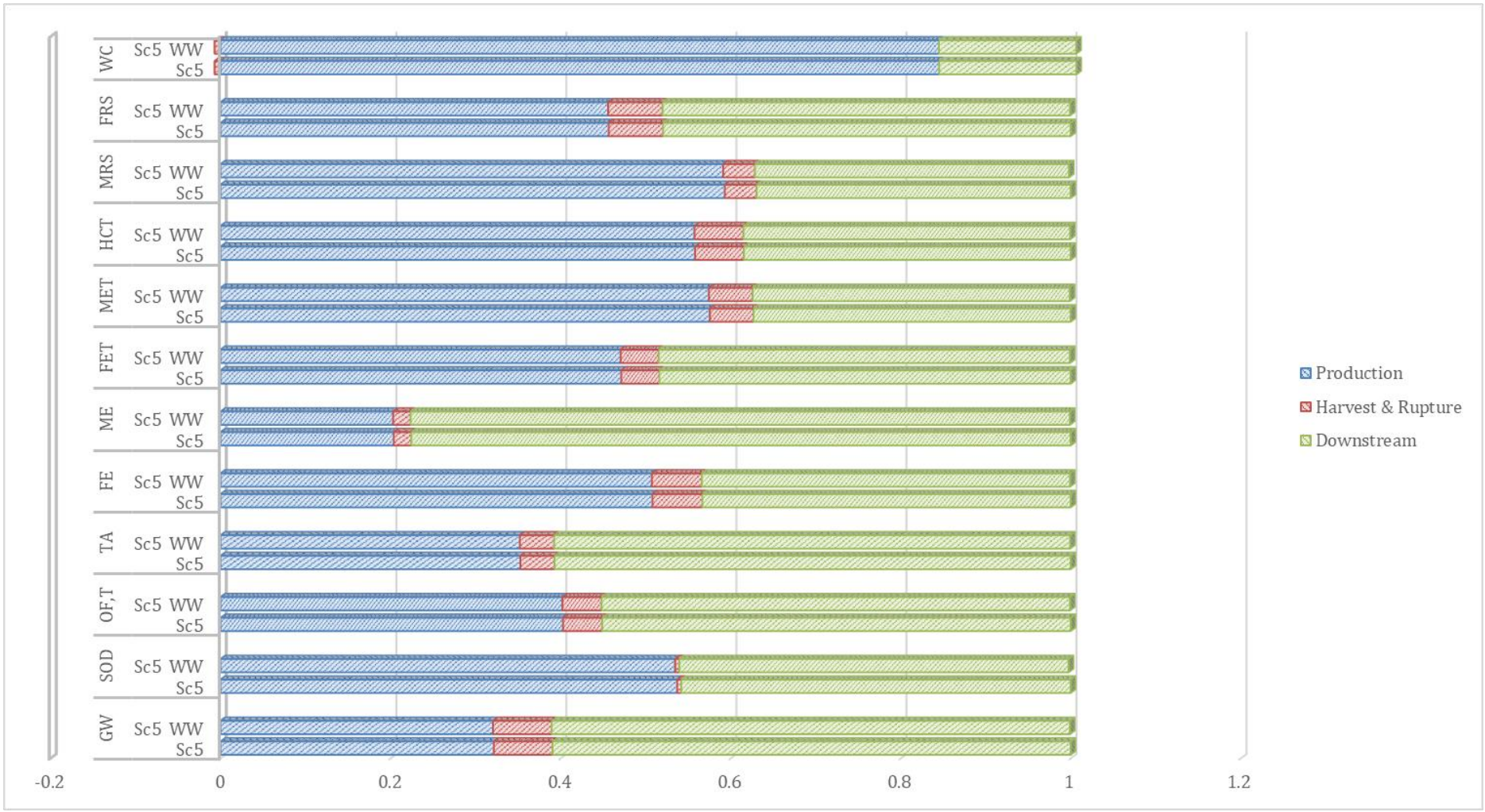

Figure 97 - Comparison of the performance of the Prorocentrum based biorefinery scenario P5 with and without the use of wastewater using normalised impact factors.

5.8.1.2 Use of renewable energy in the *Prorocentrum* based biorefinery

The use of locally produced renewable energies, like solar, wind power and others can decrease the use of electric power from the main grid, and with it the global warming effects and the fossil resources depletion. With this in mind, the energy consumed in each of the scenarios was replaced with photovoltaic energy to study the impact it would have on the proposed scenarios. The photovoltaic energy was chosen since it is the one most used in industrial facilities.

To better understand the distribution, the results of our chosen scenario, scenario P1, were compared with and without using photovoltaic energy. The results are shown in Figure 98. As expected, in the two previously mentioned categories, and many others related to the production of electricity from fossil fuels, like ozone formation, there is a large decrease in the negative impact, especially in the stages where most energy is consumed, which is the case of the extraction stage. What is also observed, is that the mineral resources depletion and marine environment toxicity increase. This is due to the use of mineral in the photovoltaic panels. The mining of those minerals also has a large impact on other categories that the electricity supplied from fossil fuels does not have. However, the use of photovoltaic modules still has a more positive performance in most categories. Further, if a different material was used, or the material of photovoltaic panels was recycled, then the impact factors could be better.

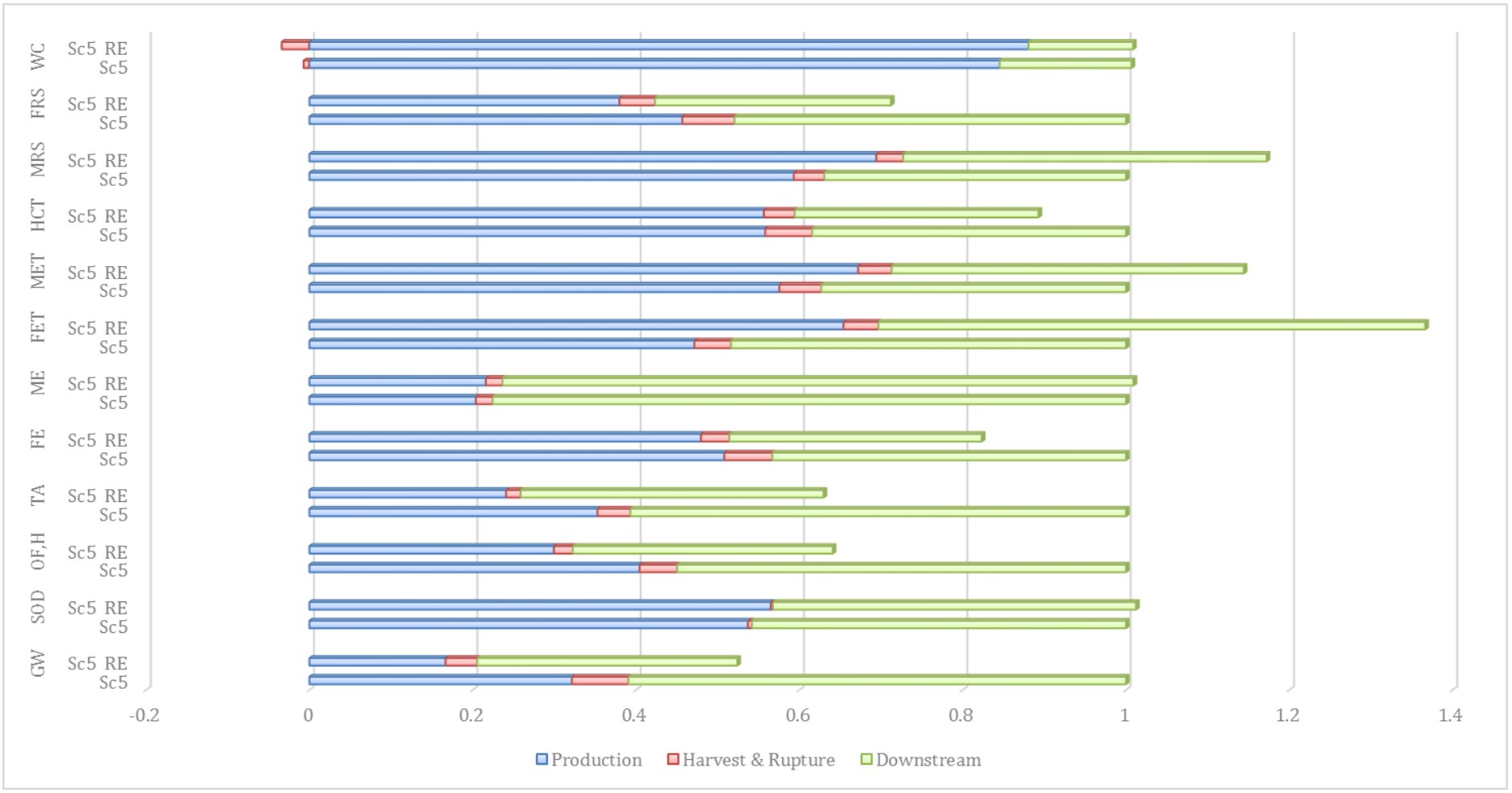

Figure 98 – Comparison of the performance of the Prorocentrum based biorefinery scenario P5 with and without locally produced renewable energy, using normalised impact factor.

Now, when looking at the changes in all scenarios in Table 109 and in *Figure 99* and *Figure 100* (some impact categories where discarded as the difference was very small), it is possible to conclude that the changes in the impact categories are different from scenario to scenario. One of the first observations is that scenario P1 and scenario P2 have the highest differences in the impact category values due to being the scenarios that consumes the biggest amount of energy per kg of EPA+DHA soap (Table 98). On the other hand, as scenario P5 consumes the lowest amount of energy per kg of EPA+DHA soap, it is the scenario with lowest absolute value changes (*Figure 99*). However, the percentage of decrease is very similar in all scenarios, around 45%, except in scenario P4 which is around 40%, as this scenario is the one with the lowest energy consumption per year.

Therefore, if taking into account the number of categories that have a better performance, it can be stated that the photovoltaic energy has a better performance, in an environmental point of view, than the energy from the national grid.

Table 109 - Total impacts of selected categories for scenarios with and without locally produced renewable energy usage.

	GW	OF	MET	HCT	MRS	FRS	WC
Scenarios without renewable energy							
P1	444.79	1.54	211258.53	2008.78	2.45	166.72	43.67
P2	470.12	1.59	211014.69	2030.86	2.40	170.13	43.03
P3	281.52	1.00	117032.15	1099.95	1.47	99.55	22.55
P4	287.93	1.05	157161.31	1458.50	1.87	119.96	34.16
P5	277.62	0.99	114167.82	1073.92	1.43	97.60	21.82
Scenarios with renewable energy							
P1 RE	238.50	0.95	241897.87	1827.31	2.86	120.07	40.38
P2 RE	245.19	1.03	244340.29	1834.06	2.84	120.61	39.63
P3 RE	154.31	0.64	135695.86	986.64	1.72	70.58	20.71
P4 RE	173.59	0.73	174274.64	1358.90	2.10	94.18	32.04
P5 RE	151.99	0.63	132596.83	961.97	1.68	68.99	20.02

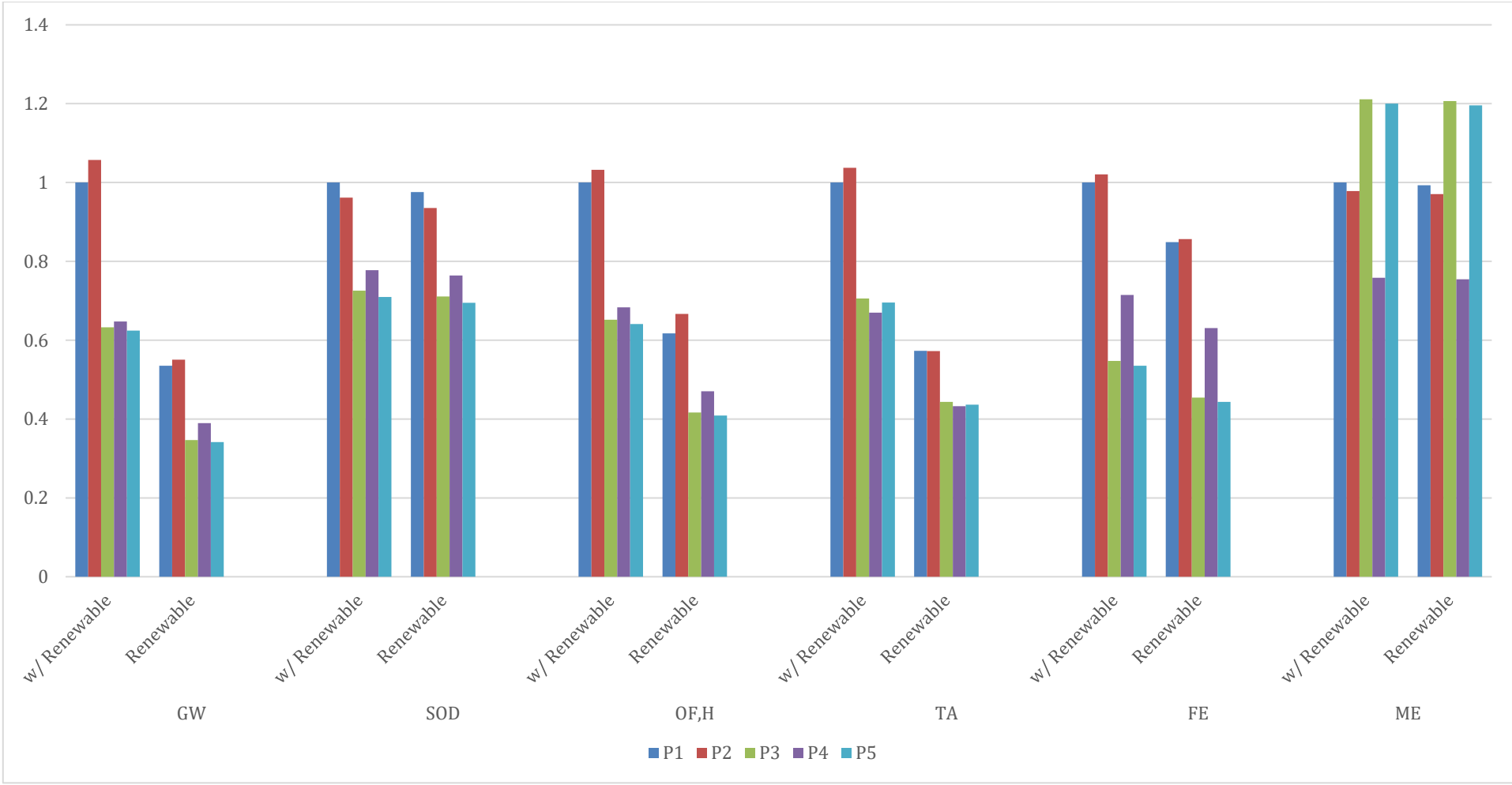

Figure 99 – Comparison of the performance of the 5 Prorocentrum based biorefinery scenarios with and without locally produced renewable energy using normalised impact factors (part 1).

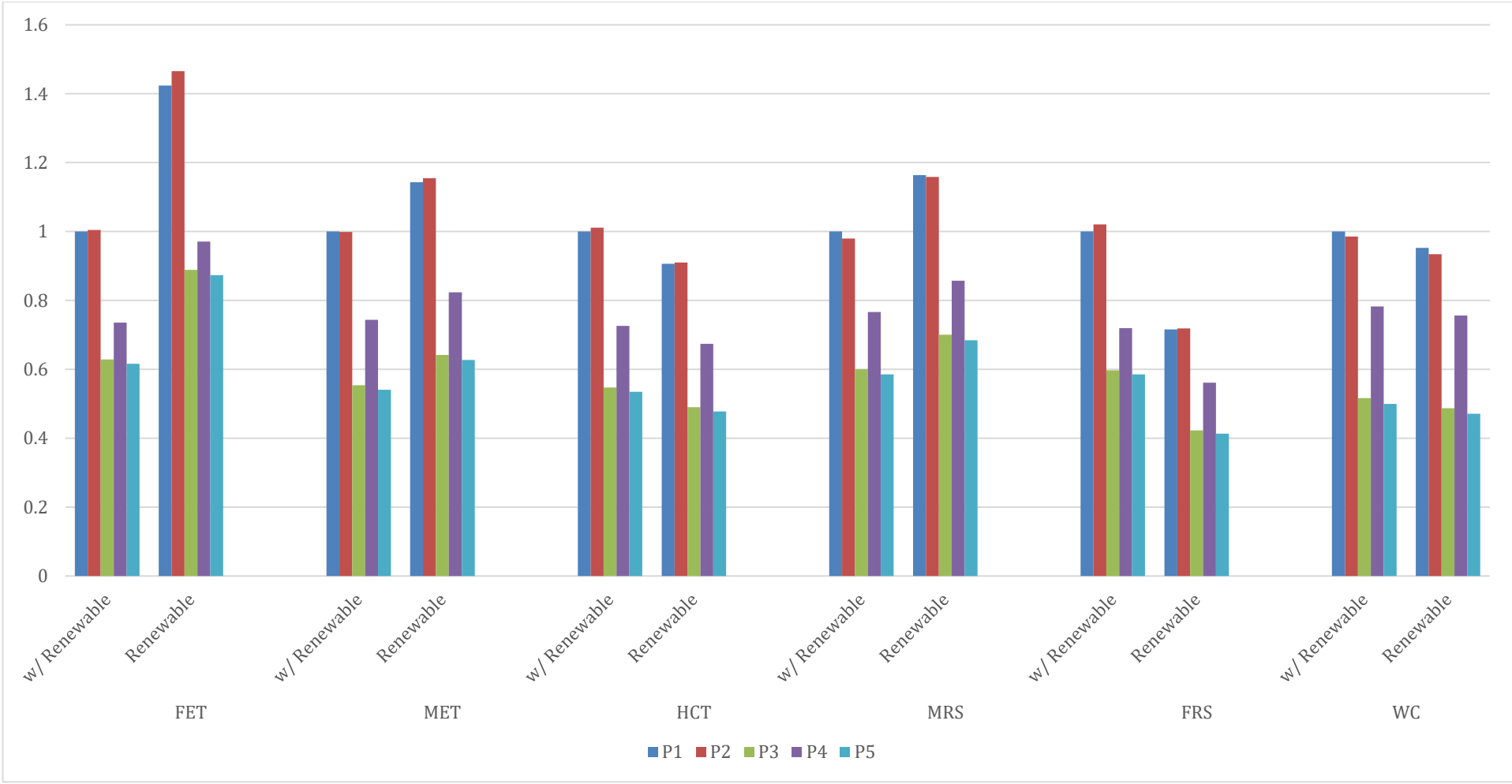

Figure 100 – Comparison of the performance of the 5 Prorocentrum based biorefinery scenarios with and without locally produced renewable energy using normalised impact factors (part 2).

5.9 Conclusion

Looking at the life cycle results, for both microalgae strains, the production process is the stage of the process where the most negative impacts occur. The main contributors are the water, CO_2 and the nutrients used for microalgae cultivation and the electricity used to power the UHT-PBRs. The second stage with the most significant impacts is the downstream stage, especially due to the use of enzymes for enzymatic hydrolysis, the use of solvents for the extraction of lipids, and chitosan for the purification of phycocyanin.

However, some of the impacts can be mitigated by the use of alternatives. In the case of the nutrients and CO_2, waste streams (wastewater for nutrients, and flue gas for CO_2) can be alternatives. The wastewater can also supply the water required for the production. On the other hand, the impacts created by the large energy consumption can be mitigated by the use of renewable energies. However, it is necessary to find an alternative for the reactor cooling, as most of the water spent in the process is used for cooling. When comparing most of the scenarios, the main factor that decided which scenario was the most sustainable, was the amount of the product considered for the functional unit produced in that scenario, with the scenario that had a higher production being the one with the lowest environmental impacts. This demonstrates the importance of selecting the functional unit. The main reason is that the impact is very dependent on the amount of the product chosen as the functional unit. This means that, even if a process does not have a large absolute environmental impact, but the amount of the product chosen as the functional unit is very small, the impact can be much higher than that of a process with a much higher absolute environmental impact. That example can be seen in the case of *Prorocentrum*, where the functional unit used was the EPA+DHA Soap, where some scenarios produced 2 times more product, and therefore have a much lower environmental impact.

Therefore, as future work, a new analysis could be performed, using as a functional unit the product with the highest production, which in both cases is the biofertilizer.

6 Business Case

6.1 *Synechocystis* based biorefinery business case

From the previous economic and life cycle analysis, scenario S5 was chosen as the most sustainable scenario as it was one of the 5 scenarios with the best economic performance, and from those 5 it was the one with the best environmental performance.

As was described previously, scenario S5 uses a membrane to harvest the biomass and the combination distillation column and pervaporation membrane to collect ethanol. Microalgal biomass is ruptured by a bead mill and two products are obtained from the biomass, phycocyanin and a protein hydrolysate. The full diagram is shown in Figure 101.

6.1.1 Economic parameters

In the previous economic analysis, a very simple look at the economic performance of the different scenarios was taken. Now, a more in depth look at this important section of the process design will be taken.

6.1.1.1 Business case assumptions

In order to perform the economic analysis of the scenario, the following general assumptions were made:

- Regarding the Net Present Value (NPV), operating cash flow is discounted using a factor of 5%
- Internal Rate of Return (IRR) of the project is calculated based on the net cash flow of the project
- The Target payback period is <10 years
- Taxes over products sales in Portugal are 23%
- Social security tax is 23.75%
- Taxes over company profits is 25%
- The year 0 is 2020

6.1.1.2 Costs

As explained before, there are different types of costs. They can be capital costs, which comprise of the initial investment required to build the processing plant and purchase the equipment required to produce the products, and operational costs, which cover the costs incurred to run the processing plant, from electric power, to pay worker salaries, etc.

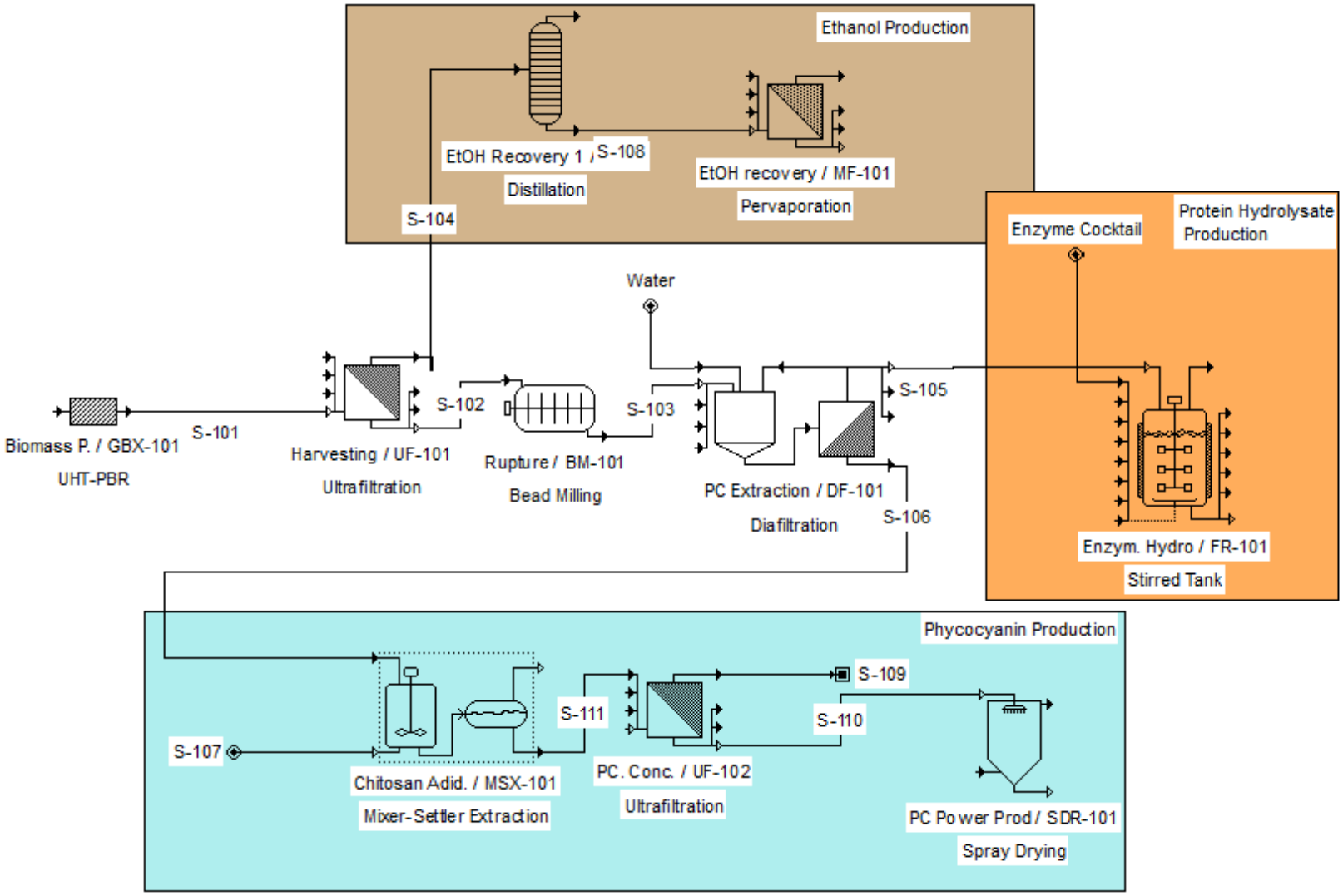

Figure 101 – PFD of scenario S5.

6.1.1.2.1 Capital costs or Capex

As mentioned before, the capital costs are the expenses related to the construction of the processing plant, from the civil engineering and building construction, to the purchase of the equipment's like the production reactors, the downstream equipment and even office and laboratory equipment.

In order to calculate the Capex the following assumptions were made:

- 75% of the Capital costs are used in the first year and 25% in the second year.
- A 10% security factor was considered
- Depreciations are considered to start in the 1st year once the unit is fully operational
- Land was considered as owned by the potential investors and therefore does not represent a cost

A small summary of the Capitals costs can be found in Table 110 and in more detail in Appendix 14.

Table 110 - Capital Costs or Capex for S5.

	Capital costs	%
Production Equipment and related systems	€9,721,993.80	66%
Harvesting	€539,580.00	4%
Processing	€746,351.41	5%
Other costs (Land purchase, facilities construction and related costs	€3,886,750.00	26%
Security	€1,512,487.21	
Total	**€16,282,939.31**	100%

Figure 102 shows the distribution of the Capex costs for the same *scenario S5*.

From the analysis of Table 110 and Figure 102, it is possible to conclude that most of the capital costs (around 65%) are used to purchase and setup the microalgae production process, especially the UHT-PBRs. These costs are followed by the "Other costs", such as land purchase, facilities construction and related (26%) and by the equipment for harvesting and downstream process (±10%).

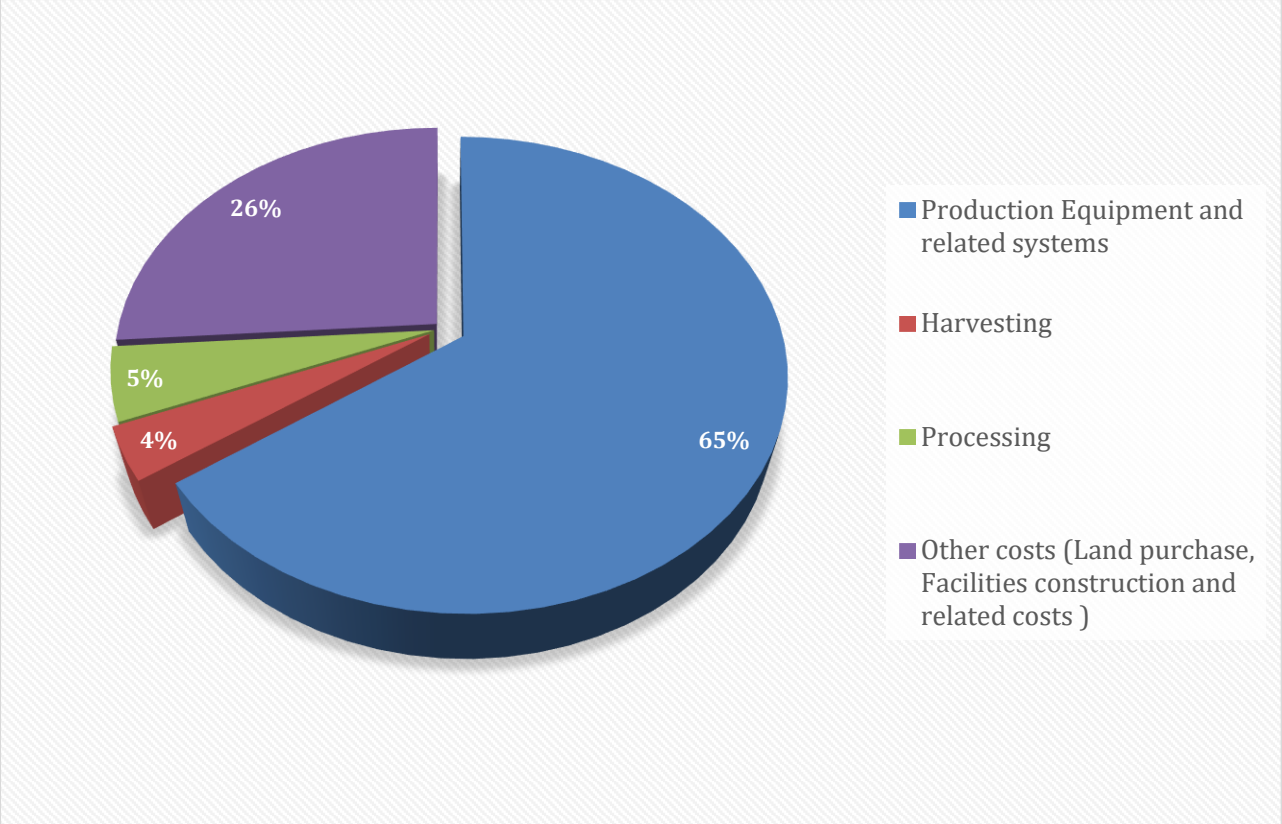

Figure 102 - Distribution of the Capex for scenario S5.

6.1.1.2.2 Operational costs or Opex

The operational costs are the expenses that a processing plant incurs to produce products and to maintain the processing plant functional. These can go from electricity costs, to consumables, to raw material and labor costs.

In order to calculate the Opex, the following assumptions were made:

- Production costs are assumed to be 30% in the first-year and production only occurs during 6 months, 75% in the second year and 100% in the following years
- Inflation on Opex is considered to be at a fixed rate of 2.5% per year
- Human resources to build the processing plant are included in the Civil Engineering prices
- Human resources distribution (see appendix 14):
    - 13% in year 0
    - 62% in year 1
    - 100% in year 2

Table 111 shows the distribution of the full operation costs for *scenario S5*. The distribution of the operation costs is shown in Figure 103.

Table 111 - Operating costs or Opex for scenario S5.

Operating Costs (€/year)	total	%
Labour	€ 1,264,725.00	29%
Electricity	€ 770,507.98	18%
Potable water	€ 384,432.00	9%
Enzymes	€ 535,504.49	12%
Maintenance and improvements	€ 755,976.12	17%
Other costs	€ 644,765.41	15%
Security factor	€ 154,559.30	
Total	€ 4,510,470.30	100%

As can be seen in Table 111 and Figure 103, labour (29%), electricity (18%) and maintenance (17%) costs are the major contributors to the operational costs, with almost 60% of the total value. Enzymes (12%) are also an important cost.

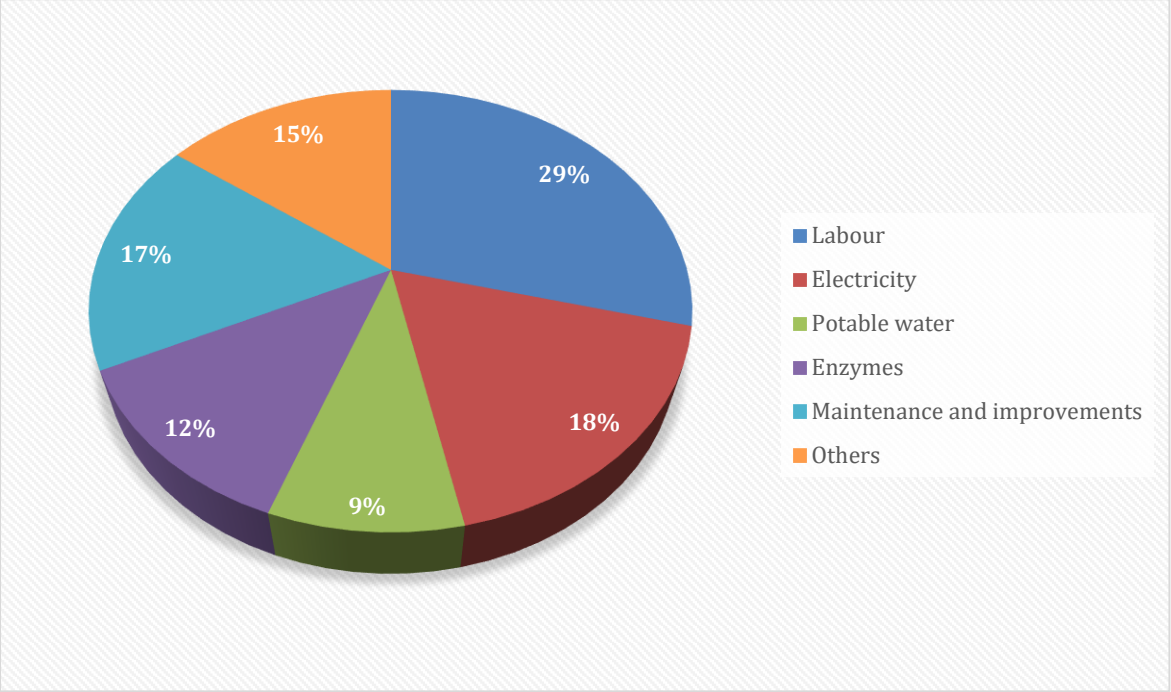

Figure 103 - Distribution of the Opex for scenario S5.

6.1.1.2.3 Revenues

The revenues are the amount of money brought in by the sale of the products, without any taxes. In this scenario, the products are ethanol, phycocyanin powder, and biofertilizer (Table 112).

In order to calculate the revenues, the following assumptions were made:

- Product sale prices are considered to increase at a fixed rate of 2.5% per year;
- Production is assumed to be 30% in the first-year of production and only occurring during 6 months, 75% in the second year and 100% in the following years.

Table 112 – Maximum possible revenue with the current prices.

Profit	Price	Prod/year	Revenue €	%
Ethanol	0.487 €/m^3	290.75 m^3	€ 141,593	2%
Phycocyanin	210 €/kg	15.9 tons	€ 3,339,360.15	41%
Protein Hydrolysate	7.2 €/m^3	638.8 m^3	€ 4,598,272.80	57%
Total Revenue			**€ 8,079,227**	

6.1.2 Economic analysis

With the previous information, a full economic analysis was performed.

From the graph in Figure 105 and from Table 113, it is possible to conclude that the payback time is on the 9th year of operation. The IRR is 7.8%, a Return on Investment (ROI) of around 13% and the NPV is of €2,240,976 . These results are due to the difference in Opex and Revenue seen in Figure 104, which allows to payback the Capex in less than the 9 years. This shows that the process has the potential of being profitable. It can be concluded from Table 112 that phycocyanin and protein hydrolysate both have a large role in this profitability, while ethanol has a very small role.

These values are lower than the ones obtained in the initial analysis. This is due to the addition of taxes, extra human resource costs (night shifts, education and extra bonuses), and the production distribution that were taken into account in this analysis and not in the previous one.

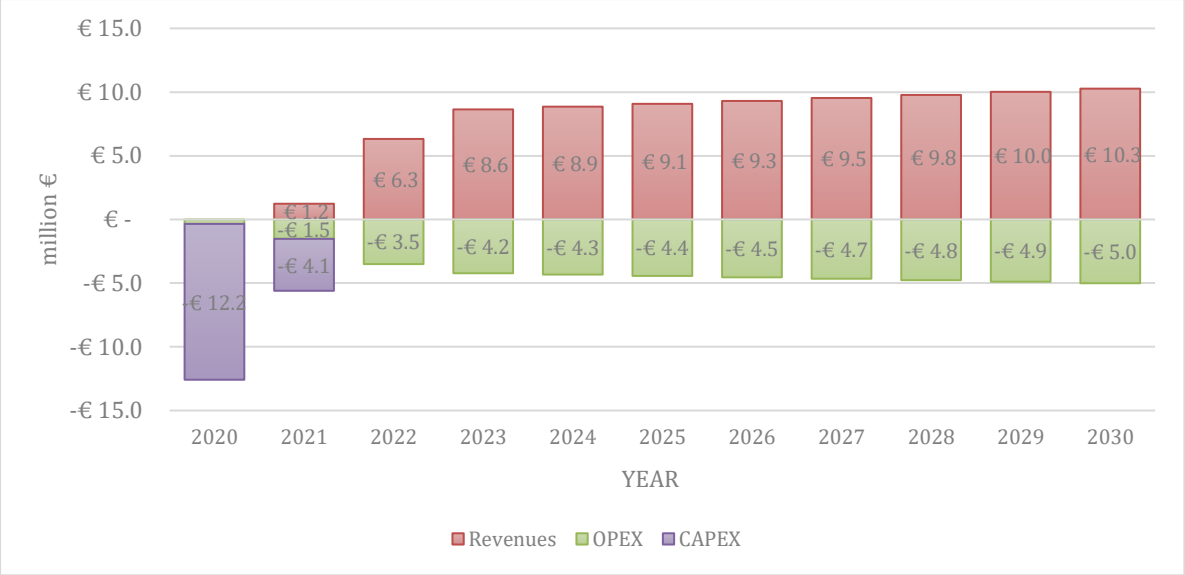

Figure 104 – Profit & Loss Summary 2020-2030.

Table 113 – Costs and Revenues variation for the period 2020-2030 and scenario S5.

year	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030
Capex	€ 12,513,344	€ 4,171,115									
Operational costs	€ -	€ 807,114	€ 2,624,413	€ 3,586,698	€ 3,676,365	€ 3,768,274	€ 3,862,481	€ 3,959,043	€ 4,058,019	€ 4,159,470	€ 4,263,457
Labour Costs	€ 348,877	€ 842,621	€ 1,650,880	€ 1,690,328	€ 1,730,761	€ 1,772,204	€ 1,814,690	€ 1,858,233	€ 1,902,868	€ 1,948,621	€ 1,995,516
Opex	€ 348,877	€ 1,649,736	€ 4,275,293	€ 5,277,025	€ 5,407,126	€ 5,540,478	€ 5,677,171	€ 5,817,276	€ 5,960,888	€ 6,108,091	€ 6,258,973
Revenues	€ -	€ 1,233,559	€ 6,321,991	€ 8,640,055	€ 8,856,056	€ 9,077,458	€ 9,304,394	€ 9,537,004	€ 9,775,429	€ 10,019,815	€ 10,270,310
Cash Flow	-€ 13,269,100	-€ 3,293,770	€ 2,334,186	€ 3,265,595	€ 3,373,789	€ 2,660,787	€ 2,728,235	€ 2,797,372	€ 2,868,235	€ 2,940,868	€ 3,015,319
NPV	-€ 13,269,100	-€ 16,398,387	-€ 14,291,636	-€ 11,491,760	-€ 8,744,087	-€ 6,685,829	-€ 4,681,294	-€ 2,729,093	-€ 827,878	€ 1,023,668	€ 2,240,976

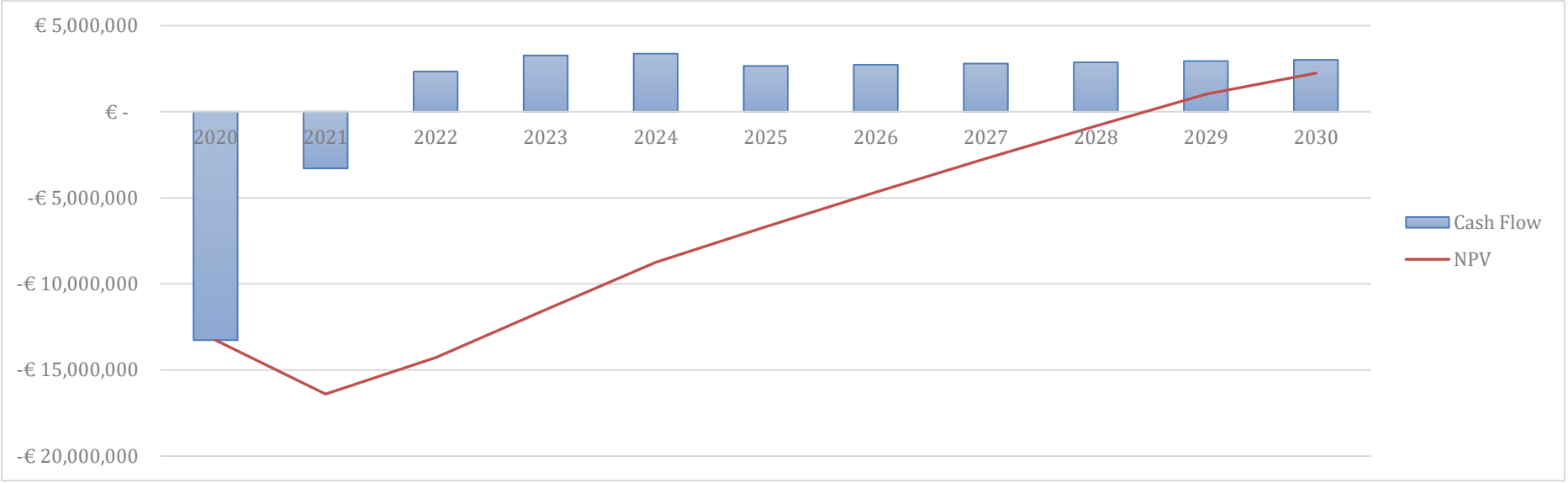

Figure 105 – Variation of the cashflow and NPV until 2030 for scenario S5.

6.1.3 Possible Improvements

As was mentioned in previous chapters, there are two possible improvements that can be done to the process: the use of wastewater to supply part of the nutrients, and the use of renewable energy to power the processing plant. The first will cause a decrease of the cost of nutrients (10%) and water for production (100%), while the second will reduce the electricity costs to 0. However, due to the necessity to build the photovoltaic power plant, the Capex will increase 8% and the maintenance costs will also increase 8%, and an additional 2 maintenance workers will be hired. However, this still means a decrease of around 16% of the Opex. The economic results for the improved scenario are shown in Figure 106 and Figure 107.

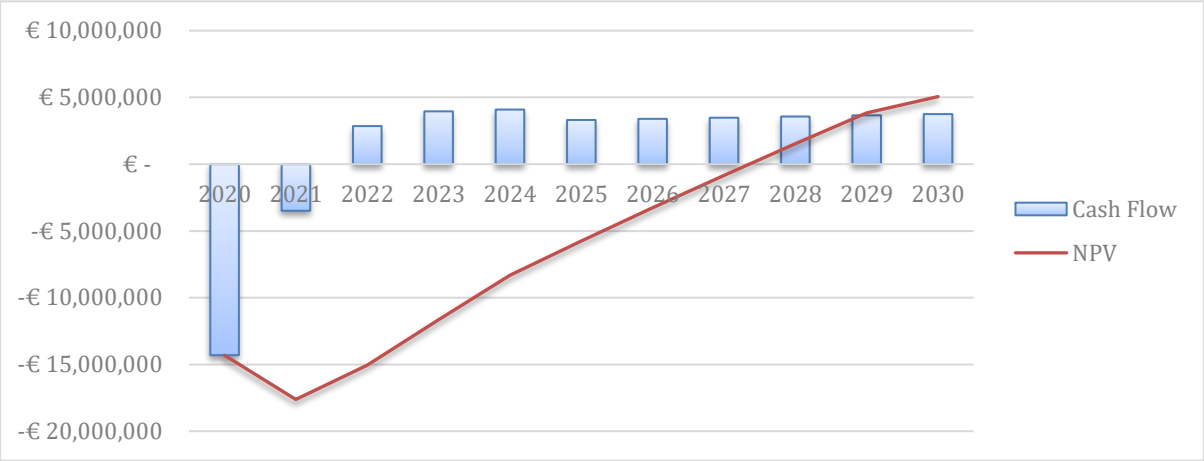

Figure 106 - Variation of the cash-flow and NPV until 2030 of improved scenario.

Figure 107 - Profit & Loss Summary 2020-2030 of improved scenario.

If these improvements were performed, then the economic results would have a better performance. The payback time would now be on the 8th year of operation. The IRR would be 8.2% (±14% increase), the ROI would be of around 28% (±100% increase) and the NPV would be €5,060,494 (±126% increase). These results are due to the large decrease in the operational costs (around 15%), compared to the small increase of the capital costs (8%). Another possible improvement, but not accounted for in this study, could be the use of steam produced in another industrial facility that could also reduce the costs of the process.

6.1.4 Scenario Conclusion

In the initial economic analysis of the *Synechocystis* based biorefinery, it was concluded that only the scenarios that had a biorefinery approach were able to have a positive economic performance (30 out of 136). The main reason behind this conclusion was that, in all scenarios that produced only phycocyanin and ethanol, the revenue was never sufficient to pay back the high investment. This high investment was especially due to the high costs of the microalgae production process that accounted for almost 60% of the initial investment. The main contributor to this high value is the price of the UHT-PBRs. Furthermore, the highest operational costs were the manpower, electricity and maintenance, adding up to almost 60% or more of the operational costs, in some scenarios. Unfortunately, it was concluded that the ethanol production, in the considered conditions, is not profitable, as the costs for extracting the ethanol from the medium were higher than the revenue generated by the sale of ethanol. Therefore, as was shown in the initial economic analysis, using a non-modified *Synechocystis*, would be more advantageous, although it would defeat the purpose of using the *Synechocystis* to produce ethanol as an alternative to biofuels. It was also concluded that the full biorefinery approach was not the best solution, as the costs incurred by the extra process to obtain carotenoids were not compensated by the extra revenue produced by the refined products. Finally, it was observed that the best scenarios are very dependent on the phycocyanin and bio-fertilizer market to have a positive economic performance.

The subsequent environmental analysis also brought to light other conclusions. As was expected, the production stage was the one with the highest impacts, due to the Nutrients, CO_2 and electricity used in that stage. Another conclusion was that the enzymes used for the protein hydrolysis and chitosan used to purify the phycocyanin stream also had a high negative impact on the environment. Although most of the scenarios had very similar environmental performance, (except the scenario with full biorefinery approach that had a much higher negative impact, due to the use of Ethanol in the lipid extraction) the tie

breaker was the phycocyanin production, as the higher the production, the lower the impacts. Therefore the scenario with the highest phycocyanin production was chosen.

In the final economic performance, the values obtained for the NPV and ROI are slightly lower and the PBP is higher than those of the initial economic analysis. This is normal as different assumptions and more costs were assumed. However, an optimistic assessment can be made as the scenario has a positive performance. These results were mostly due to the phycocyanin and biofertilizer, as the production of these components does not attain very high costs and the products have a high value. Also, if some improvements are implemented, like the use of photovoltaic panels to supply energy, use of wastewater to supply part of the nutrients and water, and use of steam produced by other factories, the economics and environmental performance of the scenario would improve, as consumables and utility costs would go down.

The final results were compared against the results obtained by other studies; one by Chaiklahan et al. (2018), which studied the production of phycocyanin from *Arthrospira* and the work by Lopes *et al*. (Lopes et al., 2019) which performed an economic analysis to the DEMA project (Table 114).

Table 114 – Economic values for the designed scenarios and two similar economic studies.

	S5	S5 Improved	Chaiklahan et al.	Lopes T. et al.
NPV (€)	2,240,976	6,781,277	30,203	16,700,000
PC yearly production (kg)	15,900	15,900	600	24,640
NPV (€/kg PC)	141	427	50	678
Production costs (€/kg PC)	388	330	197	592

As can be seen in Table 114, the NPV per kg of phycocyanin of the designed scenarios is higher than in the process proposed by Chaiklahan et al. (2018). The reason for this difference is that in the study by Chaiklahan et al. only phycocyanin is produced, and in this study more products are obtained, so a higher revenue is expected. The fact that this study includes more products also increases the production costs per kg of phycocyanin of the designed processes. Another reason for the high production costs in the designed scenarios is the use of biomass produced in house, with an average cost of 23 €/kg of biomass, while in the process by Chaiklahan et al. a biomass with an average cost of 11 €/kg was considered.

When comparing the scenario economic parameters with those of Lopes et al., we can conclude that the values per kg of phycocyanin of the improved scenario are in the same order of magnitude. The reason why Lopes at al. (2019) estimated higher production costs and NPV is the extra products explored in their work, as well as the slightly higher prices

used, respectively. The work by Lopes et al. also contemplates energy recycling and anaerobic digestion of the biomass, which decreases the costs and therefore results in a similar performance to the improved scenario.

6.2 *Prorocentrum* based biorefinery business case

From the previous economic and life cycle analysis, scenario P5 was chosen, as it was one of the 5 scenarios with the best economic performance, and from those 5 it was the one with the best environmental performance.

As was explained before, scenario P5 uses a membrane system to harvest the biomass that is then ruptured by ultrasonication, and three products are obtained from the biomass: EPA + DHA soap, a mixture of carotenoids and a protein hydrolysate. The full diagram is shown in Figure 108.

6.2.1 Economic parameters

In the previous analysis, a very simple look at the economic performance of the different scenarios was taken. A more detailed look will be taken now, at this important section of the process design.

6.2.1.1 Business case assumptions

In order to establish the economic analysis of all the scenarios that were considered, the following general assumptions were made:

- Regarding the Net Present Value (NPV), operating cash flow is discounted using a factor of 5%
- Internal Rate of Return (IRR) of the project is calculated based on the net cash flow to the project
- Target payback period is <10 years
- Taxes over products sales in Portugal is 23%
- Social security tax is 23.75%
- Taxes over company profits is 25%
- The year 0 is 2020

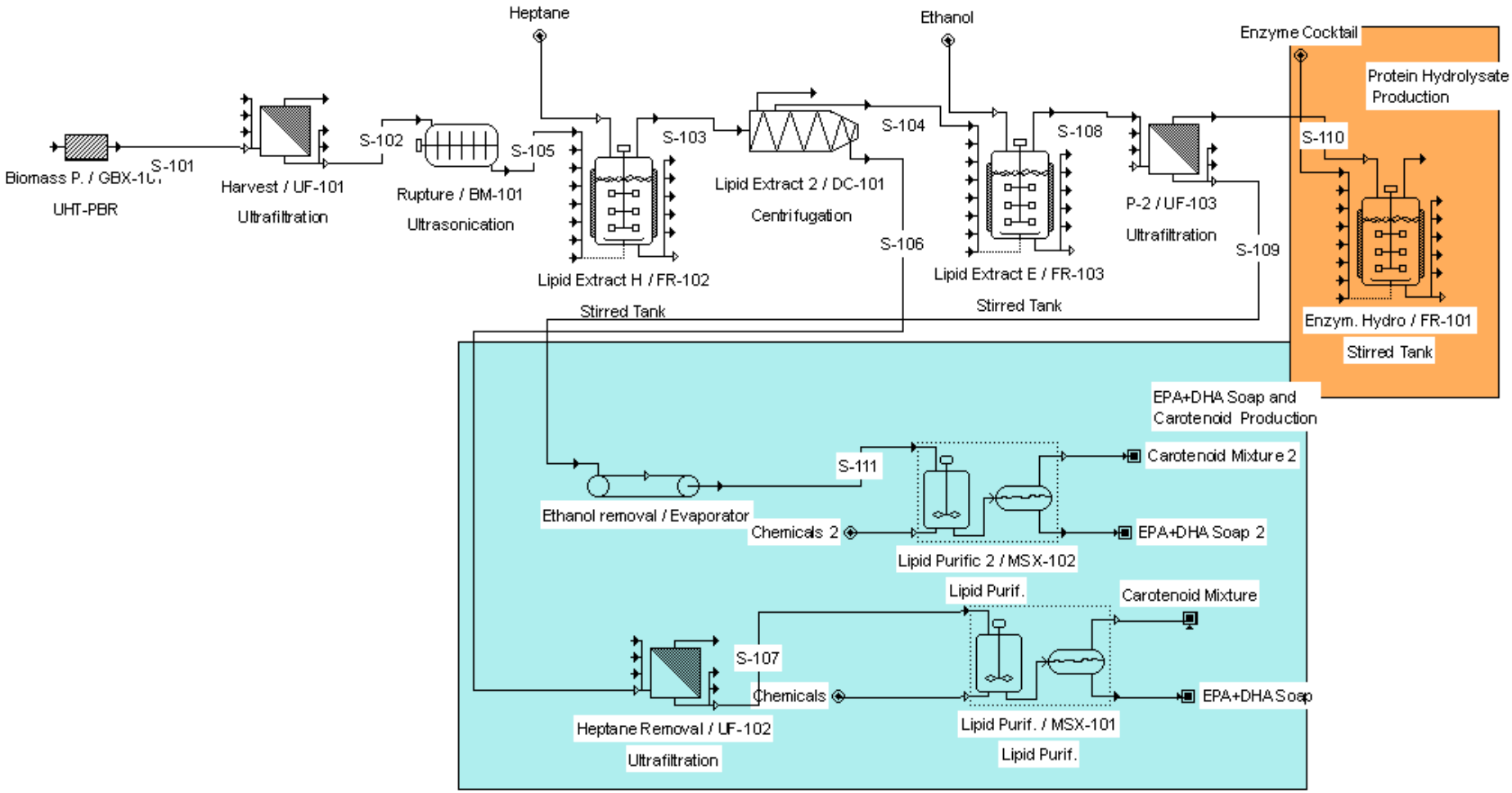

Figure 108 - PFD of scenario P5 .

The different types of costs to be considered include capital costs, which is the initial investment required to build the processing plant and purchase the equipment required to produce the products, and operational costs, which are the costs incurred to run the processing plant, from electric power, to worker salaries.

6.2.1.1.1 Capital costs or Capex for *Prorocentrum* based biorefineries

The capital costs are the expenditures related to the construction of the processing plant, from the civil engineering and building construction, to the purchase of the equipment like the production reactors, the extraction equipment and even office and laboratory equipment.

In order to calculate the Capex, the following assumptions were made:

- 75% of the Capital costs are used in the first year and 25% in the second year
- A 10% security factor was considered
- Depreciations are considered to start in the 1st year once the unit is fully operational
- Land is considered as owned by the potential investors and therefore does not represent a cost

A summary of the Capitals costs is presented in Table 115, for the scenario P5 of the *Prorocentrum* based biorefinery. The distribution of the Capex costs is shown in the graph of Figure 109.

From Figure 109 and Table 115 it is possible to see that most of the capital costs are used to purchase and setup the microalgae production (70%) and the downstream processing equipment (44%). The main contributors to the high extraction costs are the lipids purification process that accounts for 94% of the processing costs. These costs are followed by the other costs (26%).

Table 115 - Capital Costs or Capex for scenario P5 of the Prorocentrum based biorefinery.

	Capital costs	%
Production Equipment and related systems	€ 10,319,510.00	49%
Harvesting	€ 325,000.00	2%
Processing	€ 6,559,347.00	31%
Other costs (Land purchase, facility construction and related costs	€ 3,877,500.00	18%
Security	€ 1,512,487.21	
Total	**€ 22,593,844.21**	100%

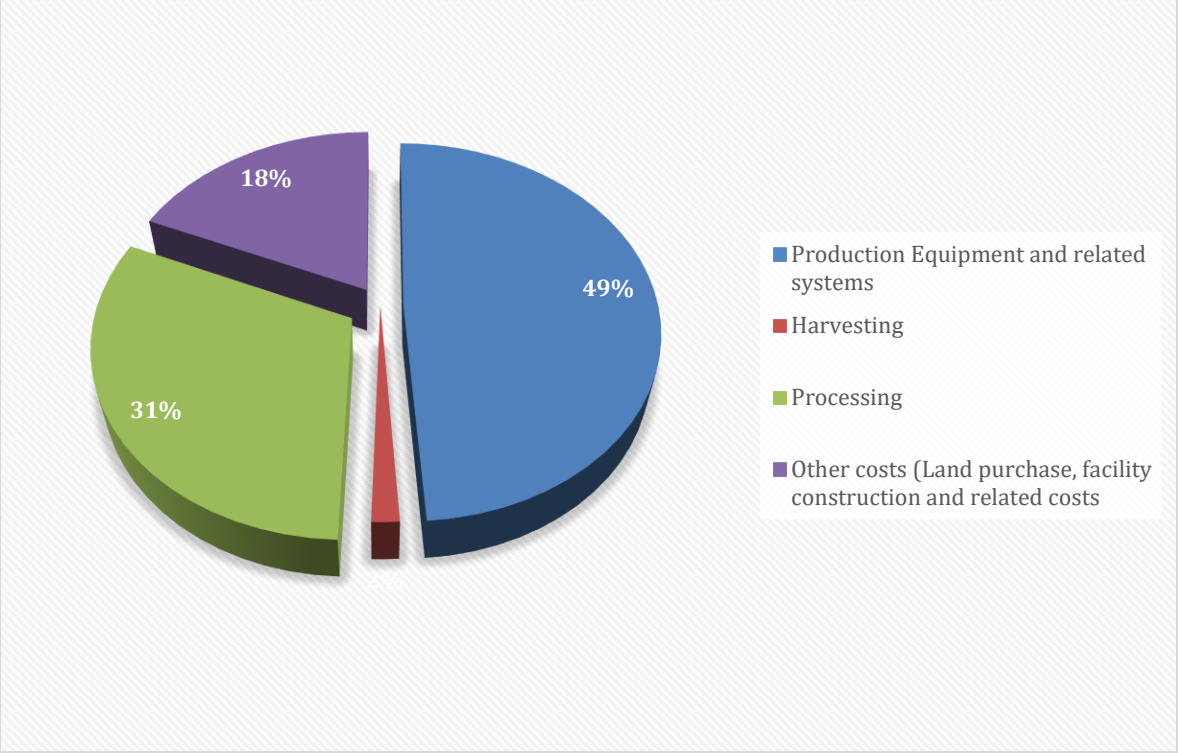

Figure 109 – Capex Distribution for scenario P5 of the Prorocentrum based biorefinery.

6.2.1.1.2 Operational costs or Opex

The operational costs are the expenses that a processing plant incurs to produce products and maintain the processing plant functional. These can go from electricity costs, to consumables, to raw material and labour costs.

In order to calculate the Opex, the following assumptions were made:

- Production costs are assumed to be 30% in the first-year and production only occurs during 6 months, 75% in the second year and 100% in the following years
- Inflation on Opex is considered to be at a fixed rate of 2.5% per year
- A security factor of 5% is considered
- Human resources to build the processing plant are included in the Civil Engineering prices
- Human resources distribution
    - 11% in year
    - 58% in year 1
    - 100% in year 2

Table 116 and Figure 110 shows the distribution of the operation costs.

As can be seen in Table 116 and Figure 110, the labor (27%), electricity (17%) and maintenance (20%) costs are the major contributors to the operational costs, with more

than 60% of the value. The enzymes (7%) also constitute an important cost along with the water consumption (7%).

Table 116 - Operational costs or Opex for scenario P5 of the Prorocentrum based biorefinery.

Operational Costs (€/year)	total	%
Labour	€ 1,437,975	27%
Electricity	€ 867,810	17%
Potable water	€ 340,145	7%
Enzymes	€ 364,591	7%
Maintenance	€ 1,050,691	20%
Others	€ 1,171,495	22%
Security factor	€ 261,635	
Total	**€ 5,494,342**	

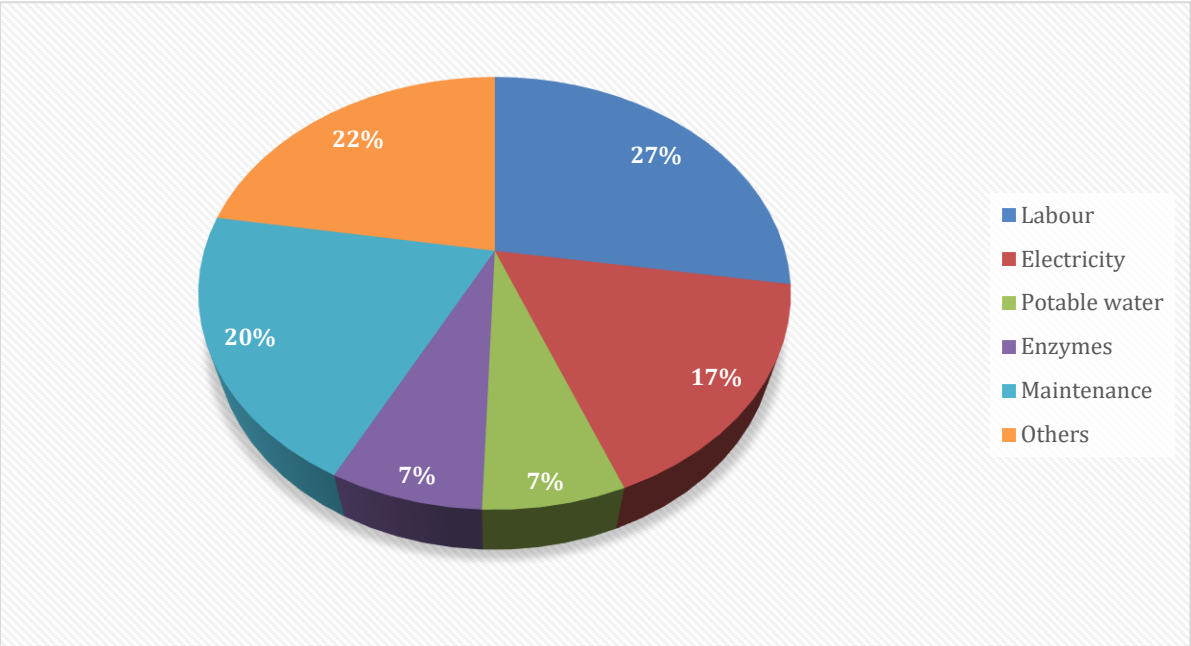

Figure 110 - Opex Distribution for scenario P5 of the Prorocentrum based biorefinery.

6.2.1.1.3 Revenues

The revenues are the amount of money obtained by the sale of the products, without any taxes. In this scenario, the products are a metal soap rich in EPA + DHA, biofertilizer, and a carbohydrate feed (see Table 117).

In order to calculate the revenues, the following assumptions were made:

- No financing rate was considered
- Product sale prices are considered to increase at a fixed rate of 2.5% per year

- Production is assumed to be 30% in the first-year production and only occurs during 6 months, 75% in the second year and 100% in the following years

Table 117 – Maximum possible revenue with the current prices.

Profit	€/kg	Prod/year	Revenue €	%
Metal Soap	60	14.49 ton	€ 869,400	9%
Protein Hydrolysate	7.2	1193.1 m3	€ 8,590,464	89%
Carbohydrate Feed	1	185.9 ton	€ 185,900	2%
Total Revenue			**€ 9,645,764**	

6.2.2 Economic analysis

With the previous information, a full economic analysis was performed.

As can be seen in Figure 112, this process has a positive but low economic performance, as it barely pays back on the 10th year of labour and only has a NPV of €552,566 , a ROI of 2% and an IRR of 5.71%. The main reason for this low performance, as can be seen is Figure 111 and Table 118, is that unlike in the previous scenario, the Opex and revenues have a small difference and therefore the cash flow is smaller and takes more time to payback the Capex, which in turn is also higher than in the previous scenario. Although the main objective of this process was to produce EPA+DHA Soap, the main revenue came from the protein hydrolysate (see Table 117). If this result was compared with the initial analysis, where the process had a better economic performance, the differences found would be due to the fact that the previous analysis had a very simple approach, without the production distribution, theextra human resources costs and taxes, which have a large impact in the project profitability.

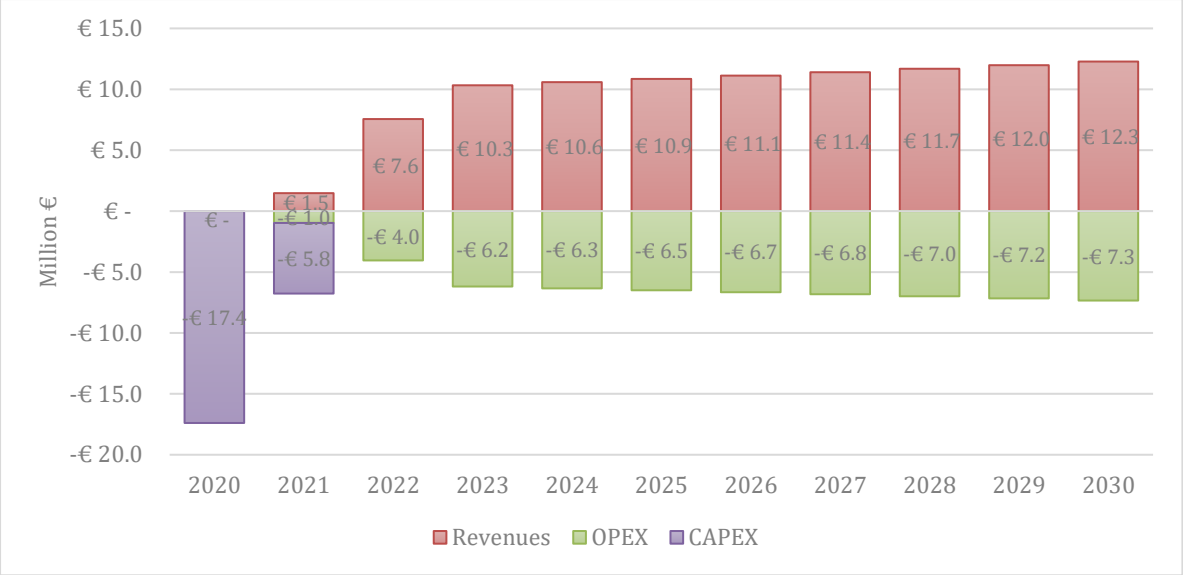

Figure 111 – Profit & Loss Summary 2020-2030.

Table 118 - Costs and Revenues variation 2020-2030 for scenario P5.

year	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030
Capex	€ 17,392,120	€ 5,797,373	€ -	€ -	€ -	€ -	€ -	€ -	€ -	€ -	€ -
Operational costs	€ -	€ 621,503	€ 3,107,515	€ 4,246,937	€ 4,353,110	€ 4,461,938	€ 4,573,486	€ 4,687,823	€ 4,805,019	€ 4,925,145	€ 5,048,273
Labour Costs	€ 348,877	€ 941,018	€ 1,943,100	€ 1,989,465	€ 2,036,988	€ 2,085,699	€ 2,135,635	€ 2,186,814	€ 2,239,277	€ 2,293,053	€ 2,348,171
Opex	€ 348,877	€ 1,482,201	€ 4,600,170	€ 5,609,668	€ 5,747,696	€ 5,889,174	€ 6,034,197	€ 6,182,840	€ 6,335,204	€ 6,491,378	€ 6,651,455
Revenues	€ -	€ 1,474,983	€ 7,559,286	€ 10,331,024	€ 10,589,300	€ 10,854,032	€ 11,125,383	€ 11,403,518	€ 11,688,606	€ 11,980,821	€ 12,280,341
Cash Flow	-€ 18,466,200	-€ 4,082,797	€ 3,173,365	€ 4,203,973	€ 4,340,648	€ 3,231,654	€ 3,313,607	€ 3,397,612	€ 3,483,715	€ 3,571,968	€ 3,662,430
NPV	-€ 18,466,200	-€ 22,349,415	-€ 19,505,951	-€ 15,929,299	-€ 12,421,644	-€ 9,948,510	-€ 7,539,893	-€ 5,194,129	-€ 2,909,597	-€ 684,720	€ 552,566

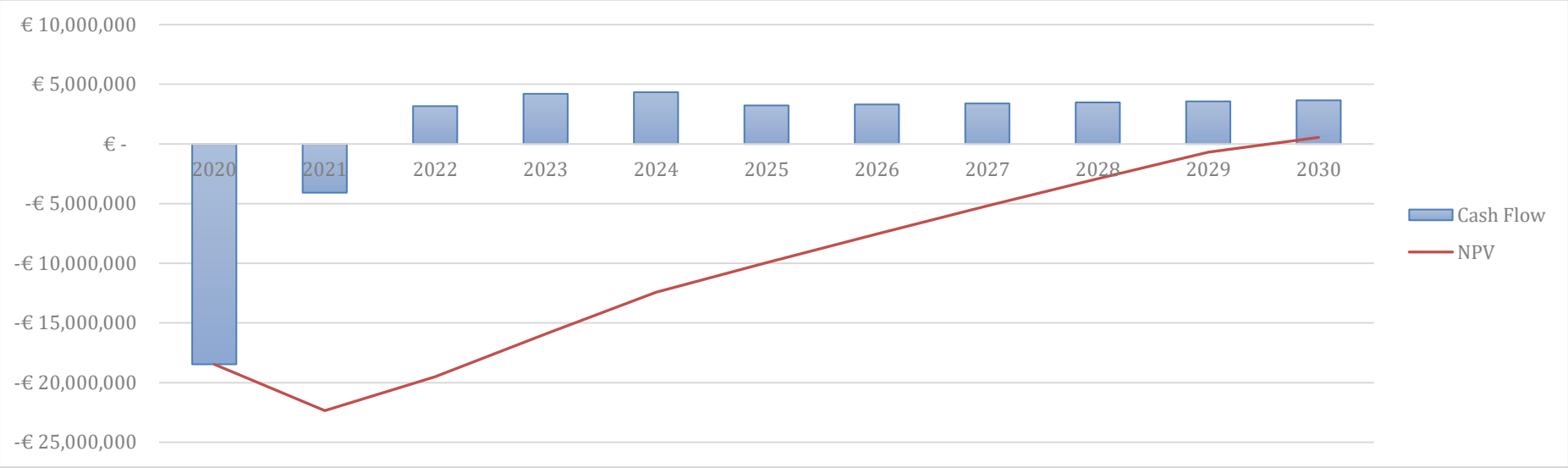

Figure 112 - Variation of the cash flow and NPV until 2030 for scenario P5.

6.2.3 Possible Improvements

As was mentioned before, there are different possibilities to improve the economic and environmental performances of the process. Three of such improvements are the inclusion of photovoltaic panels to supply the energy, the use of wastewater to supply nutrients and water to the production system and the use of flue gas from other industries to supply the CO_2 for production.

Using the previously mentioned improvements, the water, CO_2 and energy consumption costs decrease to 0% and the nutrient cost decreases 3%. However, due to the purchase, installation and maintenance of the photovoltaic panels, as well as a scrubber to treat the flue gas, the cost related to maintenance and the capex increase by 8%. In addition, two more workers were hired for panel maintenance. Although there is an increase in costs, these improvements should lead to a reduction of 18% of the Opex. Due to these changes, the difference between the Opex and Revenues is much larger than in the original scenario (see Figure 113).

As can be observed in Figure 114, the scenario now has a positive NPV of €4,444,041 , a ROI of 15% (%750 increase) and Internal rate on return of 8.2% (44% increase). The payback period is around 8 years. This result is due to the large decrease in Opex that can be observed in Figure 113. This decrease is due to the decrease in energy costs, as now all electricity is supplied by the photovoltaic panels, as well as to the decrease in water, CO_2 and nutrient costs due to the use of free waste streams. These symbioses with other industries, when related to the waste streams, are extremely important for cost savings not only for the company that receives the wastes, but also for the company that supplies them, as they also decrease the waste treatment costs, and in the case of CO_2, the carbon credits cost reduction can also be important.

Figure 113 – Profit & Loss Summary 2020-2030 in improved scenario.

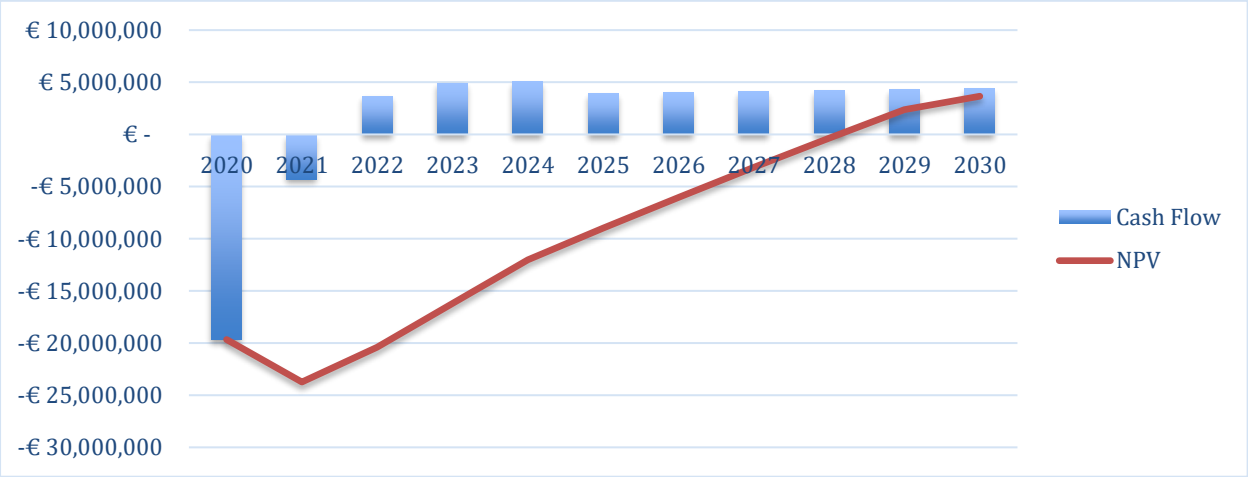

Figure 114 - Variation of the cash flow and NPV until 2030 in improved scenario.

6.2.4 Scenario Conclusion

In the initial economic analysis, it was concluded that only the scenarios that had a biorefinery approach were able to have a positive economic performance, as only 15 of the 98 scenarios had a positive NPV and all had a biorefinery approach. In all other cases, the revenue produced was never enough to pay back the high investment performed, as the products had a low value. The high investment was again due to the high costs of equipment used in the microalgae production process that accounted for almost 40% of the initial

investment, but also due to the lipid purification process that accounted for 15 to 30% of the Capital costs. Unlike in the *Synechocystis* scenario, in the *Prorocentrum* scenario the full biorefinery approach was the best solution, as the costs incurred by the extra processes were compensated by the extra revenue produced by the more refined products. Actually, by using the assumptions considered in this study, the extra products produced more income than the main product. This is due to the purification process. Unfortunately, due to the low amount of omega-3 fatty acids in *Prorocentrum*, the extraction process incurs in heavy costs per kg of EPA+DHA produced, that are not compensated by the EPA+DHA Soap sales. Due to the low amount of EPA+DHA, it was observed that the best scenarios are very dependent on the bio-fertilizer market to have a positive economic performance, as the amount of EPA+DHA Soap is not enough to balance the sales. This also shows that the *Prorocentrum* biomass has low value, as the amount of omega-3 fatty acids existent is small (around 8%) and, besides the proteins and a small quantity of carotenoids, there are not many high value components in the biomass. A possibility to make *Prorocentrum* a profitable microalga could be to only produce protein hydrolysate, as the process is cheaper (no need for the lipid purification step) and the protein hydrolysate is the main source of income of the process (from 60 to 90% of total revenues) as can be seen in Table 117.

The subsequent environmental analysis also brought to light other conclusions. As was expected the production stage was the one with the highest impacts, due to the nutrients, CO_2 and electricity used in that stage, but due to the solvents used, the extraction stage also had a high impact. Another conclusion was that the enzymes used for the protein hydrolysis had a high negative impact on the environment. During comparison, it was found that the lipid extraction process choice is important, not only due to environmental reasons but also technical reasons. It was found that, although the supercritical approach has less impacts than the solvent extraction, especially if using ethanol, as the amount of EPA+DHA Soap extracted is smaller, the impacts per kg of soap of the supercritical extraction are higher. Therefore, if the efficiency of the supercritical extraction was improved, the whole process could be more sustainable with supercritical extraction than with solvent extraction. Nevertheless, there are other improvements that can be made, such as the use of waste streams from other industries and the use of renewable energy.

In the final economic performance, the main observation is that the process has a positive economic performance, but the value is very low. This is mostly due to the low value of the biomass and the high costs incurred to produce biomass and to purify the lipids. This, however, can be improved by making some changes such as adding photovoltaic panels, using wastewater as nutrient source and flue gas as a CO_2 source. With the reduction of

energy and nutrient costs, it is possible to obtain a better economic performance of the *Prorocentrum* biorefinery.

Unfortunately, there are not many economic analysis studies on the topic of EPA+DHA from microalgae, and those that exist are either very short on information, or only contemplate the production of microalgae rich in EPA+DHA without any further processing. Therefore, a qualitative comparison of this studies results was performed against the price of 1 gram of EPA+DHA supplied by other sources like fish, eggs, milk, seaweeds, supplements and other articles that produced EPA+DHA from microalga (Table 119).

Table 119 - Cost of 1 g of EPA + DHA supplied by different products (Edmonds, 2012; Press, 2011).

Source	Cost (€/ g of EPA+DHA Consumed)	
	min	max
Cod fish Oil[*1]	0.05	0.36
Salmon[*1]	0.42	8.94
Eggs[*1]	3.84	10.45
Milk and Soy Milk[*1]	15.25	21.29
Seaweeds[*1]	4.10	52.27
Supplements [4]*[1]	0.09	0.91
EPA+DHA from microalgae (Chauton et al., 2015) [*2]	0.05	
EPA+DHA from microalgae (Zhu et al., 2017) [*2]	0.21	
EPA+DHA Soap[*2] **produced in P5**	1.5	

*1 – Sales Price

*2 - production price

As can be seen in Table 119 the price of production of EPA+DHA soap in this study is very close to that of salmon and lower than most other common products. This result should be a positive result, meaning that the product in this study could be sold at prices higher than 1.5 €/g. However, it is not possible to compare the price of those products because they are not purchased only for the EPA+DHA, but also for the other components. Therefore, when comparing to the products similar to the one in this study, like the supplements that contain EPA+DHA and other articles that produce EPA+DHA from microalga, the conclusion is that their prices are much lower.

In the case of Chauton et al. (2015) this is due to the fact that their study only assumed the production stage and no further processing was considered, and therefore the costs are much lower. In the case of the supplements, this is due to the fact that the EPA+DHA comes from cheap subtracts like fish oil, which, as can be seen in the Table 119, is very cheap. Consequently, their selling prices are quite low. In the case of Zhu et al. (Zhu et al., 2017), the microalgae used was *Schizochytrium* sp. which can have a content of DHA of around 20% of the biomass (Sahin et al., 2018) and can grow around 10 times faster. This is almost 10 times higher DHA content than EPA + DHA content considered in this study, which explains the difference of production costs between both scenarios.

Therefore, it is not possible to sell the EPA+DHA soap at production price, as the product would not be competitive enough. For this reason, it is only sold at a price of 0.17 €/g of EPA+DHA, which is the price of algal oil enriched with EPA+DHA (see Appendix 6). Due to the small amount of EPA+DHA present in the *Prorocentrum* biomass, the production of EPA+DHA is so costly that the process is only feasible due to the revenue obtained by the protein hydrolysate. This result helps us to conclude that *Prorocentrum* is not a good microalga solely or predominantly for the production of EPA+DHA soaps.

7 Conclusion

Microalgae have been the focus of different studies, due to their diverse and interesting composition. However, most economic studies have pointed out that currently microalgae production is still very expensive, and only a few special microalgae have been successfully implemented at industrial scale. These microalgae have either the capacity of being produced in large amounts for a very low price (*Arthrospira* sp. – mainly for health food applications) and/or contain very high value components that cover the production costs (*Dunaliella* sp., *Haematococcus* sp. – mainly for nutraceuticals, supplements and cosmeceuticals*)*. Other microalgae also contain high value products, but these are either more problematic and expensive to produce and process, or do not contain large amounts of one product, making extraction difficult and expensive. Nevertheless, some of these microalgae can be profitable if the right approach is chosen, using a biorefinery concept. Taking into account these considerations, this study was performed, using two microalgae that are not commonly considered for industrial scale production. The idea was to design a standard for two different species, one process representative of a downstream of a microalgae with target intracellular compounds and the other representative of a downstream of a microalgae with target extracellular compounds, so that in the future similar microalgae could be exploited within a biorefinery approach. This study also proved to be a novelty as no other work could be found in the literature that combined the technical-economic evaluation with the environmental performance evaluation of a *Synechocystis* or a *Prorocentrum* based biorefinery.

During this thesis, a large number of scenarios were created for two different microalgae species. For *Synechocystis,* 17 production, harvesting and rupture combinations were considered, and 8 extraction scenarios were designed, creating a total of 136 scenarios considered for economic analysis. In the case of *Prorocentrum*, 14 production, harvesting and rupture combinations were considered, and 7 extraction scenarios were designed, ending with a total of 98 scenarios. For economic analysis, the Capex, Opex and revenues of each scenarios were calculated. During the economic analysis, the 5 best scenarios for each microalgae species were selected based on their ROI, PBP and NPV. After the economic analysis, a life cycle assessment was performed on the 5 best economic scenarios for each microalga, and the one with the lowest environmental impact was chosen. Last but not least, a more detailed and in depth economic analysis was performed to each of the chosen scenarios in order to identify potential improvements and bottlenecks. The results showed that both scenarios proved to have economic feasibility, showing that there is potential for

these two microalgae. Furthermore, the comparison of these two different scenarios can also be useful to take some conclusions that can help in future endeavors. The first is in the economic aspect of a biorefinery. The most important starting point for a biorefinery is the microalgae biomass. In the *Synechocystis* scenario, the biomass achieved values of almost 50 €/kg due to the possible products that could be obtained from that biomass. On the other hand, the *Prorocentrum* biomass only achieved a price of around 25 €/kg which is half of the value of the *Synechocystis* biomass. This is one of the reasons why *Prorocentrum* has a worse economic performance than *Synechocystis,* even though it has 3 times higher biomass productivity. This brings us to the second important aspect, which is the maximum concentration of biomass produced. This factor will influence the biomass production price per kg of biomass as well as the following harvesting step. Comparing both scenarios, the *Prorocentrum* scenario has a lower production capital cost/kg (about 2.2 €/kg) and a harvesting step with lower operational and capital costs, than the *Synechocystis* scenario that has a production capital cost of around 5.0 €/kg and slightly higher harvesting costs. This happens because the concentration achieved during *Prorocentrum* production is higher (2.4 g/L) than the one of *Synechocystis* (0.52 g/L). These results mean that although choosing the right process is important, selecting the right species, with a high content of high value metabolites and high productivity, is even more important to achieve a profitable microalgae biorefinery. Another observation is that the microalgae production equipment is responsible for the bulk of the capital costs. Improvements in this stage might lead to better economic performance by future microalgae biorefineries. Again, the selection of the microalgae biomass to cultivate is important. For instance, extremophile species can be cultivated in open reactors, as they are less prone to contamination, decreasing the costs of the production process. Examples of such species are *Dunaliella* and *Arthrospira* that are already in use at industrial scale. Furthermore, the operational costs of the production stage, with water, nutrients and electricity at the top of the list, are among the highest of the process. However, this can be improved with the use of renewable energy and synergy with other industries.

In the environmental aspect of the biorefinery, other conclusions can also be attained. In both cases, the production process is the stage where the most negative impacts are observed. This is mostly due to the water, nutrients, CO_2 and electricity used in this stage. However, unlike in other stages, these impacts can be mitigated by the use of alternatives. In the case of the nutrients and CO_2, waste streams (wastewater for nutrients, and flue gas for CO_2) can be alternatives. The wastewater can also supply the water required for the production. However, the source of wastewater must be restricted, as wastewater from heavy industries can contain certain components like heavy metals and others that might

be hazardous to microalgae and/or to human health. Still, a solution is required to decrease the consumption of water for cooling the production reactors. On the other hand, the impacts created by the large energy consumption can be mitigated by the use of renewable energies. However, not all impact categories will be reduced, as for example with photovoltaic energy, where the impact on mineral consumptions increases due to the construction of the panels. Another negative point observed is the use of solvents in the extraction phase of lipids, which increase the negative impact of the process. When compared to other works reported in the literature, it was observed that the use of ethanol and heptane were responsible for most impacts in the extraction stage. The alternative is to use supercritical extraction. However, the method must be improved in order to achieve better extraction yield and consequently better environmental results per kg of lipids extracted.

Overall, the final conclusion is that a microalgae biorefinery can be profitable as long as certain considerations are made. These considerations go from the selection of the right microalgae biomass, to the selection of the appropriate pathway to obtain the products, to the use of renewable energy and creation of synergies with neighboring industries.

Unfortunately, when looking at the environmental sustainability, it was not possible to compare quantitatively the two scenarios with currently used processes, due to the lack of information available in the literature.

Further, when comparing the results of this study to studies performed on microalgae production of both products, most only analyze the production of one single product, making a reliable comparison very difficult.

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APPENDIX.

SUSTAINABLE MICROALGAE BIOREFINERY DEVELOPMENT THROUGH PROCESS OPTIMIZATION

7.1 Appendix 1 - *Prorocentrum* composition

Since very little information was available on the composition of *Prorocentrum* sp. biomass, the composition had to be either calculated or analysed experimentally.

7.1.1 *Prorocentrum* lipid composition

Due to the lack of information regarding the lipid profile of *Prorocentrum* some assumptions and calculations had to be performed.

A lipid composition for *Prorocentrum* was taken from Kumariah Manoharan et al. (1999), however it was only given in µmoles/10^7cell. Therefore, to calculate the mass percentage of each of the main lipid components in the cell, it was necessary to calculate the molar weight of each of the 3 components (TAGs, Phospholipids and Glycolipids). Unfortunately, the fatty acid distribution was not found so an assumption was made to calculate an average molar weight. Therefore, it was necessary to obtain the fatty acid profile of *Prorocentrum*. A fatty acid profile was obtained from an analysis performed on the biomass used for the *PUFAChain* project, and a similar profile was found and corroborated the analysis (Nichols et al., 1984).

Table 120 – Fatty acid composition of Prorocentrum cassubicum (Leblond et al., 2003).

Lipids	MW (g/mol)	% in *Prorocentrum lipids*	Average MW Contribution
C14:0	231	5%	12.5
C16:0	259	27%	70.1
C16:2	255	2%	4.4
C18:0	287	4%	10.3
C18:1	285	8%	22.2
C18:2	283	3%	8.56
C18:4	279	16%	45.9
C20:5w3	305	21%	64.8
C22:6w3	331	14%	45.2
Total		100%	279.4

Thus, now that the average molecular weight was calculated, the average molecular weight for the lipids was found by adding the molecular weight of each of the non-fatty acid groups to the average molecular weight of the fatty acids, and by multiplying it by the number of

fatty acids that exist in the lipids. Using the new molar weight, it was possible to calculate the lipid distribution.

$$Total\ MW(TAG) = 279.4 \times 3 + 92.1 = 930.4$$

$$Total\ MW(PL) = 279.4 \times 2 + 156.1 = 715$$

$$Total\ MW(GL) = 279.4 \times 2 + 92 = 715.9$$

Table 121 - Distribution of the different classes of lipids in Prorocentrum.

lipid	TAG	PL	GL	Total Lipids	units
Non-Fatty acid group MW	92.1	156.1	157		
Total MW	930.4	715.0	715.9		
µmoles of lipid (Manoharan et al., 1999)	2.1	11.5	2	15.6	µmoles
Mass of lipids	2.257E-09	1.61E-08	2.79E-09	2E-08	g
% of lipids	10.7%	76.1%	13.2%	100%	%

TAG - triacylglyceride; PL – Phospholipids; GL- Glicolipids

7.1.2 *Prorocentrum* protein composition

Another piece of information missing was the protein composition of *Prorocentrum* sp. This information was obtained through experimental data.

A sample of *Prorocentrum* supplied by A4F was added to demineralized water until a concentration of 10 g/l of sample (corresponds to 3.27 g/l of dry biomass). The sample was then ruptured by bead beating for 30 min, in order to make all the components of the biomass available. Two replicates were made.

Using the BCA (Bicinchoninic Acid) Protein Assay Method two analysis were made; one to the whole sample and afterwards the sample was centrifuged and only the supernatant was analysed. The first analysis provided the total amount of protein present in the sample while the second analysis provided the total amount of water-soluble protein. The results can be found in Table 122.

Table 122 - Protein composition of Prorocentrum.

	Concentration mg/l	% protein
Initial Sample	3,270	-
Total Protein 1	734.5	22%
Total Protein 2	714.2	22%
Water Soluble Protein 1	153.8	5%
Water Soluble Protein 2	153.7	5%

7.2 Apendix 2 – Harvesting and disruption equipment parameters

In this section it is presented in Table 123 a brief summary of the conditions considered while using different equipment for processing microalgae culture/fractions, as reported in the literature by several authors.

Table 123 - Harvesting and disruption equipment efficiencies and maximum concentrations.

Harvesting Equipment	Harvesting Efficiency	Maximum concentration achieved	Reference
Membrane Filtration	95%	100 – 150* g/l	(Bilad et al., 2014; Drexler and Yeh, 2014)
Centrifuge	90%	200 g/l	(Gerardo et al., 2015; Monte et al., 2018; Shelef and Sukenik, 1984)
Dissolved Air Flotation (with flocculant)	90%	60 g/l	(Al Hattab et al., 2015b; Kwon et al., 2014; Singh et al., 2013)
Flocculation	85%	50 g/l	(Branyikova et al., 2018; Gutiérrez et al., 2015; Martínez, 2016; Smith and Davis, 2012)
Cell Disruption Equipment	**Cell Disruption Efficiency**	**Maximum concentration for use**	**Reference**
Bead Mill	99%	200 g/l	(P. R. Postma et al., 2017; Postma et al., 2015),(Greenwell et al., 2010)
High Pressure Homogenizer	96%	200 g/l	(Patrignani and Lanciotti, 2016)
Ultrasonication	100%	100 g/l	(Kurokawa et al., 2016), (A4F, 2018)

*For *Synechocystis* sp., the value of maximum concentration used was 100 g/l due to the initial concentration being 0.5 g/l, which implies already a concentration 200 times higher.

7.3 Appendix 3 - Solvent extraction of proteins from *Prorocentrum* by heptane and Ethanol

One of the alternatives for lipid extraction from microalgae biomass is to use organic solvents like hexane and ethanol, or a mixture of both. Since these solvents have different polarities, their interaction with the components of the biomass is different. However, since most articles are focused only on lipid extraction, very little information exists regarding the behaviour of proteins when in the presence of the previously mentioned solvents. Yet, for biorefinery design, it is important to understand the effect of the extraction methods on all the components of the biomass. Therefore, the aim of this experiment was to obtain information regarding the behaviour of the different protein groups, if they can be extracted or not, when exposed to the different solvents or their mixture.

It is described in the next paragraphs the procedure used for protein extraction using solvents, and a few remarks are presented. The sample was washed until all salt was removed. Due to osmotic shock some cells might rupture, therefore a sample of each diafiltration was taken to account for the proteins lost during this step. Table 124 presents all the samples taken and the amount of protein removed during this process.

Table 124 – Protein removal by diafiltration.

	Concentration (mg/l)	Protein (mg)	Volume (ml)	Biomass (mg)	Protein remaining in sample (mg)	Protein concentration (mg/l) in biomass sample
initial sample	-	225.9	30.0	3076.0	225.9	7531.3
DS1	833.0	25.0	30.0	3051.0	200.9	6698.3
DS2	388.1	11.6	30.0	3039.4	189.3	6310.2
DS3	237.5	7.1	30.0	3032.2	182.2	6072.7
DS4	144.0	4.3	30.0	3027.9	177.9	5928.7

DS – Diafiltration Sample

After diafiltration the cells were ruptured by bead milling and afterwards the solution was split into 4 tubes and hexane was added. The amount of hexane and biomass added to each tube are shown in Table 125**Error! Reference source not found.**.

Table 125 - Diafiltrated sample distribution.

	Water phase volume (ml)	Hexane phase (ml)	Biomass (mg)	Protein (mg)	Water soluble protein (mg)
Tube 1	6.0	2.0	600	35.2	8.0
Tube 2	6.5	6.0	650	38.2	8.7
Tube 3	6.5	2.0	650	38.2	8.7
Tube 4	5.5	2.0	550	32.3	7.3

Since it is not possible to perform a direct analysis of the protein extracted by hexane, an indirect approach was used. After hexane extraction, water was added to two samples of post hexane extracted biomass (WHS) in order to access the proteins that remained in the biomass. Afterwards three samples were taken: one of the water phase of hexane extraction (S1), another of the WHS (biomass+water)(S3) and one of the water phase of the WHS (S6).

To calculate the amount of protein removed by hexane, we added the amount of proteins adding the amount of protein in S1, which are the proteins that remain in the water phase, added to the proteins present in the homogenized and supernatant of the samples washed with water after the extraction with hexane (samples S3 and S6). The results are shown in Table 126.

Table 126 - Amount of proteins after hexane extraction.

Sample	Concentration (mg/l)	Protein (mg)	Volume of solvent (ml)	Biomass dry weight (mg)
S1a	589.6	3.5	6.0	600
S1b	627.6	4.1	6.5	650
S1a	575.0	3.7	6.5	650
S2b	2108.6	12.7	6.0	600
S2a	1993.1	8.0	4.0	650
S3a	217.1	1.5	7.0	600
S3b	277.0	1.4	5.0	650

S1 – Remaining protein in the water phase after hexane extraction

S2 – Remaining protein after ethanol extraction

S3.x – Water soluble protein remaining after both extractions

Table 127 - Amount of proteins extracted by Hexane.

	% Extraction	mg Protein/ml hexane	Total protein extracted (mg)
Extracted by hexane 1	52%	10.2	17.7
Extracted by hexane 2	59%	9.4	13.4

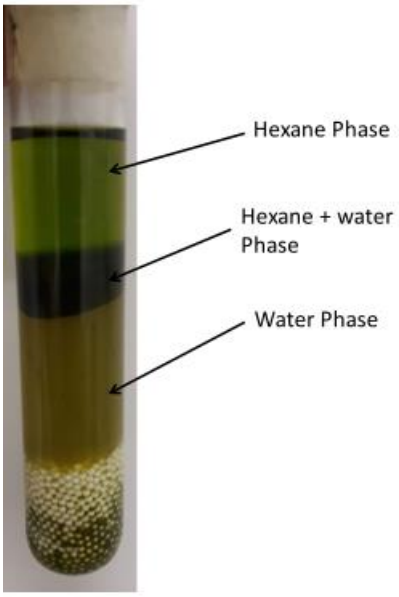

Figure 115 - Different phases of a hexane/water separation.

Ethanol was also added to two other samples of post hexane extraction biomass, to simulate the process (S2). This time the samples were analysed directly with the BCA method. The results are shown in Table 128.

As can be observed in Table 128, the amount of protein extracted (S4) is similar to the one of S3 (total water-soluble protein after hexane extraction that remains in the biomass), shown in Table 126. This means that ethanol removes all the water-soluble proteins that remain in the previously extracted microalga biomass.

Table 128 - Proteins extracted by ethanol.

Sample	Concentration (mg/l)	Protein (mg)	Volume (ml)	Biomass dry weight (mg)	Total protein (mg)
S4a	263.1	1.8	7.0	650.0	25.5
S4b	249.1	1.7	7.0	550.0	24.7
	% total protein extracted	**% of water soluble proteins extracted**	**mg protein/ml ethanol**		
Extracted by ethanol 1	23%	100%	0.3		
Extracted by ethanol 2	22%	100%	0.3		

This is corroborated by the values of S5x (which is the amount of proteins left in the biomass after both extractions steps, shown in Table 129) which is very low. In the same table it is possible to see the amount of proteins remaining after the extraction.

Table 129 - Remaining protein in the biomass after both extractions.

	Concentration	Protein (mg)	Volume (ml)	Biomass (mg)
S5a	15.4	0.1	4.0	650
S5b	21.3	0.1	4.0	550
S6a	1308.2	6.5	5.0	650
S6b	2019.6	10.1	5.0	550

From the previously described values, the recovery efficiencies for the method used were calculated as:

- 52% non-water-soluble protein extraction by hexane
- 100% water-soluble protein extraction by ethanol

7.4 Appendix 4 - Distillation

As mentioned before, in scenario 1 the microalga produces and excretes ethanol into the medium. Therefore, the ethanol needs to be recovered. However, since it cannot be done using pervaporation membrane due to the biomass present in the culture medium, this is done using distillation.

The objective is to concentrate ethanol until 85 wt% (±70 mole%) from a stream with 1 wt% ethanol.

The initial conditions for the feed stream are pressure of 1 atm, and 25 ºC temperature.

The initial concentration of ethanol in the stream is 10 g/l or 1 wt%. The objective is to recover at least 85 % of the ethanol produced.

Using the McCabe–Thiele method (Azevedo and Alves, 2009) first the q (mol fraction liquid in feed) is calculated using equation 1, where the values for h_v, h_f and h_l were obtained from Figure 116.

$$q = \frac{h_v - h_f}{h_v - h_l} \qquad (1)$$

$h_{v\ (enthalphy\ of\ the\ vapour)} = -615\ kcal/kg$

$h_{f(enthalphy\ of\ the\ feed)} = -20\ kcal/kg$

$h_{l(enthalphy\ of\ the\ liquid)} = -110\ kcal/kg$

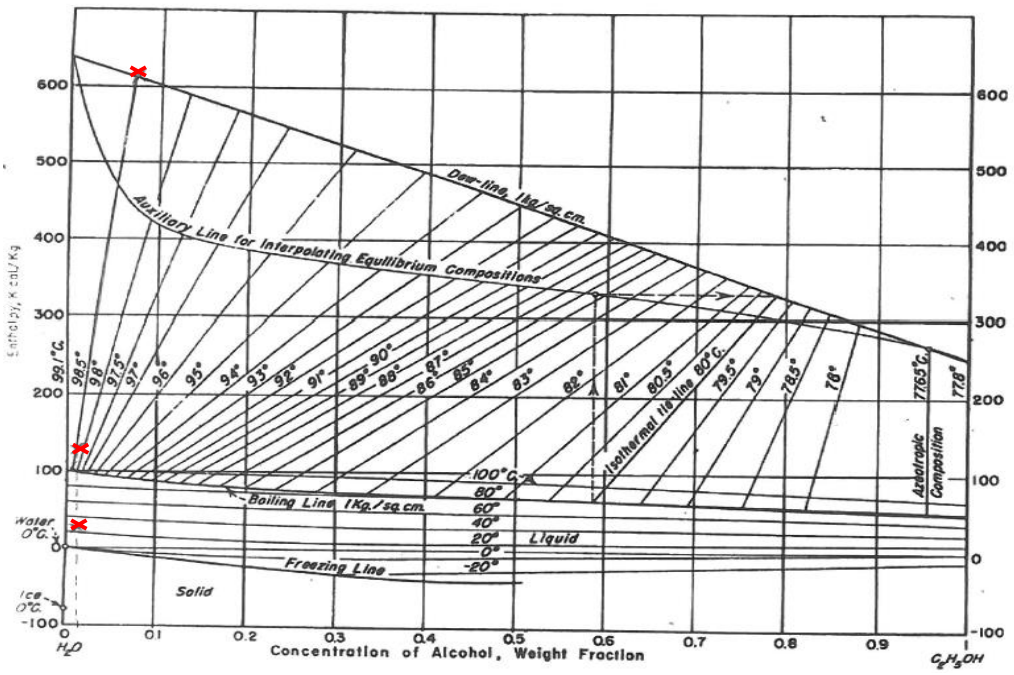

Figure 116- Ethanol-water enthalpy graph to calculate the feed conditions (q).

Under these conditions, *q* is 1.18. With q, the feed condition is obtained and in this case is a sub cooled feed as we can see in Figure 117.

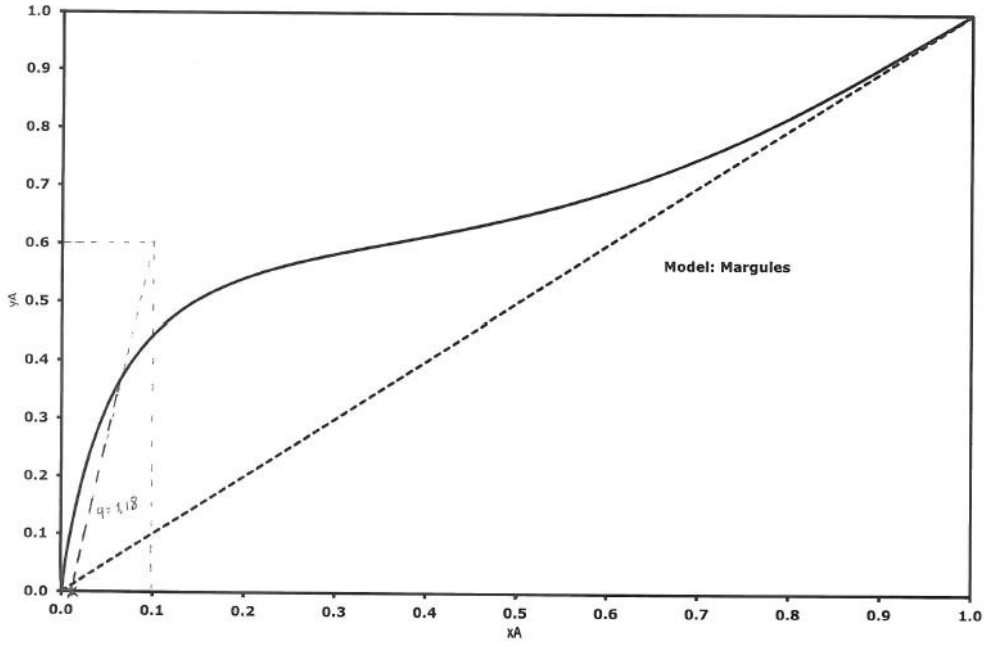

*Figure 117 – Representation of the feed conditions (q) in Water-Ethanol equilibrium graph (*ya – *vapor fraction of ethanol;* xa *liquid fraction of ethanol).*

Now that *q* is known, and the desired mol% is also known (70%) it is possible to calculate the minimum reflux rate (R_{min}) with the *q* the R_{min} is calculated using equation 2. Now we can trace the R_{min} (Figure 118).

$$D = \frac{R_{min}}{1+R_{min}} \qquad (2)$$

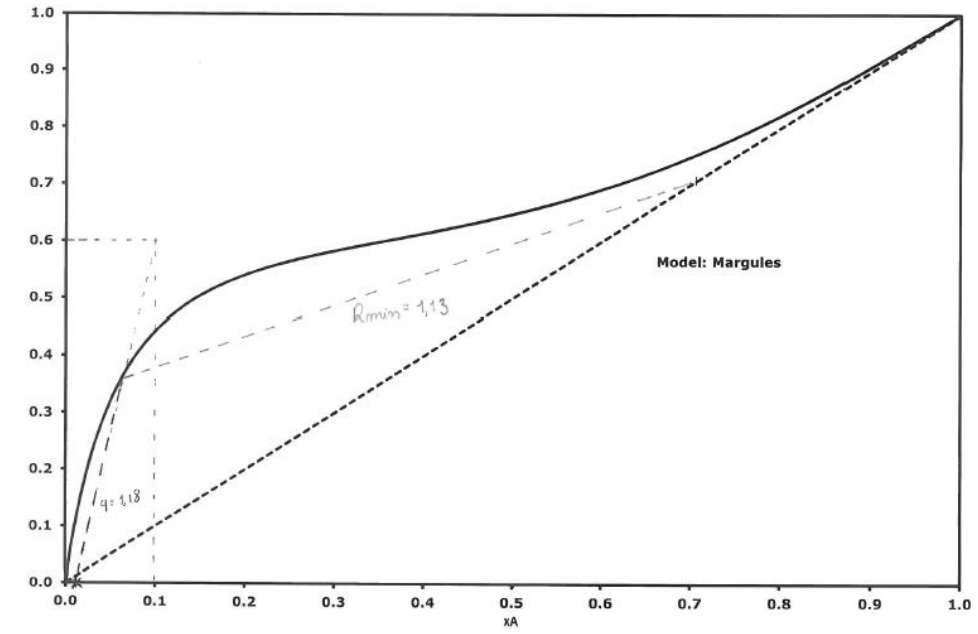

Figure 118 – Calculation of the minimum Reflux Ratio (R_{min}).

Considering that the Reflux Ratio (R) is calculated by the equation $R = 1.2 \times R_{min}$ then $R = 1.35$. With *R* value we can write the rectifying section operating line in the equilibrium graph (see Figure 119)

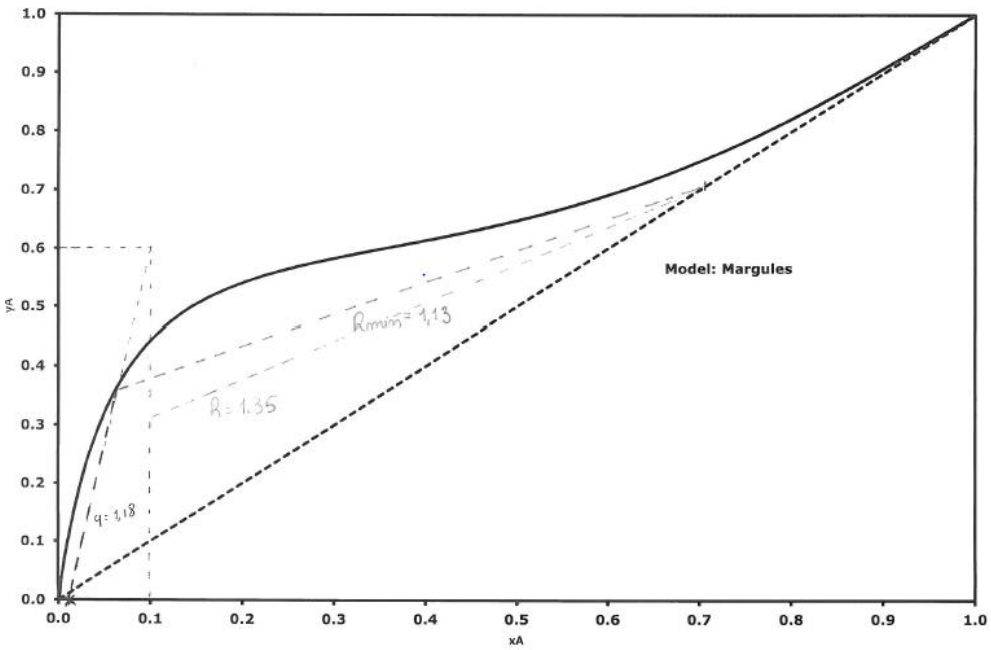

Figure 119 – Calculation of the Reflux Ratio (R).

Now it is possible to trace the operating lines and calculate the number of stages (see Figure 120).

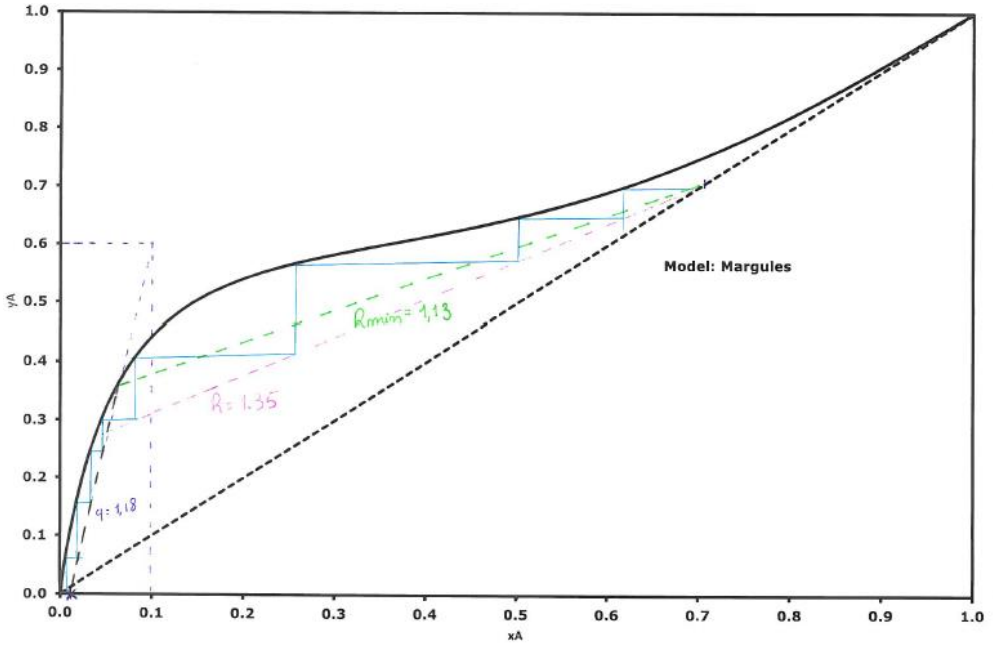

Figure 120 - Calculation of the number of stages of the distillation column.

In the end, the number of stages obtained is 8 with the feed entering in stage 5.

Since the Feed conditions are known and the objective is to obtain a purity of 85 %wt and only 10% loss of ethanol, this information was added to the program CHEMSEP (Chemsep, n.d.) in order to obtain a more precise calculation of the values. The results are shown in Table 130.

Table 130 – Results for the distillation of ethanol using Chemsep.

	Feed	Distillate	Residue
Molar Flowrates (mol/h)	231696	1106	230590
Ethanol (mol/h)	927	834	93
Water (mol/h)	230769	358	230411
Ethanol (mol%)	0.4	70	0.04
Ethanol (wt%)	10	85	0.1
Ethanol recovery		90%	
Energy added (kJ/s)		370.4	

7.5 Appendix 5 - Full Extraction Scenarios Mass balances

7.5.1 *Synechocystis* based scenarios

7.5.1.1 Mass balances for the full extraction scenario using solvent extraction as the lipid extraction method

Table 131 – Full mass balances for Synechocystis based biorefinery scenario 1.

Component	1	2	3	4	5	6	7	8	9	10	11
SQDG (kg/h)	0.71	0.00	0.67	0.00	0.00	0.67	0.28	0.37	0.19	0.03	0.37
Glycolipids (kg/h)	1.21	0.00	1.15	0.00	0.00	1.15	0.65	0.47	0.44	0.07	0.47
Phospholipids (kg/h)	0.13	0.00	0.13	0.00	0.00	0.13	0.05	0.07	0.03	0.01	0.07
Water non-Soluble protein (kg/h)	6.28	0.00	5.97	0.00	0.00	5.97	0.00	5.67	0.00	0.00	2.32
Water Soluble protein (kg/h)	4.92	4.67	0.00	4.44	0.23	0.00	0.00	0.00	0.00	0.00	0.00
Amino Acids (kg/h)	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	3.34
Phycocyanin (kg/h)	2.30	2.19	0.00	1.97	0.21	0.00	0.00	0.00	0.00	0.00	0.00
Zeaxanthin (kg/h)	0.18	0.00	0.18	0.00	0.00	0.18	0.17	0.01	0.00	0.17	0.00
Carbohydrates (kg/h)	4.27	0.00	4.27	0.00	0.00	4.06	0.00	3.86	0.00	0.00	3.86
Capacity (m^3/h)	0.40	2.32	0.33	0.06	2.25	0.33	0.25	0.10	-	0.25	0.03
Concentration (g/l)	50.0	3.0	150.0	100.0	-	0.037	0.0	100.0	-	1.14	399.7
Water (l/h)	400.00	2317.58	82.42	64.12	2253.45	82.42	56.32	26.10	-	56.32	26.10
Ethanol (l/h)	-	-	247.27	-	-	247.27	168.96	78.31	-	168.96	-
$Mg(OH)_2$ (kg/h)	-	-	-	-	-	2.32	0.07	-	-	-	-
Chitosan (kg/h)		0.14	-	-	-	-	-	-	-	-	-
Heptane (l/h)	114.08		108.59				2.2	-	-	-	-
Enzymes (l/h)	-	-	-	-	-	-	-	0.004	-	-	-
NaOH (kg/h)	-	-	-	-	-	0.24	-	-	-	-	-

7.5.1.2 Mass balances for the full extraction scenario using supercritical extraction as the lipid extraction method

Table 132 - Full mass balances for Synechocystis based biorefinery scenario 2.

Component	1	2	3	4	5	6	7	8	9	10	11
SQDG (kg/h)	0.71	0.00	0.67	0.0	0.00	0.64	0.23	0.41	0.15	0.03	0.41
Glycolipids (kg/h)	1.21	0.00	1.15	0.0	0.00	1.09	0.39	0.70	0.18	0.04	0.70
Phospholipids (kg/h)	0.13	0.00	0.13	0.0	0.00	0.12	0.03	0.09	0.01	0.00	0.09
Water non-Soluble protein (kg/h)	6.28	0.00	5.97	0.0	0.00	5.37	0.00	5.37	0.00	0.00	2.20
Water Soluble protein (kg/h)	4.92	4.67	0.00	4.4	0.23	0.00	0.00	0.00	0.00	0.00	0.00
Amino Acids (kg/h)	0.00	0.00	0.00	0.0	0.00	0.00	0.00	0.00	0.00	0.00	3.17
Phycocyanin (kg/h)	2.30	2.19	0.00	2.0	0.21	0.00	0.00	0.00	0.00	0.00	0.00
Zeaxanthin (kg/h)	0.18	0.00	0.18	0.0	0.00	0.18	0.17	0.01	0.00	0.17	0.01
Carbohydrates (kg/h)	4.27	0.00	4.27	0.0	0.00	3.63	0.00	3.63	0.00	0.00	3.63
Capacity (m3/h)	0.4	2.3	0.1	-	-	-	0.00	0.0	-	3.67	0.1
Concentration (g/l)	50	3.0	100.0	100	0.20	0.0	0.00	0.0	-	-	150.0
Water (l/h)	400.00	2276.36	123.64	64.12	2212.24	-	-	-	-	-	68.01
Heptane (l/h)	-	-	-	-	-	-	1.43	-	-	-	-
Chitosan (kg/h)	-	-	-	-	-	-	-	-	-	-	-
$Mg(OH)_2$ (kg/h)	-	-	-	-	-	-	-	0.05	-	-	-
Supercritical CO_2 (kg/h)	-	-	-	-	-	-	441.15	-	-	-	-
Enzymes (l/h)	-	-	-	-	-	-	-	-	-	-	0.003
NaOH (kg/h)	-	-	-	-	-	-	-	0.06	-	-	-

7.5.2 *Prorocentrum* based scenarios

7.5.2.1 Mass balances for the full extraction process for *Prorocentrum* using conventional solvent extraction with heptane for lipid extraction

Table 133- Full mass balances for Prorocentrum based biorefinery scenario 1.

Component	1	2	3	4	5	6	7	8(5)	9(6)	10(7)
TAG (kg/h)	0.43	0.43	0.06	0.35	0.06	0.06	0.00	0.35	0.28	0.04
Phospholipids (kg/h)	3.05	3.05	1.98	1.07	1.98	1.88	0.00	1.07	0.71	0.12
Glycolipids (kg/h)	0.53	0.53	0.42	0.11	0.42	0.40	0.00	0.11	0.07	0.01
Water non-Soluble protein (kg/h)	13.49	13.49	6.48	7.02	2.66	2.52	0.00	7.02	0.00	7.02
Water Soluble protein (kg/h)	4.96	4.96	4.96	0.00	2.03	1.29	0.68	0.00	0.00	0.00
Amino Acids (kg/h)	0.00	0.00	0.00	0.00	6.75	4.27	2.25	0.00	0.00	0.00
Carotenoids (kg/h)	0.34	0.34	0.34	0.00	0.34	0.32	0.00	0.00	0.00	0.00
Carbohydrates (kg/h)	15.45	15.45	15.45	0.00	15.45	14.68	0.00	0.00	0.00	0.00
Nucleic Acids (kg/h)	6.75	6.75	6.75	0.00	6.75	0.00	6.75	0.00	0.00	0.00
Water (l/h)	300.00	300.00	300.00	0.00	182.21	121.47	60.74	0.00	0.00	10.00
capacity (m3/h)	0.43	0.43	0.30	0.13	0.18	0.17	0.06	0.13	0.00	0.53
concentration (g/l)	150.00	104.30	121.50	6.49	200.00	150.00	159.31	6.49	0.00	0.00
Heptane (l/h)	131.58	131.58	-	131.58	-	-	-	3.49		3.49
$Mg(OH)_2$(kg/h)	-	-	-	-	-	-	-	0.11		2.43
Enzyme (l/h)	-	-	-	-	0.008	-	-	-	-	-
NaOH (kg/h)	-	-	-	-	-	-	-	0.2	-	-

*The streams in brackets correspond to the scenario where stream 3 does not go through protein hydrolysis

7.5.2.2 Mass balances for the full extraction process for *Prorocentrum* using conventional solvent extraction (Ethanol and Hexane) for lipid extraction

Table 134 - Full mass balances for Prorocentrum based biorefinery scenario 2.

Component	1	2	3	4	5	6	7	8	9	10	11	12	13	14
TAG (kg/h)	0.4	0.0	0.4	0.0	0.0	0.0	0.3	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Phospholipids (kg/h)	3.0	2.3	0.6	0.9	1.3	0.0	0.6	0.4	0.0	0.0	1.3	0.9	0.1	0.1
Glycolipids (kg/h)	0.5	0.5	0.2	0.3	0.2	0.0	0.2	0.1	0.0	0.0	0.2	0.1	0.0	0.0
Water non-Soluble protein	13.5	6.5	7.0	6.2	0.0	0.0	6.7	0.0	6.7	0.0	0.0	0.0	0.0	6.7
Water Soluble protein (kg/h)	5.0	5.0	0.0	0.7	4.0	0.0	0.0	0.0	0.0	0.0	3.2	0.0	3.2	3.2
Amino Acids (kg/h)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Carotenoids (kg/h)	0.3	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.3
Carbohydrates (kg/h)	15.5	15.5	0.0	14.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Nucleic Acids (kg/h)	6.8	6.8	0.0	0.0	6.8	0.0	0.0	0.0	0.0	0.0	5.4	0.0	5.4	5.4
capacity (m3/h)	0.43	1.04	0.13	0.152	0.88	0.13	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08
concentration (g/l)	150.00	35.53	61.73	150.00	14.22	-	-	-	-	-	-	-	>1000	187.51
water (l/h)	300.0	300.0	0.0	43.9	256.1	0.0	0.0	0.0	1.9	0.0	0.0	0.0	2.6	79.5
heptane (l/h)	131.6	0.0	131.6	0.0	0.0	129.9	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
ethanol (l/h)	-	736.6	-	107.8	628.8	-	0.0	-	0.0	628.8	0.0	0.0	0.0	0.0
MgOH (kg/h)	-	-	-	-	-	-	0.08	-	-	-	0.1	-	-	0.00
NaOH (kg/h)	-	-	-	-	-	-	0.11	-	-	-	0.15	-	-	-
Enzymes (l/h)	-	-	-	-	-	-	-	-	-	-	-	-	-	0.004
heptane (l/h)	-	-	-	-	-	-	1.67	-	-	-	3.4	-	-	-

7.5.2.3 Mass balances for the full extraction process for *Prorocentrum* using supercritical solvent extraction for lipid extraction

Table 135 - Full mass balances for Prorocentrum based biorefinery scenario 3.

Component	1	2	3	4	5	6	7
TAG (kg/h)	0.43	0.41	0.16	0.25	0.16	0.20	0.03
Phospholipids (kg/h)	3.05	2.90	2.11	0.78	2.11	0.52	0.09
Glycolipids (kg/h)	0.53	0.50	0.32	0.18	0.32	0.12	0.02
Water non-Soluble protein	13.49	12.15	12.15	0.00	4.98	0.00	0.00
Water Soluble protein (kg/h)	4.96	4.46	4.46	0.00	1.83	0.00	0.00
Amino Acids (kg/h)	0.00	0.00	0.00	0.00	9.80	0.00	0.00
Carotenoids (kg/h)	0.34	0.34	0.00	0.31	0.00	0.00	0.31
Carbohydrates (kg/h)	15.45	13.14	13.14	0.00	13.14	0.00	0.00
Nucleic Acids (kg/h)	6.75	6.08	6.08	0.00	6.08	0.00	0.00
capacity (m3/h)	0.3	0.00	0.00	-	0.19	0.00	0.03
concentration (g/l)	150.00	20000.0	19222.8	-	200.0	0.00	0.00
water (l/h)	300.00	2.00	2.00	-	192.04	0.00	10.00
Heptane (l/h)	-	-	-	2.8	-	-	2.8
$Mg(OH)_2$ (kg/h)	-	-	-	0.09	-	-	0.43
Enzyme Both (l/h)	-	-	-	-	0.008	-	-
CO_2 (kg/h)	90	-	-	-	-	-	-
NaOH (kg/h)	-	-	-	0.12		-	-
ethanol(l/h)	4.5	-	-	-	-	-	-

7.6 Appendix 6 – Products and possible replacements

One of the objectives of a microalgae based biorefinery is to produce products that can replace or compete with chemically produced products, both to improve their quality and to replace petroleum as a main feedstock.

However, one main concern must be addressed: the microalgae and respective products must be licenced for food and pharmaceutical use by the health organizations of the respective countries. Thus, several tests have to be performed to guarantee that the products are not harmful to humans and animals. Therefore, none of the proposed products is certain to reach the market before being tested.

7.6.1 Microalgae biorefinery products

In this appendix, in Table 136 and Table 137, the different products obtained from the microalga are either compared to similar products from other microalgae, or they are compared to similar products from other sources that they might replace or compete in the future.

Table 136 - Possible products from Synechocystis based biorefinery and possible replacements.

Microalgae Product				Similar Products		
Name	**Composition**	**Applications**	**Name**	**Composition**		**reference**
Phycocyanin (food Colorant)	Purity ~ 0.94	Food Colorant	-	Purity >0.7		1, 2
Amino acid Hydrolysate	hydrolysis degree 70% Amino acids	Biostimulant	Biostimulant	hydrolysis degree 60-70%		2
Metal Lipid Soap	16:0 60%	Food applications	Palm oil	16:0	44%	3
	18:1 10%			18:1	39%	
	18:2 20%			18:2	10%	
Feed	Protein 58-54%	Fish Feed	Fish meal Or soymeal	Protein	50-70%	4
	Lipids 9-15%			Lipids	6-10%	
	Carbohydrates 35-37%			Carbohydrates	~1	
Zeaxanthin	61-70% purity	Feed, Nutraceutical Cosmeceuticals	Zeaxanthin from marigold	>5% zeaxanthin		5
Bio-Ethanol	>97% purity	Pharmaceutical, Cosmetics	Ethanol	>97% purity		6

1 (Chaiklahan et al., 2018) ; 2 (Rito-Palomares et al., 2001); 2 (Acién, 2016; AgriAlgae, 2018; du Jardin, 2015); 3 (Oil Palm Knowledge Base, 2014); 4 (Oil Palm Knowledge Base, 2014);5 (Friedl, 2017); 6 (University of Limerick, 2018)

Table 137 - Possible products from Prorocentrum based biorefinery scenario and possible replacements.

Microalgae Product				Similar Products			
Name	**Composition**		**Applications**	**Name**	**Composition**		**reference**
EPA + DHA + Pigment Product	DHA	14%	Feed, Nutraceutical Cosmeceuticals	Fish oil	DHA	11%	1
	EPA	21%			EPA	17%	
	Pigments*[1]	18%			Protein	50 - 70%	
Amino acid Hydrolysate	hydrolysis degree 52- 58% Amino acids around 40g/l		Bio stimulant	Biostimulant	hydrolysis degree 60-70% and 40 g/l (>20% amino acids)		2
EPA + DHA Metal Soap	DHA	14%	Feed, Nutraceutical Cosmeceuticals	Fish oil	DHA	11%	3
	EPA	21%			EPA	17%	
Carotenoid Mix	Peridinin*[2]	52%	Feed, Nutraceutical Cosmeceuticals	Mixed Carotenoid pills	Beta Carotene	14.2 mg	4
	ß-Carotene	15%			Zeaxanthin + Lutein	150 µg	
Protein Concentrate	> 90% protein content		Feed	Fish Protein Powder	> 90% protein content		5
Carbohydrate rich Product	Lipids	6-7%	Animal Feed	Wheat grain	Lipids	-	6
	Carbohydrates	71-84%			Carbohydrates	82.50%	
	Protein	9-23%			Protein	0.43%	

1 (Lysi, 2018); 2 (Acién, 2016); 3 (Friedl, 2017) ,(Lysi, 2018); 4 (Health, 2019; Natures best, 2019); 5 (Asiedu et al., 2018); 6 (Staples, 2007)

7.6.2 Price of Products

One important factor while considering the possibility of replacing products already available in the market with those produced in a microalgae based biorefinery is their commercial value. In Table 138 to Table 139 it is shown the estimated price of such products.

7.6.2.1 *Synechocystis* based biorefinery products

Table 138 - Possible price for products from Synechosystis based biorefinery.

Product	*Price*	*reference*
Phycocyanin (food Colorant)	210 €/kg	(University of Limerick, 2018)
Amino acid Hydrolysate (+/- 35% amino acids)	7.2*[1] €/l	(University of Limerick, 2018)
Palmitic Acid Soap	0.56 €/kg	(IndexMundi, 2018)
Fish Feed	1.5 €/kg	(Index Mundi, 2018)
Zeaxanthin	500 €/kg	(University of Limerick, 2018)
Bio-Ethanol	0.49 €/l	(University of Limerick, 2018)

Notes*
*1 – The values were obtained based on (Smith and Birbeck, 2012) with 27.5% profit for the retailer and 17.5% profit for the wholesaler.

7.6.2.2 *Prorocentrum* based biorefinery products

Table 139 - Possible price for products from *Prorocentrum* based biorefinery.

Product	*Price*	*reference*
EPA + DHA + Pigment Product	41.5[*1]-45[*1] €/kg	(Friedl, 2017)
Amino acid Hydrolysate (~ 20% amino acids to > 35% amino acids)	4.1-7.2[*2] €/l	(Acién, 2016; University of Limerick, 2018)
EPA + DHA Metal Soap	120 €/kg	(Friedl, 2017)
Carotenoid Mix	25 €/kg	(Friedl, 2017)
Protein Concentrate + Carotenoids	31.5[*1] €/kg	(Asiedu et al., 2018; Friedl, 2017)
Protein Concentrate	28.9[*4] €/kg	(Asiedu et al., 2018)
Carbohydrate rich Product	3-4.5[*1] €/kg	(Dairy One, 2018)

*1 – The values were obtained based on the % of components with known prices.
*2 – The values were obtained based on (Smith and Birbeck, 2012) with 27.5% profit for the retailer and 17.5% profit for the wholesaler
*4 – The values were obtained based on (Smith and Birbeck, 2012) with 32.5% profit for the retailer and 25% profit for the wholesaler since the product is only proteins and requires extra components to be added.
*5 – The values were obtained based on (Smith and Birbeck, 2012) with 27.5% profit for the retailer and 17.5% profit for the wholesaler.
*5 – The values were obtained based on the % of components with known prices with 17.5% profit for the wholesaler

7.6.3 Products from PUFAChain

Some of the products obtained from *Prorocentrum* were based on products obtained and proposed in the PUFAChain project (Friedl, 2017). Table 140 contains those products, their prices and possible applications. Table 141 contains the conventional products already available in the market and their corresponding prices.

Table 140 - PUFAChain products, their prices and applications.

Product characteristics	Product value	Markets
PUFAmix Premium 20% EPA 20% DHA	60 €/kg	Pet Food Market Nutraceuticals Cosmeceuticals
PIGMENTmix 50% 20% chlorophyll, 10% peridinin, 1% beta-carotene	25 €/kg	Pet Food Market Other
PUFAduo 60% 30% EPA 30% DHA Veg. 100%, non-GMO, sustainable, contam. free *Prorocentrum* extract	120 €/kg	Nutraceutical Cosmeceuticals

Table 141 – Price range and average price by product, 2011 (adapted from Frost & Sullivan, 2012).

Product	Lower Price (€/kg)	Highest Price (€/kg)	Average Price (€/kg)	Key differentiating factor
Fish oil at 30% of Omega-3 EPA and DHA	2.50	12.50	8.40	Good source of EPA
Tuna	15.00	20.00	18.49	High quality source
Cod liver oil	4.00	16.00	8.78	Good source of EPA
Salmon	6.00	20.00	12.74	High quality source
Krill oil	75.00	150.00	105.72	Natural antioxidant
Concentrated oil 40-55%	8.00	32.00	16.99	High EPA/DHA concentration
Concentrated oil 60-70%	18.00	70.00	37.77	High EPA/DHA concentration
Concentrated oil 85-95%	130.00	300.00	185.40	High EPA/DHA concentration and pharmaceutical quality
Algae oil	60.00	140.00	76.51	Plant source of EPA/DHA

7.7 Appendix 7 – Economic values for each scenario

7.7.1 *Synechocystis* based biorefinery scenario

In this Appendix the Capital Costs (Capex), the Operation Costs (Opex), and the revenue of the *Synechocystis* based biorefinery scenario 1 are described, grouped per blocks of operations (phases).

7.7.1.1 Production, Harvesting and Disruption phase

7.7.1.1.1 Capital Costs (Capex)

Assumptions

- If the precise price was not available, the Capex values for the equipment were calculated using the capacity rule (Perry and Green, 2008)
- Production System values were supplied by A4F from company research projects

Table 142 - Capex values for scenario 1 (capacity 108.2 tons/year of ruptured biomass).

		Capex	Reference
Production System		€ 13,838,941	(University of Limerick, 2018)– Values A4F
Harvesting Equipment	Membrane System 100g/l	€ 600,000	(University of Limerick, 2018) – Values Pall
	Membrane System 10g/l	€ 290,000	(University of Limerick, 2018)– Values Pall
	Centrifugation System	€ 750,000	Values A4F – Westfalia Centrifuge
	Centrifugation System 2	€ 77,032	Values A4F – Westfalia Centrifuge
	Flocculation System	€ 27,283	(Qingdao All Universe Machinery Equipment Co., 2019)
	DAF System	€ 60,000	(Jiangsu Benenv Environmental Technologies Co., 2018)
Cell Disruption Equipment	Bead mill (200g/l)	€ 93,253	(Friedl, 2017)
	Bead mill (100 g/l)	€ 141,346	(Friedl, 2017)
	Bead mill (60 g/l)	€ 192,040	(Friedl, 2017)
	Bead mill (50g/l)	€ 214,240	(Friedl, 2017)
	HPH (50g/l)	€ 240,268	(Shanghai Youcan Beverage Machinery Co, 2018)
	HPH (60 g/l)	€ 215,371	(Shanghai Youcan Beverage Machinery Co, 2018)
	HPH (100 g/l)	€ 158,518	(Shanghai Youcan Beverage Machinery Co, 2018)
	HPH (200 g/l)	€ 104,583	(Shanghai Youcan Beverage Machinery Co, 2018)
	Ultrasonication	€ 62,074	(Yuhuan Clangsonic Ultrasonic Co. Ltd., 2018)
Ethanol Recovery	Pervaporation (4.2 m3/h)	€ 355,403	Dema Project – Values Pervatech
	Pervaporation (0.2 m3/h)	€ 57,199	Dema Project – Values Pervatech
	Distillation Column	€ 214,008	Calculations Appendix 10

7.7.1.1.2 The operation costs (Opex)

To simplify the calculations operation costs were divided into 3 main components; Electricity costs, Consumables and Reagent costs (C&R), and Maintenance costs.

- No Inflation on Opex is considered
- Opex value has a 5% security margin and contains maintenance costs
- Maintenance costs are considered to be 5% of the Capex– cleaning water and cleaning reagents are also included in these costs.
- Consumables costs are considered to be 2% of the Capex

Table 143 - Opex costs for production, harvesting and disruption equipment.

		Electricity	C&R	Maintenance	Opex
Production System*1		€ 366,459	€ 907,979	€ 691,947	€ 1,966,384
Harvesting Equipment	Membrane System (100 g/L)*2	€ 14,708	€ 60,000	€ 30,000	€ 139,248
	Membrane System (10 g/L)*2	€ 13,972	€ 29,000	€ 14,500	€ 90,536
	Centrifugation System (45 m3/h)	€ 44,940	€ 15,000	€ 37,500	€ 132,071
	Centrifugation System (2 m3/h)	€ 2,247	€ 1,541	€ 3,852	€ 9,211
	Flocculation System*3	€ 586	€ 54,697	€ 1,364	€ 91,103
	DAF System*3	€ 6,394	€ 17,445	€ 3,000	€ 61,227
Cell Disruption Equipment	Bead mill (200g/l)	€ 7,993	€ 1,865	€ 4,663	€ 14,520
	Bead mill (100 g/l)	€ 10,657	€ 2,827	€ 7,067	€ 20,551
	Bead mill (60 g/l)	€ 26,642	€ 3,841	€ 9,602	€ 40,085
	Bead mill (50g/l)	€ 31,970	€ 4,285	€ 10,712	€ 46,967
	HPH (50g/l)	€ 39,430	€ 4,805	€ 12,013	€ 56,249
	HPH (60 g/l)	€ 32,858	€ 4,307	€ 10,769	€ 47,934
	HPH (100 g/l)	€ 13,143	€ 3,170	€ 7,926	€ 24,240
	HPH (200 g/l)	€ 8,961	€ 2,092	€ 5,229	€ 16,282
	Ultrasonication	€ 34,102	€ 1,241	€ 3,104	€ 38,447
Ethanol Recovery	Pervaporation Membrane (4.2 m3/h)	€ 120,672	€ 17,770	€ 7,108	€ 172,206
	Pervaporation Membrane (0.2 m3/h)	€ 31,744	€ 5,720	€ 2,859	€ 40,323
	Distillation Column	€ 224,181	€ 4,281	€ 10,700	€ 239,162

Notes*
*1 - This information was provided by A4F from company research projects
*2– The consumables of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years)
*3 – In the Flocculation System and DAF systems the reagents cost includes the Chitosan cost (5g of Chitosan/kg of biomass for the DAF and 20 g of Chitosan/kg of biomass for the flocculation)
Ni – no information available – total value given by Pervatech for Dema Project

7.7.1.2 Extraction process

Assumptions

- The values for the extraction are calculated for a base of 20 kg/h.
- All equipment has a 30% extra capacity as security margin, except the saponification processes
- The Maintenance is 5% of the Capex of the equipment and takes into account cleaning water and reagents.
- The Consumables are 2% of the Capex except where stated otherwise

Depending on the type of harvesting equipment chosen, different concentrations can be achieved. Since the objective is to create as many scenarios as possible it was necessary to calculate the mass balances of the extraction scenarios for different final biomass concentrations.

7.7.1.2.1 Capex Extraction Process 1

In the following Table 144 and Table 145 the Capital Costs (Capex) for the *Synechocystis* extraction scenarios using conventional solvent extraction for lipid extraction are presented.

Table 144 - Capex values for different Synechocystis biomass concentrations (60 – 100 g/l).

	Capacity	Capex	Reference
	50 g/l		
Membrane System	3.12 m^3/h	€ 164,000	(University of Limerick, 2018)
Mixer Settler tank	3.01 m^3/h	€ 58,144	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	3.01 m^3/h	€ 102,898	(University of Limerick, 2018)
Stirred Tank	0.43 m^3/h	€ 18,045	(Qingdao All Universe Machinery Equipment Co., 2019)
Membrane System	0.43 m^3/h	€ 31,934	(University of Limerick, 2018)
Evaporator	0.25 m^3/h	€ 100,000	(University of Limerick, 2018)
Purification process	1.51 kg of biomass/h	€ 868,734	(Friedl, 2017)*1
Enzymatic reaction tank	0.54 m^3/h	€ 20,796	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
	100 g/l		
Membrane System	1.56 m^3/h	€ 108,200	(University of Limerick, 2018)
Mixer Settler tank	1.45 m^3/h	€ 37,536	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	1.45 m^3/h	€ 66,428	(University of Limerick, 2018)
Stirred Tank	0.43 m^3/h	€ 18,045	(Qingdao All Universe Machinery Equipment Co., 2019)
Membrane System	0.43 m^3/h	€ 31,934	(University of Limerick, 2018)
Evaporator	0.25 m^3/h	€ 100,000	(University of Limerick, 2018)
purification process	1.51 kg of biomass/h	€ 868,734	(Friedl, 2017)*1
Enzymatic reaction tank	0.54 m^3/h	€ 20,796	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)

Table 145 - Capex Values for different Synechocystis biomass concentrations (150 to 200 g/l).

	Capacity	Capex	Reference
		150 g/l	
Membrane System	1.04 m^3/h	€ 84,834	(University of Limerick, 2018)
Mixer Settler tank	0.93 m^3/h	€ 28,774	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	0.93 m^3/h	€ 50,922	(University of Limerick, 2018)
Stirred Tank	0.43 m^3/h	€ 18,045	(Qingdao All Universe Machinery Equipment Co., 2019)
Membrane System	0.43 m^3/h	€ 31,934	(University of Limerick, 2018)
Evaporator	0.25 m^3/h	€ 100,000	(University of Limerick, 2018)
Purification process	1.51 kg of biomass/h	€ 868,734	(Friedl, 2017)*1
Enzymatic reaction tank	0.54 m^3/h	€ 20,796	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
		200 g/l	
Membrane System	0.78 m^3/h	€ 71,385	(University of Limerick, 2018)
Mixer Settler tank	0.67 m^3/h	€ 23,652	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	0.67 m^3/h	€ 41,857	(University of Limerick, 2018)
Stirred Tank	0.43 m^3/h	€ 18,045	(Qingdao All Universe Machinery Equipment Co., 2019)
Membrane System	0.43 m^3/h	€ 31,934	(University of Limerick, 2018)
Evaporator	0.25 m^3/h	€ 100,000	(University of Limerick, 2018)
purification process	1.51 kg of biomass/h	€ 868,734	(University of Limerick, 2018)*1
Enzymatic reaction tank	0.54 m^3/h	€ 20,796	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)

Notes*: *1 – the saponification values were taken from the PUFAChain Project and given by IOI oleo

7.7.1.2.2 Opex Extraction Process 1

In the following Table 146 and Table 147 the Operational Costs (Opex) for the *Synechocystis* extraction scenarios using conventional solvent extraction for lipid are presented.

Table 146 - Opex values for different Synechocystis biomass concentrations (50 to 100 g/l).

	Energy costs	Consumables and reagents	Maintenance costs	Opex
		50 g/l		
Membrane System	€ 1,009	€ 42,118*1	€ 8,200	€ 51,327
Mixer Settler tank	€ 32,478	€ 22,720 *2	€ 2,927	€ 58,105
Membrane System	€ 974	€ 10,290 *4	€ 5,145	€ 16,409
Stirred Tank	€ 4,620	€ 70,809 *3	€ 902	€ 76,332
Membrane System	€ 139	€ 3,193 *4	€ 1,597	€ 4,929
Evaporator	€ 51,106	€ 10,000	€ 5,000	€ 66,106
Purification process	€ 17,375	€ 29,325 *5	€ 43,437	€ 90,136
Enzymatic reaction tank	€ 252	€ 13,568 *5	€ 3,569	€ 17,390
		100 g/l		
Membrane System	€ 505	€ 23,679 *1	€ 5,410	€ 29,593
Mixer Settler tank	€ 15,662	€ 22,308 *2	€ 1,877	€ 39,846
Membrane System	€ 470	€ 6,643 *4	€ 3,321	€ 10,434
Stirred Tank	€ 4,620	€ 70,809 *3	€ 902	€ 76,332
Membrane System	€ 139	€ 3,193 *4	€ 1,597	€ 4,929
Evaporator	€ 51,106	€ 10,000	€ 5,000	€ 66,106
purification process	€ 17,375	€ 29,325 *5	€ 43,437	€ 90,136
Enzymatic reaction tank	€ 252	€ 13,568 *5	€ 3,569	€ 17,390

*1- The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years). Also included is the cost for water for the diafiltration
*2 – The C&R include the cost for the chitosan
*3 – The C&R also include the cost for Ethanol. It is considered that 90% of the ethanol spent in the ethanol assisted solvent extraction is recycled into the system
*4 - The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years).
*5- The C&R include the cost for the Water, Heptane, $Mg(OH)_2$, and NaOH used for the process (the reagents consumption was obtained from PUFAChain Project)
*6- The C&R include the cost of the enzymes and water added

Table 147 - Opex Values for different Synechocystis biomass concentrations (150 to 200 g/l).

	Energy costs	Consumables and reagents	Maintenance costs	Opex
		150 g/l		
Membrane System	€ 336	€ 17,056 *1	€ 4,242	€ 21,634
Mixer Settler tank	€ 10,056	€ 22,132 *2	€ 1,439	€ 33,627
Membrane System	€ 302	€ 5,092 *4	€ 2,546	€ 7,940
Stirred Tank	€ 4,620	€ 70,809 *3	€ 902	€ 76,332
Membrane System	€ 139	€ 3,193 *4	€ 1,597	€ 4,929
Evaporator	€ 51,106	€ 10,000	€ 5,000	€ 66,106
Purification process	€ 17,375	€ 29,325 *5	€ 43,437	€ 90,136
Enzymatic reaction tank	€ 252	€ 13,568 *5	€ 3,569	€ 17,390
		200 g/l		
Membrane System	€ 252	€ 13,568 *1	€ 3,569	€ 17,390
Mixer Settler tank	€ 7,253	€ 22,030 *2	€ 1,183	€ 30,466
Membrane System	€ 218	€ 4,186 *4	€ 2,093	€ 6,496
Stirred Tank	€ 4,620	€ 70,809 *3	€ 902	€ 76,332
Membrane System	€ 139	€ 3,193 *4	€ 1,597	€ 4,929
Evaporator	€ 51,106	€ 10,000	€ 5,000	€ 66,106
purification process	€ 17,375	€ 29,325 *5	€ 43,437	€ 90,136
Enzymatic reaction tank	€ 252	€ 13,568 *5	€ 3,569	€ 17,390

*1- The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years). Also included is the cost for water for the diafiltration
*2 – The C&R include the cost for the chitosan
*3 – The C&R also include the cost for Ethanol. It is considered that 90% of the ethanol spent in the ethanol assisted solvent extraction is recycled into the system
*4 - The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years).
*5 - The C&R include the cost for the Water, Heptane, $Mg(OH)_2$, and NaOH used for the process (the reagents consumption was obtained from PUFAChain Project)
*6 - The C&R include the cost of the enzymes and water added

7.7.1.2.3 Capex extraction Process 2

In the following Table 148 the Capital Costs (Capex) for the *Synechocystis* extraction scenarios using supercritical solvent extraction for lipid are presented.

Table 148 - Capex Values for different Synechocystis biomass concentrations (50 to 200 g/l).

	Capacity	Capex	Reference
50 g/l			
Membrane System	3.12 m^3/h	€ 164,000	(University of Limerick, 2018)
Spray Dryer	160.73 kg water evaporated/h	€ 82,520	A4F
Supercritical Reactor	11.03 kg/h	€ 767,167	(Friedl, 2017)
Membrane System	2.96 m^3/h	€ 101,796	(University of Limerick, 2018)
Mixer settler tank	2.96 m^3/h	€ 57,522	(University of Limerick, 2018)
Saponification process	1.07 m^3/h	€ 709,139	(Friedl, 2017)
Enzymatic reaction tank	0.35 m^3/h	€ 16,080	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
100 g/l			
Membrane System	1.56 m^3/h	€ 108,200	(University of Limerick, 2018)
Spray Dryer	160.73 kg water evaporated/h	€ 82,520	(A4F, 2018)
Supercritical Reactor	11.03 kg/h	€ 767,167	(Friedl, 2017)
Membrane System	1.40 m^3/h	€ 64,947	(University of Limerick, 2018)
Mixer settler tank	1.40 m^3/h	€ 36,700	(University of Limerick, 2018)
Saponification process	1.07 m^3/h	€ 709,139	(Friedl, 2017)
Enzymatic reaction tank	0.35 m^3/h	€ 16,080	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
150 g/l			
Membrane System	1.04 m^3/h	€ 84,834	(University of Limerick, 2018)
Spray Dryer	160.73 kg water evaporated/h	€ 82,520	(A4F, 2018)
Supercritical Reactor	11.03 kg/h	€ 767,167	(Friedl, 2017)
Membrane System	0.88 m^3/h	€ 49,147	(University of Limerick, 2018)
Mixer settler tank	0.88 m^3/h	€ 27,771	(University of Limerick, 2018)
Saponification process	1.07 m^3/h	€ 709,139	(Friedl, 2017)
Enzymatic reaction tank	0.35 m^3/h	€ 16,080	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
200 g/l			
Membrane System	0.78 m^3/h	€ 71,385	DEMA Project
Spray Dryer	160.73 kg water evaporated/h	€ 82,520	(A4F, 2018)
Supercritical Reactor	11.03 kg/h	€ 767,167	(Friedl, 2017)
Membrane System	0.62 m^3/h	€ 39,824	(University of Limerick, 2018)
Mixer settler tank	0.62 m^3/h	€ 22,504	(University of Limerick, 2018)
Saponification process	1.07 m^3/h	€ 709,139	(Friedl, 2017)
Enzymatic reaction tank	0.35 m^3/h	€ 16,080	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)

7.7.1.2.4 Opex extraction process 2

In the following Table 146 and Table 147, the Operational costs (Opex) for the *Synechocystis* extraction scenarios using supercritical solvent extraction for lipid are presented.

Table 149 - Opex Values for different biomass concentrations (60 to 150 g/l).

	Energy costs	Consumables and reagents	Maintenance costs	Opex
50 g/l				
Membrane System	€ 1,009	€ 42,118 *1	€ 8,200	€ 51,327
Spray Dryer	€ 228,706	€ 1,650	€ 4,126	€ 234,483
Supercritical Reactor	€ 11,414	€ 66,297 *2	€ 38,358	€ 116,069
Membrane System	€ 957	€ 10,180 *3	€ 5,090	€ 16,226
Mixer settler tank	€ 31,901	€ 22,708 *4	€ 2,876	€ 57,485
Saponification process	€ 14,183	€ 22,102 *5	€ 35,457	€ 71,742
Enzymatic reaction tank	€ 3,813	€ 295,786 *6	€ 804	€ 300,403
100 g/l				
Membrane System	€ 505	€ 23,679 *1	€ 5,410	€ 29,593
Spray Dryer	€ 228,706	€ 1,650	€ 4,126	€ 234,483
Supercritical Reactor	€ 11,414	€ 66,297 *2	€ 38,358	€ 116,069
Membrane System	€ 453	€ 6,495 *3	€ 3,247	€ 10,195
Mixer settler tank	€ 15,084	€ 22,292 *4	€ 1,835	€ 39,211
Saponification process	€ 14,183	€ 22,102 *5	€ 35,457	€ 71,742
Enzymatic reaction tank	€ 3,813	€ 295,786 *6	€ 804	€ 300,403
150 g/l				
Membrane System	€ 336	€ 17,056 *1	€ 4,242	€ 21,634
Spray Dryer	€ 228,706	€ 1,650	€ 4,126	€ 234,483
Supercritical Reactor	€ 11,414	€ 66,297 *2	€ 38,358	€ 116,069
Membrane System	€ 284	€ 4,915 *3	€ 2,457	€ 7,656
Mixer settler tank	€ 9,479	€ 22,113 *4	€ 1,389	€ 32,980
Saponification process	€ 14,183	€ 22,102 *5	€ 35,457	€ 71,742
Enzymatic reaction tank	€ 3,813	€ 295,786 *6	€ 804	€ 300,403

*1– The consumables of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years). Also included is the cost for water for the diafiltration

*2 – The C&R also include the cost for the CO_2. It is considered that 90% of the CO_2spent in the supercritical extraction is recycled into the system.

*3 – The C&R include the cost for the chitosan

*4 - The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years).

*5- The C&R include the cost for the Water, Heptane, $Mg(OH)_2$, and NaOH used for the process (the reagents consumption was obtained from PUFAChain Project)

*6- The C&R include the cost of the enzymes and water added

Table 150 - Opex values for different biomass concentration (200 g/l).

	energy costs	consumables and reagents	maintenance costs	Opex
		200 g/l		
Membrane System	€ 252	€ 13,568 *1	€ 3,569	€ 17,390
Spray Dryer	€ 228,706	€ 1,650	€ 4,126	€ 234,483
Supercritical Reactor	€ 11,414	€ 66,297 *2	€ 38,358	€ 116,069
Membrane System	€ 200	€ 3,982 *3	€ 1,991	€ 6,174
Mixer settler tank	€ 6,676	€ 22,008 *4	€ 1,125	€ 29,809
Saponification process	€ 14,183	€ 22,102 *5	€ 35,457	€ 71,742
Enzymatic reaction tank	€ 3,813	€ 295,786 *6	€ 804	€ 300,403

*1- The consumables of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years). Also included is the cost for water for the diafiltration
*2 – The C&R also include the cost for the CO_2. It is considered that 90% of the CO_2spent in the supercritical extraction is recycled into the system.
*3 – The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years).
*4 - The C&R include the cost for the chitosan
*5- The C&R include the cost for the Water, Heptane, $Mg(OH)_2$, and NaOH used for the process (the reagents consumption was obtained from PUFAChain Project)
*6- The C&R include the cost of the enzymes and water added

7.7.1.3 Extra Equipment

Some products require an extra step for their formulation. This means that they require extra equipment with respective Opex and Capex as shown in Table 151.

Table 151 – Capex and Opex values for extra equipment potentially needed.

Product	Equipment	Capex	Opex*1
Feed 1 (Scenario 1)	Spray Dryer	€ 55,277	€ 123,822
Feed 2 (Scenario 1)	Spray Dryer	€ 27,727	€ 39,930
Phycocyanin (Scenario 1)	Spray Dryer	€ 47,546	€ 96,646
Phycocyanin (Scenario 2)	Spray Dryer	€ 47,546	€ 96,646

*1 – Accounts for 7% (maintenance and consumables) and the energy costs

7.7.2 *Prorocentrum* based biorefinery scenario

In this Appendix the Capital Costs (Capex), the Operation Costs (Opex), and the revenue of *Prorocentrum* based biorefinery scenario 1 are described.

7.7.2.1 Production, Harvesting and Disruption phase

7.7.2.1.1 Capital costs (Capex)

Assumptions

- If the precise capacity was not available, the Capex values for the equipment were calculated using the capacity rule (Perry and Green, 2008)
- Production System values were supplied by A4F from company research projects

Table 152 - Capex values for scenario 2 (capacity 345 tons/year of ruptured biomass).

		Capex	Reference
Production System		€ 14,197,010	(Friedl, 2017) – Values A4F
Harvesting Equipment	Membrane System (150 g/L)	€ 285,000	(University of Limerick, 2018)– Values Pall
	Membrane System (100 g/L)	€ 112,775	(University of Limerick, 2018)– Values Pall
	Centrifugation System (20.9 m^3/h)	€ 450,000	Values A4F – Westfalia Centrifuge
	Centrifugation System (9.2 m^3/h)	€ 186,842	Values A4F – Westfalia Centrifuge
	Flocculation System	€ 18,000	(Qingdao All Universe Machinery Equipment Co., 2019)
	DAF System	€ 40,000	(Jiangsu Benenv Environmental Technologies Co., 2018)
Cell Disruption Equipment	Bead mill (200 g/l)	€ 141,105	(Friedl, 2017)
	Bead mill (150 g/l)	€ 173,219	(Friedl, 2017)
	Bead mill (60 g/l)	€ 324,175	(Friedl, 2017)
	Bead mill (50 g/l)	€ 280,787	(Friedl, 2017)
	HPH (50 g/l)	€ 363,559	(Shanghai Youcan Beverage Machinery Co, 2018)
	HPH (60 g/l)	€ 314,899	(Shanghai Youcan Beverage Machinery Co, 2018)
	HPH (100 g/l)	€ 153,452	(Shanghai Youcan Beverage Machinery Co, 2018)
	HPH (200 g/l)	€ 158,248	(Shanghai Youcan Beverage Machinery Co, 2018)
	Ultrasonication	€ 62,074	(Yuhuan Clangsonic Ultrasonic Co. Ltd., 2018)

7.7.2.1.2 The operation costs (Opex)

To simplify the calculations operation costs were divided into 3 main components; Electricity costs, Consumables and Reagent costs (C&R), and Maintenance costs.

- No Inflation on Opex is considered
- Opex value has a 5% security margin and contains maintenance costs
- Maintenance costs are considered to be 5% of the Capex– cleaning water and cleaning reagents are also included in these costs.
- Consumables costs are considered to be 2% of the Capex

Table 153 – Opex values for the Production, Harvesting and Disruption Steps of scenario 2.

		Electricity	C&R	Maintenance	Opex
Production System*1		€ 312,579	€ 889,743	€ 709,851	€ 1,912,173
Harvesting Equipment	Membrane System (150 g/L)*2	€ 6,682	€ 28,500	€ 14,250	€ 65,970
	Membrane System (100 g/L)*2	€ 4,069	€ 11,277	€ 5,639	€ 20,985
	Centrifugation System (20.9 m^3/h)	€ 22,273	€ 9,000	€ 22,500	€ 70,386
	Centrifugation System (9.2 m^3/h)	€ 9,839	€ 18,684	€ 9,342	€ 37,865
	Flocculation System*3	€ 586	€ 110,566.24	€ 900	€ 128,115
	DAF System*3	€ 4,263	€ 28,351.56	€ 2,000	€ 50,833
Cell Disruption Equipment	Bead mill (200 g/l)	€ 7,992.6	€ 2,822.11	€ 7,055.27	€ 17,869.97
	Bead mill (150 g/l)	€ 10,656.8	€ 3,464.38	€ 8,660.95	€ 22,782.13
	Bead mill (60 g/l)	€ 26,642.0	€ 6,483.50	€ 16,208.75	€ 49,334.25
	Bead mill (50 g/l)	€ 31,970.4	€ 5,615.73	€ 14,039.34	€ 51,625.47
	HPH (50 g/l)	€ 1,9715.1	€ 7,271.18	€ 18,177.94	€ 45,164.20
	HPH (60 g/l)	€ 32,858.5	€ 6,297.99	€ 15,744.97	€ 54,901.42
	HPH (100 g/l)	€7,886.0	€ 3,069.04	€ 7,672.60	€ 18,627.68
	HPH (200 g/l)	€8961.4	€ 3,164.96	€ 7,912.41	€ 20,038.77
	Ultrasonication	€3,4101.8	€ 1,241.48	€ 3,103.69	€ 38,446.93

*1 - This information was provided by A4F from company research projects
*2– The consumables of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years)
*3 – In the Flocculation System and DAF systems the reagents cost include the Chitosan cost

7.7.3 Extraction processing

Assumptions:

- The values for the extraction are calculated for a base of 45 kg/h
- All equipment has a 30% extra capacity as security margin, expect the saponification processes
- The Maintenance is 5% of the Capex of the equipment and takes into account cleaning water and reagents
- The Consumables are 2% of the Capex except where stated otherwise

Depending on the type of harvesting equipment chosen, different concentrations can be achieved. Since the objective is to create as many scenarios as possible, it was necessary to calculate the mass balances of the extraction scenarios for different final biomass concentrations.

7.7.3.1 Capex Process 1

In the following Table 154 and Table 156 the Capital costs (Capex) for the *Prorocentrum* scenarios using conventional solvent extraction for lipid are presented.

Table 154 - Capex Values for different Prorocentrum biomass concentrations (50 to 100 g/l).

	Capacity	Capex	Reference
		50 g/l	
Stirred Tank	1.34 m^3/h	€ 25,043	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Decanter centrifuge	1.34 m^3/h	€ 73,674	(Nanjing Kingreat Machinery Co., 2018)
Stirred Tank	1.93 m^3/h	€ 31,184	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	1.93 m^3/h	€ 78,838	(University of Limerick, 2018)
Hexane recovery Membrane System	0.17 m^3/h	€ 18,403	(University of Limerick, 2018)
Evaporator	1.49 m^3/h	€ 250,000	(University of Limerick, 2018)
Purification process	10.03 m^3/h	€ 2,708,898	(Friedl, 2017)
Purification process	13.84 m^3/h	€ 3,286,598	(Friedl, 2017)
Purification process	11.10 kg/h	€ 2,878,875	(Friedl, 2017)
Stirred tank	0.41 m^3/h	€ 12,358	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Stirred Tank	4.68 m^3/h	€ 53,011	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
		100 g/l	
Stirred Tank	0.76 m^3/h	€ 17,756	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Decanter centrifuge	0.76 m^3/h	€ 52,237	(Nanjing Kingreat Machinery Co., 2018)
Stirred Tank	1.35 m^3/h	€ 25,101	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	1.35 m^3/h	€ 63,459	(University of Limerick, 2018)
Membrane System	0.17 m^3/h	€ 18,403	(University of Limerick, 2018)
Evaporator	1.04 m^3/h	€ 250,000	(University of Limerick, 2018)
Purification process	10.03 kg/h	€ 2,708,898	(Friedl, 2017)*1
Purification process	13.66 kg/h	€ 3,260,199	(Friedl, 2017)*1
Purification process	11.10 kg/h	€ 2,878,875	(Friedl, 2017)
Stirred tank	0.41 m^3/h	€ 12,358	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Stirred Tank	2.34 m^3/h	€ 34,974	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)

*1 – the saponification values were taken from the PUFAChain Project and given by IOI oleo

Table 155- Capex values for different Prorocentrum biomass concentrations (150 to 200 g/l).

	Capacity	Capex	Reference
		150 g/l	
Stirred Tank	0.56 m^3/h	€ 14,846	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Decanter centrifuge	0.56 m^3/h	€ 43,677	(Nanjing Kingreat Machinery Co., 2018)
Stirred Tank	1.15 m^3/h	€ 22,842	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	1.15 m^3/h	€ 57,747	(University of Limerick, 2018)
Membrane System	0.17 m^3/h	€ 18,403	(University of Limerick, 2018)
Evaporator	0.88 m^3/h	€ 250,000	(University of Limerick, 2018)
Purification process	10.03 kg/h	€ 2,708,898	(Friedl, 2017)
Purification process	13.56 kg/h	€ 3,246,249	(Friedl, 2017)
Purification process	11.10 kg/h	€ 2,459,556	(Friedl, 2017)
Stirred tank	0.04 m^3/h	€ 12,358	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Stirred Tank	1.56 m^3/h	€ 27,422	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
		200 g/l	
Stirred Tank	0.46 m^3/h	€ 13,240	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Decanter centrifuge	0.46 m^3/h	€ 38,950	(Nanjing Kingreat Machinery Co., 2018)
Stirred Tank	1.05 m^3/h	€ 21,653	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Membrane System	1.05 m^3/h	€ 54,742	(University of Limerick, 2018)
Membrane System	0.17 m^3/h	€ 18,403	(University of Limerick, 2018)
Evaporator	0.81 m^3/h	€ 250,000	(University of Limerick, 2018)
Purification process	10.03 kg/h	€ 2,708,898	(Friedl, 2017)
Purification process	13.50 kg/h	€ 3,237,622	(Friedl, 2017)
Purification process	11.10 kg/h	€ 2,878,875	(Friedl, 2017)
Stirred tank	0.04 m^3/h	€ 12,358	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Stirred Tank	1.17 m^3/h	€ 23,074	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)

*1 – the saponification values were taken from the PUFAChain Project and given by IOI oleo

7.7.3.2 Opex Process 1

In the following Table 156 and Table 157, the Operational costs (Opex) for the *Prorocentrum* scenarios using conventional solvent extraction for lipid are presented.

Table 156 - Opex values for different Prorocentrum biomass concentrations (50 to 100 g/l).

	Energy costs	Consumables and Reagents	Maintenance costs	Opex
		50 g/l		
Stirred Tank	€ 8,150	€ 101,671	€ 888	€ 110,709
Decanter centrifuge	€ 2,038	€ 1,045	€ 2,612	€ 5,694
Stirred Tank	€ 14,512	€ 210,369	€ 1,255	€ 226,136
Membrane System	€ 726	€ 6,346	€ 3,173	€ 10,244
Membrane System	€ 92	€ 1,840	€ 920	€ 2,853
Evaporator	€ 242,530.27	€ 25,000	€ 12,500	€ 280,030
Purification process	€ 54,178	€ 54,676	€ 135,445	€ 244,299
Purification process	€ 65,204	€ 91,989	€ 163,010	€ 320,203
Purification process	€ 57,577	€ 85,370	€ 143,944	€ 286,891
Stirred tank	€ 4,455 *1	€ 364,838 *10	€ 617.91	€ 369,911
Stirred tank	€ 50,450 *1	€ 790,250 *10	€ 2,651	€ 843,351
		100 g/l		
Stirred Tank	€ 8,150	€ 101,671	€ 888	€ 110,709
Decanter centrifuge	€ 2,038	€ 1,045	€ 2,612	€ 5,694
Stirred Tank	€ 14,512	€ 210,369	€ 1,255	€ 226,136
Membrane System	€ 726	€ 6,346	€ 3,173	€ 10,244
Membrane System	€ 92	€ 1,840	€ 920	€ 2,853
Evaporator	€ 242,530.27	€ 25,000	€ 12,500	€ 280,030
Purification process	€ 54,178	€ 54,676	€ 135,445	€ 244,299
Purification process	€ 65,204	€ 91,989	€ 163,010	€ 320,203
Purification process	€ 57,577	€ 85,370	€ 143,944	€ 286,891
Stirred tank	€ 4,455 *1	€ 364,838 *10	€ 617.91	€ 369,911
Stirred tank	€ 25,225*1	€ 789,889*10	€ 1,749	€ 816,863

*1 – Stirred tanks consume 10 kWh/m3 (Jordan, 1996)
*2 – The Decanter centrifuge consumes 2.5 kWh/m3
*3 - Membranes consume 0.3 kWh/m3 (A4F from company research projects)
*4 - The amount of energy consumed was calculated based on the energy necessary for the evaporation of the water and ethanol removed from the biomass
*5 – This information was supplied by a partner in the PUFAChain project, IOI Oleo
*6 – The C&R include the cost for the Heptane
*7 - The C&R include the cost for the Ethanol
*8- The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years).
*9- The C&R include the cost for the Water, Heptane, $Mg(OH)_2$, and NaOH used for the process (the reagents consumption was obtained from PUFAChain Project)
*10- The C&R include the cost of the enzymes and water added

Table 157 – Opex values for different Prorocentrum biomass concentrations (150 to 200 g/l).

	Energy costs	Consumables and reagents	Maintenance costs	Opex
		150 g/l		
Stirred Tank	€ 6,048	€ 101,613	€ 742	€ 108,403
Decanter centrifuge	€ 1,512	€ 874	€ 2,184	€ 4,569
Stirred Tank	€ 12,401	€ 210,323	€ 1,142	€ 223,867
Membrane System	€ 620	€ 5,775	€ 2,887	€ 9,282
Membrane System	€ 92	€ 1,840	€ 920	€ 2,853
Evaporator	€ 202,728.91	€ 25,000	€ 12,500	€ 240,229
Purification process	€ 54,178	€ 54,676	€ 135,445	€ 244,299
Purification process	€ 64,925	€ 91,710	€ 162,312	€ 318,947
Purification process	€ 57,577	€ 85,370	€ 143,944	€ 286,891
Stirred tank	€ 4,455 *1	€ 364,838 *10	€ 618	€ 369,911
Stirred tank	€ 16,817*1	€ 789,738 *10	€ 1,371	€ 807,926
		200 g/l		
Stirred Tank	€ 4,997	€ 101,581	€ 662	€ 107,240
Decanter centrifuge	€ 1,249	€ 779	€ 1,948	€ 3,976
Stirred Tank	€ 11,345	€ 210,300	€ 1,083	€ 222,727
Membrane System	€ 567	€ 5,474	€ 2,737	€ 8,779
Membrane System	€ 92	€ 1,840	€ 920	€ 2,853
Evaporator	€ 182,904.48	€ 25,000	€ 12,500	€ 220,404
Purification process	€ 54,178	€ 54,676	€ 135,445	€ 244,299
Purification process	€ 64,752	€ 91,537	€ 161,881	€ 318,171
Purification process	€ 57,577	€ 85,370	€ 143,944	€ 286,891
Stirred tank	€ 4,455 *1	€ 364,838 *10	€ 618	€ 369,911
Stirred tank	€ 12,613 *1	€ 789,651 *10	€ 1,154	€ 803,418

Notes*
*1 – Stirred tanks consume 10 kWh/m3 (Jordan, 1996)
*2 – The Decanter centrifuge consumes 2.5 kWh/m3
*3 - Membranes consume 0.3 kWh/m3 (A4F from company research projects)
*4 - The amount of energy consumed was calculated based on the energy necessary for the evaporation of the water removed from the biomass
*5 – This information was supplied by a partner in the PUFAChain project, IOI Oleo
*6 – The C&R include the cost for the Heptane
*7 - The C&R include the cost for the Ethanol
*8- The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years).
*9- The C&R include the cost for the Water, Heptane, $Mg(OH)_2$, and NaOH used for the process (the reagents consumption was obtained from PUFAChain Project)
*10- The C&R include the cost of the enzymes and water added

7.7.3.3 Capex Process 2

In the following Table 158, the Capital costs (Capex) for the *Prorocentrum*m scenarios using supercritical solvent extraction for lipid are presented.

Table 158 - Capex values for different Prorocentrum biomass concentrations (50 to 200 g/l).

	Capacity	Capex	Reference
		50 g/l	
Spray Dryer	860.04 kg of water evaporated/h	€ 225,746	(Friedl, 2017)
Supercritical Reactor	51.95 kg of biomass/h	€ 1,944,127	(Friedl, 2017)
Stirred Tank	1.00 m^3/h	€ 20,983	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Purification process	1.9 kg of biomass/h	€ 1,021,490	(Friedl, 2017)
		100 g/l	
Spray Dryer	410.04 kg of water evaporated/h	€ 144,745	(Friedl, 2017)
Supercritical Reactor	39.96 kg of biomass/h	€ 1,660,58	(Friedl, 2017)
Stirred Tank	1.00 m^3/h	€ 20,983	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Purification process	1.9 kg of biomass/h	€ 1,021,490	(Friedl, 2017)
		150 g/l	
Spray Dryer	260.04 kg of water evaporated/h	€ 110,136	(Friedl, 2017)
Supercritical Reactor	39.96 kg of biomass/h	€ 1,660,58	(Friedl, 2017)
Stirred Tank	1.00 m^3/h	€ 20,983	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Purification process	1.9 kg of biomass/h	€ 1,021,490	(Friedl, 2017)
		200 g/l	
Spray Dryer	185.04 kg of water evaporated/h	€ 89,798	(Friedl, 2017)
Supercritical Reactor	39.96 kg of biomass/h	€ 1,660,58	(Friedl, 2017)
Stirred Tank	1.00 m^3/h	€ 20,983	(Guangzhou Fuluke Cosmetics Equipment Co ltd, 2018)
Purification process	1.9 kg of biomass/h	€ 1,021,490	(Friedl, 2017)

*1 – the saponification values were taken from the PUFAChain Project and given by IOI oleo

7.7.3.4 Opex Process 2

In Table 159, the Operational costs (Opex) for the *Prorocentrum* scenarios using supercritical solvent extraction for lipid are presented.

Table 159 - Opex Values for different Prorocentrum biomass concentrations (50 to 200 g/l).

	Energy costs	Consumables and reagents	Maintenance costs	Opex
		50 g/l		
Spray Dryer	€ 1,222,564*1	€ 4,515	€ 11,287	€ 1,238,366
Supercritical Reactor	€ 53,762 *2	€ 49,278 *5	€ 97,206	€ 200,246
Stirred Tank	€ 10,765*3	€ 832,215*6	€ 1,049	€ 844,029
Purification process	€ 20,430 *4	€ 42,282 *7	€ 51,075	€ 113,787
		100 g/l		
Spray Dryer	€ 582,879 *1	€ 2,895	€ 7,237	€ 593,011
Supercritical Reactor	€ 53,762 *2	€ 49,278 *5	€ 97,206	€ 200,246
Stirred Tank	€ 10,765*3	€ 832,215*6	€ 1,049	€ 844,029
Purification process	€ 20,430*4	€ 42,282 *7	€ 51,075	€ 113,787
		150 g/l		
Spray Dryer	€ 369,650 *1	€ 2,203	€ 5,507	€ 377,360
Supercritical Reactor	€ 53,762 *2	€ 49,278 *5	€ 97,206	€ 200,246
Stirred Tank	€ 10,765*3	€ 832,215 *6	€ 1,049	€ 844,029
Purification process	€ 20,430*4	€ 42,282 *7	€ 51,075	€ 113,787
		200 g/l		
Spray Dryer	€ 263,036 *1	€ 1,796	€ 4,490	€ 269,322
Supercritical Reactor	€ 53,762 *2	€ 49,278 *5	€ 97,206	€ 200,246
Stirred Tank	€ 10,765 *3	€ 832,215*6	€ 1,049	€ 844,029
Purification process	€ 20,430 *4	€ 42,282 *7	€ 51,075	€ 113,787

Notes*
*1 – The amount of energy consumed was calculated based on the energy necessary for the evaporation of the water removed from the biomass
*2 – This information was supplied by a partner in the PUFAChain project, Natex
*3 - Stirred tanks consume 10 kWh/m3 (Jordan, 1996)
*4 - This information was supplied by a partner in the PUFAChain project, IOI Oleo
*5 – The C&R also include the cost for the CO_2. It is considered that 90% of the CO_2 spent in the supercritical extraction is recycled into the system.
*6 - The C&R include the cost of the enzymes and water added
*7 - The C&R of the membrane system are 10% instead of 2% to include the price of the Membranes (change membranes 2 in 2 years).

7.7.3.5 Extra Equipment

Some products require an extra step for their formulation. This means that they require extra equipment with respective Opex and Capex.

Table 160 – Capex and Opex of extra equipment potentially needed.

Product	Equipment	Capex	Opex*1
Feed (Process 2 scenario 1)	Spray Dryer	€ 6,337	€485
Feed (Process 2 scenario 3)	Spray Dryer	€ 86,633	€ 9285
Concentrated protein (process 2)	Spray Dryer	€ 41,630	€ 3520

*1 – Accounts for 7% (maintenance and consumables) and the energy costs

7.8 Appendix 8 - Distillation Column Costs

Equations 1 to 5 were taken from http://www.matche.com in order to calculate the cost of the distillation column:

$$Weight(W) = \rho_{steel}\left(\pi \times D_c \times e \times H + \frac{4}{3} \times \pi\left[\left(\frac{D_c}{2} + e\right)^3 - \left(\frac{D_c}{2}\right)^3\right]\right) \quad (1)$$

$$Cost\ Plates(€) = n_{plats} \times 315 \times e^{0.72Dc} \quad (2)$$

$$C_{column}(€) = 56.181 \times W^{0.878} + cost\ of\ plates \quad (3)$$

$$t(m) = 0.0023 + 0.003D_c \quad (4)$$

$$Height = (n_{plates} \times 0.6) + 4 \times 1.5 \quad (5)$$

Using the previous equation and the column properties in Table 161, the column costs were calculated.

Table 161 - Distillation column properties.

Parameter	Number	Unit
n of plates	8	-
Height	10.8	m
Diameter$_{internal}$	2.5	m
Thickness	0.0098	m
Diameter$_{external}$	2.4804	m
Volume	0.828007	m3
Weight	6624.055	kg
Cost$_{plate}$	15245.11	€
Cost$_{column}$	142459.5	€

7.9 Appendix 9 - Price of utilities and reagents and manpower

The prices used to calculate the costs for the Opex of the processes are shown in Table 162.

Table 162 - Utilities prices and references.

Utilities	price	Unit	REF
Water	1.67	€/ m^3	(EPAL, 2019)
Ethanol	0.487	€/kg	(University of Limerick, 2018)
Electricity	0.14	€/kWh	(PORDATA, 2018)
CO_2	0.15	€/kg	(University of Limerick, 2018)
Chitosan	20	€/kg	(Xu et al., 2013)
Steam	60	€/ton	(U.S. Department of Commerce, 2019)
Heptane	0.684	€/kg	(ICIS, 2018)
NaOH	0.57	€/kg	(EChemi, 2018)
$Mg(OH)_2$	0.21	€/kg	(KEMCORE, 2018)
Enzyme Cocktail	13500	€/m^3	(University of Limerick, 2018)

7.10 Appendix 10 – GAMS code

The following code was used in the program GAMS to calculate the values used in the economic analysis.

7.10.1 GAMS code *Synechocystis* based biorefinery

```
Sets
i Harvest Rupture opt /HR1*HR17/
j Downstream Process/ proc1*proc8/
k cenas    /concentration,capex, opex, final_biomass, ethanol/
l cenas2 /capexp1, capexp2, opexp1, opexp2, income/;

Table a (i,k)
          capex          opex      concentration     final_biomass       ethanol
HR1       15164883.7      2382259.758       200       152.7869868       141949.9228
HR2       15176251.7      2382948.95        250       148.1570781       142023.9292
HR3       15133742.93      2406189.063       100       154.3302897       141579.8909
HR4       14989883.7      2378547.437       200       161.2751527       141949.9228
HR5       15001251.7      2380311.997       200       156.3880269       141949.9228
HR6       14958742.93      2402476.743       100       162.9041947       141579.8909
HR7       15244373.94      2115210.865       200       161.2751527       134063.816
HR8       15267784.47      2116975.425       200       156.3880269       134063.816
HR9       15114487.57      2139140.17        100       162.9041947       133714.3414
HR10       14648629.3      2333531.313       60        144.2988209       141086.5151
HR11       14672039.83      2341386.517       60        139.9261293       141086.5151
HR12       14518742.93      2331899.027       60        145.7563847       141086.5151
HR13       14586004.99      2065665.983       80        152.7869868       141394.875
HR14       14619010.34      2083549.347       80        148.1570781       141394.875
HR15       14538294.22      2071384.51        80        154.3302897       141394.875
HR16       15076964.26      2143891.409       200       145.1476375       141949.9228
HR17       15109969.61      2152079.171       200       140.7492242       141949.9228
;
```

```
Table  b (j,l)
           capexp1    capexp2       opexp1     opexp2     Income

proc1        -253492    1651918       -49666   1524488     21.6
proc2        -253492    1656098       -49530    1887288     47.0
proc3        -401802    3514424       -55809    2153490     24.4
proc4        -401802    3518605       -55672     2516290    50.9
proc5        -122498    1630847       -227392    3019224     22.6
proc6        -122498    1634969       -227392    3378614     47.5
proc7        -122498    2422882       -227392    3256629     24.5
proc8        -122498    2427005       -227392    3637777   49.4       ;

Parameter d(i,j)   Downstream Capex ;
        d(i,j) = ( b(j,'capexp1')*log(a(i,'concentration')))+b(j,'capexp2')    ;

Parameter t(i,j)   total capex   ;
        t(i,j) = a(i,'capex') + d(i,j);

Parameter t1(i,j)   total capex  + security  ;
        t1(i,j) = t(i,j) * 1.1;

Parameter x(i,j)   Downstream opex ;
        x(i,j) =  (b(j,'opexp1')*log(a(i,'concentration')))+b(j,'opexp2')    ;

Parameter o(i,j)   "total opex"  ;
        o(i,j) = a(i,'opex')+ x(i,j)      ;

Parameter o1(i,j)   total opex + security     ;
        o1(i,j) = o(i,j)*1.05

Parameter r(i,j)   income  ;
        r(i,j) =  (b(j,'Income')*a(i,'final_biomass')*1000)+ a(i,'ethanol') ;
```

```
Parameter e(i,j)   profit    ;
        e(i,j) =  r(i,j)-o1(i,j);

Parameter m(i,j) Net present value  ;
        m(i,j) =
((e(i,j)/(1.05**1))+(e(i,j)/(1.05**2))+(e(i,j)/(1.05**3))+(e(i,j)/(1.05**4))+(e(i,j)/(1.05**5))+(
e(i,j)/(1.05**6))+(e(i,j)/(1.05**7))+(e(i,j)/(1.05**8))) -t1(i,j)

Parameter roi(i,j) Return on Investment ;
        roi(i,j)
=(((e(i,j)/(1.05**1))+(e(i,j)/(1.05**2))+(e(i,j)/(1.05**3))+(e(i,j)/(1.05**4))+(e(i,j)/(1.05**5))
+(e(i,j)/(1.05**6))+(e(i,j)/(1.05**7))+(e(i,j)/(1.05**8)))-t1(i,j))/t1(i,j) ;

Parameter pp (i,j) payback period      ;
        pp (i,j) =  t1(i,j) / e(i,j)  ;

Variables
    z    NPV       ;
Equations
    NPV          define objective function  ;
NPV(i,j)                              ..                              z=e=
((e(i,j)/(1.05**1))+(e(i,j)/(1.05**2))+(e(i,j)/(1.05**3))+(e(i,j)/(1.05**4))+(e(i,j)/(1.05**5))+(
e(i,j)/(1.05**6))+(e(i,j)/(1.05**7))+(e(i,j)/(1.05**8)) -t1(i,j))  ;

Model Optimization/all/ ;
Solve Optimization using minlp maximizing z ;
Display  t,t1 ,r,o,o1, e,m,roi, pp;
```

7.10.3 GAMS code *Prorocentrum* based biorefinery

```
Sets
i Harvest Rupture opt /HR1*HR14/
j Downstream Process/ proc1*proc5/
k cenas    /concentration,capex, opex, final_biomass/
l cenas2 /capexp1, capexp2, opexp1, opexp2, income/
                        ;
Table a (i,k)
            capex        opex          concentration   final_biomass
HR1       14788115.75      2000428.695       200       345.496575
HR2       14805258.56      2013351.171       200       335.0269818
HR3       14709084.22      2021005.648       100       348.9864394
HR4       14670229.34      2003782.554       150       364.6908292
HR5       14655258.56      2011792.879       150       353.6395919
HR6       14559084.22      2019447.356       100       368.3745749
HR7       14495797.12      2091912.816       50       345.496575
HR8       14578569.19      2105166.621       50       335.0269818
HR9       14277084.22      2078734.273       50       348.9864394
HR10       14561185.36       2012339.971       60       326.3023208
HR11       14551909.74       2017907.145       60       316.4143717
HR12       14299084.22       2001452.651       60       329.5983039
HR13       14641391.14       1989102.794       200       328.2217462
HR14       14863844.59       1997974.631       200       314.9602615
;
```

Table b (j,l)

	capexp1	capexp2	opexp1	opexp2	Income
proc1	-129518	3763833	-721590	5750338	7.2
proc2	-55815	3300062	-40300	2770719	22.6
proc3	-94168	6849279	-154608	3451672	9.5
proc4	-93646	6926368	-154641	3909229	26.1
proc5	-99056	2552943	-710081	5339885	2.8
Proc6	-99056	3574434	-710081	5540297	2.9
Proc7	-99056	3595416	-710081	6470951	23.9

```
Parameter d(i,j)   Downstream Capex ;
        d(i,j) = ( b(j,'capexp1')*log(a(i,'concentration')))+b(j,'capexp2')    ;
Parameter t(i,j)   total capex   ;
        t(i,j) = a(i,'capex')+ d(i,j);
Parameter t1(i,j)   total capex  + security  ;
        t1(i,j) = t(i,j) * 1.1;
Parameter x(i,j)   Downstream opex ;
        x(i,j) =  (b(j,'opexp1')*log(a(i,'concentration')))+b(j,'opexp2')    ;
Parameter o(i,j)   "total opex"  ;
        o(i,j) = a(i,'opex')+ x(i,j)      ;
Parameter o1(i,j)   total opex + security     ;
        o1(i,j) = o(i,j)*1.05
Parameter r(i,j)   income  ;
        r(i,j) =  (b(j,'Income')*a(i,'final_biomass')*1000)  ;
Parameter e(i,j)   profit    ;
        e(i,j) =  r(i,j)-o1(i,j);
Parameter m(i,j) Net present value  ;
```

```
        m(i,j)                                                                                                  =
((e(i,j)/(1.05**1))+(e(i,j)/(1.05**2))+(e(i,j)/(1.05**3))+(e(i,j)/(1.05**4))+(e(i,j)/(1.05**5))+(
e(i,j)/(1.05**6))+(e(i,j)/(1.05**7))+(e(i,j)/(1.05**8))) -t1(i,j)

Parameter roi(i,j) Return on Investment ;

        roi(i,j)
=(((e(i,j)/(1.05**1))+(e(i,j)/(1.05**2))+(e(i,j)/(1.05**3))+(e(i,j)/(1.05**4))+(e(i,j)/(1.05**5))
+(e(i,j)/(1.05**6))+(e(i,j)/(1.05**7))+(e(i,j)/(1.05**8)))-t1(i,j))/t1(i,j) ;

Parameter pp (i,j) payback period      ;

        pp (i,j) =  t1(i,j) / e(i,j)  ;

Variables

    z     NPV        ;

Equations

    NPV          define objective function ;

NPV(i,j)                                    ..                                                          z=e=
((e(i,j)/(1.05**1))+(e(i,j)/(1.05**2))+(e(i,j)/(1.05**3))+(e(i,j)/(1.05**4))+(e(i,j)/(1.05**5))+(
e(i,j)/(1.05**6))+(e(i,j)/(1.05**7))+(e(i,j)/(1.05**8)))-t1(i,j)  ;


Model Optimization/all/ ;

Solve Optimization using minlp maximizing z ;

Display  t,t1 ,r,o,o1, e,m,roi, pp;
```

7.11 Appendix 11 – Biomass value for the sensitivity analysis

7.11.1 *Synechocystis* based biorefinery

In Table 163, the *Synechocystis* biomass value used to calculate the revenues of the different processes is presented for different variations in the phycocyanin price due to market price fluctuations.

Table 163 - Biomass value with changes in Phycocyanin price.

Phycocyanin prices change	-20%	-10%	10%	20%
Process 1	€ 16.8	€ 8.7	€ 22.5	€ 24.3
Process 2	€ 43.7	€ 45.5	€ 49.3	€ 51.2
Process 3	€ 19.6	€ 21.5	€ 25.3	€ 27.1
Process 4	€ 46.2	€ 48.0	€ 51.8	€ 53.7
Process 5	€ 17.8	€ 19.7	€ 23.5	€ 25.3
Process 6	€ 42.7	€ 44.6	€ 48.4	€ 50.2
Process 7	€ 19.8	€ 21.7	€ 25.4	€ 27.3
Process 8	€ 44.7	€ 46.6	€ 50.3	€ 52.2

In Table 164, the *Synechocystis* biomass value used to calculate the revenues of the different processes is presented for different variations in biofertilizers and zeaxanthin market prices.

Table 164 – Biomass value with changes in biofertilizer and zeaxanthin prices.

Biofertilizer price change								
Zeaxanthin prices change	0%	10%	20%	-10%	0%	10%	-10%	10%
Process 1	€ 21.6	€ 21.7	€ 21.8	€ 21.5	€ 21.6	€ 21.7	€ 21.5	€ 21.7
Process 2	€ 44.1	€ 44.1	€ 44.1	€ 45.5	€ 45.5	€ 45.5	€ 48.4	€ 48.4
Process 3	€ 24.4	€ 24.8	€ 25.2	€ 24.0	€ 24.4	€ 24.8	€ 24.0	€ 24.8
Process 4	€ 46.8	€ 47.2	€ 47.6	€ 47.8	€ 48.2	€ 48.6	€ 50.5	€ 51.3
Process 5	€ 22.6	€ 22.8	€ 23.0	€ 22.4	€ 22.6	€ 22.8	€ 22.4	€ 22.8
Process 6	€ 44.9	€ 45.1	€ 45.3	€ 44.7	€ 44.9	€ 45.1	€ 44.7	€ 45.1
Process 7	€ 24.5	€ 25.0	€ 25.4	€ 24.1	€ 24.5	€ 25.0	€ 24.1	€ 25.0
Process 8	€ 46.9	€ 47.3	€ 47.7	€ 46.5	€ 46.9	€ 47.3	€ 46.5	€ 47.3

7.11.2 *Prorocentrum* based biorefinery

In Table 165, the *Prorocentrum* biomass value used to calculate the revenues of the different processes is presented for different changes in the EPA + DHA price due to market price fluctuations.

Table 165 - Biomass value with changes in EPA + DHA prices.

EPA + DHA prices change	-20%	-10%	10%	20%
Process 1	€ 7.0	€ 7.1	€ 7.4	€ 7.5
Process 2	€ 22.4	€ 22.5	€ 22.8	€ 22.9
Process 3	€ 8.9	€ 9.1	€ 9.6	€ 9.8
Process 4	€ 25.7	€ 26.0	€ 26.4	€ 26.7
Process 5	€ 2.8	€ 2.8	€ 2.8	€ 2.8
Process 6	€ 2.7	€ 2.8	€ 3.0	€ 3.1
Process 7	€ 23.7	€ 23.8	€ 24.0	€ 24.2

In Table 166, the *Prorocentrum* biomass value used to calculate the revenues of the different processes is presented for different changes in the biofertilizer and protein concentrate prices, due to market price fluctuations.

Table 166 – Biomass value with changes in biofertilizer and protein concentrate prices.

Biofertilizer price change								
Protein Concentrate Price Change	**-10%**	**0%**	**10%**	**-10%**	**10%**	**-10%**	**0%**	**10%**
Process 1	€ 6.4	€ 7.2	€ 8.2	€ 6.4	€ 8.2	€ 6.4	€ 7.2	€ 8.2
Process 2	€ 20.1	€ 21.0	€ 21.9	€ 21.8	€ 23.6	€ 23.4	€ 24.3	€ 25.3
Process 3	€ 8.7	€ 9.3	€ 10.0	€ 8.7	€ 10.0	€ 8.7	€ 9.3	€ 10.0
Process 4	€ 23.8	€ 23.8	€ 23.8	€ 26.1	€ 26.1	€ 28.4	€ 28.4	€ 28.4
Process 5	€ 2.8	€ 2.8	€ 2.8	€ 2.8	€ 2.8	€ 2.8	€ 2.8	€ 2.8
Process 6	€ 2.9	€ 2.9	€ 2.9	€ 2.9	€ 2.9	€ 2.9	€ 2.9	€ 2.9
Process 7	€ 21.7	€ 21.7	€ 21.7	€ 23.9	€ 23.9	€ 26.2	€ 26.2	€ 26.2

7.12 Appendix 12 – Global Inventories

All equipment material is per year so the total value was divided by 20 years (assumed equipment lifetime) except for membrane material that was divided by two (as 2 years was the lifetime assumed for the membranes)

7.12.1 *Synechocystis* Inventory

Table 167 – LCI Data for the Synechocystis production.

Material	amount per year	units
UHT-PBR		
Glass	32707.77	kg
Wood	3311.00	kg
NBR	289.72	kg
PP	439.12	kg
Stainless steel	4040.00	kg
Harvesting Pump		
Cast Iron	890	kg
Chemical/Nutrients		
Water	218732.19	m^3
Chlorine	1514.25	kg
Sodium thiosulfate	474.15	kg
NaNO3	117325.00	kg
KH_2PO_4	9927.50	kg
CO2	1083.02	ton
Utilities		
Electricity	2410560.00	**kWh**

7.12.2 *Prorocentrum* based biorefinery Inventory

Table 168 – LCI Data for the Prorocentrum production.

Material	amount per year	units
UHT-PBR		
Glass	32707.77	kg
Wood	3311.00	kg
NBR	289.72	kg
PP	439.12	kg
Stainless steel	4040.00	kg
Harvesting Pump		
Cast Iron	890	kg
Chemical/Nutrients		
Water	227412.27	m^3
Chlorine	48315.27	kg
Sodium thiosulfate	1.51	ton
NaNO3	219328.56	kg
KH_2PO_4	18558.57	kg
CO2	1184.37	ton
NaCl	484118.98	kg
Utilities		
Electricity	2258519.40	**kWh**

7.13 Appendix 13 – Life Cycle Assessment Values

In this appendix, the material, energy and consumables inventories used in the LCA calculations, and their impacts are accounted for.

7.13.1 *Synechocystis* based biorefinery

The Inventories used for the LCA calculations of the different *Synechocystis* based biorefineries are found in Table 169 and Table 170 while the impacts calculated are found in Table 171 and Table 172.

Table 169- LCI Data for the Synechocystis harvesting and rupture.

	Scenario S1	Scenario S2	Scenario S3	Scenario S4	Scenario S5
Biomass Produced (kg)	180500.00	180500.00	180500.00	180500.00	180500.00
Ruptured biomass (kg)	161275.15	162904.19	154330.29	152786.99	162904.19
Polyethersulfone (kg)	126.00	126.00	0.00	0.00	126.00
Stainless steel 316 (kg)	688.22	616.40	390.60	651.42	785.60
Cast iron (kg)	0	0	0	111.00	0.00
Titanium alloy (kg)	0.00	0.22	221.00	0.00	0.22
Glass fiber reinforced polyester (kg)	0.00	0.00	257.81	0.00	0.00
Carbon steel (kg)	0.00	0.00	165.40	0.00	0.00
PDMS (kg)	26.52	26.25	5.25	5.25	26.25
Energy (kWh)	1023086.63	1211736.63	454909.95	590 968.95	561178.35
Water (m^3)	10198.79	10214.40	1478.40	5208.00	10214.40
Wastewater (m^3)	34947.32	34540.69	34456.45	34630.94	34540.69
Cleaning agent (m^3)	424.95	425.60	61.60	217.00	425.60
Ethanol (m^3)	368.46	368.46	367.18	369.43	368.46
Steam (t)	0.00	0	0	3742.40	3742.40
transport equipment (tkm)	**1514.08**	**1356.08**	**2276.59**	**1433.13**	**1728.81**
transport chemicals (tkm)	42282.47	42347.20	6129.20	21591.50	42347.20

Table 170 - LCI Data for the Synechocystis extraction processing.

	Scenario S1	Scenario S2	Scenario S3	Scenario S4	Scenario S5
Ruptured biomass (kg)	161275.15	162904.19	154330.29	152786.99	162904.19
Phycocyanin (kg)	51688.69	52210.79	49462.86	48968.23	52210.79
Amino acid Hydrolysate (m^3)	99990.59	84710.18	95684.78	94727.93	101000.60
Lipids soap (kg)	0	5701.65	0	0	0
Carotenoids (kg)	0	2443.56	0	0	0
Water (m^3)	8063.76	8145.21	7716.51	7639.35	4072.60
Wastewater (m^3)	0	13.81	0	0	0
Chitosan (g)	35077.35	41947.83	33566.8	33231.17	35431.66
Enzyme (l)	17740.27	15029.23	16976.33	16806.57	17919.46
Stainless Steel 316 L (kg)	113.60	14916.90	117.65	110.73	113.60
PES (kg)	21	52.5	21	21	21
Ethanol (l)	0	197781.98	0	0	0
$Mg(OH)_2$ (m^3)	0	2.43	0	0	0
NaOH (m^3)	0	0.35	0	0	0
Heptane (m^3)	0	26.20	0	0	0
Water Purification l	0	13032.34	0	0	0
Cleaning agent (m^3)	48.87	51.68	51.14	44.75	48.87
Cleaning water (m^3)	1172.90	1240.3	1074.11	1146.69	1172.90
Energy (kWh)	822865	980899	952995	757723	822865
Packages 25 kg polypropylene (kg)	59.54	69.53	56.98	56.41	60.15
Packages 200 l polyethylene (kg)	5447.49	4615.01	5212.91	5160.78	5502.51
Transport equipment (kg)	249.93	32817.19	243.61	258.84	249.93
Transport chemicals (kg)	4924.93	29247.91	5148.34	10354.11	11154.41

Table 171 -Total impacts for Synechocystis based biorefinery scenarios normalized for all scenarios and per stage.

		GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
SC1	Production	0.21	0.87	0.64	0.36	0.45	0.35	-0.04	0.34	0.07	0.80	0.00	0.72	0.71	0.72	0.00	0.79	0.65	0.54
	Harvesting and Rupture	0.11	0.03	0.19	0.10	0.13	0.10	-0.01	0.09	0.00	0.09	0.00	0.14	0.15	0.14	0.00	0.11	0.16	-0.02
	Extraction	0.69	0.10	0.17	0.54	0.41	0.54	1.05	0.56	0.93	0.11	1.00	0.14	0.14	0.14	1.00	0.10	0.19	0.48
	Total	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
SC2	Production	0.20	0.86	0.63	0.35	0.45	0.35	0.04	0.34	0.07	0.80	0.00	0.72	0.70	0.72	0.00	0.78	0.64	0.54
	Harvesting and Rupture	0.12	0.03	0.20	0.11	0.15	0.11	0.01	0.10	0.00	0.09	0.00	0.15	0.17	0.15	0.00	0.11	0.17	-0.02
	Extraction	0.86	0.49	0.29	0.72	0.57	0.72	-1.23	0.70	1.23	0.35	1.18	0.25	0.23	0.27	1.19	0.29	0.32	0.61
	Total	1.18	1.38	1.13	1.18	1.17	1.19	-1.18	1.14	1.30	1.23	1.18	1.12	1.10	1.13	1.19	1.18	1.13	1.13
SC3	Production	0.21	0.91	0.67	0.37	0.47	0.37	-0.04	0.36	0.07	0.84	0.00	0.76	0.74	0.76	0.00	0.83	0.68	0.57
	Harvesting and Rupture	0.28	0.03	0.06	0.09	0.12	0.09	0.11	0.08	0.10	0.03	0.11	0.05	0.06	0.04	0.11	0.05	0.45	-0.01
	Extraction	0.70	0.10	0.18	0.56	0.43	0.56	1.05	0.57	0.93	0.12	1.00	0.15	0.15	0.15	1.00	0.11	0.21	0.48
	Total	1.20	1.05	0.91	1.02	1.02	1.02	-1.11	1.01	1.10	0.98	1.11	0.95	0.95	0.95	1.11	0.98	1.34	1.04
SC4	Production	0.22	0.92	0.68	0.37	0.48	0.37	0.04	0.36	0.07	0.85	0.00	0.76	0.75	0.76	0.00	0.83	0.69	0.57
	Harvesting and Rupture	0.24	0.04	0.11	0.06	0.11	0.07	0.01	0.05	0.00	0.05	0.00	0.08	0.09	0.08	0.00	0.07	0.48	-0.05
	Extraction	0.69	0.11	0.17	0.55	0.42	0.55	-1.04	0.56	0.93	0.11	0.99	0.14	0.14	0.14	0.99	0.10	0.20	0.49
	Total	1.14	1.05	0.95	0.98	1.00	0.98	0.99	0.97	0.99	1.01	0.99	0.98	0.98	0.98	0.99	1.01	1.36	1.00
SC5	Production	0.20	0.86	0.63	0.35	0.45	0.35	0.04	0.34	0.07	0.80	0.00	0.72	0.70	0.72	0.00	0.78	0.64	0.54
	Harvesting and Rupture	0.24	0.04	0.15	0.07	0.12	0.07	0.01	0.07	0.00	0.07	0.00	0.12	0.12	0.11	0.00	0.10	0.47	-0.03
	Extraction	0.69	0.10	0.17	0.54	0.41	0.54	-1.05	0.56	0.93	0.11	1.00	0.13	0.14	0.13	1.00	0.10	0.19	0.48
	Total	1.13	1.00	0.96	0.96	0.98	0.97	1.00	0.97	1.00	0.98	1.00	0.97	0.96	0.97	1.00	0.98	1.31	0.99

Table 172 - Total impacts for Synechocystis based biorefinery scenarios.

	GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
Sc1	293.7	0.0	39.1	0.8	0.5	0.8	-17.4	0.2	0.5	805.9	2315318.0	58137.4	627.9	47839.5	8782.7	0.6	50.2	29.1
Sc2	347.7	0.0	44.0	1.0	0.6	1.0	-20.5	0.2	0.7	994.0	2741123.7	65138.3	689.9	54095.6	10440.1	0.8	56.9	32.9
Sc3	351.0	0.0	35.6	0.8	0.5	0.8	-19.4	0.2	0.6	793.6	2564321.7	55092.4	595.9	45333.4	9725.3	0.6	67.4	30.3
Sc4	333.6	0.0	37.2	0.8	0.5	0.8	-17.2	0.2	0.5	813.2	2292164.7	56919.6	613.6	46848.6	8694.6	0.6	68.3	29.2
Sc5	331.2	0.0	37.4	0.8	0.5	0.8	-17.4	0.2	0.5	788.8	2315317.8	56191.0	601.8	46258.1	8782.2	0.6	65.8	28.9

In Table 174 to Table 176 are presented the impact values for the different improvement scenarios considered for the *Synechocystis* biorefinery.

Table 173 – Total Impact values for scenario 5 with and without wastewater.

		GW	SOD	OF,H	TA	FE	ME	FET	MET	HCT	MRS	FRS	WC
S5 normal scenario	Production	18.36	0.00	0.09	0.20	0.02	0.01	1.22	12801.62	135.26	0.15	9.93	4.81
	Harvesting & Rupture	21.58	0.00	0.02	0.06	0.00	0.00	0.21	2063.89	23.10	0.02	7.31	-0.25
	Extraction	60.96	0.00	0.13	-5.61	0.03	0.15	711570.46	2134.20	23.50	0.02	2.38	4.22
	Total	100.91	0.00	0.24	-5.35	0.05	0.16	711571.89	16999.71	181.86	0.19	19.62	8.78
S5 with waste water	Production	16.92	0.00	0.08	0.19	0.02	0.01	1.17	12198.57	125.70	0.14	9.56	4.12
	Harvest & Rupture	21.58	0.00	0.02	0.06	0.00	0.00	0.21	2063.89	23.10	0.02	7.31	-0.25
	Extraction	60.96	0.00	0.13	-5.61	0.03	0.15	711570.46	2134.20	23.50	0.02	2.38	4.22
	Total	99.47	0.00	0.24	-5.36	0.05	0.16	711571.84	16396.66	172.30	0.18	19.25	8.09

Table 174 – Normalized Impact values for scenario 5 with and without wastewater.

		GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
S5 normal scenario	Production	0.18	0.86	0.67	0.37	0.46	0.37	-0.04	0.35	0.07	0.82	0.00	0.75	0.74	0.75	0.00	0.81	0.51	0.55
	Harvesting & Rupture	0.21	0.04	0.16	0.07	0.12	0.07	-0.01	0.07	0.00	0.08	0.00	0.12	0.13	0.12	0.00	0.10	0.37	-0.03
	Extraction	0.60	0.10	0.17	0.56	0.42	0.56	1.05	0.58	0.93	0.10	1.00	0.13	0.13	0.13	1.00	0.09	0.12	0.48
S5 with waste water	Production	0.17	0.77	0.64	0.35	0.44	0.35	-0.04	0.33	0.06	0.80	0.00	0.72	0.69	0.72	0.00	0.75	0.49	0.47
	Harvesting & Rupture	0.21	0.04	0.16	0.07	0.12	0.07	-0.01	0.07	0.00	0.08	0.00	0.12	0.13	0.12	0.00	0.10	0.37	-0.03
	Extraction	0.60	0.10	0.17	0.56	0.42	0.56	1.05	0.58	0.93	0.10	1.00	0.13	0.13	0.13	1.00	0.09	0.12	0.48

Table 175 – Total impact values of all Synechocystis scenarios without and with photovoltaic panels.

		GW	SOD	OF,H	TA	FE	ME	FET	MET	HCT	MRS	FRS	WC
No renewable Energy	S1	89.42	11.96	0.25	-5.35	0.05	244.87	711571.96	17622.03	2699.18	0.20	14.85	8.94
	S2	106.81	13.38	0.30	-6.31	0.06	304.39	842435.81	19693.40	3208.29	0.23	17.03	10.09
	S3	107.03	10.88	0.26	-5.96	0.05	241.06	788098.83	16682.07	2988.85	0.19	20.11	9.29
	S4	101.69	11.37	0.25	-5.29	0.05	247.34	704456.21	17248.81	2672.11	0.20	20.39	8.97
	S5	100.91	11.41	0.24	-5.35	0.05	239.67	711571.89	16999.71	2699.02	0.19	19.62	8.78
Renewable Energy	S1	66.73	8.90	0.19	-5.51	0.05	362.42	711572.93	20915.96	2698.09	0.24	9.67	8.72
	S2	82.52	10.11	0.23	-6.48	0.05	430.24	842436.84	23219.98	3207.13	0.28	11.48	9.85
	S3	85.76	8.02	0.20	-6.11	0.05	351.25	788099.74	19769.98	2987.83	0.23	15.25	9.08
	S4	80.58	8.53	0.19	-5.44	0.05	356.70	704457.12	20313.36	2671.10	0.24	15.57	8.76
	S5	80.88	8.71	0.19	-5.50	0.05	343.41	711572.75	19906.82	2698.06	0.23	15.05	8.58

Table 176 – Normalized impacts of all scenarios with and without photovoltaic panels.

		GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
w/ Renewable	S1	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
	S2	1.19	1.38	1.12	1.19	1.17	1.19	1.18	1.14	1.29	1.24	1.18	1.12	1.10	1.13	1.19	1.20	1.15	1.13
	S3	1.20	1.05	0.91	1.02	1.02	1.02	1.11	1.01	1.10	0.98	1.11	0.95	0.95	0.95	1.11	0.98	1.35	1.04
	S4	1.14	1.05	0.95	0.98	1.00	0.98	0.99	0.97	0.99	1.01	0.99	0.98	0.98	0.98	0.99	1.01	1.37	1.00
	S5	1.13	1.00	0.95	0.96	0.98	0.96	1.00	0.97	1.00	0.98	1.00	0.96	0.95	0.97	1.00	0.98	1.32	0.98
Renewable	S1	0.75	0.96	0.74	0.74	0.68	0.75	1.03	0.91	1.00	1.48	1.00	1.19	0.89	1.18	1.00	1.22	0.65	0.97
	S2	0.92	1.34	0.85	0.92	0.83	0.92	1.21	1.04	1.29	1.76	1.18	1.32	0.98	1.32	1.19	1.44	0.77	1.10
	S3	0.96	1.01	0.67	0.78	0.73	0.78	1.14	0.93	1.10	1.43	1.11	1.12	0.85	1.11	1.11	1.19	1.03	1.02
	S4	0.90	1.02	0.71	0.73	0.70	0.74	1.02	0.89	0.99	1.46	0.99	1.15	0.88	1.15	0.99	1.21	1.05	0.98
	S5	0.90	0.97	0.73	0.74	0.70	0.74	1.03	0.89	1.00	1.40	1.00	1.13	0.86	1.12	1.00	1.18	1.01	0.96

7.13.2 *Prorocentrum* based biorefinery

The Inventories used for the LCA calculations of the different *Prorocentrum* based biorefineries are found in Table 177 and Table 178 while the impacts calculated are found in Table 179 and Table 180.

Table 177 - LCI Data for Prorocentrum harvesting and rupture.

	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Biomass produced (kg)	408200.00	408200.00	408200.00	408200.00	408200.00
Ruptured biomass (kg)	345496.57	364690.83	353639.59	345496.57	368374.57
Stainless steel 316 (kg)	53.57	53.57	25.42	42.45	25.42
Cast iron (kg)	239.22	333.22	252.50	333.22	261.40
Titanium alloy (kg)	74.00	0.00	0.00	0.00	0.00
Polyethersulfone (kg)	0.00	63.00	63.00	63.00	0.00
Energy (kWh)	161.00	129.50	114.10	129.50	298.90
Titanium alloy (kg)	0.00	0.00	0.00	0.00	0.22
Wastewater (m^3)	16613.49	16538.66	16538.66	16538.66	16409.41
Cleaning agent (m^3)	140.70	280.70	280.54	280.70	285.60
Water for cleaning (m^3)	1696.80	6736.80	6732.93	6736.80	6854.40
Transport equipment (tkm)	689.08	733.08	555.50	733.08	575.08

Table 178 - LCI Data for the Prorocentrum based biorefinery extraction process.

	Scenario 1	Scenario 2	Scenario 3	Scenario 4	Scenario 5
Ruptured biomass (kg)	345496.57	364690.83	353639.59	345496.57	368374.57
EPA + DHA (kg)	6449.27	6807.56	13909.82	8138.36	14489.40
Amino acid Hydrolysate (m^3)	1881036.91	1985538.96	1145399.34	1295612.16	1193124.32
Carotenoids (kg)	3378.19	3565.87	0.00	0.00	0.00
Feed (kg)	0.00	0.00	176348.28	0.00	183696.12
Protein Concentrate (kg)	0.00	0.00	0.00	55125.90	0.00
Water (l)	1475270.38	1557229.84	589399.32	20653.02	613957.62
Wastewater (l)	17311.98	18273.76	37271.21	36413.00	38824.18
Enzyme (l)	61421.61	64833.93	27488.43	58350.53	28633.78
Stainless steel 316 (kg)	736.02	760.51	1344.74	786.02	1344.74
PES (kg)	0.00	0.00	10.50	0.00	10.50
Titanium (kg)	20.00	20.00	0.00	0.00	0.00
Supercritical CO_2 (kg)	69099.31	72938.17	0.00	0.00	0.00
Ethanol (l)	4378.92	4622.19	578868.72	0.00	602988.25
$Mg(OH)_2$ (kg)	689.58	727.89	1484.60	1450.41	1546.46
NaOH (kg)	943.63	996.05	2031.55	1984.78	2116.20
Heptane (l)	2122.42	2240.34	106909.91	103649.08	111364.49
Water for purification (l)	16332.06	17239.40	35161.52	34351.88	36626.59
Cleaning agent (l)	46106.36	70426.81	75173.74	4622.80	75173.74
Water for cleaning (l)	1106552.58	1690243.40	75173.74	110947.20	75173.74
Energy MJ	8792273.40	11533884.14	14650331.81	3676521.10	14650331.81
Packages 25 kg polypropylene (kg)	11.32	11.95	219.18	9.38	228.31
Packages 200 l polyethylene (kg)	93863.74	99078.39	57155.43	64651.05	59536.90
Transport equipment (tkm)	1619.24	1673.11	2958.43	1729.24	2958.43
Transport chemicals (tkm)	24218.92	27642.40	76108.57	11149.90	78980.63

Table 179 - Total impacts for Prorocentrum based biorefinery scenarios normalized for scenario 1 and per stage.

		GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
SC1	Production	0.44	0.86	0.58	0.57	0.57	0.57	0.55	0.61	0.55	0.73	0.64	0.69	0.66	0.69	0.15	0.77	0.57	0.95
	Harvesting and Rupture	0.06	0.00	0.04	0.04	0.04	0.04	0.04	0.04	0.05	0.03	0.04	0.04	0.05	0.04	0.01	0.03	0.06	-0.03
	Extraction	0.49	0.14	0.38	0.38	0.39	0.38	0.42	0.35	0.40	0.24	0.32	0.27	0.29	0.27	0.84	0.19	0.36	0.09
	Total	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
SC2	Production	0.42	0.81	0.55	0.54	0.54	0.54	0.52	0.57	0.52	0.69	0.61	0.65	0.63	0.65	0.14	0.73	0.54	0.90
	Harvesting and Rupture	0.07	0.01	0.06	0.05	0.05	0.05	0.04	0.06	0.05	0.04	0.05	0.05	0.06	0.05	0.01	0.04	0.06	-0.01
	Extraction	0.57	0.14	0.42	0.44	0.45	0.44	0.48	0.39	0.41	0.25	0.34	0.29	0.33	0.30	0.87	0.21	0.41	0.10
	Total	1.06	0.96	1.02	1.03	1.03	1.03	1.04	1.02	0.98	0.98	1.00	1.00	1.01	1.00	1.02	0.98	1.02	0.99
SC3	Production	0.20	0.40	0.27	0.27	0.26	0.27	0.25	0.28	0.25	0.34	0.30	0.32	0.31	0.32	0.07	0.36	0.27	0.44
	Harvesting and Rupture	0.03	0.00	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.01	0.02	0.03	-0.01
	Extraction	0.39	0.33	0.27	0.36	0.34	0.36	0.43	0.24	0.93	0.28	0.30	0.21	0.21	0.21	1.91	0.22	0.29	0.08
	Total	0.63	0.73	0.56	0.65	0.63	0.65	0.70	0.55	1.21	0.64	0.62	0.55	0.54	0.56	1.98	0.60	0.58	0.52
SC4	Production	0.35	0.68	0.46	0.45	0.45	0.45	0.43	0.48	0.44	0.58	0.51	0.55	0.52	0.55	0.12	0.61	0.46	0.75
	Harvesting and Rupture	0.06	0.01	0.05	0.04	0.04	0.04	0.04	0.05	0.04	0.03	0.04	0.04	0.05	0.04	0.01	0.04	0.05	-0.01
	Extraction	0.24	0.09	0.22	0.19	0.18	0.19	0.20	0.19	0.28	0.14	0.18	0.15	0.15	0.15	0.58	0.12	0.20	0.04
	Total	0.64	0.78	0.73	0.68	0.67	0.68	0.67	0.71	0.76	0.75	0.73	0.74	0.72	0.74	0.71	0.76	0.71	0.78
SC5	Production	0.20	0.38	0.26	0.25	0.25	0.25	0.24	0.27	0.24	0.33	0.29	0.31	0.29	0.31	0.07	0.34	0.26	0.42
	Harvesting and Rupture	0.04	0.00	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.03	0.03	0.03	0.01	0.02	0.04	0.00
	Extraction	0.38	0.33	0.26	0.35	0.33	0.35	0.42	0.23	0.93	0.28	0.30	0.20	0.21	0.21	1.91	0.22	0.28	0.08
	Total	0.62	0.71	0.55	0.64	0.62	0.64	0.69	0.53	1.20	0.63	0.61	0.54	0.53	0.54	1.98	0.58	0.57	0.50

Table 180 - Total impacts for Prorocentrum based biorefinery scenarios.

	GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
Sc1	455.5	0.0	129.9	1.6	1.2	1.6	3.6	0.3	0.3	3025.9	21.2	214385.9	2037.2	177317.6	89.1	2.5	174.2	43.8
Sc2	480.9	0.0	133.1	1.6	1.3	1.6	3.7	0.3	0.3	2972.0	21.3	214142.1	2059.3	177047.5	90.6	2.4	177.7	43.1
Sc3	284.7	0.0	73.1	1.0	0.8	1.0	2.5	0.2	0.3	1937.6	13.3	117970.1	1108.5	98969.8	176.7	1.5	101.8	22.6
Sc4	293.3	0.0	94.4	1.1	0.8	1.1	2.4	0.2	0.2	2266.5	15.5	158735.3	1472.8	131436.4	63.2	1.9	123.7	34.2
Sc5	280.8	0.0	71.7	1.0	0.8	1.0	2.5	0.2	0.3	1895.1	13.0	115105.7	1082.5	96603.0	176.4	1.4	99.9	21.9

In Table 181 to Table 184 are presented the impact values for different improvements considered for the *Prorocentrum* scenario.

Table 181 - Total impacts for Prorocentrum Scenario P5 and P5 using wastewater as nutrient source.

			GW	SOD	OF,H	TA	FE	ME	FET	MET	HCT	MRS	FRS	WC
P5 without using wastewater	Production	89.25	0.00	0.40	0.87	0.08	0.07	6.08	65720.60	599.46	0.85	44.57	18.44
	Harvesting & Rupture	19.18	0.00	0.05	0.10	0.01	0.01	0.58	5855.73	61.76	0.05	6.25	-0.15
	Extraction	169.19	0.00	0.54	1.50	0.07	0.25	6.24	42591.49	412.69	0.53	46.79	3.52
	Total	277.62	0.00	0.99	2.48	0.16	0.32	12.90	114167.82	1073.92	1.43	97.60	21.82
P5 using wastewater	Production	89.00	0.00	0.40	0.87	0.08	0.07	6.07	65601.75	598.83	0.85	44.50	18.44
	Harvesting & Rupture	19.18	0.00	0.05	0.10	0.01	0.01	0.58	5855.73	61.76	0.05	6.25	-0.15
	Extraction	169.19	0.00	0.54	1.50	0.07	0.25	6.24	42591.49	412.69	0.53	46.79	3.52
	Total	277.37	0.00	0.99	2.47	0.16	0.32	12.89	114048.97	1073.29	1.43	97.54	21.81

Table 182 - Total impacts for Prorocentrum based biorefinery scenarios without and wth renewable energy.

		GW	SOD	OF,H	TA	FE	ME	FET	MET	HCT	MRS	FRS	WC
without renewable energy	P1	444.79	0.00	1.54	3.56	0.30	0.27	20.94	211258.53	2008.78	2.45	166.72	43.67
	P2	470.12	0.00	1.59	3.69	0.30	0.26	21.03	211014.69	2030.86	2.40	170.13	43.03
	P3	281.52	0.00	1.00	2.51	0.16	0.32	13.17	117032.15	1099.95	1.47	99.55	22.55
	P4	287.93	0.00	1.05	2.38	0.21	0.20	15.40	157161.31	1458.50	1.87	119.96	34.16
	P5	277.62	0.00	0.99	2.48	0.16	0.32	12.90	114167.82	1073.92	1.43	97.60	21.82
With renewable energy	P1	238.50	0.00	0.95	2.04	0.25	0.27	29.86	241897.87	1827.31	2.86	120.07	40.38
	P2	245.19	0.00	1.03	2.04	0.25	0.26	30.74	244340.29	1834.06	2.84	120.61	39.63
	P3	154.31	0.00	0.64	1.58	0.14	0.32	18.63	135695.86	986.64	1.72	70.58	20.71
	P4	173.59	0.00	0.73	1.54	0.19	0.20	20.36	174274.64	1358.90	2.10	94.18	32.04
	P5	151.99	0.00	0.63	1.56	0.13	0.32	18.30	132596.83	961.97	1.68	68.99	20.02

Table 183 - Impact values for scenario 5 with and without improvements.

		GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
Sc5	Production	0.32	0.54	0.47	0.40	0.41	0.40	0.35	0.51	0.20	0.52	0.47	0.58	0.56	0.57	0.03	0.59	0.46	0.85
	Harvesting & Rupture	0.07	0.07	0.00	0.06	0.05	0.05	0.05	0.04	0.06	0.02	0.03	0.04	0.05	0.06	0.05	0.00	0.04	0.06
	Extraction	0.61	0.46	0.47	0.55	0.54	0.55	0.61	0.43	0.78	0.45	0.48	0.37	0.38	0.38	0.96	0.37	0.48	0.16
Sc5 wastewater	Production	0.32	0.53	0.47	0.40	0.41	0.40	0.35	0.51	0.20	0.52	0.47	0.57	0.56	0.57	0.03	0.59	0.46	0.85
	Harvesting & Rupture	0.07	0.00	0.06	0.05	0.05	0.05	0.04	0.06	0.02	0.03	0.04	0.05	0.06	0.05	0.00	0.04	0.06	-0.01
	Extraction	0.61	0.46	0.47	0.55	0.54	0.55	0.61	0.43	0.78	0.45	0.48	0.37	0.38	0.38	0.96	0.37	0.48	0.16
Sc5 Photovoltaic Panels	Production	0.17	0.57	0.41	0.30	0.31	0.30	0.24	0.48	0.22	0.68	0.65	0.67	0.56	0.66	0.02	0.69	0.38	0.88
	Harvesting & Rupture	0.04	0.00	0.03	0.02	0.02	0.02	0.02	0.03	0.02	0.03	0.04	0.04	0.04	0.04	0.00	0.03	0.04	-0.03
	Extraction	0.32	0.44	0.32	0.32	0.30	0.32	0.37	0.31	0.77	0.61	0.67	0.43	0.30	0.44	0.94	0.44	0.29	0.13

Table 184 - Normalized impact values for all scenarios with and without photovoltaic panels.

		GW	SOD	IR	OF,H	FPM	OF,T	TA	FE	ME	TET	FET	MET	HCT	HNCT	LU	MRS	FRS	WC
without photovoltaic panels	SC1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	SC2	1.80	1.31	1.90	1.67	1.75	1.66	1.55	1.95	0.81	1.53	1.64	1.84	1.92	1.82	0.51	1.64	1.79	1.92
	SC3	1.08	0.99	1.05	1.06	1.06	1.06	1.06	1.05	1.00	1.01	1.02	1.02	1.04	1.02	1.00	1.00	1.05	1.01
	SC4	1.11	1.06	1.35	1.11	1.14	1.11	1.00	1.37	0.62	1.17	1.20	1.37	1.38	1.35	0.36	1.28	1.26	1.53
	SC5	1.07	0.97	1.03	1.04	1.04	1.04	1.04	1.03	0.99	0.98	1.00	1.00	1.01	1.00	1.00	0.98	1.02	0.98
with photovoltaic panels	SC1	0.57	0.98	0.78	0.67	0.66	0.67	0.66	0.84	1.00	1.30	1.37	1.14	0.90	1.13	0.97	1.15	0.73	0.95
	SC2	0.93	1.28	1.46	1.00	1.06	1.00	0.86	1.64	0.80	2.14	2.39	2.13	1.72	2.09	0.45	1.94	1.25	1.82
	SC3	0.59	0.97	0.80	0.68	0.67	0.68	0.66	0.87	0.99	1.35	1.45	1.19	0.93	1.17	0.97	1.17	0.74	0.96
	SC4	0.66	1.04	1.13	0.76	0.79	0.77	0.65	1.21	0.62	1.48	1.58	1.52	1.28	1.49	0.33	1.43	0.98	1.47
	SC5	0.58	0.95	0.78	0.66	0.66	0.67	0.65	0.85	0.98	1.32	1.42	1.16	0.91	1.15	0.97	1.15	0.72	0.92

7.14 APPENDIX 14 – Improved Business case

In this section, it is presented the financial data concerning the improved scenarios for both microalgae based biorefineries.

7.14.1 *Synechocystis* based biorefinery improved scenario economic information

The improved scenario considers the full use of renewable energy and the use of wastewater to supply part of the nutrients and water for microalgae production.

Table 185 – Capex for Synechocystis based biorefinery improved scenario.

	Capital costs	%
Production Equipment and related systems	€ 9,952,190	61%
Harvesting	€ 539,580	3%
Processing	€ 746,351	5%
Other costs (Land purchase, facilities construction and related costs	€ 3,976,750	24%
Photovoltaic Power Plant	€ 1,229,134	7%
Security	€ 1,635,401.00	
Total	€ 18,079,406	

Table 186 – Opex for *Synechocystis* based biorefinery improved scenario.

	Operating Costs	%
Labour	€ 1,294,725	36%
Electricity	€ 0	0%
Potable water	€ 326,767	9%
Enzymes	€ 535,504	15%
Maintenance and improvements	€ 817,433	23%
Other costs	€ 636,513	18%
Security factor	€ 180,547	
Total	€ 3,791,489.00	100%

7.14.2 *Prorocentrum* based biorefinery improved scenario economic information

The improved scenario considers the full use of renewable energy, CO_2 from industrial flue gas, and the use of wastewater to supply part of the nutrients and water for microalgae production.

Table 187 - Capex for Prorocentrum based biorefinery improved scenario.

	Capital costs	%
Production Equipment and related systems	€ 10,629,510	47%
Harvesting	€ 325,000	1%
Processing	€ 6,559,347	29%
Other costs (Land purchase, facilities construction and related costs	€ 3,877,500	17%
Photovoltaic Power Plant	€ 1,384,353	6%
Security	€ 2,277,571.00	
Total	€ 25,053,281	

Table 188 – Opex for Prorocentrum based biorefinery improved scenario.

	Operating Costs	%
Labour	€ 1,437,975.00	34%
Electricity	€ 0.00	0%
Potable water	€ 316,335.00	7%
Enzymes	€ 364,591.00	9%
Maintenance and improvements	€ 1,135,408.00	27%
Other costs	€ 968,655.00	23%
Security factor	€ 211,148.00	
Total	€ 4,434,112.00	