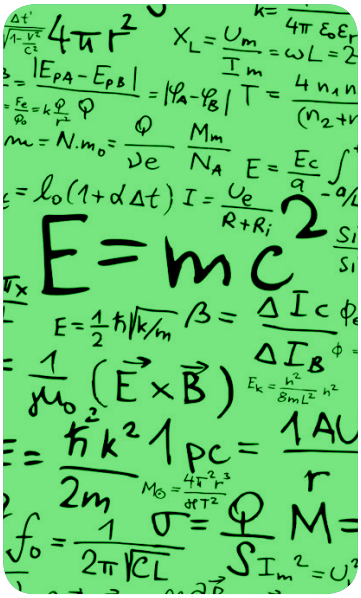

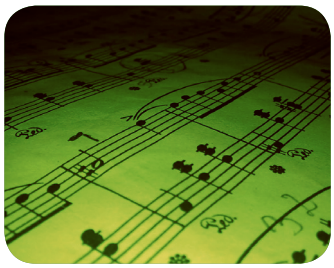

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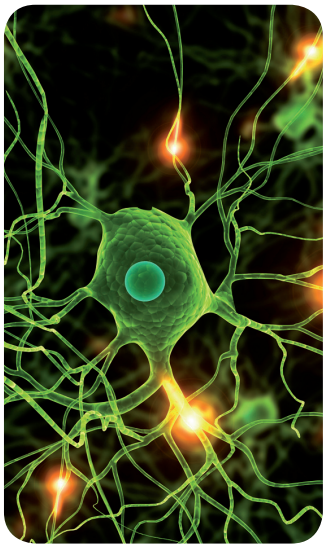

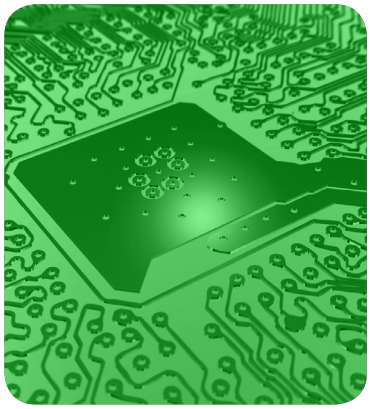

Scale-up and design of an optimised spirulina microalgae process for lipid extraction and biogas production

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UAM EDICIONES

MASTER UNIVERSITARIO EN INGENIERÍA QUÍMICA

Curso académico 2015-16

Trabajo Fin de Master

SCALE-UP AND DESIGN OF AN OPTIMISED SPIRULINA MICROALGAE PROCESS FOR LIPID EXTRACTION AND BIOGAS PRODUCTION

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"Imagine an economy in which today's goods are tomorrow's resources, forming a virtuous cycle that fosters prosperity in a world of finite resources."

Ellen MacArthur Foundation

Acknowledgements

This work has been carried out within the framework of INSPIRA1 project, funded by Community of Madrid (P2013/ABI2783). I would like to thank Dr Luis Fernando Bautista, Dr Victoria Morales and Alejandra Sánchez-Bayo for their support during its development.

Table of Content

Table of Figures

Table of Tables

List of symbols and abbreviation

AACE	American Association of Cost Engineering
C	Concentration; Carbon
CS	Carbon Steel
DFC	Direct Fixed Capital
DNS	3,5-Dinitrosalicylic acid
EDTA	Ethylenediaminetetraacetic acid

I	Intensity
k_1	Growth rate constant,
k_2	Dead rate constant,
k_a	Algal turbidity constant
k_n	Non-algal turbidity constant
k^h	Half-saturation constant
MP	Main Product
MT	Metric Ton
N	Nitrogen
P	Biomass production attenuation coefficient; Phophorous
PMSF	Phenylmethanesulfonyl fluoride
SPD	SuperPro Designer
STD	Standard
t	Time
T	Temperature
TRL	Technological Readiness Level
TS	Total Solids
UDCM	User-Defined Cost Model
VS	Volatile Solids
X	Biomass
α	Total extinction coefficient
λ	pH-effect coefficient

List of subscripts

0	Incident at its maximum
D	Depth of pond
L	Life
r	Temperature in the pond
β	Incident

Abstract

The recognized drawbacks of fossil fuels have led to an intensive search of other types of energies. One of the most promising renewable energies is biofuels obtained from biomass. Biofuels have attracted the attention of the scientific community and three (or even four) generations of them have been studied. The first generation, obtained from crops, is being limited due to its competition with the food and arable land and the second generation, from lignocellulosic biomass, brings negative impacts on the biodiversity and ecosystems and contributes to soil erosion. The third generation, from aquatic autotrophic organism, seems not to have these problems and might even be used as CO_2 and wastewater remediation.

The present work establishes a plausible case for achieving a modelled process of lipid and biogas production, via *Spirulina maxima* algal cultivation, lipid solvent extraction and biogas production from the remaining fraction, mainly formed by carbohydrates and proteins. The pathway is based on own laboratory research data described and presented here from cultivation experiments of the algae under optimised conditions and analysis of lipid, carbohydrate and protein content. Experimental biogas yield has been also used to model the anaerobic digestion of the algae. The cultivation has been then modelled in order to up-scale it to an industrial process where the most relevant parameter used have been: nutrients, light intensity, CO_2 (through pH) and temperature.

The up-scaled process discussed here uses 140 ha of raceway ponds with a surface area of 0.15 ha each to obtain 3.2 MT/h of algae. Using 416 kg/h and 21 kg/h of nitrogen and phosphorus, CO_2 from the flue gas obtained from a cogeneration and the conditions of Almeria (light and temperature), the biomass is able to grow from 0.01 g/L to 0.4 g/L. This algal biomass needs to be dewatered in two steps: a clarifier to 50 g/L and a centrifuge to 150 g/L. Then, the algae is ready to be disrupted in order to obtain the lipids from one side using Soxhlet method and the rest from the other, which is used for biogas production. Lipids would be used in a biodiesel production plant whilst most of the biogas, in the own plant aiming to meet the needs of heat and electricity and the excess would be sold or used for biomethane from biogas upgrading.

Total capital investment of the whole plant is \$216 M. The estimated annual operating cost is \$82.7 M, which results in a unit production cost of \$7.18/kg of algal oil. Using a saving and selling of \$0.15/m^3(STD) of biogas, the selling price has been obtained as \$8.04/kg of algal oil. This price does not seem to be competitive in comparison with other similar techno-economic analysis for a similar technology which showed a price of \$1.8/kg. Therefore, further improvements of the process such as water and nutrient recycling has to be done.

1. Introduction

1.1. Energy from biomass

In the last years, the interest in renewable energy has increased due to the concern about the problems related to the fossil fuel both economic and environmental. As seen so far, the **instability in the oil prices** has led to negative effects for oil-dependent countries such as USA and EU, since it affects to their economies and might derivate in severe financial problems. By contrast, political systems of supplier countries such as Middle East Countries, Venezuela or Russia can be destabilized because of the interests-driven. In regards to the **environmental problems**, it is well-known the impacts derived from an energetic system based on fossil fuels: air, water and soil pollution, health diseases, global warming, etc. And those problems are far from being solved.

Thus, in the recent years there has been a great research about renewable energy sources which could change the global energy system, reducing the pressure on the environment and locating and diversifying energy production so that countries would not be energetically dependents.

One of the most promising sources is the biomass (Figure 1.1), which can be converted into solid, liquid and gaseous **biofuel**, as well as into some chemicals [1] and has gained much interest from business, consumers, society and politicians who have stimulated it with new regulations, restrictions and incentive programs.

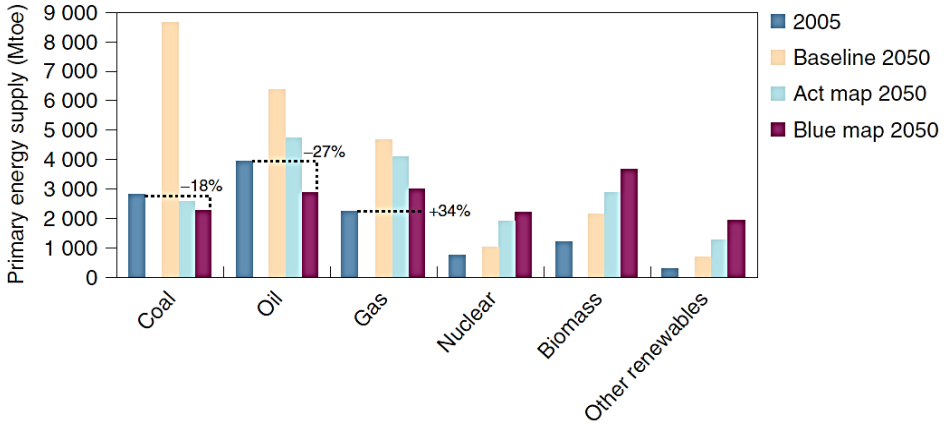

Figure 1.1. World fuel supply for Baseline and Blue Map 2050 [Source: IEA – Energy Technology and Perspective 2008. In support of the G8 Plan of actions. Scenarios and strategies to 2050]

One of the major causes is its null contribution to the greenhouse effect since it may be considered **CO_2 neutral**, i.e. its emissions are used to produce new biomass which is one of the objectives for EU to move towards the so-called **Circular Economy** [2] and a **Bioeconomy** [3] contributing to achieve the 2030 EU target to reduce greenhouse gas emissions by 40% – 62 Mt of CO_{2eq} per year and would be avoided in 2030 [4].

The Renewable Energy Directive (2009/28/EC) (RED, 2009) states that biofuels must achieve at least a **60 % reduction of greenhouse gas emissions** compared to the fossil fuel displaced on a whole life cycle basis by 2018. As of 2015, (European Parliament News, 2013) indirect land use change (ILUC) must be considered in assessing sustainability of the biofuel system. In February 2015, the Environment Committee of the EU Parliament stated that "Advanced biofuels sourced from algae or certain kinds of wastes should account for at least **1.25 % of energy consumption in transport** by 2020" (European Parliament News, 2015).

Besides the biomass and biofuel **advantages** mentioned, there are others such as [5]: great availability and relatively cheap resource; rural revitalization with creation of new jobs; potential use of oceans and low-quality soils and the possibility of restoration of those contaminated; reduction of biomass-containing residues; typically low contents of ash, C, S, N, and trace elements; good reactivity during conversion; mitigation of hazardous emissions (CH_4, CO_2, NO_X, SO_X, trace elements) and wastes separated and the capture of some hazardous components by ash during combustion. But it has **drawbacks** as well, such as [5]: insufficient knowledge and variability of composition, properties and quality; great collection, transportation, storage and pre-treatment costs; low energy density; potential competition with food and feed production of some sources; possible soil damage and loss of biodiversity; possible hazardous emissions during heat treatment; among others.

The lack of knowledge on the **composition, variability** and **properties** of the biomass and how to apply this knowledge for obtaining the **most advanced and sustainable utilisation** of biomass is one of the challenges for the next years.

Several **varieties of biomass** exist that can be used for biofuels and biochemical: woody biomass, herbaceous and agricultural biomass, animal and human biomass wastes, aquatic biomass, semi-biomass (contaminated biomass and industrial biomass wastes such as municipal solid waste, refuse-derived fuel, sewage sludge, demolition wood and other industrial organic wastes) and their mixtures [5]. Biofuels are generally classified into four groups:

- **1st Generation (G1)**: The source of carbon for the biofuel is sugar, lipid or starch directly extracted from a plant. The crop is considered to be in competition with food [6].
- **2nd Generation (G2)**: The biofuel carbon is derived from cellulose, hemicellulose, lignin or pectin. For example, this may include agricultural, forestry wastes or residues, or purpose-grown non-food feedstock (e.g. Short Rotation Coppice, Energy Grasses) [7].
- **3rd Generation (G3)**: The biofuel carbon is derived from aquatic autotrophic organism (e.g. algae). Light, carbon dioxide and nutrients are used to produce the feedstock "extending" the

carbon resource available for biofuel production. This means that a heterotrophic organism (using sugar/cellulose to produce biofuels) would not be considered as G3 [8]. Other researchers propose the G3 as a designed oilier crops which could greatly boost yield [9].

- **4rd Generation (G4):** The technology combines genetically optimized feedstock, which are designed to capture large amounts of carbon, with genomically synthesized microbes which are made to efficiently make fuels. Capture and sequestration of CO_2 are the keys to the process, which renders fourth-generation biofuels a carbon negative source of fuel [9].

1.2. Third generation of biofuels

Third generation has some **advantages over the first two generations**: high surface productivity as shown in the comparison with various plant oils in the Table 1.1 [10]; it can be cultivated on non-arable land and therefore, does not compete with food production; being a photosynthetic organism, they fix the CO_2 and provide greenhouse mitigation. Some species are able to be cultivated on fresh, brackish, sea or even wastewater and can amass up to 60 % oil per dry weight under stressful conditions. Moreover, it has been further technically demonstrated than the fourth generation does not have a TRL higher than 3.

Table 1.1. Yield of various plant oil

Crop	Oil yield (L/ha)	Land area needed (ha)[1] [11]
Algae (70% wt. oil)	136,900	1
Algae (30% wt. oil)	58,700	2.3
Oil Palm	5,950	23
Coconut	2,689	50
Jatropha	1,892	70
Canola	1190	112
Soybean	446	297
Corn	172	770

The third generation is mainly divided into **microalgae** and **macroalgae** (or seaweed). Compared with macroalgae, microalgae have advantages such as simpler structures, faster growth rate and higher oil content [12]. Therefore, most of the industrial companies prefer to use microalgae as the feedstocks to produce biomass energy.

The three critical aspects of algae biofuel production are [13]: **production, harvesting and processing** (Figure 1.2). By using wastewater, flue gases and waste heat, production cost may be reduced; for recovering, a separation system is needed and processing would aim to produce biofuels and chemicals.

[1] Taking as reference Algae (70% wt. oil) for the same oil production.

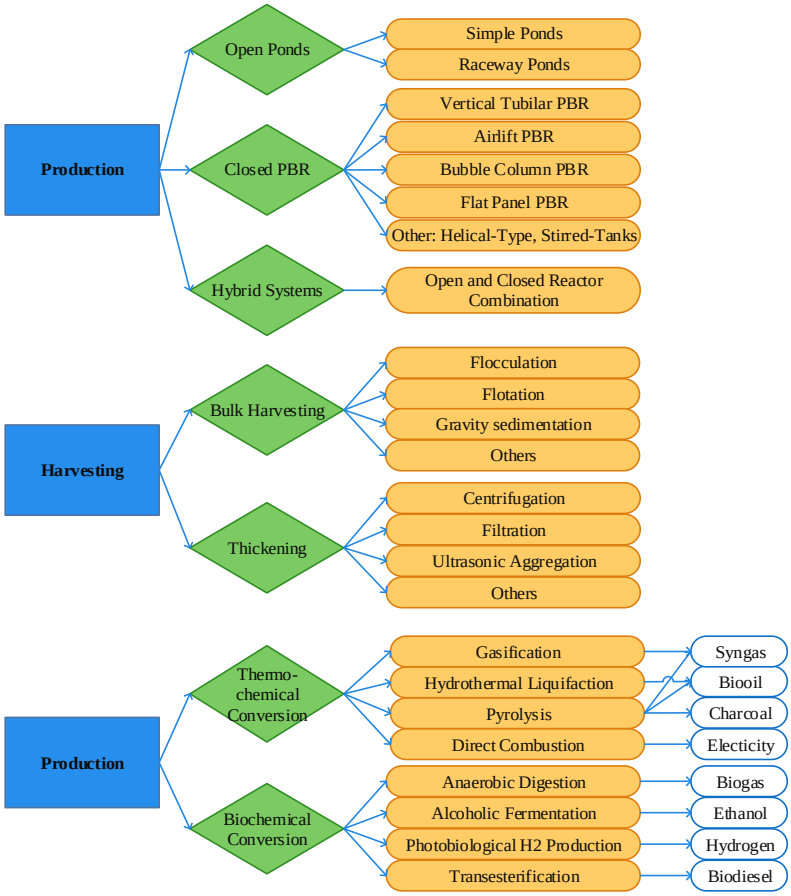

Figure 1.2. Production, Harvesting, Processing technologies for algal biomass and its products

The cultivation of microalgae as part of biotechnology has received researcher attention. Algal autotrophic growth is based on photosynthesic, converting light energy into chemical energy. The two main types of autotrophic cultivation systems are **Open Ponds** and **Closed Photobioreactor (PBR)**. Both types have their advantages and disadvantages. Open Ponds are generally shallow raceways constructed as concrete, clay or plastic-lined ponds and its capital expenses for construction and operational cost are lower than for PBRs and for that reason, they have been more industrially used [14]. By contrast, operation conditions and contamination are easier controlled in PBRs, therefore, biomass productivity is higher but its scalability has not been carried out nowadays and is one of the challenges to be addressed [15]. A combination of these two production system has been proposed: firstly closed PBRs would be used to culture the initial inoculum with robust growth characteristics and minimum contamination and a second stage of an Open Pond for maximizing the biomass growth and lipid accumulation [16]. Cultivation of rapidly grown microalgae may require only 1 % of land area needed for conventional crop-based farmlands. A microalgae production scenario estimated the use of only 121,000 ha of open pond or 58,000 ha of photobioreactor footprint to meet global annual gasoline requirements [11].

Table 1.2. Comparison between the Production of Microalgae in Open and Closed Bioreactors

Characteristic	Open System	Closed system
Required space	High	For PBR itself low
Evaporation	Very high, may also cause salt precipitation	No evaporation
CO_2 loss	High, depending on pond depth	Low
Weather dependence	High (light intensity, temperature, rainfall)	Low (light intensity, cooling required)
Contamination risk	High (limiting the number of species that can be grown)	Low (Medium to Low)
Cleaning	No issue	Required (wall-growth and dirt reduce light intensity), but causes abrasion, limiting PBR lifetime
Max. daily growth	22.7 g/sq m-day	45.9 g/sq m-day
Operating costs	Low (paddle wheel, CO_2 addition)	High (CO_2 addition, oxygen removal, cooling, cleaning, maintenance)
Capital investments	High (~€0.1 M per hectare)	Very high (~€0.3-1 M per hectare)
Harvesting cost	High, species dependent	Lower due to high biomass concentration and better control over species and conditions
Current commercial application	5,000 (8-10,000) t of algal biomass per year	Limited to processes for high added value compounds or algae used in food and cosmetics

The type and design of reactors for large-scale cultivation represent a compromise between the cost of investment and establishment of optimal conditions for maximum productivity [13]. The most commonly cultivated microalgae in open ponds are ***Spirulina*** and *Chlorella* (Table 1.3) [17], [18].

Table 1.3. Estimation of the worldwide microalgal biomass production

Algae	Production, t dry/year	Strain
Spirulina	10,000	Platensis, Maxima
Chlorella	4,000	Vulgaris, Pyrenoidosa
Dunaliella	1,000	Bioculata, Salina

From cultivation reactors, a dilute solution is obtained with an algae content of 0.1-10 g/L (0.1-0.5 g/L for Open Ponds and 0.5-8 g/L for Closed PBR) and its efficient **harvesting** and dewatering technology is critical for the economic viability of algae biofuels. Aside conventional technologies (sedimentation, flocculation, dissolved air flotation, filtration, centrifugation, hydrocyclones), others have been recently proposed such as electroflocculation and ultrasonic-assisted algae concentration techniques [19]. Most of them are expensive or unreliable in a continuous and large-scale operation for biofuel production and the suitable technology is highly dependent on the algae strain. Hence, pilot scale tests are necessary before selecting the optimum harvesting technology [13].

Regarding **processing** technology, many have been investigated to obtain liquid, gas or solid biofuel from algae as seen in Figure 1.2, and some of them are compared in Table 1.4.

Table 1.4. Products from Different Processing Technologies

Processing	Fuel	Max yield, kg or L/kg	Efficiency	HHV, MJ/L or MJ/kg
Direct combustion	Biomass	1.0 of biomass	80	18.15
Solvent extraction	Biodiesel	1.0 of lipid content	80	35.7
Anaerobic digestion	Biogas (62% CH_4)	475.8 L/kg of biomass	95	2.375×10^{-2}
Fermentation	Ethanol	0.51 of carbohydrate	85	23.4
Thermochemical conversion (fast pyrolysis)	Bio-oil	0.553 of biomass	90	33.64

Fast pyrolysis and gasification have been suggested as thermochemical processes utilising microalgae which have the advantage of varying composition can be used. However, these processes produce not only usable biofuels but they also result in a wide range of products and require additional processing. Ethanol is obtained through fermentation of carbohydrate (which is a significant fraction of algal biomass) using yeast. **Solvent extraction** is the most common technology for lipid recovery which is used for biodiesel production [13].

One of the most direct approaches for the utilization of algae biomass is **methane production in anaerobic digester**. This approach has the advantage of utilizing wet algae without the need for additional drying. Many researchers have tried anaerobic digestion of both micro- and macroalgae biomass since 1950s [20], [21], [22].

1.3. Solvent extraction

For liquid transportation biodiesel production, algae cells must be disrupted and oil present extracted. The most common technology for lipid recovery is solvent extraction using one of the many polar solvents such as hexane, chloroform, petroleum ether, butanol and methanol. The **indirect method** (disruption and lipid recovery) is often used to obtain biodiesel by subsequent transesterification [23] (Figure 1.3). The solvents of choice are usually hexane in the case of **Soxhlet** and Goldfish methods [24]; chloroform/methanol or chloroform/ methanol/ water in the case of the **Folch Method** [25]; or modified **Bligh and Dyer** Procedure [26]. Thermal pre-treatment of the algae cells has been recently demonstrated to enhance solvent recovery [27]. Among the different methods for cell disruption such as autoclaving, bead-beating, sonication and microwave heating prior to solvent extraction using chloroform and methanol. The last two methods were identified as the most effective and simple methods of cell disruption [28]. The direct method uses direct transesterification just after pretreatment.

Lipids are soluble in organic solvents but sparingly soluble or insoluble in water. Solubility of lipids is an important criterion for their extraction and typically depends on the type of lipid present and the proportion of nonpolar lipids (principally triacylglycerols) and polar lipids (mainly phospholipids and glycolipids) in the sample [29].

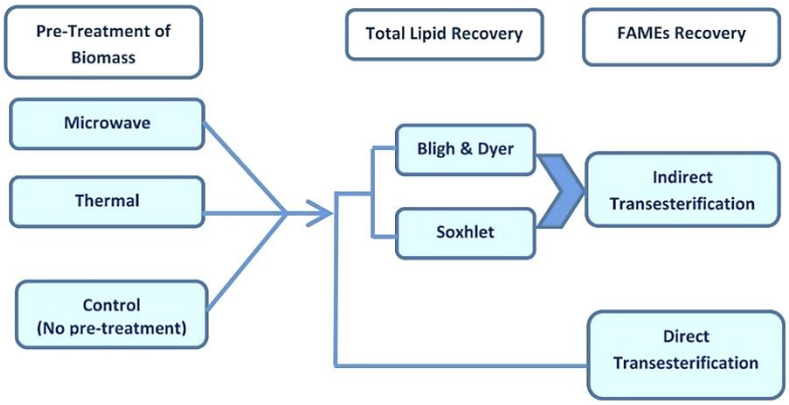

Figure 1.3 Schematic experimental flow of pre-treated or untreated (control) algal biomass for lipid extraction followed by direct or indirect transesterification techniques [Source Ghasemi Naghdi et al. (2014)]

While conversion of oil in algae to biodiesel can be accomplished using standard commercially available technology, **extraction of oil is one of the key challenges**. Remaining rich in protein and carbohydrates solids can be used as **feedstock for biogas** production.

1.4. Anaerobic digestion of microalgae

The use of anaerobic digestion technology gets rid of some of the issues associated with a high cost in algae biofuel: drying, extraction and fuel conversion, therefore, it is considered a cost-effective and a state-of-the-art methodology. This approach has been studied in order to demonstrate its potential. A study of Vergana-Fernández at al. (2008) indicated that biogas production levels of **180.4 m^3/t·day** and up to 500 L/kg VS can be reached from a two-stage anaerobic digestion process using different strains [30], which is a rate production much higher than a wastewater digestion plant and at the level of high-productivity crop sources (Table 1.5).

As mentioned before, this approach could be used in situations such as **integrated wastewater treatment** and **CO_2 fixing**. In addition, sludge from the anaerobic digestion can be used as a nitrogen-rich organic fertilizer [31] or recycled for algae growth. Besides, the combination with lipid extraction seems to be a promising technology.

As drawbacks, mainly two can be mentioned: the process cannot produce liquid transportation fuels and takes longer time for algae processing in comparison with other technologies. Moreover, such an industrial-scale anaerobic facilities that can handle millions of tons of biomass annually can pose significant challenges.

Generally, **the theoretical biogas yield is higher from lipids** (1,390-1,014 L/kg VS) than proteins (851-446 L/kg VS) or carbohydrates (746-415 L/kg VS) [32], [33]. However, accumulation of ammonia and long chain fatty acid, which are significant inhibitors of anaerobic microorganism, are produced by an excess lipid and/or protein content [34]. Thus, **it**

Table 1.5. Methane and biogas production from different microalgae species measured by BMP tests

Species	T, °C	Biogas prod., L/kg VS	CH_4 prod., L/kg VS	CH_4 content, %	Literature
Arthrospira platensis		481 ± 14	293	61	[35]
Chlamydomonas reinhardtii		587 ± 9	387	66	[35]
Chlorella kessleri		335 ± 8	218	65	[35]
Chlorella vulgaris	28 – 31		310 – 350	68 – 75	[36]
Dunaliella salina		505 ± 25	323	64	[35]
Dunaliela	35		420		[37]
Euglena gracilis		485 ± 3	325	67	[35]
Nanochloropsis spp.	38	388	312	80.5	[38]
Scenedesmus obliquuus		287 ± 10	178	62	[35]
Spirulina	35		320 – 310		[37]
	38	556	424	76.3	[38]
Spirulina maxima	35		190 – 340		[39]
Mixed algae sludge *(Clorella-Scenedesmus)*	35 – 50		170 – 320	62 – 64	[20]
	50	500	not specified		[20]
	35	405	not specified		[22]
	45	611	not specified		[20]
	35		100 – 140		[40]
Green algae	38	420	310	73.9	[38]
Other sources					
Sunflower			300		[41]
Corn			360		[41]
Oilseed			420		[42]
Municipal Solid Waste			30		[43]

is important to know the composition of the algae and to optimize it in order to obtain a high biogas yield.

On the other hand, pre-treatment steps may also allow higher biogas production rates and yields. Some of these methods are: Thermal hydrolysis, mechanical treatment (e.g. ultrasound, lysis-centrifuge), chemical (e.g. oxidation, alkali treatment) or biological (e.g. enzymes) [44]. The pre-treatment steps have been studied in microalgae and their highest increase was achieved by thermal hydrolysis and the temperature depended on the microalgae strain [45]. For digestion with 2-12 % TS, continuously stirred tank reactors (CSTRs) are commonly used. Filtration and centrifugation are suitable harvesting methods since they are in the range 0.5-27 % [46].

It could be said that the **TRL for microalgal biogas is below 4** [47]. Energy and carbon balances and cost of produced biogas are unknown. It may well be that biorefineries are required to allow financially sustainable algal biofuel systems. The undoubted benefit of algal biofuels is the high energy yields per unit of area (not land), the separation of bioenergy from agricultural land and the lack of indirect land use change effects.

2. Specific objectives

The present work focuses on two main objectives, the first is obtaining experimental data from *Spirulina* microalgae in order to **characterize and model the feedstock** for lipid extraction and biogas production and the second, **up-scaling, design and simulate a process for lipid and biogas production** through the software tool SuperPro Designer v9 which also provides an estimation of the capital and operating costs. The aims are represented in the Figure 2.1

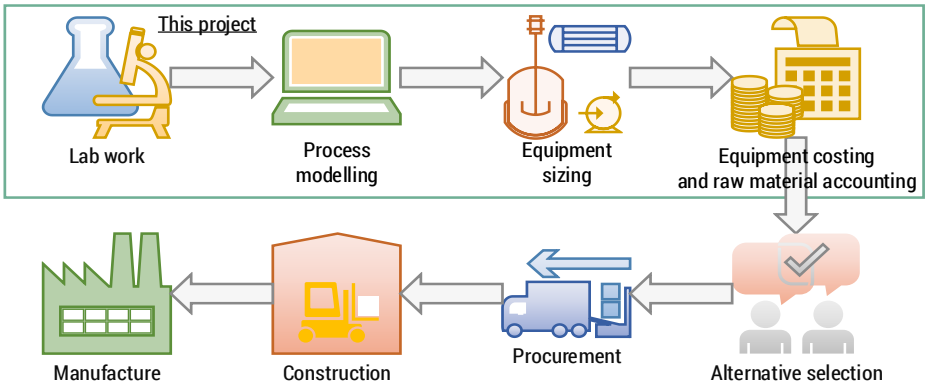

Figure 2.1. Industrial project developing and aims of this study (above)

This research has been carried out within the framework of INSPIRA1 project (http://inspira-cm.org/), funded by Community of Madrid (P2013/ABI2783). Scientific and technical objectives related to this project are: (1) optimizing spirulina cultivation in photobioreactors; (2) evaluation of the residue after extraction of value-added products: biogas production; (3) rescaling the process and study of technical, economic and environmental viability.

The specific objectives of this work are as follows:

- To obtain the growth rate of the algae. Its concentration has been measured for two weeks.
- To obtain the composition of the algae along the growth. Proteins, lipids, total carbohydrates and soluble carbohydrates have been evaluated the days which growth was exponential.
- To scale-up, design and simulate a process for the production optimal biogas through:
  - An optimized open pond system to obtain a rich-lipid *Spirulina* microalga.
  - An evaluated method of algae harvesting.
  - An evaluated lipid extraction process by Soxhlet method.
  - An optimized digester using mainly protein and carbohydrate yields: system configuration and composition of produced biogas (methane and CO_2) will be studied.
- To acquire the material and heat balances from the simulation models.
- To estimate the capital and operating costs through the balances and design of the equipment.
- To obtain a minimum lipid selling price for a complete economic evaluation.

3. Methodology and Calculation Tools

3.1. Microalgae culture

Spirulina maxima (*Arthrospira maxima*) was obtained inoculated from University Göttingen (Germany). It was made to grow in an air bubbled vessel of 1 L in a culture medium solution of 33.3 g salt/L and 1 mL of f medium (f/2) Guillard from AlgaEnergy S. A. (Spain) and was maintained at its maximum concentration.

Twenty-four Falcon tubes were filled by 5 mL of the algae broth and 45 mL of the culture medium. They were bubbled using M-104 IPX4 pumps with a 3.5 L/min flow of air which was passed through filters Agilent Nylon 0.45 um and needles until the bottom of the vessels. The illumination by two files of LEDs were installed in the walls with a schedule of 12 h:12 h. The final configuration is shown in Figure 3.1.

Figure 3.1. Configuration of the algae culture vessel

The microalgae concentration was measured on the days 3, 5 and 7. For that, the optical density of the culture was evaluated those days by a Jasco V-730 Spectrophotometer at a fixed wavelength of 540 nm. Besides, eight of the tubes were filtered under vacuum each day, removing the medium and obtaining the microalgae mass. The mediums were taken to a Varian 720-ES ICP with the propose of obtaining the concentration of a large quantity of elements.

3.2. Microalgae growth modelling

A robust kinetic model has been developed that takes the stages of algae growth along with nutrient consumption. The most influencing factors of algae growth are light intensity, photosynthetic rate, temperature, nutrient availability, and pH [48]. The model has been adapted from the one used by Jayaraman and Rhinehart (2015) [49]. Volume was supposed as completely mixed batch (of uniform concentration) but with spatially dependent light intensity.

The algal dead concentration and rate were not considered (k_2X_L), therefore, only exponential algal growth is modelled as was also done by Jayaraman and Rhinehart (2015). The time development of live algae biomass is represented by conventional kinetic models in eq 1, where the parameter P (eq 2) is formed by the product of functions for light intensity, system temperature and the availability of nutrients like phosphate, nitrate and CO_2.

$$\frac{dX_L}{dt} = k_1 P X_L - \cancel{k_2 X_L} \qquad \textit{eq 1}$$

$$P = f(I)f(T)f(P)f(N)f(C) \qquad \textit{eq 2}$$

3.2.1. *Light Dependence Modelling*

The variation in light intensity depends on depth of water, suspended particles and biomass, and between day and night [50]. The day and night cycle was modelled by the eq 3.

$$I_\beta = max\left[0, sin\left(\frac{(t-7)2\pi}{24}\right)\right] \qquad \textit{eq 3}$$

The total light extinction in a pond system is calculated as a linear function of non-algal turbidity and algal turbidity as shown in eq 4.

$$\alpha = k_n + k_a X \qquad \textit{eq 4}$$

The parameter k_n and the constant k_a have been reported as $2.22 - 7.13$ and 0.014 ± 0.003, respectively [51]. Light intensity can be calculated using the Beer-Lambert model in eq 5.

$$I = I_\beta e^{-\alpha D} \qquad \textit{eq 5}$$

Being a perfect mixed reactor, the model uses an average light intensity to obtain the effect of light source on growth of algae as shown in eq 6.

$$I_{avg} = \left(\frac{I_\beta}{\alpha D}\right)(1 - e^{-\alpha D}) \qquad \textit{eq 6}$$

The average intensity is used in the photosynthetic rate modelling [52] to calculate the photosynthetic rate *f(I)* as shown in eq 7.

$$f(I) = 9.34\left(1 - e^{-0.0044 I_{avg}}\right) - 1.60 \qquad \textit{eq 7}$$

3.2.2. *Temperature Dependence Modelling*

In regards to the effect of temperature on the growth, the function is centred on the optimal temperature for the specie as shown in eq 8.

$$f(T) = e^{\left(-k_t (T_r - T_{opt})^2\right)} \qquad \textit{eq 8}$$

Most strains used for biofuel have an optimal temperature between 20 and 30 ºC. As an approximation, a temperature-effect coefficient of 0.0001 has been chosen corresponding to data from *Galdiera sulphuaria* algae [53].

3.2.3. *Nutrient Dependence Modelling*

Nutrient uptake has been modelled in several forms using Michaelis-Menten model [54], Droop model [55], Monod model [49]. The latter is to be used in this study assuming that algal growth is limited by nitrogen and phosphate availability as shown in eq 9 and eq 10.

$$\frac{dC_A}{dt} = -k_A r_A P X_L \qquad \textit{eq 9}$$

$$f(C_A) = \frac{C_A}{k_A^h + C_A} \qquad \textit{eq 10}$$

Finally, the CO_2 was modelled following the literature [56], through medium pH and optimum pH using the probit function, which monotonically decreases with pH as CO_2 concentration increases, eq 11.

$$f(CO_2) = \frac{1}{(1 + e^{\lambda(pH - pH_{opt})})} \qquad \textit{eq 11}$$

3.3. Protein analysis

Firstly, proteins must be extracted from biomass. With this aim, a lysisbuffer solution was prepared using 0.5 ml tryton-x, 0.03722 g Na-EDTA and 0.0035 g PMSF and 100 mL of distilled water. Then, 0.05 g of each biomass (3, 5 and 7 days) was dissolved in 3 mL of the lysisbuffer prepared and assisted with a vortex 4-5 min each. As a result, microalgae cell wall was broken. For quantification of proteins, Bradford assay was used [57].

3.4. Carbohydrate analysis

3.4.1. *Total carbohydrate*

In order to measure the total carbohydrate contained in algae, Phenol–sulfuric acid method was used [58] from the ruptured cell algae solution utilised in protein analysis.

3.4.2. *Soluble carbohydrate*

The carbohydrate soluble fraction was measured from the ruptured cell algae solution using a DNS solution made by: 1 g of 3,5-dinitrosalicylic acid, 30 g of sodium potassium tartrate tetrahydrate, 50 ml of water and 20 ml of 2 N NaOH. The procedure was: 1 ml of each day sample and each calibration solution was taken into a test tube, 1 ml of DNS was added and the resulted solution was vortexed and then heated with a water bath at 100 ºC for 10 min and

cooled quickly in ice. Finally, 10 ml of distilled water was added and the solution was measured by Spectrophotometer at 540 nm.

3.5. Lipid and fatty acids analysis and methyl esters preparation

3.5.1. Total lipids

For lipid quantification, 0.5004 g, 0.4628 g and 0.3700 g of dry biomass from days 3, 5 and 7, respectively, were collected. Lipids were extracted from biomass by a ratio methanol: algae of 20 mL/g at 60 °C during 2 hours in a 50 mL stirred round flask at 350 rpm.

The resulting solution was filtered under vacuum and the liquid was taken to a rotary evaporator with the bath at 80 °C. Lipids were separated (stuck to walls) from the methanol and weighted. In order to collect the lipids, a ratio hexane:lipid of 40 mL/g was used.

3.5.2. Fatty acids

Fatty acids content was obtained through Thin-Layer Chromatography (TLC). A small spot of hexane-lipid solution was applied to the plate, about 1 centimetre from the bottom edge. Each sample is punctured 3 times. A solution of 80 % hexane, 20 % diethyl ether was used as eluent and iodine vapours as colour reagent. The plate was scanned and through Un Scan It gel 6.1 software, types of lipids and their percentages were calculated.

3.5.3. Methyl esters preparation

Aiming to prepare methyl esters from the lipid fraction obtained in previous stages, the boron trifluoride method and iso-octane solution from ISO 5509:2000 standard (Preparation of methyl esters of fatty acids) was used.

3.6. Anaerobic digestion of algae

Results obtained by Department of Chemical and Energy Technology of Universidad Rey Juan Carlos, were used in this work wherein biodiesel and biogas production from *Spirulina* microalgae were explored. Two biodiesel production pathways were evaluated: an indirect method with a previous oily fraction extraction using methanol as solvent and a direct method using the whole dried biomass. Then, biogas from the remaining fraction was assessed and compared with the yield of the biogas from raw algal biomass. This method was described in the paper from Mendoza *et al.* (2015) where *Nannochloropsis gaditana* was used as feedstock for biodiesel and biogas production using these two approaches [59].

3.7. Software modelling

This project has been modelled by SuperPro Designer (SPD) version 9 Build 2 which is a very reliable simulation tool due to its large and strong database of specific chemical compounds and unit operations. This database is helpful to facilitate the calculation of physical, chemical

and biological processes [60]. SPD has been already used as a modelling tool for different integrated processes, evaluation and optimization in a wide range of industries (Pharmaceutical, Biotechnology, Specialty Chemical, Biofuels, Food, Consumer Goods, etc.) [60], [61].

Specifically, microalgae processes have been modelled using SPD for a process for capture of CO_2 from power plant flue gas in alkaline solutions to produce biodiesel from algal oil [62]; for a method of microbial lipid production and to evaluate the manufacturing costs of microbial lipids and biodiesel produced at a commercial scale [63]; for a simulation and energy integration for a biorefinery of valuable substances and biofuel from microalgae [64]; for comparing two renewable approaches for isoprene production: by photosynthetic organisms (autotrophic microalgae / cyanobacteria) and by heterotrophic organisms (bacteria) [65]; among other works.

The proposed process is divided into four sections: Algae Ponds, Algae Harvesting, Lipid Extraction and Anaerobic Digestion. Flowsheet sections in SuperPro are simply sets of related unit procedures (i.e., processing steps). The purpose and basic steps associated with these sections are described in Technical Solution. The flowsheet is entirely in continuous mode of operation. Therefore, scheduling information is not specified and all operations are assumed to run at steady state. The heat and material balances from the simulation models are used to estimate the capital and operating costs.

4. Technical Solution and Results

4.1. Algae kinetic and characterization results

4.1.1. Algae growth

The results from the described algae culture condition are shown in the Figure 4.1. The maximum concentration achieved was near 1 g/L (dry weight) from the day 7, when stationary phase is achieved. The initial N:P ratio in the medium was 8. The concentration profile of nitrogen and phosphate content in the supernatant of the days 0, 3, 5 and 7 is displayed in the Figure 4.2 which shows a rapid depletion of the phosphate within the first three days. The phosphorus consumption rate starts in 0.9 mg P/L·day and decreases to 0.025 mg P/L·day after the 3rd day. On the other hand, nitrogen does not seem to be affected in the first three days but from the third day it decreases in a linear way with a rate of 5.5 mg N/L·day until it reaches a low concentration in the culture medium the 7th day. The lack of nitrogen in the broth seems to be the reason for the stationary phase apparition. This was also noticed by Mohite and Wakte (2011) where *Spirulina* cultures were tested under both N sufficiency and N limitation [66].

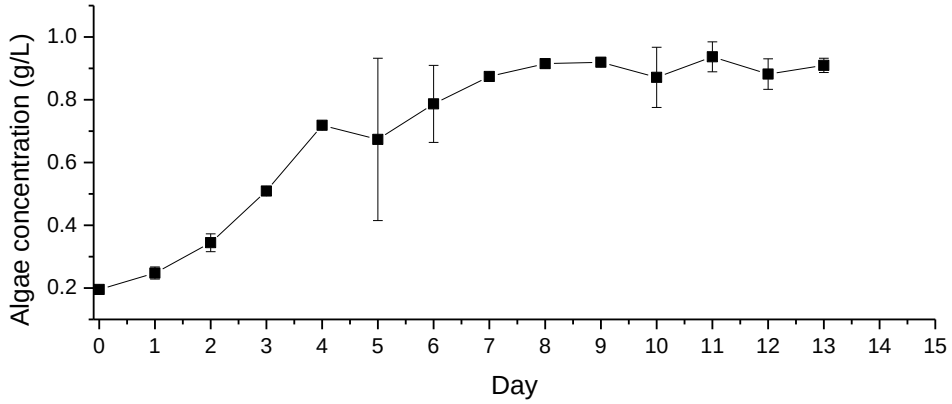

Figure 4.1. Algae culture result

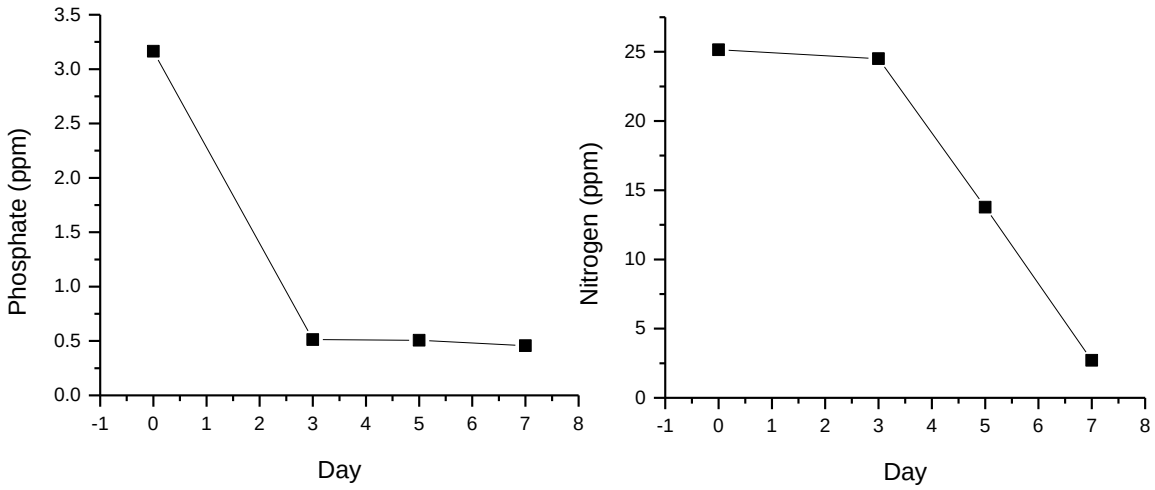

Figure 4.2. Concentration of phosphate and nitrogen in the broth

4.1.2. Model regression results

The results from the parameter modelling for the calculation of *P* and its value are shown in the Figure 4.3, CO_2 and T parameters have been considered as 1 due to its constant pH near its optimum (9.3) [67] and room temperature at laboratory conditions. The decrease of *P* along the days can be noticed, mainly due to the decrease of N and P nutrient.

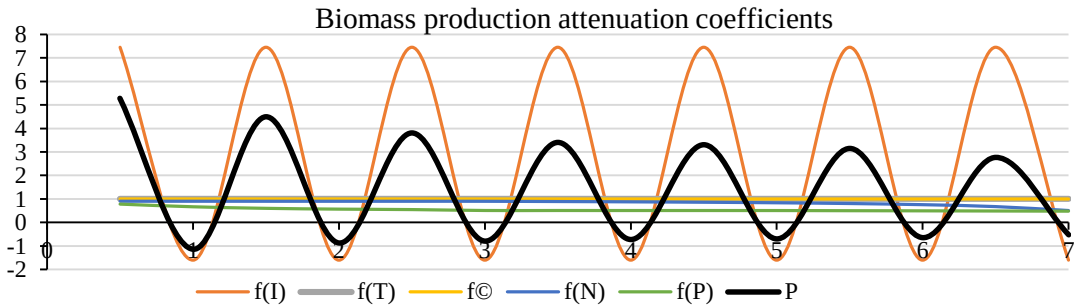

Figure 4.3. Biomass product attenuation coefficients

The model regression has been carried out by the simple finite element method using eq 12.

$$X_{i+1} = X_i + k_1 P_i X_i \Delta t \qquad \textit{eq 12}$$

Due to the difficulty of using mathematic software for parameter calculation (k_1, k_N^h, k_P^h), literature has been used. According to Levert and Xia (2001) who modelled a batch culture of *Spirulina maxima* under limitations of light and nutrients nitrogen and sulphur, nitrogen half-saturation constant was found as 0.0204 mmol/L (0.29 ppm) [68]. Baldia *et al.* (1994) results showed a phosphorus half-saturation constant of 0.02-0.07 ppm for *Spirulina* [69]. The paper from Bamba *et al.* (2014) collected a comparison of *Spirulina* biomass cultivation in various production systems and operating conditions. Specific growth rate (day^{-1}) is usually between 0.03-0.49 [70]. Figure 4.4 shows the comparison of measured and simulated biomass concentration of algae and in Table 4.1 the parameter results are found.

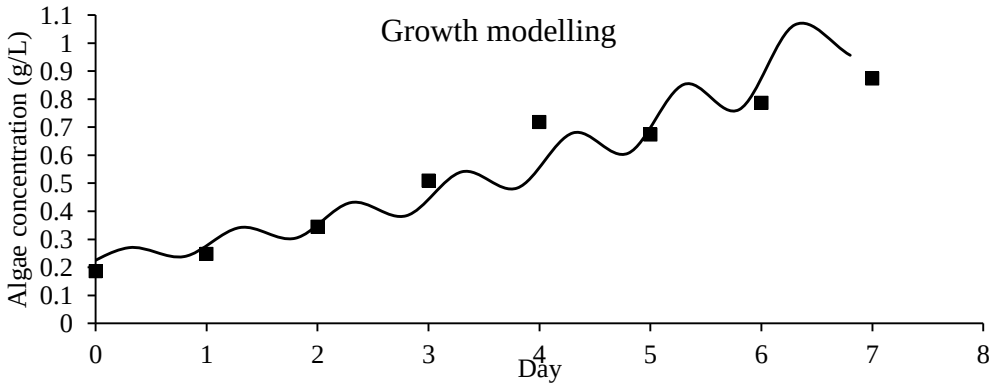

Figure 4.4. Algal growth modelling

Table 4.1. Parameter result of algal modelling

Parameter	Value in this work	Value in literature	Reference
k_1 (day^{-1})	0.312	0.03-0.49	[68]
k_N^h (mg/L)	0.29	0.29	[69]
k_P^h (mg/L)	0.07	0.02-0.07	[70]
Biomass concentration (d.w.g/L)	1	0.4-2.6	[70]

Specific growth rate was obtained through a simple minimization of residual sum of squares (RSS), which measures the discrepancy between the real algal concentration data and the estimation from the model using Excel solver tool. RSS resulted in 0.0849.

4.1.3. Biomass characterization

The results from algal component quantification (proteins, carbohydrates: total and soluble, lipids) are displayed in the Figure 4.5. Proteins, 20.1-20.9 %, total carbohydrates, 22.4-24.7 %, and soluble carbohydrates, 14.8-16 %, seems not to be dependent on day. However, lipids content at day 3 is 33.5 % and it increases up to 42 % at day 7.

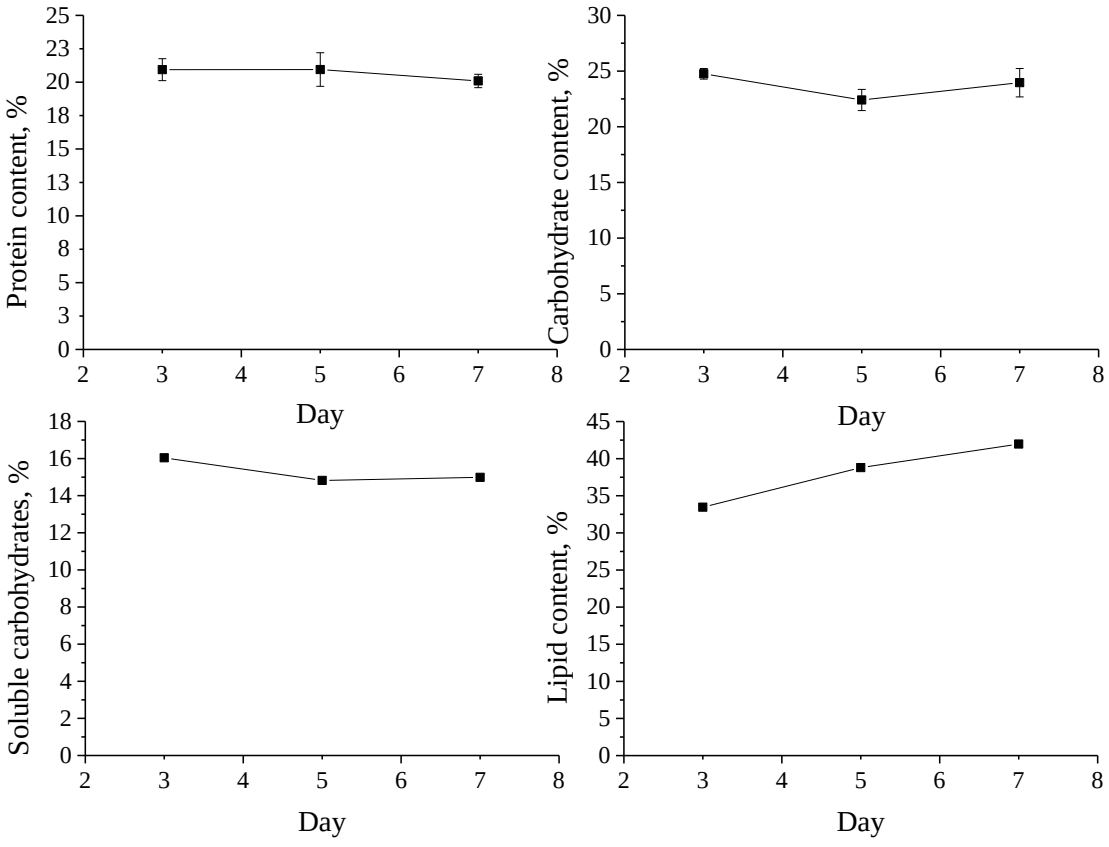

Figure 4.5. Biomass characterization results

The low protein content, high lipid content and the latter variation may be explained by the phosphate nutrient starvation from day 3 and high decreasing nitrogen level. As mentioned by Bhakar *et al.* (2014), the conditions of culture and nutrition greatly influence the composition

and physiological state of these organisms, and nutrient starvation in particular is the main strategy for enhancing lipid accumulation [71]. They found that lipid content in *Spirulina* was four folds under nutrient lack. Moreover, the results are consistent with other reported oil content values for microalgae (12-53 % wt.) [72]. Concretely, Goldberg and Cohen (2006) found that under phosphorus limitation, the total cellular lipid content of starved cells increased, mainly due to the dramatic increase in triglyceride levels from 6 % to 39 % of total lipids [73]. Nitrogen limitation likely caused photo-assimilated C to be redirected towards the synthesis of carbohydrates instead of proteins and chlorophyll. This response has been widely observed in many algal species and justifies the abnormal low protein content in *Spirulina* algae [74].

Results from the Un Scan It Gel are displayed in the Figure 4.6, and the final composition of the lipid is shown in the Table 4.2.

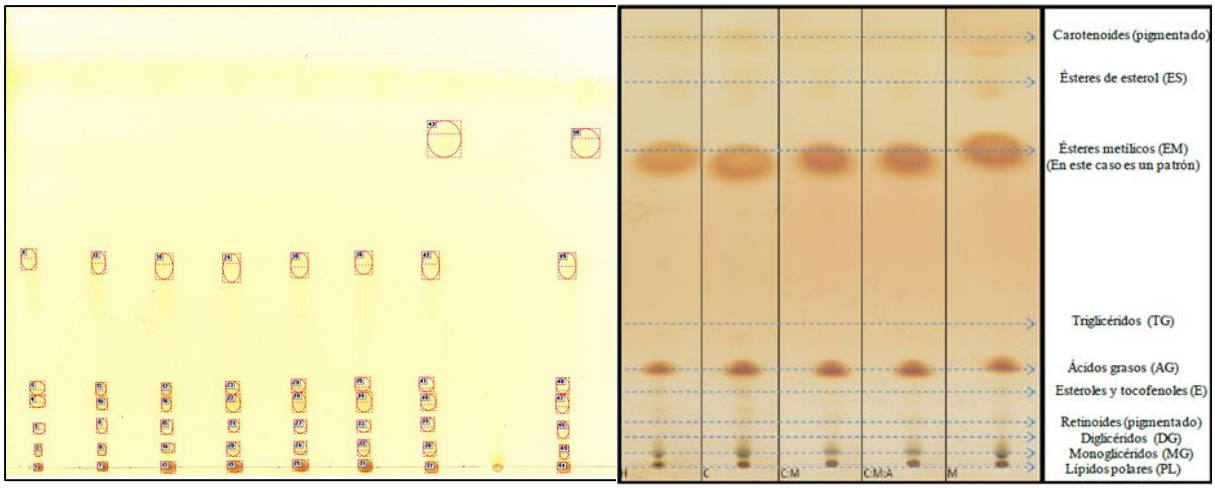

Figure 4.6. Results from the lipid chromatography (left) and the pattern (right)

Table 4.2. Lipid analysis result

Lipid type	Day 3	Day 5	Day 7
Polar Lipids	28.5	32.1	35.2
Mono-/Di-glycerides	15.6	11.2	14.4
Sterols and tocopherols	5.0	3.8	3.3
Tri-glycerides	21.0	35.4	36.9
Methyl Esters	17.1	9.8	2.3
Sterolesters	12.9	7.7	7.0

Tryglycerids increased as expected from the results of Goldberg and Cohen (2006). Polar lipids also increased, likely formed by glycolipids as phospholipids are not expected to be produced from day 3 due to the lack of phosphorus nutrient.

No results from the methyl esters preparation were successfully achieved likely due to the small amount of biomass used for this task.

4.1.4. *Results of algae digestion*

For **not-treated** *Spirulina* microalgae biomass, methane yield reached 400 mL CH_4/g VS on the 36th day. With regard to lipids-extracted biomasses using methanol as solvent, the biomass resulting from the **indirect process** achieved similar methane yield, 395 mL CH_4/g VS on the 36th day, while the **direct process** obtained lower yields, 310 mL CH_4/g VS. In the three cases, the slope changes and it becomes flatter from day 17. *Spirulina* composition may vary from what is obtained in this work due to the different cultivation conditions. Nevertheless, these results will be used for the SPD modelling and validation as an approximation.

4.2. Process description

4.2.1. *Overall process*

The process that will be proposed and designed will produce **biogas from spent microalgae fraction after lipid extraction** for biodiesel production which obtained a higher energy potential (26.5 MJ/kg) by comparison with a whole algal (after cell disruption) digestion (25.9 MJ/kg), represented in Figure 4.7[2], based on Torres *et al*. (2013) estimations [75].

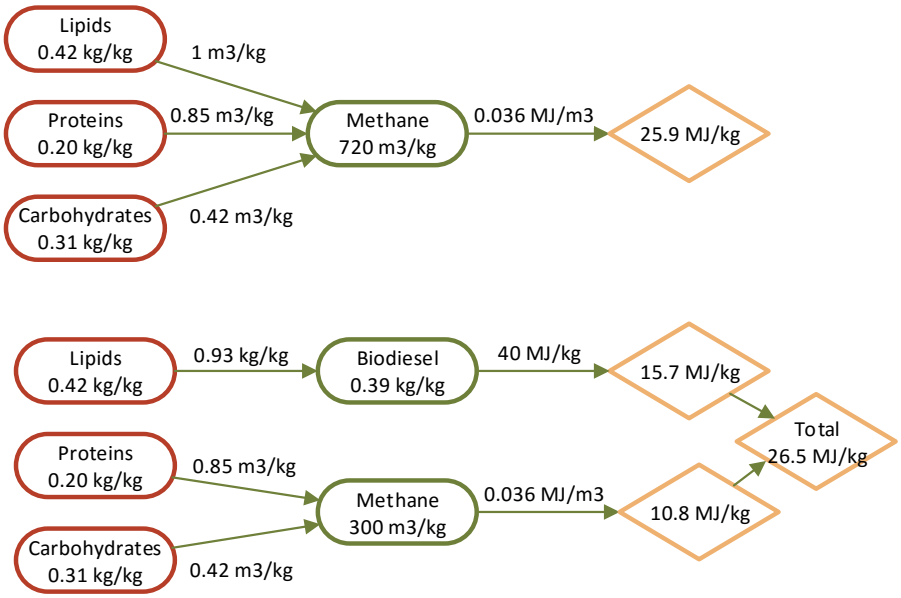

Figure 4.7. Energy potential of microalgae considering: Anaerobic digestion of whole microalgae only for biogas production (above) or biodiesel production and further anaerobic digestion of microalgae residues for biogas production (below)

Therefore, the stages of the process to be designed and represented by the Figure 4.8, are: algae growth in open pond reactors, where a final algal concentration of 0.4 g/L and with a composition showed in Figure 4.5 (day 7) is achieved, harvesting, formed by a clarifier and a

[2] Yield of fraction not considered in this work was evaluated as carbohydrates and a ~7 % of ash was assumed.

centrifuge, sonication for cell disruption and lipid extraction by solvent and the anaerobic digestion of the remaining fraction (mainly composed by proteins and carbohydrates).

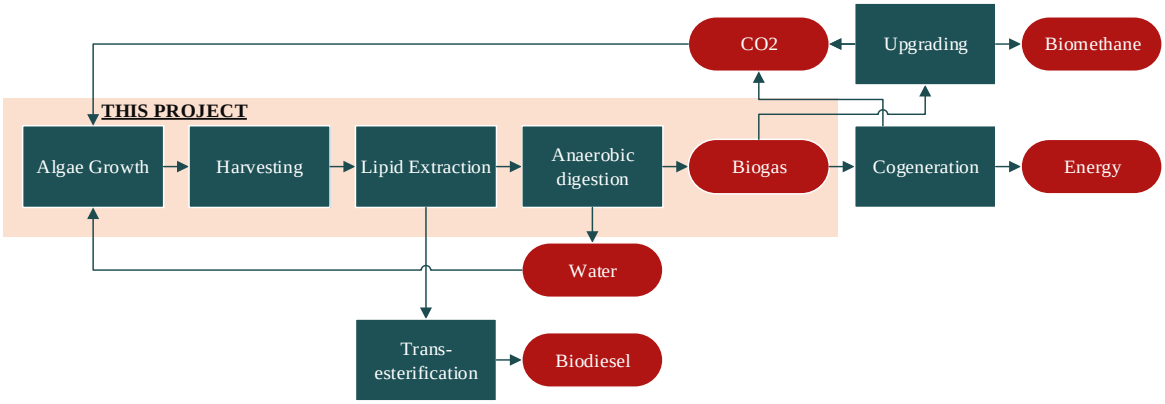

Figure 4.8. Algal Process Block Diagram

The SPD model is based on a related model developed by the own company as an example for the version 9.5 [76] which in turn was created by modifying the model created by Daniel Klein-Marcuschamer at the Joint BioEnergy Institute in Emeryville, CA, focused on aviation biofuel production [77]. This process modelled *Nannochloropsis* microalgae cultivation, harvest, oil solvent extraction, triacylglyceride oil degumming and refining into a combination of fuel products. On the other hand, SPD model is formed by the same steps except the refining, which is not modelled.

The SPD Process Flow Diagram of this work is represented in the Figure 4.9. The inputs were based on the previous experimental work developed in this study, on literature and on the SPD's example. **Process recycles have not been taken into account** as shown in Figure 4.8, except the solvent recycle for lipid extraction.

Commonly, biogas plants have an installed potential of 200-2,000 kW. In this work, a plant of approximately **1,200 kW will be designed**, which will need approximately a production of **3,200 kg algae/hour (42 % oil)**.

4.2.2. Raceway algae reactor design

This section aims to produce algae in raceway ponds as well as nutrient and water supply. The nutrients include phosphate (sodium dihydrogen orthophosphate) and nitrate (sodium nitrate), corresponding with the compounds included in the f medium used in the laboratory work. The water supply includes **Seawater** with a salt concentration of 33.3 g/L as was used in the experiments. In regards to pond sizing, the common dimensions [78] are shown in the Table 4.3. Dimension of this work are assumed as 1,500 m^2 of area and length/width ratio of 10.

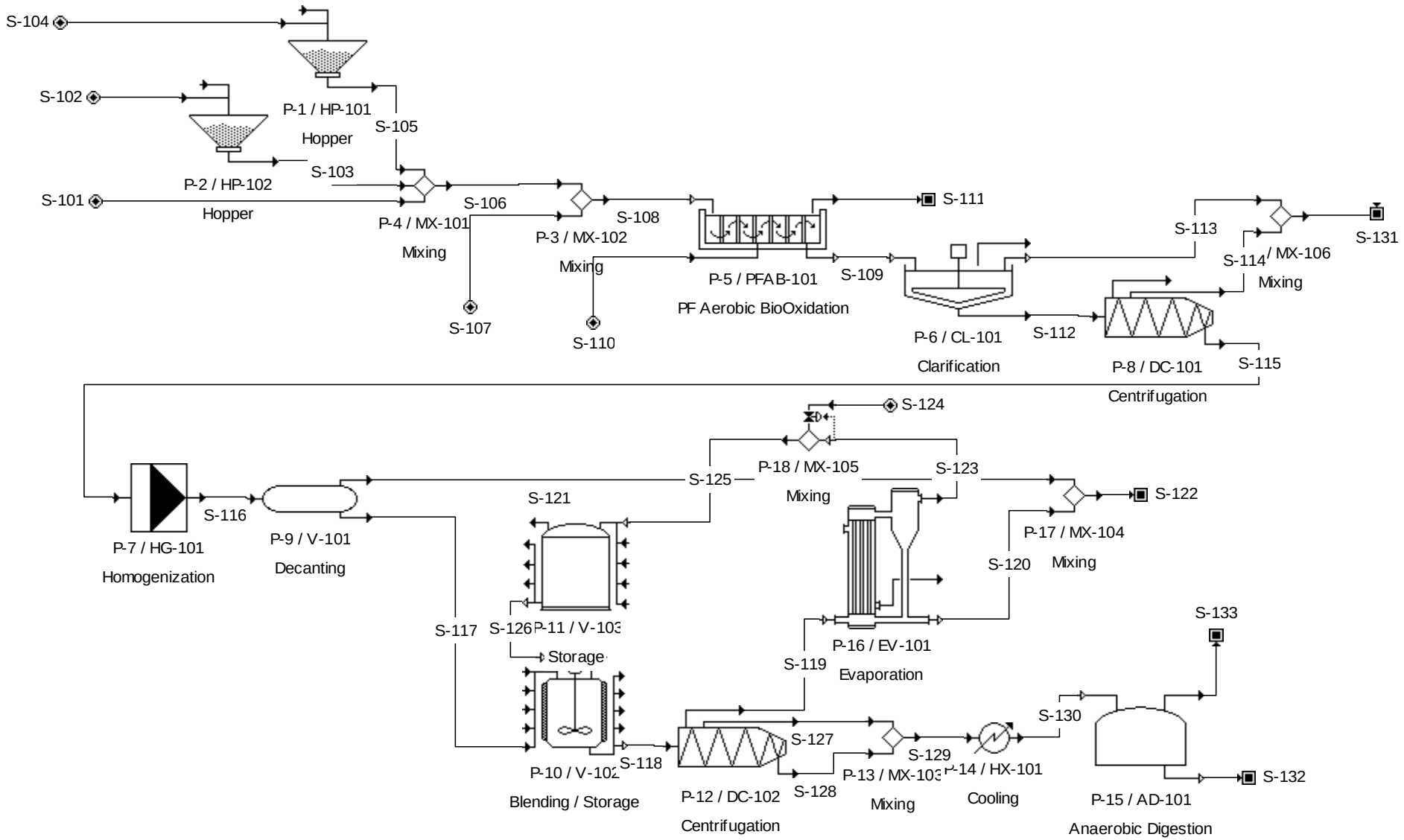

Figure 4.9. Algal Process Flow Diagram

Table 4.3. Dimension of algae ponds

Parameter, unit	Typical range [78]	This work
Length, m	10-300	274
Width, m	1-30	27.4
Area, m^2	300-2,000	1,500
Depth, m	0.05-1	0.2
Flow velocity, m/s	0.05-0.4	0.3

An average residence time of 6.5 days of the liquid in the algae ponds was assumed. Resulting surface area for each pond is approximately 0.15 hectares. The present work aims to study the production on roughly 140 ha, therefore, 929 pond will be needed. Total pond volume is 300 m^3 and 278,700 m^3 for the 929 reactors.

The pond configuration is displayed in Figure 4.10. In 6.5 days, as an average, each cell will go throughout the paddlewheel and nutrient feeding around 562 times (each 17 min). Thus, composition is supposed as constant along the reactor. Nutrient concentration is maintained at 24 mg N/L and 0.5 mg P/L which lead to a phosphorus scarcity such as studied previously. Water inlet flow (S-101) (33.3 g salt/L) is 8,155 m^3/h.

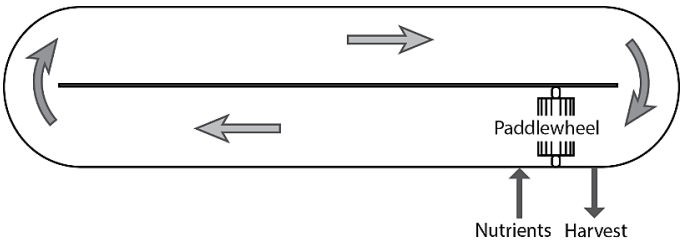

Figure 4.10. Raceway pond configuration

Algae production is modelled using a **PF Aerobic BioOxidation** unit procedure (PFAB-101). The biomass formation parameters are specified through a reaction. CO_2, sodium di-hydrogen orthophosphate, sodium nitrate, sulphate and water are consumed within the raceway ponds, and biomass, oxygen, and salts are produced. The elemental composition of the *Spirulina* was supposed as the Biomass composition included in SPD (C:N = 5) and the phosphorus content through a N:P ratio of 40 which indicates the P limitation (Figure 4.11) [79]. Thus, the algal stoichiometric ratio is C:N:P = 200:40:1.

From microalgae elemental composition, a stoichiometrically-adjusted reaction has been developed:

$$100\ CO_2 + 0.5\ H_2PO_4^- + 20\ NO_3^- + 89.5\ H_2O \rightarrow 100\ CH_{1.8}O_{0.5}N_{0.2}P_{0.005} + 148.75\ O_2$$

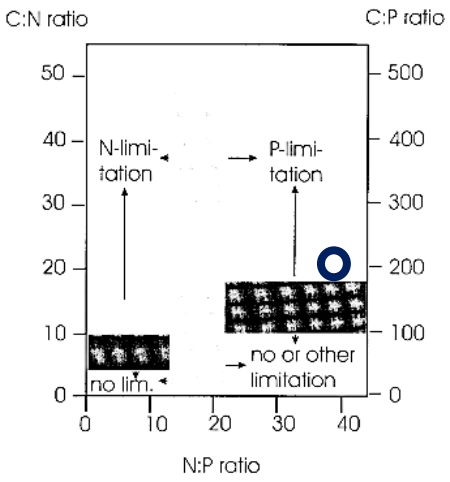

Figure 4.11. Schematic diagram on the use of nutrient ratios as indicator of nitrogen or phosphorus limitation. Shaded bars represent the range of optimal ratios. [Source: Hillebrand, 1999]

The outlet algal concentration in the ponds was calculated using the **previous developed modelling** and taking into account variations in parameters such as temperature during the day (an average in **Almeria**, Spain), light intensity and sun incidence and continuous supply of nutrients and flue gas which maintains the broth pH in 9.3, same as in the experiments. **From an inoculum concentration of 0.01 g/L, the outlet biomass concentration obtained was 0.40 g/L.** This concentration is within the common range for Open Pond, as mentioned before (0.1-0.5 g/L).

The outlet flowrate of algal biomass is 3,217 kg/h and about 81 kg/h would recycled back to the reactor (S-107). Assuming that each pond is operational and productive for 330 days/year (or 7,920 h/year), this leads to an annual biomass production of 24,840 MT.

From a nutrient consumption rate of 5.5 mg N/L·day and 0.025 mg P/L·day as commented before and a constant concentration in the reactor (and in the outlet stream) of 24 mg N/L and 0.5 mg P/L (N:P = 48), 416 kg/h and 21 kg/h of nitrogen and phosphorus respectively are fed into the reactor (S-102 and S104). That means 2,360 kg/h of $NaNO_3$ and 80 kg/h of NaH_2PO_4. Nutrient supply is modelled by **Hoppers** (HP-101 and HP-102) with a residence time of 5 min (by default) which means a volume of 300 and 8 L each one.

Inlet gas (S-110) has been configured as a flue gas from the cogeneration (not modelled in this work), with a mass composition of: 18 % CO_2, 70 % N_2, 2 % O2 and 10 % water. Gas requirement is supposed as 2.5 STD m3 per kilogram of inlet stream [76], which means a 0.6 % wt. of CO_2 excess for algae growth. The emission (S-111) performing in the Open Ponds was set at 100 % emitted for CO_2 (non-reacted) and O_2, and 1 % for water. The emission composition is shown in the Table 4.4.

Table 4.4. Open pond emissions

Component	Flowrate (kg/h)	Mass Comp. (%)	Concentration (g/L)
Carb. Dioxide	48	0.04	0.00
Nitrogen	21,980	20.43	0.93
Oxygen	6,689	6.22	0.28
Water	78,871	73.31	3.34

4.2.3. Harvesting method

The purpose of this section is to concentrate the algae. The first concentration step to 5 % (dry cell mass) (50 g/L) takes place in three circular **Clarification unit** (CL-101), where the algae settles to the bottom of the tanks with the aid of the flocculant that was added immediately upstream (not modelled in this work). The removal of the algae is 98.4 %.

The set overflow rate was fixed at 1,333 $L/m^2 \cdot h$ as SPD default settings. The volume of the clarifiers is thus 6,000 m^3. The depth is fixed in 3 m and the diameter of the tank is 50 m.

The algae-rich heavy stream (S-112) which exits the clarification tanks is then sent to the **Centrifuge** (DC-101), where it is further concentrated from 5 % (dry cell wt.) to 15 % (150 g/L). Algae removal was set as 99 %, producing a clarified stream of 99.9 % of sea water.

The two harvest units produce a mixed clarified effluent (S-131) form by 81 kg/h of algae, that would be used as inoculum, and 7,900 m^3 of salt water, 4 kg/h of NaH_2PO_4 and 195 kg/h of $NaNO_3$ (Table 1.1) as water and nutrient recycling, which **must be studied in further works and will perform a high decrease of operating costs**.

Table 4.5. Clarified effluent (S-131)

Component	Flowrate (kg/h)	Mass Comp. (%)	Concentration (g/L)
Biomass	81	0.00	0.01
Na	701	0.01	0.09
NaH_2PO_4	4	0.00	0.00
$NaNO_3$	195	0.00	0.02
Sodium Chloride	268,347	3.33	33.78
Water	7,790,689	96.66	980.84

4.2.4. Lipid extraction method

The aim of this section is to break algae up in order to allow the oils to be recovered in the extraction section. The concentrated biomass stream (S-115) is sent to the Sonicators (HG-101) modelled by a **High Pressure Homogenization** for cell disruption, which is represented by the following mass stoichiometry obtained in the experiments:

$$100\ \text{Biomass} \rightarrow 31\ \text{Carbohydrates} + 20\ \text{Proteins} + 42\ \text{Lipids} + 7\ \text{Ash}$$

The assumed extent of cell disruption is 95 %. Consequently, for every 100 kg of biomass entering the Sonicator, heated to 35 ºC using low pressure steam, 0.95 x 42 = 39.9 kg of lipid is released and available for further processing downstream.

Lipid extraction follows the **Soxhlet extraction method** [24]. The mixture of disrupted biomass (S-116) is sent to a **Decanter** (V-101) where the lipid-rich oil phase (99.85 % lipid) (S-125) is separated as the light phase at atmosphere pressure with a residence time of 10 min. Within this unit, lipid separation is 50 % and the other 50 % continue with the aqueous fraction. The heavy phase (S-117) leaving the decanter is combined with hexane in a **Blending Tank** (V-102) with a ratio hexane:lipid of 6.67 which has been reported by Belarbi *et al.*, (2000) [66], being three times lower than the ratio used in the experiments. The remaining oil components are extracted from the aqueous phase into the organic phase. Residence time is 6 h (by default) and its volume is 175 m^3. Using a common height/diameter ratio of 3, measures are 12.6 m x 4.2 m.

Hexane (S-126) is continuously fed by a storage tank modelled by a **Flat Bottom Tank** (V-103). Residence time is 8 hours (by default) and its volume is 51 m^3. Using a common height/diameter ratio of 3, measures are 8.4 m x 2.4 m.

The contents of this Blending Tank (S-118) are then introduced in a **Centrifuge** (DC-102) in order to separate the aqueous phase (S-127) and the 100 % remaining solids (S-128) from the oil-rich organic phase (S-119) with an efficiency of 99 % for the lipids and the hexane [76]. The separated solids and bulk aqueous phase are then sent to the Anaerobic Digestion Section.

The abovementioned oily fraction is separated from the solvent by evaporation, modelled by a **Multieffect Evaporator** (EV-101) which removes the 99.8 % of the hexane being recycled (S-123) and heated up by steam to 152 ºC with a calculated heat transfer area of 1.17 m^2. A stream of solvent make-up (S-124) is mixed in a **Custom Mixer** (MX-105) that sets the output flow at the fixed ratio. This stream (S-125) is sent to the solvent storage tank.

The two oily streams (S-125 and S-120) from the decanter and the evaporator are mixed (S-122) and the composition is shown in the Table 4.6.

Table 4.6. Lipid product stream (S-122)

Component	Flowrate (kg/h)	Mass Comp. (%)	Concentration (g/L)
Ash	0.0	0.00	0.01
Carbohydrates	9.5	0.65	5.85
Fats	1244.8	85.49	764.63
Hexane	7.3	0.50	4.50
Na	0.0	0.00	0.01
NaH_2PO_4	0.0	0.00	0.00
$NaNO_3$	0.0	0.00	0.00
Proteins	6.1	0.42	3.77
Sodium Chloride	6.2	0.43	3.83
Water	182.0	12.50	111.78

4.2.5. *Biodigester design*

Waste streams from the Lipid Extraction section are sent to the **Anaerobic Digestion** (AD-101) in order to turn the remaining biomass and other organic components into methane fuel. The combined inlet streams are conditioned at 30 ºC in a **Heat Exchanger** (HX-101) used prior to digestion. Residence time was set as 17 days from the experiment works which means a working volume of 8,875 m^3. The reactions are defined from literature [32][33]. Extent of the reaction means to assume a percentage of completion for the limiting reactant.

$$100\ \text{Lipids} \rightarrow 70\ CH_4 + 30\ CO_2\ (\text{Extent} = 60\ \%)$$

$$100\ \text{Protein} \rightarrow 60\ CH_4 + 40\ CO_2\ (\text{Extent} = 50\ \%)$$

$$100\ \text{Carbohydrate} \rightarrow 50\ CH_4 + 50\ CO_2\ (\text{Extent} = 40\ \%)$$

$$100\ \text{Biomass} \rightarrow 60\ CH_4 + 40\ CO_2\ (\text{Extent} = 20\ \%)$$

$$100\ \text{Hexane} \rightarrow 70\ CH_4 + 30\ CO_2\ (\text{Extent} = 10\ \%)$$

The generated gaseous stream (S-133) is **rich in methane** (65 % wt.). The yield toward methane from the biomass is **~400 L/kg VS**, which is a similar result from the obtained experimental work described above.

4.3. Process simulation results

4.3.1. *Material and energy balances*

This plant produces approximately 2,490 MT of algal biomass per year which is 1,046 MT of oil and 1,444 MT of remaining fraction to produce 158,875 MT of biogas (181,943 m^3) 65 % wt. of methane. The quantities of each raw material needed to produce this amount of biofuels are displayed in the Table 4.7, which shows the material requirements.

Table 4.7. Process bulk material

Material		kg/yr	kg/h	kg/kg MP
NaNO3	kg	18,691,200	2,360	1.62
NaH2PO4	kg	633,996	80	0.06
Sodium Chloride	kg	2,130,099,048	268,952	184.71
Water	m^3(STD)	62,482,369	7,889	5.42
Carb. Dioxide	kg	44,763,840	5,652	3.88
Nitrogen	kg	174,081,600	21,980	15.10
Oxygen	kg	4,973,760	628	0.43
Hexane	kg	351,062	44	0.03

The process report is provided in Table 4.8 which provides detailed stream information (e.g., flowrate, composition, temperature, pressure, etc.).

Table 4.8. Material Balance of the Process

		S-101	S-102	S-103	S-104	S-105	S-106	S-107	S-108	S-109	S-110	S-111	S-112	S-113	S-114	S-115	S-116
Type		Salt water	P source		N source			Inoculum			Flue gas	Emission					
Total Flow	kg	8155000	80	80	2360	2360	8157440	81	8157521	8081334	31400	107588	64385	8016949	43068	21317	21317
Temperature	℃	15	25	25	25	25	15	25	15	15	32	15	15	15	16	16	35
Pressure	bar	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Liq Density	g/L	1015	2040	2040	2106	2106	1015	1050	1015	1015	992	998	1016	1015	1015	1020	1007
Total Contents	kg/h	8155000	80	80	2360	2360	8157440	81	8157521	8081334	31400	107588	64385	8016949	43068	21317	21317
Ash	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	209
Biomass	kg/h	0	0	0	0	0	0	81	81	3217	0	0	3167	50	32	3136	157
Carb. Dioxide	kg/h	0	0	0	0	0	0	0	0	0	5652	48	0	0	0	0	0
Carbohydrates	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	923
Lipids	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1251
Hexane	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Methane	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Na	kg/h	0	0	0	0	0	0	0	0	703	0	0	5	697	4	2	2
NaH_2PO_4	kg/h	0	80	80	0	0	80	0	80	4	0	0	0	4	0	0	0
$NaNO_3$	kg/h	0	0	0	2360	2360	2360	0	2360	195	0	0	1	194	1	0	0
Nitrogen	kg/h	0	0	0	0	0	0	0	0	0	21980	21980	0	0	0	0	0
Oxygen	kg/h	0	0	0	0	0	0	0	0	0	628	6689	0	0	0	0	0
Proteins	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	596
NaCl	kg/h	268952	0	0	0	0	268952	0	268952	268952	0	0	2038	266914	1433	605	605
Water	kg/h	7886048	0	0	0	0	7886048	0	7886048	7808264	3140	78871	59173	7749090	41599	17574	17574

Table 4.8. Material Balance of the Process (continuation)

		S-117	S-118	S-119	S-120	S-121	S-122	S-123	S-124	S-125	S-126	S-127	S-128	S-129	S-130	S-131	S-132	S-133
Type							Lipids		Hexane									Biogas
Total Flow	kg	20691	24391	4485	830	626	1456	3656	44	3700	3700	17336	2569	19906	19906	8060017	19206	699
Temperature	°C	35	36	37	40	35	39	40	40	40	40	37	37	37	30	15	30	30
Pressure	bar	1	0	1	0	1	0	0	1	0	0	1	1	1	1	1	1	1
Liq Density	g/L	1011	931	681	905	880	894	1	643	643	643	1008	1055	1014	1017	1015	1016	1000
Total Contents	kg/h	20691	24391	4485	830	626	1456	3656	44	3700	3700	17336	2569	19906	19906	8060017	19206	699
Ash	kg/h	209	209	0	0	0	0	0	0	0	0	0	209	209	209	0	209	0
Biomass	kg/h	157	157	0	0	0	0	0	0	0	0	0	157	157	157	82	125	0
Carb. Dioxide	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	249
Carbohydrates	kg/h	923	923	10	10	0	10	0	0	0	0	811	103	914	914	0	548	0
Lipids	kg/h	626	626	619	619	626	1245	0	0	0	0	6	0	6	6	0	3	0
Hexane	kg/h	0	3700	3663	7	0	7	3656	44	3700	3700	37	0	37	37	0	33	0
Methane	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	450
Na	kg/h	2	2	0	0	0	0	0	0	0	0	1	0	2	2	701	2	0
NaH_2PO_4	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0
$NaNO_3$	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	195	0	0
Nitrogen	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oxygen	kg/h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Proteins	kg/h	596	596	6	6	0	6	0	0	0	0	523	67	590	590	0	295	0
NaCl	kg/h	605	605	6	6	0	6	0	0	0	0	531	68	599	599	268347	599	0
Water	kg/h	17574	17574	181	181	1	182	0	0	0	0	15426	1966	17392	17392	7790689	17392	0

4.3.2. Economic evaluation

- *Capital Expenditure*

Within microalgae processes, equipment cost is the main driver, in particular the cost of raceways ponds and harvesting facilities. The purchase cost of equipment is an important parameter that affects the direct fixed capital investment of a project and indirectly affects the annual operating cost [80].

SPD is equipped with correlations for estimating the purchase cost of equipment based on its type and size. The Built-In Cost Models for most types of equipment are more suitable for fine chemical and pharmaceutical types of facilities than for large-scale algae production plants [76]. User-defined costs may either be specified for equipment of a certain size or as a User-Defined Cost Model (UDCM). In this case, a UDCM has been chosen for the equipment cost estimate. The UDCM allows to specify a cost vs. size correlation that is used by the tool to estimate the cost of a piece of equipment.

For these types of processes, the own equipment cost data based on literature [76] [77] have been used for better accuracy. Data are entered in the form of the power-law equation (eq 13).

$$C = C_0 \cdot \left(\frac{Q}{Q_0}\right)^a \qquad \textit{eq 13}$$

In Table 5.9, equipment size obtained before appearing and the obtained unit cost and total cost per type of equipment are gathered.

Table 4.9. Major equipment specification and FOB cost (2016 prices)

Equipment	Quantity	Name	Description	Unit Cost, ($)	Cost, ($)
Hopper	1	HP-101	Vessel Volume = 296 L	7,000	7,000
Hopper	1	HP-102	Vessel Volume = 8 L	2,000	2,000
Open Pond	929	PFAB-101	Vessel Volume = 1,500 m^3	167,000	155,143,000
Clarifier	3	CL-101	Surface Area = 1,991 m2	1,024,000	3,072,000
Homogenizer	1	HG-101	Rated Throughput = 20,660 L/h	126,000	126,000
Decanter Centrifuge	1	DC-101	Throughput = 625,92 L/h	288,000	288,000
Decanter Tank	1	V-101	Vessel Volume = 3,201 L	73,000	73,000
Blending Tank	1	V-102	Vessel Volume = 172,943 L	664,000	664,000
Flat Bottom Tank	1	V-103	Vessel Volume = 51,187 L	108,000	108,000
Decanter Centrifuge	1	DC-102	Throughput = 25,945 L/h	288,000	288,000
Anaerobic Digester	1	AD-101	Vessel Volume = 8,875 m^3	6,099,000	6,099,000
Heat Exchanger	1	HX-101	Heat Exchange Area = 16.7 m^2	46,000	46,000
Evaporator	1	EV-101	Evaporation Area = 1.2 m^2	121,000	121,000
TOTAL	943				**166,036,000**

The direct fixed capital (DFC) is calculated as the sum of direct, indirect, and miscellaneous costs associated with that section's capital investment. The direct costs include elements that

are directly related to an investment such as the cost of equipment, process piping, instrumentation, buildings, facilities, etc.

By default, the DFC is estimated based on the purchase costs of all major process equipment multiplied by cost factors that are applied to the purchase costs. The cost factors include installation factors which are equipment-specific, as well as other factors specified at the section level. Process-specific cost factors for piping, instrumentation, buildings, etc., are more appropriate for high-value chemical and biochemical plants and they will greatly overestimate the total capital cost associated with an algae production facility. In order to more-accurately estimate the total DFC of the facility, the equipment-specific installation factors were increased (Table 4.10) and zeroed all the section specific multipliers. The increased installation cost factor accounts for the costs of foundation, piping, instrumentation, insulation, buildings, engineering costs, etc. For units that are constructed on-site (such as Raceway Ponds), the installation factor is much smaller.

Table 4.10. Capital Cost Adjustments

Equipment		Number of Units	Installation Factor	Maintenance Factor	Material of Construction	Material factor
HP-101	(Hopper)	1	2	0.1	CS	1
P-102	(Hopper)	1	2	0.1	CS	1
PFAB-101	(Open Pond)	929	0.2	0.02	Concrete	1
CL-101	(Clarifier)	3	0.2	0.15	Concrete	1
HG-101	(Homogenizer)	1	2	0.1	SS316	1
DC-101	(Centrifuge)	1	2	0.1	SS316	1
V-101	(Decanter Tank)	1	0.5	0.1	SS316	1
V-102	(Blending Tank)	1	2	0.1	SS316	1
V-103	(Flat Bott. Tank)	1	0.5	0.1	SS316	1
DC-102	(Centrifuge)	1	2	0.1	SS316	1
AD-101	(Anaer. Digester)	1	0.1	0.1	Concrete	1
HX-101	(Heat Exchanger)	1	0.5	0.1	CS	1
EV-101	(Evaporator)	1	2	0.1	SS316	1
V-102	(Blending Tank)	1	2	0.1	SS316	1

Using the cost models for each equipment unit and their respective cost adjustments, the total equipment cost is calculated based on the quantity of each required equipment item. From total equipment costs, **93.4 % is associated with the algae ponds** due to the very large number of ponds required, 3.6 % with the digester and 1.9 % with the clarifier.

The indirect costs include elements that are indirectly related to an investment, such as the costs of engineering and construction.

Once the equipment purchase cost is calculated, the miscellaneous costs associated with that section's capital investment is estimated. The working capital will cover expenses for 30 days

of labour, raw material and utilities whilst the start-up and validation cost will be 5 % of the DFC.

Following the AACE, the expected accuracy range is commonly between -20% to +30% for a study of feasibility of production plants.

Table 4.11. Total Investment

Departure	Cost (€)
Equipment Purchase Cost	166,036,000
Installation	34,864,000
Total Plant Direct Cost	201,392,000
Working Capital	4,062,000
Start-up Cost	10,070,000
Total Investment	**215,524,000**

- *Operational expenditure*

Resource requirements associated with materials are determined based on the mass and composition specifications for each of the flowsheet's input streams. To determine material costs, the annual amounts of each raw material are calculated by SPD and multiplied by the unit costs specified of each material. The material costs are shown in the Table 4.12.

The price of sodium di-hydrogen orthophosphate and sodium nitrate was obtained from Alibaba suppliers. Hexane price is already available in SPD and sea water cost was estimated as \$0.5/ m^3(STP). Water is the main material cost and its recycling would reduce it.

Table 4.12. Material cost

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
NaNO3	0.40	18,691,200	kg	7,476,480	18.93
NaH2PO4	1.20	633,996	kg	760,795	1.93
Sodium Chloride	0.00	2,130,099,048	kg	0.00	0.00
Water	0.50	62,244,792	m^3(STP)	31,122,396	78.79
Biomass	0.00	639,540	kg	0.00	0.00
Carb. Dioxide	0.00	44,763,840	kg	0.00	0.00
Nitrogen	0.00	174,081,600	kg	0.00	0.00
Oxygen	0.00	4,973,760	kg	0.00	0.00
Hexane	0.40	351,062	kg	140,425	0.36
TOTAL				39,500,096	100

Utility requirements associated with change of pressure and temperature such as power for the pumps, water for cooling and steam for heating. They are calculated using the energy balances and the SPD specifications for each utility. Costs of utilities are already introduced by defect from SPD. The estimated results costs are shown in the Table 4.13.

Table 4.13. Utility costs

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0.10	6,731,071	kW-h	673,107	90.4
Steam	12.00	5,075	MT	60,905	8.2
Cooling Water	0.05	211,221	MT	10,561	1.4
TOTAL				**744,573**	100

The labour requirement for each resource in each operation is estimated by the SPD default setting which gives a total of 13.7 operators where the direct time utilisation is 70 % as default for continuous processes (Table 4.14). Then, it is multiplied by its cost to compute the associated operating costs.

Table 4.14. Operators for each unit and labour cost

Equipment		Operators
HP-101	(Hopper)	-
P-102	(Hopper)	-
PFAB-101	(Open Pond)	7.3
CL-101	(Clarifier)	0.1
HG-101	(Homogenizer)	0.5
DC-101	(Centrifuge)	1
V-101	(Decanter Tank)	-
V-102	(Blending Tank)	1
V-103	(Flat Bott. Tank)	1
DC-102	(Centrifuge)	1
AD-101	(Anaer. Digester)	0.5
HX-101	(Heat Exchanger)	0.1
EV-101	(Evaporator)	1
V-102	(Blending Tank)	1
Total	Operators	13.7
Cost	€/hour per operator	57.5
TOTAL	€	**4,359,000**

Facility dependent costs are related to the total investment and the life time of the plant. For this work, a life time of 20-years has been chosen. These costs are calculated based on estimations of depreciation. The summary of the operational costs and the total cost are shown in the Table 4.15. Unit operation cost is **$7.18/kg of produced lipid**.

Mainly, they are obtained from raw material and facility-dependent costs which gives an idea of where the effort has to be made. Material recycling, water and nutrients, has to be studied as well and decreasing the pond costs.

- *Minimum lipid selling price*

The minimum lipid selling price is determined using a **Discounted Cash Flow Rate of Return Analysis**. The methodology was used by Phillips *et al.* (2007) to calculate a minimum ethanol

Table 4.15. Operational costs

Cost Item	Cost ($)	%
Raw Materials	39,500,000	47.74
Labour-Dependent	4,359,000	5.27
Facility-Dependent	38,143,000	46.10
Consumables	-	-
Waste Treatment/Disposal	-	-
Utilities	738,000	0.89
Transportation	-	-
Miscellaneous	-	-
Advertising/Selling	-	-
Running Royalties	-	-
Failed Product Disposal	-	-
TOTAL	**82,740,000**	100.00

selling price [81] and has been used in subsequent techno-economical works for other advanced biofuels, biochemicals and hydrocarbon chemicals.

The minimum lipid selling price is the selling price of the lipid that makes the Net Present Value (NPV) of the process equal to zero with an **8 % discounted cash flow rate of return over a 20-year plant life** for this study.

Biogas production in this kind of plants is not the main target as its revenue is not comparable to the lipids for liquid biofuels production [82]. The price of the biogas is obtained from the electricity that could be saved from every cubic meter. A generator uses 0.62-0.81 cubic meters of biogas per kWh and the price of the electricity is $0.1/kWh, therefore, the final price of the biogas is around **$0.15 /m^3(STD).** Total electricity production would be around 9,000 MWh per year (1,200 kW) and would overcome the electricity needs. Revenues for electricity excess or even biogas sales for biomethane are taken into consideration.

The Table 4.16 displays the key economic evaluation. Summarizing for a facility of 140 ha of pond surface area, the total capital investment is roughly $213 million. The estimated annual operating cost is $82.7 million which results in a unit production cost of $7.18/kg of algal oil.

The results calculated for the Return-On-Investment, Payback Time, etc. are based on minimum lipid selling price of $8.04/kg of Algal Oil and saving and selling of $0.15/m^3(STD) of Biogas. **Payback Time** obtained is **8.4 years** and **Return-On-Investment**, **12 %.** However, this price is not competitive neither with current fuel prices nor in comparison to other similar tech-economic analysis [82] carried out by the Department of Energy of USA for a similar technology that showed a price of ~$1.8/kg. This study used:

- A large 4-hectare ponds with paddle-wheel mixing, consuming at a total installed cost of $34,000/ha while this process used $111,000/ha.

Table 4.16. Economic results

Totals	Value	Unit
Total Capital Investment	215,524,000	$
Capital Investment Charged to This Project	215,524,000	$
Operating Cost	82,752,000	$/yr
Net Operating Cost	82,751,781	$/yr
Main Revenue	92,833,159	$/yr
Other Revenues	909,670	$/yr
Total Revenues	93,627,508	$/yr
Cost Basis Annual Rate	11,532,069	kg MP/yr
Unit Production Cost	7.18	$/kg MP
Net Unit Production Cost	7.18	$/kg MP
Unit Production Revenue	8.22	$/kg MP
Gross Margin	11.33	%
Return On Investment	11.90	%
Payback Time	8.41	years
IRR (After Taxes)	8	%
NPV (at 8.0% Interest)	0	$

- Plant life as 30 years instead of 20.
- A hexane solvent ratio of 5:1 kg solvent per kg dry biomass while this process, 6:1 kg/kg.
- Material recycling and therefore reducing one of the higher operational costs, raw materials.
- A process heat integration.
- The digestate material is sold as a fertilizer co-product.
- A much larger production scale.

The Table 4.17 shows the final associated revenue sources. Unit operation revenue is $8.04/kg of produced lipid and the Unit Production Revenue is $8.22/kg MP.

Table 4.17. Process revenues

Revenue/Savings Rates	Production		Price		Revenue	
Lipids	11,392,508	kg /yr	8.04	$/kg	92,833,159	$/yr
Biogas	5,992,914	m^3(STP) /yr	0.15	$/$m^3$(STP)	909,670	$/yr
TOTAL					**93,627,508**	$/yr

Biogas revenues/saving is just around 1 % of the total in this case. Nevertheless, under a competitive lipid price of $1.8/kg the share of biogas would increase to a 5 %.

5. Conclusions and recommendations

The conclusions of this work are listed below:

- *Spirulina maxima* algae under phosphate scarcity and the described condition has shown very high lipid and low protein content. On the other hand, nitrogen has seemed to be the nutrient limiting growth.
- Algae cultivation has been modelled by several equations which use nutrient concentration and limitation parameters, temperature, CO_2 through pH, and light intensity. This model seems to be suitable for the upscaling that has been carried out here.
- The model showed an achievable concentration of algae of 0.4 g/L under Almeria weather conditions and using flue gas as CO_2 feedstock.
- An algal process has been satisfactorily modelled using SuperPro Designer, a software that has been extensively used in biochemical processes.
- To obtain the lipids from the modelled algae and biogas from the remaining fraction, a four-stage process was needed: algae cultivation, harvesting, lipid extraction and anaerobic digestion.
- The economic evaluation of the process showed a total investment cost of $210 M and an operational cost of $83 M per year ($7.18/kg of produced lipid).
- A discounted cash flow rate of return analysis using a biogas price (and save) of $0.15/m^3(STD), showed a minimum lipid selling price of $8.04/kg of Algal Oil. This price does not seem to be competitive in comparison with other similar techno-economic analysis.

Moving forward, to reduce uncertainty in key areas for the algal process in the context of a fully integrated process, a number of important bottlenecks, uncertainties and areas for further development are summarized below:

- Validate algae growth rates and biomass compositional analysis based on data from larger scale demonstrations.
- Confirm Anaerobic Digestion performance.
- Design the process taking into consideration the recycling of the nutrients and water which will lead to a reduction of costs.
- Consider variations in algal production along the seasons.
- Reduce cost and increase performance for cultivation and dewatering. Substantial improvements in both performance (e.g. cultivation productivity) and cost (e.g. alternative or lower-cost designs) will be required.

6. References

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