DEVELOPMENT OF A TOPOLOGY OF BIOREFIENRY FOR OBTAINING BIOFUELS AND BIOPRODUCTS FROM MICROALGAE BIOMASS

ANGEL DARIO GONZALEZ DELGADO

UNIVERSIDAD INDUSTRIAL DE SANTANDER
FACULTY OF PHYSICHOCHEMICAL ENGINEERINGS
SCHOOL OF CHEMICAL ENGINEERING
DOCTORATE IN CHEMICAL ENGINEERING
BUCARAMANGA
2014

DEVELOPMENT OF A TOPOLOGY OF BIOREFIENRY FOR OBTAINING BIOFUELS AND BIOPRODUCTS FROM MICROALGAE BIOMASS

ANGEL DARIO GONZALEZ DELGADO

Submitted to the School of Chemical Engineering of
Universidad Industrial de Santander
In partial fulfillment to the requirements for the degree of
Doctor en Ingeniería Química

DIRECTOR

Dr. Sc. Viatcheslav Kafarov
Universidad Industrial de Santander

UNIVERSIDAD INDUSTRIAL DE SANTANDER
FACULTY OF PHYSICHOCHEMICAL ENGINEERINGS
SCHOOL OF CHEMICAL ENGINEERING
DOCTORATE IN CHEMICAL ENGINEERING
BUCARAMANGA
2014

Table of Contents

List of Tables

List of Figures

List of Annexes

PREFACE

The change of the focus of the chemical engineering, from traditional transformation processes, to sustainable utilization of resources, has promoted a revolution in the way of designing novel chemical processes, being in general terms, more dedicated to find the industrial application than to the phenomenological science, but still built over the basic concepts of the thermodynamics, transport phenomena, advanced mathematics, and chemical reaction engineering. If the goal of reach sustainability is clear, the technical issues for transforming the ideas into viable actions is still under construction, and involve a wide number of aspects that includes but is not limited to the integration of interdisciplinary knowledge, the development of novel technologies, and the efficient use of renewable feedstocks.

In this book is presented the development of a topology of microalgae-based biorefinery, considered as a scheme or flowsheet with the sequence necessary for the total use of microalgae biomass and its transformation into a set of products. This development was supported on three pillars: the experimentation, as the primary technical information source, the Computer Aided Process Engineering as decision-making support tool for technologies evaluation, and the process synthesis as the crucible where are put together separate elements into a coherent whole.

First chapter of the book provides an overview of the main issues addressed in the rest of the book, such as the generations of biofuels, the potential of microalgae as a feedstock for biofuels and other products, the most studied alternatives production and processing, the biorefinery concept and their similarities and differences with traditional refineries and of linear biomass transformation chains, and finishing with an overview of process synthesis describing the two major

approaches (Hierarchical and Superstructure optimization-based) and the recent advances in process design focused on the development of biorefineries.

Chapter two shows the experimental adjustment of three solvent-based methods for microalgae oil extraction coupled with cell wall disruption, in order to increase the extraction efficiency and to generate primary information for further experimental and computer-aided studies. Chapter three shows the comparison of five previously developed oil extraction methods in lab-scale, using five microalgae strains from a national bioprospecting study, comparison was made in terms of toxicity, energy requirements, oil extraction efficiency and extraction costs and allowed to identify the most promising methods under several parameters and reduce the number of scenarios for a further process simulation and superstructure optimizations.

In Chapter four is presented a study of reducing sugars production and oil extraction from microalgae, evaluating in lab-scale four routes, two sequential routes in which fermentable sugars are obtained in first stage and the microalgae lipids are obtained in the second stage; and two multifunctional routes in which sugars and oil are obtained simultaneously in the same reaction unit. For multifunctional routes also was made the modelling of reducing sugars production and degradation.

Chapter five is dedicated to show the utilization of Computer Aided Process Engineering for the evaluation of emerging technologies for microalgae processing, and for evaluation of technology behavior at large scale. Several comparative studies are shown taking as common starting point the robust modelling of a microalgae strain and the simulation of alternatives to evaluate based on both information obtained in lab-scale and reported in scientific literature. Three cases of study are shown for evaluation of processing alternatives in which are combined

process simulation and evaluation methodologies under sustainability criteria as Exergy Analysis, Life Cycle Assessment and Energy Integration.

Chapter six is dedicated to the development of a hybrid methodology for microalgae biorefinery synthesis integrating process design concepts as hierarchical analysis, forward-backward branching, superstructure optimization and in-depth analysis, once methodology is developed, is shown its application for the development of a microalgae-based biorefinery, taking as main product the diesel-like biofuel. As result, are shown two feasible topologies of biorefineries. Next section shows the general conclusions of the research and gives some recommendations for future work.

The scientific novelty of the research presented in this book is measured in terms of number of publications generated from the work, and the quality of the journals in which documents were published, this quality was evaluated taking into account as the national category of the journal according to the Colombian Administrative Department of Science, Technology and Innovation COLCIENCIAS, as the international visibility of the journal in SCOPUS database. Other criteria of research novelty used were the number of citations of the author in the last three years, the *h-index*, and the number of presentations in scientific events.

ABSTRACT

TITLE: DEVELOPMENT OF A TOPOLOGY OF BIOREFIENRY FOR OBTAINING BIOFUELS AND BIOPRODUCTS FROM MICROALGAE BIOMASS*

AUTHOR: ANGEL DARIO GONZÁLEZ DELGADO**

KEYWORDS: MICROALGAE, BIOREFINERY, TOPOLOGY, BIOFUELS

DESCRIPTION:

The technical issues for transforming the ideas into viable actions is still under construction, and involve a wide number of aspects that includes but is not limited to the integration of interdisciplinary knowledge, the development of novel technologies, and the efficient use of renewable feedstocks. In this book is presented the development of a topology of microalgae-based biorefinery, considered as a scheme or flowsheet with the sequence necessary for the total use of microalgae biomass and its transformation into a set of products. This development was supported on three pillars: the experimentation, as the primary technical information source, the Computer Aided Process Engineering as decision-making support tool for technologies evaluation, and the process synthesis as the crucible where are put together separate elements into a coherent whole.
The big-picture approach developed allowed to obtain from the enormous number of possibilities of existing and emerging technologies for microalgae processing, two feasible topologies of biorefineries with positive objective values (annual revenue) taking as main product diesel-like biofuel methodology proposed is useful owing to their hierarchical characteristics, and also can be re-focused to other main product, or objective function (e.g., maximum yield, maximum profit, minimum processing steps, minimum waste, minimum emissions, maximum feedstock flexibility, highest energy or exergy efficiency).

*Doctoral thesis
**Faculty of Physicochemical Engineerings. School of Chemical Engineering
Director: Dr.Sc. Viatcheslav Kafarov

RESUMEN

TÍTULO: DESARROLLO DE UNA TOPOLOGÍA DE BIOREFINERÍA PARA LA OBTENCIÓN DE BIOCOMBUSTIBLES Y PRODUCTOS DE ALTO VALOR AGREGADO A PARTIR DE BIOMASA DE MICROALGAS*

AUTOR: ANGEL DARIO GONZÁLEZ DELGADO**

PALABRAS CLAVES: MICROALGAS, BIOREFINERÍA, TOPOLOGÍA, BIOCOMBUSTIBLES

DESCRIPCIÓN:

Los aspectos técnicos para transformar las ideas en acciones viables aún están en construcción, e involucran una gran cantidad de aspectos que incluyen pero no se limitan a la integración de conocimientos interdisciplinarios, el desarrollo de nuevas tecnologías y el uso eficiente de materias primas renovables. En este libro se presenta el desarrollo de una topología de biorrefinería basada en microalgas, considerada como un esquema o diagrama de flujo con la secuencia necesaria para el uso total de biomasa de microalgas y su transformación en un conjunto de productos. Este desarrollo fue apoyado en tres pilares: la experimentación, como la fuente de información técnica primaria, la ingeniería de procesos asistida por ordenador como herramienta de apoyo a la toma de decisiones para la evaluación de tecnologías y la síntesis de procesos como el crisol donde se juntan elementos separados en un conjunto coherente.
El enfoque del marco general desarrollado en esta tesis permitió obtener de la enorme cantidad de posibilidades de tecnologías existentes y emergentes para el procesamiento de las microalgas, dos topologías posibles de bio-refinerías con valores objetivos positivos (ingresos anuales), teniendo como principal producto de la metodología al biodiesel, La metodología desarrollada en la parte final es útil para otros procesos y productos debido a sus características jerárquicas, y también puede ser reorientada a otro producto principal o función objetivo (por ejemplo, el rendimiento máximo, el máximo beneficio, pasos mínimos de procesamiento, un mínimo de residuos, emisiones mínimas, máxima flexibilidad de materia prima, la más alta energía o eficiencia exergética)

*Tesis doctoral
**Facultad de Ingenierías Fisicoquímicas. Escuela de Ingeniería Química.
Director: Dr.Sc. Viatcheslav Kafarov

1. CHAPTER I. INTRODUCTION TO BIOFUELS, MICROALGAE, BIOREFINERIES AND PROCESS SYNTHESIS[1]

1.1. OVERVIEW OF BIOFUELS

The continued use of fossil-derived fuels is recognized as unsustainable due to the depletion of supplies and the associated environmental impact. Therefore, there is a growing interest in the identification of cost-effective, clean, and renewable sources of energy. Biofuels are among the most promising alternatives as they offer many benefits related to energy security, economic stability and reduction of the environmental impact of greenhouse gases.

First-generation biofuels are derived from crops such as sugarcane [1], sugar beet [2], potato [3], corn [4], sorghum [5] among others, for the case of bioethanol production, and oils from soybean [6], rapeseed [7], palm oil [8], among others. The advantages of first-generation biofuels include relatively high yields, reliable conversion technologies (especially to bioethanol and biodiesel), and known supplies of biomass. On the other hand, the use of crops as feedstocks create competition between food and fuel that ultimately hurts both markets and calls into questions several social and ethical issues [9].

Second-generation biofuels are produced from a variety of raw materials that do not compete with food sources. These include lignocellulosic materials

[1] This chapter is based on the papers "*Microalgae based biorefinery: Issues to consider*" by Angel Darío González Delgado & Viatcheslav Kafarov, published in CT&F Journal Vol. 4 (4), 47 – 60 (2011). "*Development of a methodology of microalgae oil extraction in the biodiesel from microalgae production chain*" by Angel Darío González Delgado, Alexander Guzmán and Viatcheslav Kafarov, published in Prospectiva Journal Vol. 7 (2) p.53 – 60 (2009), and "*Development of a topology of microalgae-based biorefinery: process synthesis and optimization using a combined forward-backward screening and superstructure approach*" by Angel Darío González Delgado, Viatcheslav Kafarov and Mahmoud El-Halwagi, under evaluation in Applied Energy Journal (2014).

resulting from agro-industrial activities such as the extraction of sugar, as corn stover [10), sugarcane bagasse [11], coffee pulp [12], palm fruit fiber [13]. rice husks [14], among other lignocellulosic wastes and perennial crops for the case of bioethanol production, and non-edible oils as herbaceous oils [15], jatropha curcas [16], castor oil [17], among others for the case of biodiesel production. Second-generation biofuels promise to be more beneficial than first-generation biofuels in terms of efficient use of land and proper environmental management. Most processes and technologies for the production of second-generation biofuels are still in the pre-commercial stage (pilot plants, demonstration plants). These biofuels have not faced the problems mentioned earlier for first-generation biofuels. Nonetheless, there is major concern about competition in the use of land and the impact on crops [18].

Third-generation biofuels are produced from non-conventional feedstocks as microorganisms (yeast, fungi and microalgae) which can biosynthesize and accumulate large amounts of lipids and/or sugars [18]. Among the most attractive feedstocks for third-generation biofuels production are microalgae. They have recently been rediscovered as promising candidates for biochemical applications and efficient energy production systems [19]. Depending on the strain, microalgae can grow in a wide range of temperatures, pH and nutrients availability. Some microalgae species feature growth rates between 20 and 30 times higher than other sources for biofuels and can produce up to 20 times more oil per unit area than palm under approipiate cultivation conditions [20]. It has been reported that oil content of microalgae can surpass 80% in dry weight biomass [21].

1.2. OVERVIEW OF MICROALGAE

Microalgae can grow in a wide variety of climates requiring only water, some nutrients, a carbon source and sufficient solar energy. As such, the development of microalgae cultivation systems (open or closed) can be made using non-arable lands. Another advantage of microalgae cultivation is the potential of utilization of wastewater as culture media, which peresents a benefit in use of residues for biomass production and wastewater treatment [22]. Microalgae can be also cultivated in freshwater, hypersaline water or sea water [23]. Due to its high growth rate, microalgae biomass can be harvested throughout the year, presenting a theoretical potential to become a viable alternative to replace petroleum-based liquid fuels in the future without the disadvantages associated with food versus fuel discussion and use of land.

Microalgae has been used as a source of several products in commercial scale, anutritional supplement for humans and animals, and a feedstock for pharmaceutical and cosmetic products [24]. These processes do not involve significant chemical processing of biomass. On the other hand, the use of microalgae for biofuel production requires more chemical processing. At present, numerous research efforts focus on developing microalgae processing technologies for biofuels production to pursue the goal of a sustainable third generation biofuels production.

For sustainable utilization of the enormous potential of microalgae as a source of biofuels, technologies for cultivation and biomass processing must be efficient from technical, economic, environmental, and energetic points of view. A wide variety of novel technologies for microalgae cultivation and processing are emerging, and others are being adapted to microalgae biomass and derived metabolites from processes used in other biomasses- or hydrocarbon-processing industries.

1.2.1. Microalgae production and dewatering

Transformation of microalgae to biofuels and other products is composed by several stages, starting with microalgae cultivation, Microalgae can be cultured in photobioreactors which offer high biomass productivities and an adaptable illumination, open ponds which can be natural systems (e.g. lagoons and lakes) or artificial systems (e.g. stirred tanks and raceway ponds) which require low energy consumption and are easy to maintain. This condition makes feasible the use of non-arable lands for photobioreactor assembly or open pond building. Microalgae can also be cultivated using as culture media waste water or sea water.

Exists also the two-stage cultivation of microalgae which is a culturing process that manipulates the culture conditions and nutrient feed in terms of the frequency and concentration in order to increase the biomass production rate and lipid content of the microalgae, first stage is the development of the cell numbers during the zoospore settlement, and the second stage is a process where the cell number or zoospore of the microalgae are increased while also increasing their size. However, during the second stage, more attention is given to how to enrich each cell with lipids rather than increasing the cell number. This idea based on microalgal nature, which actively responds to nutrient starvation or excess. The light intensity has different effects on microalgal species, as some species require more or less light energy to conduct the photosynthesis process. A light intensity between 76 and 600 $\mu mol/m^2.s$ can be applied to culture microalgae, obtaining better productivities when a light intensity of 76 $\mu mol/m^2.s$ is applied in first stage and a higher intensity (around of 240 $\mu mol/m^2.s$) is used for second stage [25].

A high nitrogen concentration is important during the first stage of the cultivation process to support the reproduction of microalgal cells, but nitrogen concentration should be decreased in second stage to carbon transformation in lipids rather than proteins. The depletion of the nitrogen source affects the intracellular consumption of the nitrogen pool to support the synthesis of cell material for further cell division. Thus, culturing under depleted nitrogen levels also inhibits microalgal growth. An organic nitrogen source can be an alternative to reduce cost of microalgal cultivation; this source can be obtained from other biomass. Urea can be also used as nitrogen source, according to results reported by Shi, Zhang and Chen [26].

Microalgal biomass production can be made absorbing energy from light (phototrophic) or consuming an organic carbon source (heterotrophic) independent of a light source, for phototrophic culture, microalgal cells depend on light to reproduce. The absorbed energy from light is stored and use in the Calvin cycle to produce glucose. Phototrophic cultivation of microalgae produces a lower lipid percentage in comparison to heterotrophic culture because of the limited acyl groups between the chloroplast lipids. Additional CO_2 supply can increase the lipid and biomass productivity of microalgae. Some microalgae strains as *Chlorella* can grow as phototrophic as heterotrophic conditions. In heterotrophic culture, the cost of the carbon source is one of the most discussed issues in heterotrophic growth, lipid content and biomass yield depend on the carbon type and concentration in the culture medium. Some carbon sources used includes glycerol, glucose and sweet sorghum. Wu, Yu and Lin [27], showed that a glucose concentration of 0.5–8% as the carbon source resulted in a lipid content of up to 44% in microalgae, finally, lipid contents of 73.4% can be achieved when 50% sweet sorghum juice is added into the culture medium instead of pure glucose [28].

Open ponds for microalgae cultivation can be divided in raceway, circular, inclined and unmixed ponds, raceway ponds are the most applicable for the pilot-plant scale and commercial scale because of their easy operation, the productivities of raceway ponds have been reported to be 14–50 g/m^2/d. The productivity of a raceway pond can be increased by improving the CO_2 mass transfer [29]. Circular ponds, however, are capable of achieving algal growth rates as high as 21 g/m^2/d [30]. With the addition of organic carbon, higher algal growth rates can be achieved. Inclined ponds are rarely selected for microalgal cultivation, most likely because of their difficult operation in comparison to other types of open ponds. However, the literature shows that this culture system is capable of achieving a microalgal growth rate of up to 31 g/m^2/d [31]. Unmixed open ponds are generally used to culture *Dunaliella salina* and have low productivities of (less than 1 g/m^2/d), for this reason, unmixed open ponds are unsuitable for the cultivation of most algal species. Most studies have shown that open ponds do not require high maintenance or set-up costs. However, open ponds are susceptible to contaminates and other fast-growing heterotrophic organisms, in addition, open ponds also require large areas of land.

Microalgae cultivation in a closed system can be conducted in photobioreactor, which can be categorized into many types including tubular, vertical, flat-plate, annular, fermenter and internally illuminated photobioreactors. The vertical tubular reactor is the most popular type of photobioreactor that has high surface to volume ratios, low shear forces, low cost, absence of wall growth, high efficiency of CO_2 use, and the ability to use sunlight. Tubular photobioreactors can be used individually or arranged in parallel for better CO_2 consumption. Flat-plate photobioreactors required a lower power supply for mass transfer compared to tubular photobioreactors, the fundamental principle in all of photobioreactor designs is to reduce the light path and thus increase the light available to each cell. However, the

design of a photobioreactor is more complicated compared to an open pond. According to Grima et al. [32], the recommended pipe diameter to culture microalgae of approximately 0.1 m, with a flow velocity of 0.3–0.5m/s. A diameter greater than 0.1m will require an unrealistic culture velocity, which could damage the microalgal cells, and a diameter less than 0.03m resulted in lower productivity, this is associated with the light distribution and the mixing gas transfer between O_2 and CO_2. In order to achieve a higher flow and volume, multiple pipes can be arranged with the same common headers. CO_2 removal efficiency of up to 82.3% can be achieved in an airlift bioreactor [33]. This type of photobioreactor was also reported to be suitable for batch, continuous and semi-continuous culture of microalgae [34].

As comparison, photobioreactor has a more complicated system compared to raceway pond. Closed system requires a degassing column, for removing O_2 produced during photosynthesis, cultivation in a closed system has less contamination with the surroundings and can be easily controlled, recently, cultivation in photobioreactors has attracted more attention, as it is easy to control and is promising for higher productivities compared with open systems. However, cultivation in closed systems is more expensive compared with open ponds. The additional costs include the light illumination, the CO_2 feed, the cultivation medium feed and the circulator system, from the other hand, microalgal productivity in a photobioreactor is higher and presents less contamination, annual biomass productivity of a closed system is higher than an open system. The overall energy requirement for an open system (450 GJ/yr) is lower than for a closed system (729 GJ/yr) [35].

Microalgae harvesting can be difficult because of their small cell size. Usually involves flocculation followed by harvesting either by filtration, centrifugation, sedimentation or flotation, other techniques as ultrasound are still in development. Harvesting microalgae at the commercial scale usually involves a flocculant to reduce the time required to separate the medium from the algal

cells. Flocculants with higher molecular weights are generally more effective, organic flocculants can be obtained naturally or synthetically. Organic flocculants have an advantage over non-organic flocculants with regard to the dosage used to flocculate the particle. Chitosan, for example, has been reported to be capable of harvesting up to 98% of the microalgae, and the reported compatibility dosage varied from 0.2 to 0.4 g/l [36]. Another natural cationic polymer that is commercially available is Greenfloc120, which is made from starch and was reported to be efficient as a flocculant to harvest freshwater microalgae [37]. Inorganic flocculants are another type of flocculant that are made from the combinations of salts and metals such as ferric chloride or alum. Separation efficiencies of up to 90% were reported when using ferric chloride as a flocculant [38]. A flocculation technique called electrolytic flocculation that only requires electricity as low as 0.3 kWh/m^3 was also reported in the literature by Poelman et al. [39], obtaining an efficiency of 90%. After the flocculation process, the separated algal cells then continue to filtration, centrifugation, floatation or sedimentation before a further drying process.

Centrifugation is the most preferred method to harvest microalgae for laboratory study. This is because this technique does not required additional chemicals; however, this method requires more electrical energy compared to flocculation. In large-scale harvesting processes, centrifugation provided good recovery and thickened the slurry, but the currently available equipment for centrifugation processes is too expensive. This delays the application of this technique at large-scale. Direct filtration process harvests microalgal biomass by using a microbial membrane which only allows algal cells to pass through. However, this technique requires backwashing to maintain the efficiency of the membrane filter and is time-consuming.

1.2.2. Separation of microalgal metabolites

There are several methods used to extract specific components from microalgae biomass, which can be broken down into chemical methods, mechanical methods, and enzymatic methods. The extraction of lipids with solvents has been used traditionally to obtain lipids from animal and plant sources. In the case of microalgae, the solvent selective toward the metabolite of interest is usually added to the dry biomass, although in some cases a certain amount of water is allowed in order to reduce the overall costs of the process, and also diminishing extraction efficiency, extraction combined with *in-situ* transesterification can also be performed [40].

A wide variety of organic solvents are often used to extract oil from microalgae, where hexane and ethanol are the most popular. A hexane-ethanol mix can be used to extract more than 98% of the fatty acids present in the biomass [41]. However, since ethanol is relatively polar, its selectivity to lipids is relatively low compared to other solvents, so in extractions with ethanol, other microalgae components may also appear, such as sugars, pigments or amino acids. A solvent-based methodology was proposed by Folch, Lees and Stanley [42] to extract both polar and non-polar lipids, due to the use of an apolar solvent that dissolves neutral lipids, in combination with a relatively polar solvent, which dissolves the polar lipids present in the sample undergoing extraction. The original method was based on the methanol/chloroform mix, followed by a purification of the extracts with a KCl solution. After that, Bligh and Dyer [43] modified Folch's method, and obtained a quick lipid extraction method, which is currently being used and has been tested successfully in extracting oil from algae [44]. This method has obtained good results in extracting oil from microalgae and is often used as a complement to mechanical destruction methods or biomass treatment with autoclave, although it poses the disadvantage of not being very

environmentally friendly due to the toxicity of the solvents used, therefore preventing its utilization at an industrial scale.

The soxhlet extraction system has been widely used to extract oil from algae [45,46]. Petroleum ether and ethyl ether have been used with this system to extract non-polar lipids from microalga *Neochloris oleobundans* [47]. The inconvenience with ethers is their volatility, which leads to a significant loss of solvent during the extraction process. Hexane has also been evaluated as an extraction solvent for microalgae in the soxhlet system with interesting results [48]. The advantage of hexane is that it is three times cheaper under local market conditions than other non-polar solvents as cyclohexane, easy-to-recover after extraction and it is selective to neutral lipids. In addition, it can be used in mixture with isopropanol [47], which is considered safe in an industrial scale and is used for lipid extraction from soybean, efficient in the extraction of fatty acids and has a low level of toxicity. The dichloromethane/hexane mixture allows the increase in the amount of total lipids extracted, if the objective is high efficiency and selectivity is not a priority. Another mixture that has been used successfully in soxhlet extraction for microalgae, is the combination of dichloromethane/methanol [48], which recovers a large amount of neutral lipids. Soxhlet extraction is a typical lab-scale extraction method and is not applied in big scale itself. However, it is considered a method for the simulation of a multi-step solvent extraction with continuous reflux.

Microwave-assisted extraction is characterized for being a technique that reduces process time and increases process efficiency. This method was compared with other methods that included a pre-treatment or biomass conditioning stage by cell destruction procedures such as autoclave, ball mill, induced resonance and osmotic shock, all followed by extraction using the methanol-chloroform mixture for the species *Chlorella vulgaris*, *Scenedesmus*

sp. and *Botryococcus* sp. [49], the results shown that the highest oil yield increase for all microalgae strains evaluated was reached when the cells were disrupted using the microwave oven method. OriginOil company exhibits a harvesting/oil extraction technology based on applying the electromagnetic fields that currently is scaling up in Australia.

The ultrasound technique consists of exposing the microalgae to sound waves of a specific frequency (low), to destroy the cell wall [50]. Cravotto et al., [51] developed an extraction technique with ultrasound and assisted by microwaves simultaneously, working at frequencies between 19 and 300 kHz, with which they obtained a significant decrease in extraction time, reducing it up to 10-fold and increasing oil extraction yield by 50–500% for seaweed oil extraction in comparison with conventional methods as soxhlet. A technology based in this extraction method is used at big scale for microalgal lipid extraction by the Fox Oil Company in Argentina.

There are also methods to extract components from microalgae called supercritical, as an alternative to the traditional use of large quantities of toxic solvents to perform extractions. Among this kind of processes, the most promising ones are supercritical fluid extraction (SFE), and subcritical water extraction (SWE), which are characterized by short extraction times and high selectivities [52]. In addition, they are eco-friendly and present high efficiencies in the extraction of solid samples. One characteristic that makes the use of SFE interesting is the possibility of combining the extraction system with in line characterization systems such as gas chromatography, or supercritical fluid chromatography [53]. This method has been tested in lab-scale with good results; however, the high costs associated with operation conditions for the extraction makes difficult the scaling-up of this technology.

SFE has been used with several species of microalgae to obtain different substances: Cheung [54] used supercritical CO_2 to obtain Omega-3 fatty acids from *Hypnea charoides*; Mendes et al. [55, 56], applied the technique to extract carotenoids from *Chlorella vulgaris*, b-carotene from *Dunaliella salina* and diolefins from *Botryococcus braunii.* In extraction using subcritical water (SWE), water is used at temperatures between 100 and 374 °C, and pressures between 10 and 60 bar [57], to keep it in a liquid state. Under these conditions, the dielectric constant of the water decreases considerably, approaching the dielectric constant of ethanol at room temperature. This method of extraction has been used in microalgae by Herrero, Ibáñez, Señoráns and Cifuentes [58], who obtained antioxidative components from the microalga *Spirulina platensis*.

Use of an autoclave to extract metabolites from microalgae is a variable methodology and used in lab-scale: Minowa, Yokoyama, Kishimoto and Okakurat [59], used an aqueous saline solution as a working fluid in an autoclave at 300 °C, and a pressure of 100,000 kPa, residence times between 5 and 60 minutes, and they used nitrogen to purge residual air.

In enzyme-assisted extraction, the cell wall of the microalgae is degraded with enzymes, which facilitates the withdrawal of the oils in the cell. Enzymes can also be used to transform the fatty acids present in the microalgae in lipids suitable for subsequent transesterification [60]. However, enzymatic activity is affected by many variables, such as the nature and concentration of the enzyme, the concentrations and ratios of the reactants, the composition of the oils or fatty acid mixtures, the composition of the cell wall, the initial water content and temperature, among others [61].

Mechanical destruction as a tool to extract components from microalgae, covers several classes of mechanical devices such as cell homogenizers, ball

mills and pressing systems, among others. Lee, Yoon and Oh [62], evaluated several mechanical destruction systems to extract lipids from the microalga *Botryococcus braunii* concluding that a higher oil extraction percentage is obtained by using a mill with glass balls 1 mm in diameter, for one minute. This method is not suitable for using at lab-scale because of the high biomass losses during its utilization and low selectivity to lipids, however, the use of mechanical destruction becomes convenient in bigger scale, the disadvantage of mechanical destruction methods is the difficulty to recover the extracted oil, and the release of other substances present within the cell. These methods should be used in combination with extraction methods using solvents.

The extraction of pigments from microalgae is achieved by breaking the cells, extraction using solvent or buffer solution, followed by centrifugation to separate the extract from the residual biomass. This filtering can be purified and sterilized partially by microfiltration and spray-dried or freeze-dried [63]. The carotenoid pigments from the biomass of *Dunaliella salina* have been obtained through saponification of the alcoholic extract (to separate them from the chlorophyll), followed by extraction with an apolar solvent. The biomass of *Chlorella vulgaris* has been extracted with 95% alcohol and acetone, and separation of the components of these extracts has been carried out by thin plate column chromatography, using different adsorbents: dextran T40, hydrolyzed starch, sucrose and cellulose [64].

As regards enzyme-assisted extractions, the two-phase biocatalysis of whole cells is a very interesting method to extract bioactive metabolites of high-values present in the microalgae cells. In this procedure, the cells take the dodecane (up to 13 pg/cell), an organic solvent added to stimulate the continued release in vitro and in vivo of β-carotene and its biosynthesis. Due to this "milking" process, larger quantities of β-carotene can be produced than

in the traditional process of commercial production. This method was applied on *Dunaliella salina*, a single-cell microalgae known as one of the richest sources of β-carotene. Mendes-Pinto, Raposo, Bowen, Young and Morais [65], extracted the carotenoids from the microalgae *Haematococcus pluvialis* in autoclave at 121 °C and 1 atm (1 bar), for 30 minutes, obtaining a higher extraction percentage than the other techniques evaluated, such as Spray Drying or enzymatic treatments.

1.2.3. Transformation of microalgae to biofuels

The conversion of microalgae to biofuel can be classified in either a biochemical and thermochemical conversion process. The biochemical conversion processes of biofuel are transesterification and fermentation, which produce biodiesel and ethanol as main products, respectively. The thermochemical processes can be categorized as pyrolysis, liquefaction, gasification and hydrogenation. The pyrolysis and liquefaction processes produces bio oil fuel as the main product, whereas gasification produces syngas and hydrogenation is a process for improving the biofuel or feedstock properties.

Biodiesel is one of the most well-known biofuel products from microalgae. Biodiesel is produced by transesterification with glycerol as co-product. Biodiesel produced from microalgae complies with the US standard for biodiesel, ASTM 6571 [66]. Yields of more than 90% of crude oil can be achieved with conversion conditions of 35–60 ◦C at atmospheric pressure, where the molar ratio of oil to alcohol is 3:1–6:1 [67, 68]. In acid and basic transesterification, the methanol and catalyst are blended before being pumped into a reactor tank, the amounts of the methanol and the catalyst are controlled to avoid excess amounts, which reduces the quality of final product and increases the energy required to remove the excess alcohol.

When enzymatic catalysis is used for biodiesel production, the excess alcohol inhibits the enzyme activity and thus decreases the catalytic activity, this enzymatic process is influenced by the pH of the enzyme itself, the substrates concentration, and the spacing between the enzyme molecules and the substrate. The enzymatic catalyst does not change during the process, and it is effective to reuse it, which could reduce the cost of the process. However, if the enzyme mixes with the product or the solvent, it will require more downstream processing to separate them. In addition, free alcohols such as excess methanol and the produced glycerol, which is insoluble with the crude oil, promote dehydrogenases during the process and thus inhibit the catalytic activity. The methanol to oil ratio can be determined by conducting a laboratory scale experiment before advancing into larger scales to avoid a high excess of methanol. To avoid direct contact with free glycerol, the enzyme catalyst must be immobilized.

Microalgae can be also potentially used for bioethanol production owing to the presence of carbohydrates within their composition and very low lignin percentage, cellulosic material must be removed from the cell wall before they can be used as a feedstock for fermentation; this can be accomplished by ultrasonic and explosive integration [69] or by hydrolytic enzymatic conversion of the biomass into a suitable fermentable feedstock [70]. Acid hydrolysis of microalgae has been also used for reducing sugars release [71], such as a multifunctional process using methanol and ethanol [72]. The most effective enzyme concentration for a good ethanol yield was 0.001–0.05%, based on the volume unit of the enzyme for every weight unit of the feedstock [70].

The pretreatment of microalgal biomass can be carried out with the aid of sulfuric acid, hydrochloric acid or acetic acid. More than 50% of the glucose in a microalgal biomass slurry can be released by using sulfuric acid during the

pretreatment process [73]. Ethanol yields of up to 0.26 g of ethanol per 1 g of microalgal biomass can be achieved [74]. Reducing sugars yields of 2.63 mg/ml were obtained using the microalgae strain *Amphiprora* sp. [75]. The ethanol production of microalgae can be improved by using yeast and an immobilized fermenter. The most preferable yeast for ethanol production is Saccharomyces cerevisiae, which has yields as high as 70 g/l. Engineered yeast can also produce up to 61.8 g of ethanol from 1 l of cornstarch over a 72 h fermentation process [76]. During the fermentation process, the pH is maintained in the range of 6–9. A pH that is below 6 or over 9 could slow down the ethanol formation because of an excess of alkali.

Microalgal pyrolysis has also been recently used for oil biofuel production, The fast pyrolysis of biomass resulted in the production of bio oil (19–57.9% of the final product) and bio char, the slow pyrolysis of biomass, resulted in the production of pyrolysis gas and bio char. Methane and carbon dioxide are the main components of the resulting gaseous product. The bio oil produced from microalgae is more stable than the bio oil produced from traditional crops such as wood, although it is not as stable as fossil fuel, the heating value of bio oil produced from microalgal biomass ranges from 30.7 to 41 MJ/kg. Higher oil yields with less oxygenic compound can be achieved in catalytic pyrolysis. The amount of oxygenic compounds in catalytic bio oil is 19.5% compared to 30.1% in bio oil obtained by direct pyrolysis. An energy recovery of bio oil of approximately 40% can be achieved under catalytic pyrolysis using $NaCO_3$ [77], bio char products of fast pyrolysis have a higher heating value than the bio char products of slow pyrolysis, The overall pyrolysis gas produced was 13–25% of the original biomass, had a heating value of 1.2–4.8 MJ/kg and was mainly composed of 9–17.5% CO_2 followed by 1–1.9% CH_4.

Hydrothermal liquefaction of biomass is a process that requires heating the biomass at high temperatures ranging from 200 to 500 ◦C with pressures greater than 20 bar in the presence of a catalyst. This process resulted in the production of bio oil yields ranging from 9 to 72% and gas mixture yields ranging from 6 to 20% [78]. The product of the liquefaction process is also comparable with crude fuel, where the energy content of the bio oil ranges from 30 to 39 MJ/kg and the gaseous product also contain an energy content of more than 21 MJ/kg. One advantage of liquefaction compared to other thermochemical process is its high tolerance of feedstock moisture content, which can be up to 65%. As liquefaction is the only thermochemical process that does not require a complex drying mechanism, this process is recommended for converting microalgal biomass to bio oil. The optimum reaction temperature for liquefaction suggested by Yang et al. [79] is 340 ◦C, with a residence time of 30 min and a catalyst dosage of 5%. This process can be improved pretreating the microalgae biomass with a catalyst in a surge container before sending it into a blender to make a biomass liquefaction results in the production of bio oil, synthesis gas, vapor, hydrogen and other hot gases.

Gasification of microalgae is a process in which the carbon-based components of the biomass react with air, steam or oxygen at high temperature ranging from 200 to 700 ◦C in a gasifier and involves other thermochemical process such as pyrolysis and combustion. This resulted in production of H_2 with yield ranging from 5 to 56% and CO with yield ranging from 9 to 52%. Methane can be considered to be a co-product and is only produced in small amounts of approximately 2–25% [80]. However, the production of clean methane-rich gas can be achieved in catalyzed supercritical water gasification process where approximately 60–70% of the heating value from the microalgal biomass can be recovered as methane. The hydrocarbon products of gasification can be further processed to produce

methanol. At 1000 ◦C, the methanol production is approximately 64% (w/w) based on the biomass weight. The ratio of energy produced to energy required to produce the methanol from the gasification process is 1.1.

Biomass gasification also produces unwanted products in small quantities such as water, ash and tar, which cause various problems with the main product yield. Moisture contents of up to 40% in microalgal biomass were reported to be tolerable for the gasification process [81]. Increasing moisture content degrades the gasifier performance and the energy content of the syngas produced. The high heating value (HHV) of the produced syngas at 5 and 30% moisture is 5.138 and 3.338 MJ/kg, respectively, showing that the moisture content of biomass has a strong effect on the syngas produced. By increasing the gasification temperature and the catalyst concentration to aid the gasification process, a higher H_2 yield can be achieved. Among the catalysts that are usually used are dolomite, alkali catalysts such as nickel, and potassium carbonate, the catalyst addition also increased the gasification efficiency of microalgal biomass, specifically *Chlorella vulgaris*, up to 84% [82]. The gasification agent also affects the syngas yield. The energy content of the produced syngas was estimated to be 8000 kJ/N m^3.

Hydrogenation process can also be applied directly to convert biomass into bio oil. This process has been used in microalgae [83] achieving 50% oil yield in a batch autoclave with a hydrogen pressure of 0.98–147 bar. In the normal hydrogenation process of an unsaturated substrate, fat and mineral oil are usually reacted with hydrogen in a catalytic fixed-bed reactor. The biomass or unsaturated hydrocarbons are fed into a catalytic fixed bed, resulting in the production of steam. A flash separator is used to separate the hydrogenated feeds into two components: light gasses, such as untreated hydrogen, methane and propane, and a liquid fraction which is then further separated through a fractionation column, resulting in the production of gasoline,

kerosene and biodiesel. However, by today there is limited information about hydrogenation of microalgal biomass.

1.3. OVERVIEW OF BIOREFINERIES

One alternative proposed by researchers for achieving a feasible microalgae use for biofuels production is the incorporation of the biorefinery concept. The term biorefinery has been a part of scientific literature since 2001 [84]. However, in the year 2007, it began to take on an increased significance in publications and reports of scientific events. This term has been defined in several ways. The International Energy Agency describes biorefining as a framework to produce several products including biofuels from a definite feedstock, giving economic competitiveness to the low value biofuels with high value co-products [85]. This concept can be extended, according to Cherubini [86], to a system or a set of systems that can integrate biomass transformation processes and equipment for the production of fuels for transportation, energy and chemicals. The palette of products from a biorefinery not only includes the products obtained in an oil refinery, but also products that cannot be obtained from crude oil. Bio-refineries can produce energy in the form of heat or by producing biofuels, molecules for fine chemistry, cosmetics or medicinal applications, materials such as plastics and sources of human food and animal feed.

This concept can be compared to the current concept of oil refineries with respect to the fractionation of a complex mixture. However, there are two major elements that make them different. The first is the formation of raw materials; because those used in a biorefinery have not undergone the long-time biodegradation leading to crude oil. Therefore, the possibilities of obtaining more products using biomass as a feedstock are greater (see Annex A), the second distinction stems from the application of different

existing and emerging technologies in order to obtain bioproducts. Biorefining involves assessing and using a wide range of technologies to separate biomass into its principal constituents (carbohydrates, proteins, triglycerides, etc.), which can subsequently be transformed into value-added products and biofuels through the application of other processes. Table 1 shows some elements that differentiate the industrial processes that are currently used without applying this concept and the impacts of including biorefining complexes to said processes.

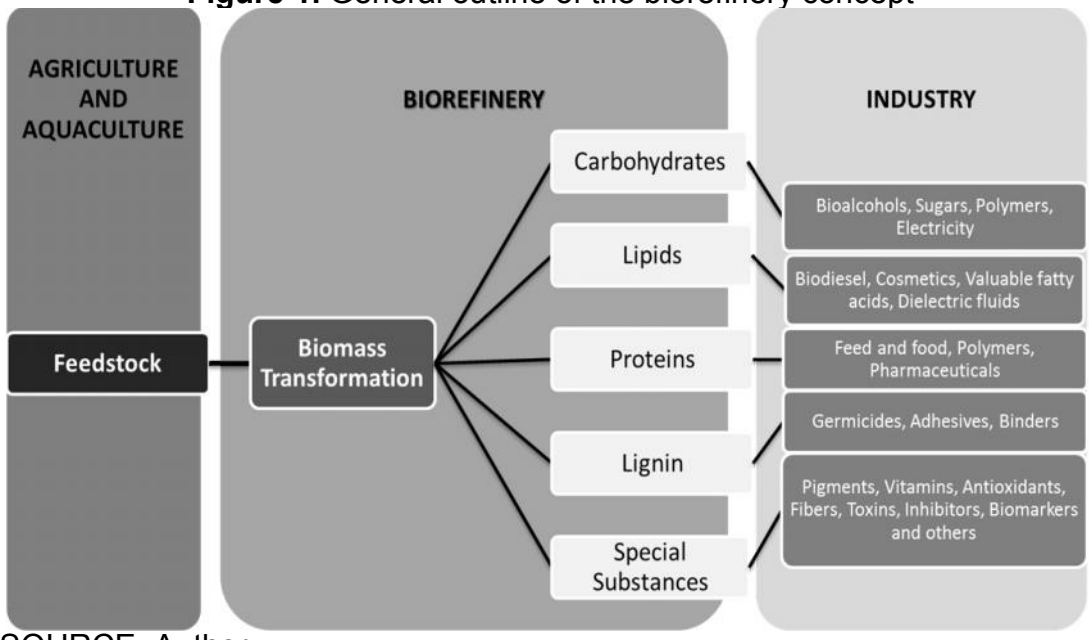
Table 1. Differentiation between traditional transformation processes and biorefineries

Traditional transformation processes	Biorefineries
Linear production chains	Combination of flows of matter from several bioindustries
Materials in competition with food	Reduction in the competition with fertile land by making use of waste
Higher use of fertile land	High productivity of bioproducts per unit area
Limited exploitation	Consumption of a high percentage of energy from biomass
Consumption of non-renewable energy	Positive environmental impacts
Urban industrial zones	Expectation of revitalizing rural areas
Major industrial complexes (oil refinery)	

SOURCE: Author

The palette of products from a biorefinery not only includes the products obtained in an oil refinery, but also products that cannot be obtained from crude. Bio-refineries can produce energy in the form of heat or by producing biofuels, molecules for fine chemistry, cosmetics or medicinal applications, materials such as plastics and sources of human food and animal feed. Figure 1 illustrates the generalized outline of a biorefinery.

Figure 1. General outline of the biorefinery concept



SOURCE: Author

A major impact generated by implementing this concept is related to the sustainable use of local biodiversity, because in the case of the combined production of biofuels and co-products, the sustainable production of biofuels can be achieved by using waste, which reduces fuel imports, leading to self-sufficiency based on raw materials available at the local level. This directly involves research based on the availability and characteristics of biomass sources specific to each region and the assessment of technologies for the sustainable separation and transformation of biomass components.

If the goal of biorefineries is to transform biomass into biofuels and high value-added products, the process synthesis methodologies and the existing and emerging technologies for these transformations have to be reviewed, because in biorefining, these technologies must be applied together. Among the advances necessary for the operation of a biorefinery, Taylor [87], suggests understanding the mechanisms of construction and destruction of the cell walls of the raw materials, the development of new plants with different characteristics, the implementation of biomass transformation

processes as a function of the composition thereof, interdisciplinary tasks and the development of new technologies focused on the raw materials.

1.4. OVERVIEW OF PROCESS DESIGN FOR BIOREFINERIES

The conceptual design of processes belongs to the area of chemical process synthesis, which was pioneered by authors as Douglas [88] and Rudd & Watson [89], process synthesis has had an important impact in the development, of chemical processes, providing systematic methodologies for identifying flowrates, design conditions and optimal networks. Several advances in process synthesis has been achieved in last decades, according to Yuan et al. [90], taking into account proposed strategies, tools and frameworks for process synthesis, three types of approaches can be differenced: heuristic-based approaches which uses specialized knowledge of a process and specific experience, mathematical programming-based approaches which uses a superstructure optimization formulation with an objective function desired, and hybrid approaches, which combines both hierarchical decomposition and mathematical programming. Table 2 shows some characteristics of first two approaches, when compared, each approach presents their own advantages and disadvantages, and selection of approach must depend of additional criteria, as the number of possible solutions to the problem, or the availability of information, more detailed discussions related to aims and scopes of process synthesis approaches, and development of hybrid methodologies can be found in literature [91, 92].

Table 2. Differentiation between most common process synthesis approaches

Hierarchical decomposition based approach	Mathematical programming based approach
Decomposition of a complex problem into several smaller easier problems	Consider explicitly economic exchanges and interactions among subsystems
Five hierarchical levels 1. Batch vs. Continuous 2. Input-output structure 3. Recycle structure 4. Separation and recovery systems 5. Heat exchanger network	Three major steps 1. Postulation of a set of superstructures 2. Formulation of a mathematical programming problem 3. Determination of the optimal solution
Heuristics are used in each level	Based on superstructure optimization formulation
Produces a base-case design	Produces an optimal process topology structure with operating parameters
Does not consider interaction between subsystems	Algorithms limited to moderate-sized problems

SOURCE: Author

According to the tendence of searching total use of a feedstock and the incorporation of With the consideration of various biomass feedstocks, process synthesis techniques have been extended from conventional chemical processes to biorefinery processes [90]. Reviews of designing biorefineries are available in literature (e.g., Stuart and El-Halwagi [93]). Several approaches have been developed for the synthesis and assessment of biorefining pathways. Alvarado-Morales et al., [94] developed a metodology to synthesize and screen processing alternatives in already established biorefinery production routes, taking as case study bioethanol production Tay, et al. [95], proposed a graphical targeting approach for the synthesis of an integrated biorefinery via the C-H-O ternary diagram, taking as case study the biomass gasification.

Optimization-based approaches have also been used for the design of biorefineries, Pham, and El-Halwagi [96] presents a "forward-backward" approach which involves forward synthesis of biomass to intermediate products and reverse synthesis starting with the desired products and identifying necessary intermediate species and pathways leading to them, after that, an optimization formulation is utilized to determine the optimal configuration based on screening and connecting the optimal intermediates generating the biorefinery flowsheet, [97], Ng et al. [98], presented a method based on the screening of competing technologies taking into account thermodynamic and economic criteria, Tan et al. [99], described a methodology based on a fuzzy linear programming for the optimization of multifunctional biofuel systems with flexible targets taking into account production levels and environmental sustainability. Ng [100], presented a procedure for automated targeting for the synthesis of an integrated biorefinery. Martín and Grossmann [101], presented a superstructure optimization for the production of lignocellulosic ethanol via gasification of switchgrass, taking into account energy and economic issues, Ojeda et al. [102], proposed a combination of computer-aided process engineering and exergy analysis for the evaluation of different routes for the production of second generation biofuels from lignocellulosic biomass, Kokossis et al. [103], developed a methodology for synthesizing complex manufacturing chains or networks in biomass based manufacturing systems, considering manufacturing process models, manufacturing performance models, logistics performance models and superstructure, Bao et al. [104], proposed a shortcut method for the preliminary synthesis of process-technology pathways for the conceptual design of integrated biorefineries based on a superstructure representation with layers of chemical species and conversion operators using an optimization function for obtaining a desired biorefinery pathway, Tay, Ng, & Ng, [105], proposed a modular optimization approach for biorefinery optimization decomposing the large optimization problem into

small models composed of a process unit and its alternatives in different degree of modeling details, in the field of feedstock supply, Čuček, Martín, Grossmann & Kravanja [106], performed a multi-period synthesis of supply networks for an optimally-integrated regional biorefinery.

Over the last few years, several important contributions have been made in the design and analysis of microalgae to biodiesel production chains from techno-economic, energetic and environmental perspectives, Pardo-Cárdenas, et al. [107], performed an environmental assessment of several alternatives for microalgae biodiesel production using the methodology of life cycle assessment (LCA), Pokoo-Aikins et al. [108], assessed the design from techno-economic point of view of an integrated system for biodiesel production from microalgae oil with sequestration of CO_2 from a power plant, Ofori-Boateng et al. [109], used exergy analysis to study the feasibility of microalgal and jatropha biodiesel production plants using three triglycerides as representative microalgae oil, Sánchez et al. [110], analyzed biodiesel production from microalgae with two reaction stages (esterification and transesterification) using heat integration techniques, Davis et al. [111] made a techno-economic analysis of autotrophic microalgae for production of “green diesel”, Peralta et al. [68] evaluated biodiesel production from microalgae oil from the exergy perspective. Delrue et al., [113], developed a model of biodiesel production from microalgae taking into account the net energy ratio (NER), production costs, greenhouse gases (GHG) emission rate and water footprint.

2. CHAPTER II. DESIGN AND ADJUSTMENT OF METHODS FOR MICROALGAE OIL EXTRACTION IN LAB-SCALE[2]

2.1. INTRODUCTION

Microalgae has the potential to produce a big amount of oil per area unit owing to its high lipid content which in some strains under appropriate cultivation conditions exceeds the lipid content of all biodiesel sources used currently [113], in addition, microalgae are cultivated in photobioreactors and open ponds which only needs water, some nutrients and sunlight to stimulate growing, these culture conditions makes feasible the using of non-crop lands for photobioreactor or open pond assembly. The use of microalgae for biodiesel production is an advantageous alternative because of the high lipid content and fatty acid profiles that suitable offers.

The biorefinery concept has been identified as the most promising way for the creation of an industry based on biomass. This concept can be applied microalgae biomass for the production of biofuels and high added value products based on the composition of promising species, a microalgae based biorefinery must take into account several issues for its sustainability as water requirements, production costs, environmental impacts and process efficiency [114].

Studies about microalgae oil extraction for biodiesel production are taking significance because the efficiency of biodiesel production chain from microalgae based on oil transesterification depends in a great way of the oil

[2] This chapter is based on the paper "*Design and adjustment of coupled microalgae oil extraction methods for the development of a topology of biorefinery*" by Angel Darío González Delgado & Viatcheslav Kafarov, published in Prospectiva Journal Vol. 10 (1) 113-123 (2012).

extraction efficiency. Oil extraction methods can be divided into physical, chemical and enzymatic [115]. Solvent-based lipid extraction methods as Folch, and Bligh and Dyer's method [116], has been used for obtaining lipids from microalgae. Using a mixture hexane-ethanol, can be extracted around of 80% of fatty acids presents into biomass [117], hexane is frequently used for soxhlet extraction using microalgae biomass as a raw material [118], hexane is cheap, easy to recovery after extraction and is selective to neutral lipids, Ethanol with acid has been used for simultaneous cell disruption and lipid extraction using microalgae strains *Amphiprora* sp. and *Navicula* sp. [119].

The main objective of this work is to establish different solvent based high detailed methodologies for the cell wall disruption and lipid extraction of microalgae for the development of a topology of biorefinery through the evaluation of operating conditions for each step in each method, after that, best operating conditions as cell disruption as solvent extraction are assembled and adjusted to a coupled extraction method for future integration in a microalgae based biorefinery concept.

2.2. METHODOLOGY

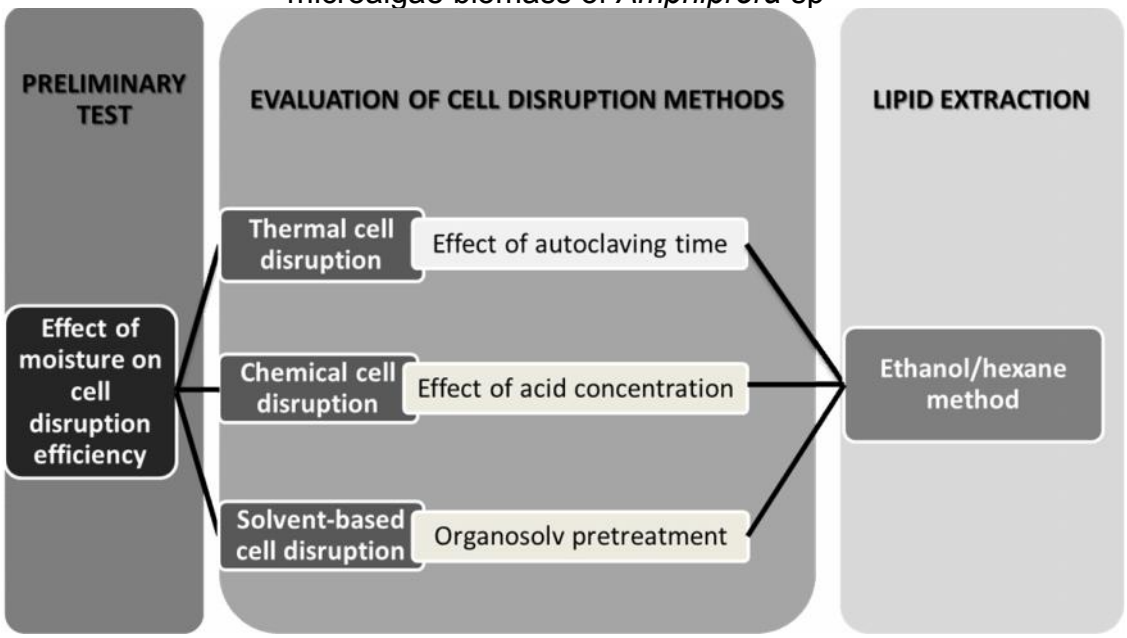
Microalgae biomass was provided by Corporación Instituto de Morrosquillo (Punta Bolivar, Colombia), algae was cultured in F/2 medium, grown in open ponds and harvested by flocculation (150 ppm $FeCl_3$), biomass was sun-dried and frozen until using, all experiments reported were made by triplicate, values in figures corresponds to mean value of measurements.

2.2.1. Cell disruption experiments

General methodology for cell disruption experiments is shown in Figure 2, all raw materials supplied were dried in a convection digital oven (make WTC

binder) at 105 °C for 4 hours, based on the standard NREL/TP-510-42621, and then, the sample was homogenized. A 25 L Autoclave was used for thermal cell disruption experiments, microalgae biomass was exposed at autoclaving conditions of 394.15 K and 103,410 Pa. by 1 and 3 hours.

Figure 2. Methodology for evaluation of cell disruption methods using microalgae biomass of *Amphiprora* sp

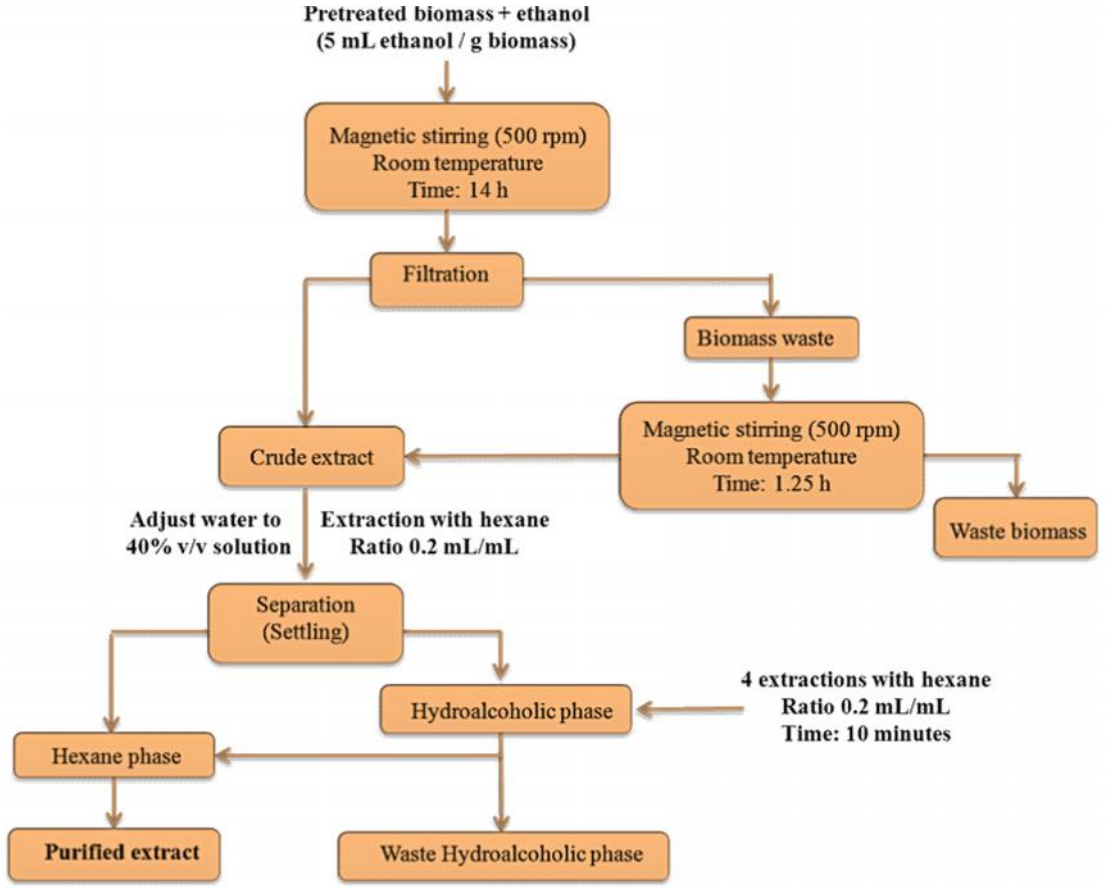


SOURCE: Author

For solvent-based cell disruption, an organosolv pretreatment previously developed by authors was performed using a mixture of water, an organic solvent and an acid at high temperatures [120]. For cell disruption using acid hydrolysis, microalgal biomass were dried in an oven at 378 K for 4 hours, after that, 3 g of biomass were mixed with different HCl solutions at concentrations of 0.1 mol L^{-1}, 0.5 mol L^{-1}, 1 mol L^{-1} and 3 mol L^{-1} with an exposure time of 0.5 hours with magnetic stirring at room temperature. Solid and liquid phases were separated by filtration and biomass is washed with distilled water, biomass was dried and submitted to lipid extraction, all cell disruption experiments were made by triplicate.

After pretreatment, biomass was separated from the liquor by vacuum filtration. Separated biomass was washed with distilled water and dried in oven at 378.15 K for 4 hours. For measurements of the effect of pretreatments on oil yield, lipid extraction using the mixture ethanol/hexane method (EHE) described in Figure 3 was used, biomass was mixed with ethanol using a ratio of 1:5, mixture was stirred by 14 hours at 500 rpm. After that, the mixture was filtered and solid phase was stirred again with fresh ethanol, two liquid phases were combined, hexane and water were added for two liquid phases formation, the phases were separated and fresh hexane is added again to hydroalcoholic phase, this process is repeated three times, the four hexane phases were mixed and lipid extract is separated of hexane by distillation, the quantification of lipid extract was determined with the aim of evaluating the performance of the process and obtains an indirect measure of the effect caused by pretreatment of cells.

Figure 3. Lipid extraction methodology for cell disruption experiments on microalgae biomass (EHE method)



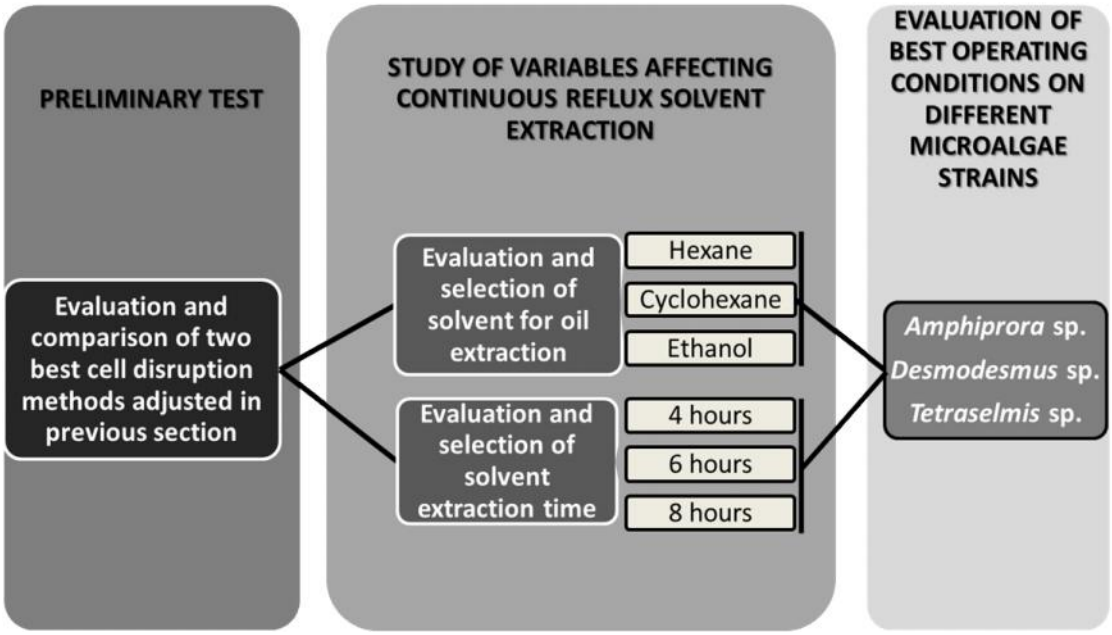
SOURCE: Author

2.2.2. Continuous reflux solvent extraction (CSE)

For continuous reflux solvent extraction evaluation, a typical Soxhlet extractor with 45/50 outer/upper and 24/40 lower/inner joint, for 250 mL capacity was used, each experiment was performed with 5 gr of dry treated biomass, three commonly used extraction solvents were evaluated; hexane, cyclohexane and ethanol. These solvents were chosen taking into account their low boiling point, costs, safety factors and toxicity. In the next phase, after selecting the cell disruption method and the solvent for lipid extraction, the extraction time was evaluated, using values of 4, 6 and 8 hours (based on literature review [118]).

During solvent extraction, the amount of biomass and the ratio biomass/solvent were kept constant. After extraction, extract-solvent mixture was filtered, distilled and the remnant solvent was evaporated. Total lipids were also quantified gravimetrically, in the final phase, the best experimental conditions for the oil yield were applied to the three genera studied, Figure 4 shows the methodology proposed.

Figure 4. Methodology for continuous reflux solvent extraction adjustment (CSE)



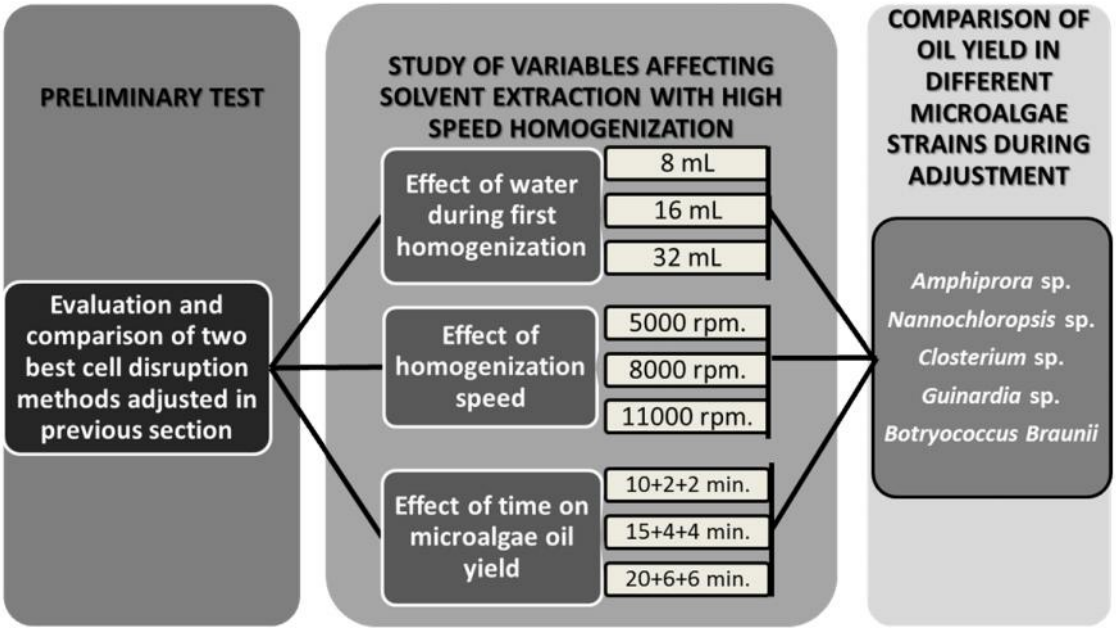
SOURCE: Author

2.2.3. Solvent extraction with high speed homogenization (SHE)

The solvent extraction method combined with high speed homogenization is based Folch and Bligh & Dyer's method, solvents chosen were methanol and chloroform, the methodology to adjust is shown in Figure 5, and includes the steps of stirring, centrifugation, separation and volatilization. In the stirring phase, two rates of biomass/ solvent were evaluated 1:10 and 1:20 based on preliminary test results, the effect of adding water in the first part of homogenization and the effect of time and frequency of homogenization,

accord to an experimental design. Centrifugation was carried out for 15 minutes and it was assessed a frequencies of 2500 and 3400 rpm. The phase separation was performed by removing the upper phase methanol/water from the centrifuge tube while lower biomass/lipids Chloroform, was filtered by gravity. Finally, the lipid extract was allowed to volatilize to constant weight for its measurement.

Figure 5. Methodology for adjustment of Solvent extraction with high speed homogenization (SHE)



SOURCE: Author

For each experiment, 5 g of disrupted biomass were mixed with methanol and chloroform in a ratio 2:1 three homogenization frequencies were evaluated (5000, 8000 and 11000 rpm.) using a Heidolph® SilentCrusher homogenizer. variables were evaluated following a 2^2 central composite experimental design, phases were separated by centrifugation and filtration and lipids were recovered from chloroform phase by evaporation, statistical analysis of main effects was made using STATISTICA 7.0 software taking as a response variable the lipid extract yield concentration. Oil yield for all experiments was

measured by gravimetric method. Each experiment was performed by triplicate in order to give reproducible results.

2.3. RESULTS AND DISCUSSION

2.3.1. Design and adjustment of cell disruption methods

2.3.1.1. Effect of moisture on cell disruption efficiency. Table 3 shows the yields obtained from the lipid extraction process using wet and dry biomass. These tests were performed with a mixture of *Navicula* sp*. and Amphiprora* sp., it is shown that the water content in the sample is not favorable for the extraction of lipids due to two reasons; Presence of water in the sample decreases the concentration of ethanol in the biomass / solvent mixture during the first stage of the process, reducing the efficiency of solvent extraction of crude oil.

Table 3. Effect of moisture on cell disruption: L_R: (%) lipid recovery in the total biomass; Y: (%) losses of biomass by handling (strains: mixture of *Navicula* sp. and *Amphiprora* sp.)

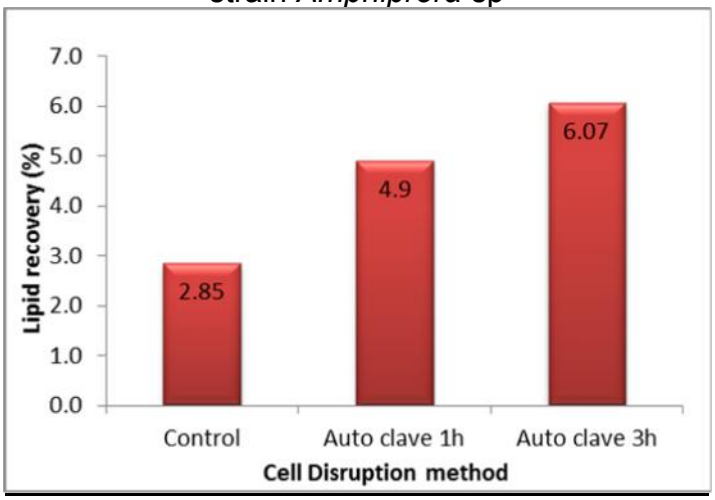
Moisture (%)	Extracts Weight (g)	Standard deviation	L_R (%)	Y (%)
80	0.0337	0.0028	1.5	30.8
>5	0.0629	0.0033	2.8	9.4

SOURCE: Author

2.3.1.2. Effect of autoclaving time. Thermal pretreatment results for microalgae strain *Amphiprora* sp. are shown in Figure 6. Although cell disruption process shows a significant increase in the recovery rate of lipids for an autoclaving time of 3 hours, failed to overcome any of the results obtained with the other pretreatments. The recovery percentages for autoclave times evaluated do not differs more than 1.2 % w/w despite

increased exposure time to 2 hours. This allows inferring that long times represents large and unnecessary energy expenditure.

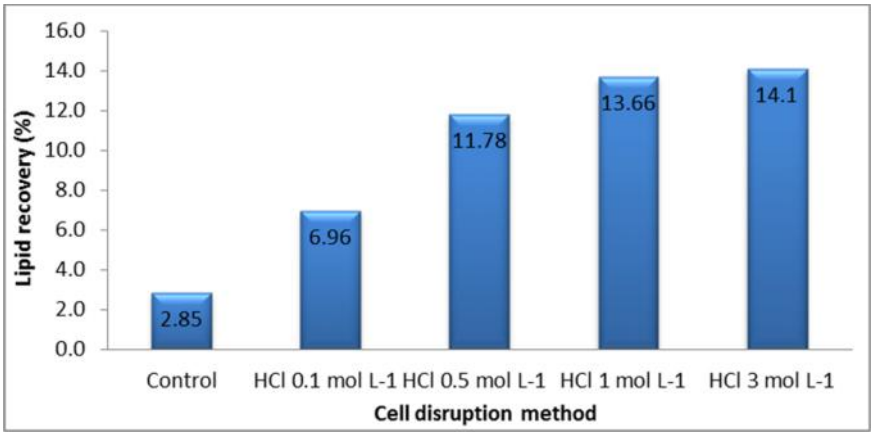
Figure 6. Effect of autoclave time on the recovery rate of lipids for microalgae strain *Amphiprora* sp



SOURCE: Author

2.3.1.3. Effect of HCl concentration. Effects of hydrochloric acid concentration on the extraction yield were also evaluated, using concentrations of 0.1 mol L^{-1}, 0.5 mol L^{-1}, 1 mol L^{-1} and 3 mol L^{-1}. Figure 7 shows that the extraction yield increases when acid concentration is also increased within the range set but at concentrations higher than 0.5 mol L^{-1}, this effect is less pronounced with a tendency to stabilize.

Figure 7. Effect of acid concentration on the recovery rate of lipids using microalgae biomass of *Amphiprora* sp



SOURCE: Author

Cell disruption with HCl 3 mol L^{-1} presents the highest percentage of lipids recovery, however, this concentration involves the increase of acid amount several times for very little yield increase in comparison with the oil yield obtained with an acid concentration of 0.5 mol L^{-1}, corresponding to 11.78 % w/w. In addition, higher concentrations of hydrochloric acid might increase the levels of corrosion in the equipment involved throughout the process. Therefore, a solution of 0.5 mol L^{-1} hydrochloric acid was the most suitable for pretreatment of biomass, 250 % decline in spending on chemical agent worked to the maximum concentration, without affecting performance deeply.

2.3.1.4. Organosolv Pretreatment. Results obtained of applying organosolv pretreatment to microalgae biomass of *Amphiprora* sp. are shown in Table 4, although organosolv pretreatment increased the recovery rate of lipids in more than 3 % w/w over 3 hours of autoclave treatment, did not surpass the results obtained with HCl 0.5 mol L^{-1}. In addition, this pretreatment involves high energy costs, a longer exposure time and use of more chemicals making it inconvenient to use as pretreatment method prior to cell disruption with ethanol-hexane if the only one product desired is microalgae crude oil.

Table 4. Organosolv pretreatment results for *Amphiprora* sp. strain: L_R: % w/w lipid recovery in the total biomass; L_R: % w/w % w/w Biomass unrecovered

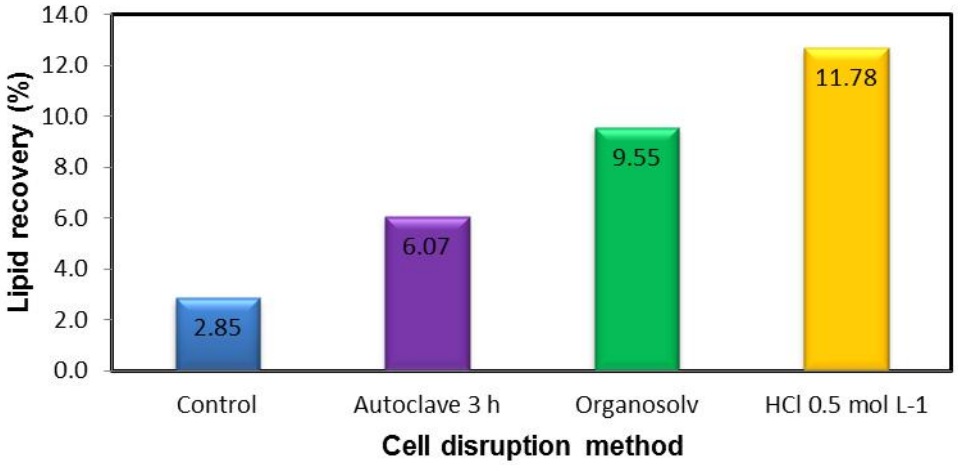
Pretreatment	L_R (%)	B_N (%)
Organosolv	9.55	71.6

SOURCE: Author

2.3.2. Comparison of cell disruption methods

Values obtained in the response variable (L_R) shows clearly that the chemical treatment with HCl 0.5 mol L^{-1} and organosolv pretreatment gives the highest oil yield when ethanol/hexane method is used for microalgae oil extraction (Figure 8).

Figure 8. Comparison of cell disruption methods for *Amphiprora* sp. biomass



SOURCE: Author

Method selected for oil extraction in this section presents lower yields compared to traditional procedures for the recovery of lipids, but the product obtained is mainly composed of neutral lipids due to the selectivity of hexane, this being the most suitable fraction for later processes of esterification, transesterification or hydro-treatment, taking into account this results, acid

hydrolysis and organosolv pretreatment were taken into account as cell disruption methods in further sections of this work.

2.3.3. Adjustment of continuous reflux solvent extraction coupled with cell disruption (CSE).

Best cell disruption methods obtained in previous section were applied to microalgae biomass and a continuous reflux solvent extraction was applied for lipids recovery. Highest oil yield was obtained with organosolv pretreatment (6.8%) in comparison with HCl 0.5 mol $L^{-}1$. In addition, when organosolv pretreatment was used, the oil yield was increased three times in comparison with the control. This difference can be attributed by the degree of hydrolysis of the cellulosic cell wall components of microalgae according to each disruptor agent and operation conditions of treatment. Then, the lipids are exposed to higher or lower proportion to the solvent extraction and the oil yield is affected. Efficiencies of the extraction process using cell disruption methods are shown in Table 5, it can be seen also that all extraction efficiencies using continuous reflux solvent extraction are higher than efficiencies obtained using ethanol/hexane method.

Table 5. Comparison of best adjusted cell disruption methods using continuous reflux solvent extraction (CSE) for microalgae biomass of *Amphiprora* sp

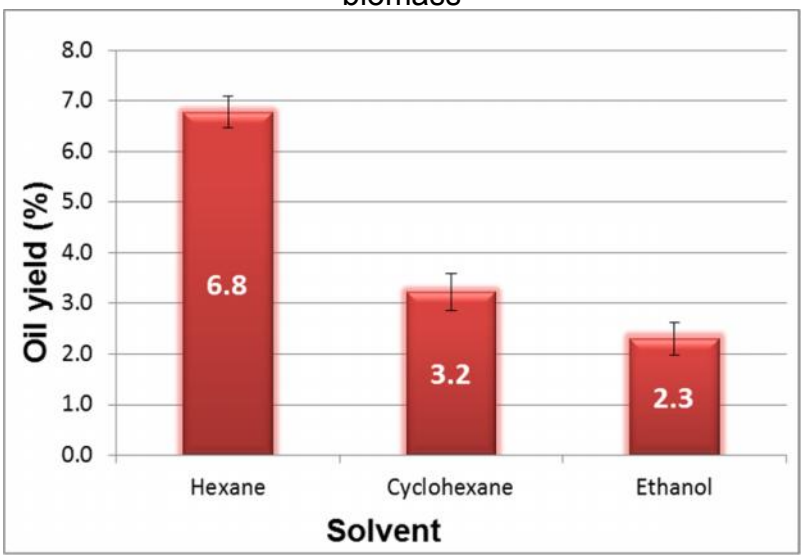
Cell disruption method	Extraction efficiency (%)	Standard deviations
Control	18.0	2.49
Organosolv	56.5	2.54
Hydrochloric acid 0.5 mol L^{-1}	37.9	1.78

SOURCE: Author

2.3.3.1. Solvent Selection. By using hexane, cyclohexane and ethanol as solvents in extraction process, it was shown that the hexane presents higher

loss of solvent. However, as evidenced in Figure 8, this solvent produced the greatest oil yield (6.8%) relative to cyclohexane (3.2%) and ethanol (2.3%). It is also the cheapest solvent of the three tested, also is selective to neutral lipids and commonly used in solvent extraction processes chemicals. Besides, when performing the extraction with cyclohexane was obtained the second highest oil yield (3.2%), but this is the solvent most expensive of the three solvents studied.

Figure 9. Oil yield with different solvents for *Amphiprora* sp. microalgae biomass



SOURCE: Author

Table 6. Effect of solvent on extraction efficiency using microalgae strain *Amphiprora* sp

Solvent	Extraction efficiency (%)	Standard deviations
Hexane	56.5	2.54
Cyclohexane	26.9	3.03
Ethanol	19.3	2.74

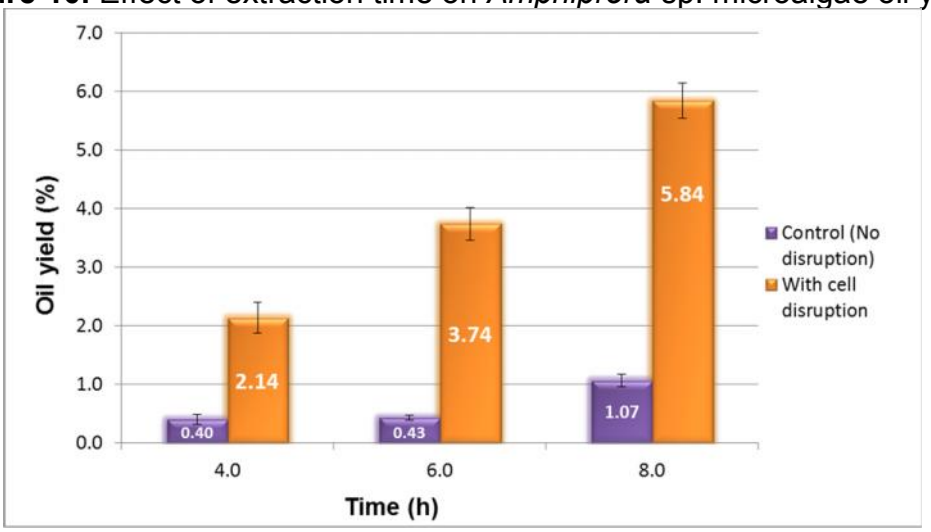
SOURCE: Author

Ethanol is known to be a good solvent for extraction, but its selectivity towards the lipids is relatively low compared with hexane and cyclohexane, and it is necessary to perform a purification process (e.g. treating the crude

extract with non-polar solvents) to obtain the extracts. Ethanol had the lowest oil yield (2.3%). Also, as shown in Table 6 with the use of hexane was achieved, the highest extraction efficiency (56.5%) is reached, and solvent hexane shown higher reproducibility of the data according to the standard deviation calculated.

2.3.3.2. Effect of extraction time. Effect of extraction time is observed clearly in Figure 10, when the contact time between solvent and biomass was increased, there was a significant impact on the oil yield, because it promotes the mass transfer of lipid components into the solvent, reaching a higher oil yield (5.8%) when the sample was extracted for eight hours and with an increasing trend for higher times. In the same way when compared the results with (dark bars) and without cell disruption method (white bars), there was an increase of more than five times in the oil yield, for all operation times evaluated.

Figure 10. Effect of extraction time on *Amphiprora* sp. microalgae oil yield



SOURCE: Author

Furthermore, the extraction time of eight hours produced the best extraction efficiency of 53.6% as is reported in Table 7.

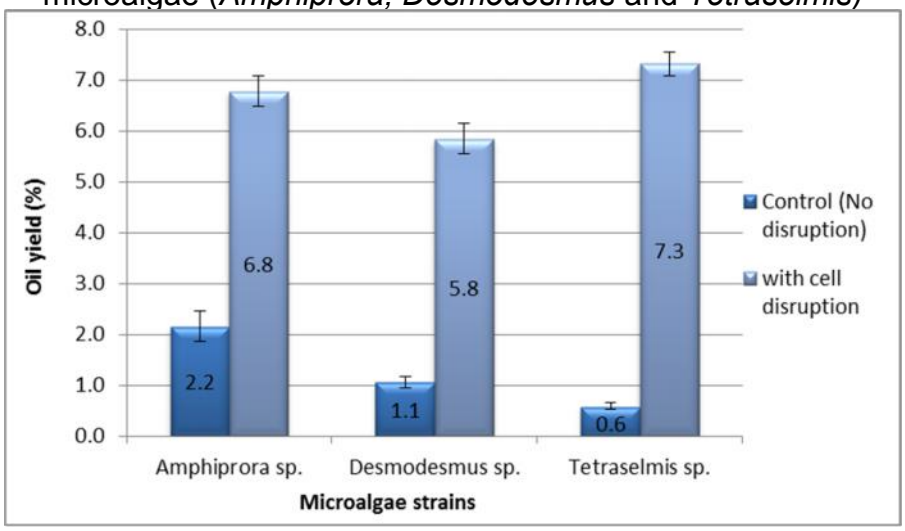
Table 7. Lipid extraction efficiency in relation to operation time for *Amphiprora* sp. strain

Cell disruption method	Extraction time (h)	Extraction efficiency (%)	Standard deviations
Biomass without disruption (control)	4	3.7	0.83
	6	3.9	0.36
	8	9.8	0.98
Biomass with organosolv pretreatment	4	19.6	2.38
	6	34.3	2.57
	8	53.6	2.74

SOURCE: Author

2.3.3.3. Best experimental conditions obtained. When the best experimental conditions according to the higher oil yield (organosolv pretreatment as cell disruption method, hexane as solvent and 8 hours of operation time) were applied, results obtained were the shown in Figure 11 for the three genera studied.

Figure 11. Oil yield with the best (CSE) conditions for the three genera of microalgae (*Amphiprora, Desmodesmus* and *Tetraselmis)*



SOURCE: Author

These results confirm the advantage of applying a cell disruption method before extraction process, it can achieve significant increases in the oil yield for biomass without disruption, and increments of three, five and twelve times

oil yield for genera *Amphiprora, Desmodesmus* and *Tetraselmis* respectively. Also as shown in Table 8, for all genera of microalgae was obtained a superior process efficiency to 50% using the best conditions of the variables analyzed, getting the highest value 57.6% for the genus *Tetraselmis*.

Table 8. Oil extraction efficiency with adjusted (CSE) method for several microalgae strains

Strain	Extraction efficiency (%)	Standard deviation
Amphiprora sp.	56.5	2.54
Desmodesmus sp.	53.6	2.74
Tetraselmis sp.	57.6	1.86

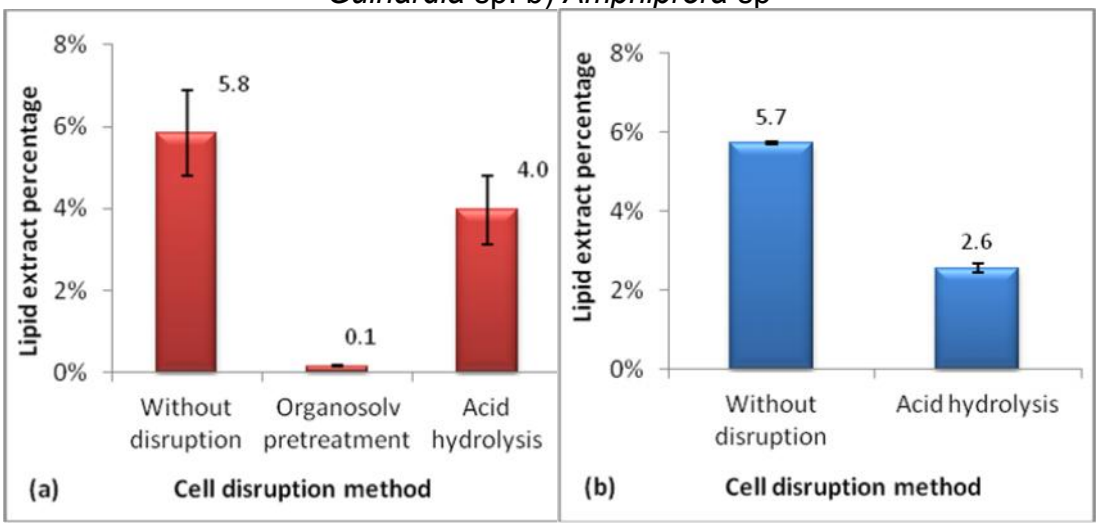
SOURCE: Author

2.3.4. Adjustment of solvent extraction with high speed homogenization (SHE).

In the stirring stage, when the biomass/solvent ratio 1:10 was initially evaluated, there was no lipid extract obtained because the rate of volatilization of chloroform was higher than the rate of filtration of the mixture, leaving all biomass retained in the filtration stage. While performing the extraction at a biomass/solvent ratio of 1:20 this problem was overcome and it was decided to maintain this ratio for further experiments. On the other hand, in the stage of centrifugation, when the frequency was adjusted according with literature in 2500 *rpm,* there was no a complete separation of the solvents mixture, for this reason, centrifugation frequency was increased to 3400 *rpm,* in this case, it was identified the biphasic system composed by a methanol and water in the upper phase and lower chloroform-lipids-biomass. Therefore, for the development of the extraction method, centrifugation stage was tuned in 3400 *rpm* during a time of 15 minutes.

2.3.4.1. Effect of cell disruption. Given that cell wall of microalgae is destroyed by the degradation of the polysaccharides present in biomass, and these and other components of the solid matrix are soluble in liquor of hydrolysis, a large percentage of the biomass subjected to the cell disruption process becomes part of the liquor, reducing the biomass used for extraction.

Figure 12. Effect of cell disruption on the percentage of extraction for. a) *Guinardia* sp. b) *Amphiprora* sp



SOURCE: Author

Figure 12a shows that acid hydrolysis and organosolv pretreatment did not increase the percentage of lipid extract in this extraction method. Based on these results, the organosolv pretreatment was discarded for further testing due to low percentages of lipid extracts obtained an the difficulty in the development of extraction. While the cell disruption with acid hydrolysis even when it reported a 32% decrease in performance continued to be the subject of study because it made easier the steps of centrifugation and filtration when extracting. After that, new tests were performed using acid hydrolysis in *Amphiprora* specie (Figure 12b) to verify that the negative effect of this method to other specie was still getting a 55% reduction in the yield of extraction.

The low extraction yields using biomass with cell disruption respect to biomass without cell disruption are due to the microalgae solvent extraction with high speed homogenization in particular, in addition to lipids, it also extracts significant amounts of non-lipid components. By previously applying cell disruption method this lipid components become part of hydrolysis liquor thus obtaining a purer lipid extract compared to the extraction using biomass without cell disruption. That is, the application of a cell disruption method allows obtaining purer extracts after lipid extraction performed with the solvent extraction with high speed homogenization, but decreases the percentages of extraction. For third method can be concluded that use of acid hydrolysis or organosolv pretreatment is not necessary because cell disruption is performed by the high speed homogenization process.

2.3.4.2. Effect of water addition during first high speed homogenization. The percentage of lipid extract obtained for two different microalgae genera with and without addition of water in the first part of the stage of agitation is shown in Table 9, where it is observer that the addition of water decreased the rate of extraction for *Amphiprora* sp., *Botryococcus* sp. and *Nannochloropsis* sp., by 15% and 40% respectively. This is because water is soluble in methanol and insoluble in chloroform and lipids, which affects the solubility of chloroform-methanol and make it difficult to extract lipids. Based on these results it was decided to avoid water addition during first part of the stirring.

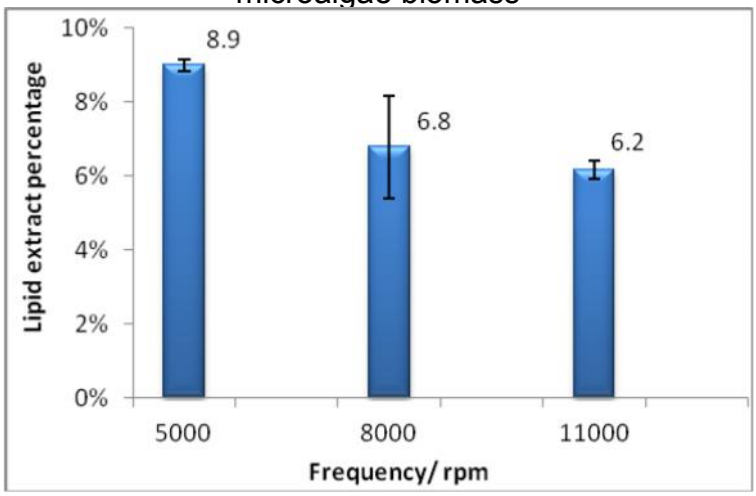
Table 9. Effect of water addition in the first stirring step during the solvent extraction with high speed homogenization (SHE) for three microalgae strains

Microalgae genera	Water (*mL*)	Oil yield (%)
Amphiprora sp.	–	8.83
	8	8.19
Botryococcus sp.	–	5.60
	16	4.74
Nannochloropsis sp.	–	1.45
	32	0.89

SOURCE: Author

2.3.4.3. Effect of shaking rate. In order to study the effect of shaking rate on extraction yield, cell disruption was performed by organosolv pretreatment and the extraction was carried out homogenizing the biomass/solvent mixture for 14 minutes at frequencies of 5000, 8000 and 11000 *rpm.*

Figure 13. Effect of shaking frequency on oil yield using *Amphiprora* sp. microalgae biomass



SOURCE: Author

Figure 13 shows that an increase in the shaking frequency decreases the percentage of lipid extract, this result agrees with that reported by Cravotto et al., [121], who evaluated the ultrasound-assisted extraction using frequencies between 19 and 300 *kHz* obtaining higher extraction yields at lower

frequencies. For that reason it was proposed an experimental design in order to examine together the variables time and frequency of shaking.

Table 10. Values and levels of the studied variables

	Levels		
Factor	-1	0	1
Time / *min*	14	23	32
Frequency / *rpm*	5000	8000	11000

SOURCE: Author

The variables studied in the experimental design were: the total of homogenization time (*min*) and the frequency of shaking (*rpm*). Table 10 shows the values of the levels selected for each of the variables of experimental design.

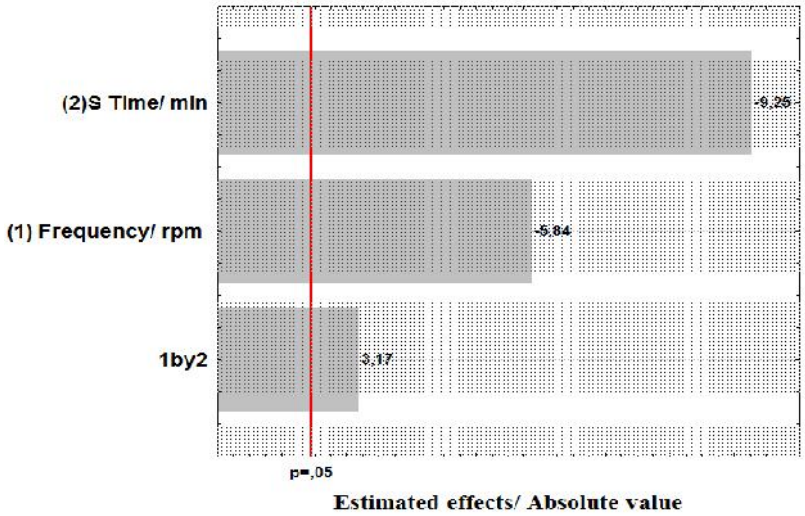
Table 11. Experimental design matrix and oil yield obtained during extraction of microalgae oil from *Amphiprora* sp

N° Experiment	Frequency	Time	Oil yield (%)
1	-1	-1	9.13
2	1	-1	6.34
3	-1	1	5.30
4	1	1	4.46
5	0	0	6.43
6	-1	-1	8.86
7	1	-1	5.98
8	-1	1	4.87
9	1	1	4.03
10	0	0	5.21

SOURCE: Author

The experimental design matrix and its respective percentages of lipid extract obtained are shown in Table 11. The best results are at the lowest level of each variable and are corresponding to experiments 1 and 6.

Figure 14. Effect of frequency and shaking time on the percentage of lipid extract from *Amphiprora* sp. strain

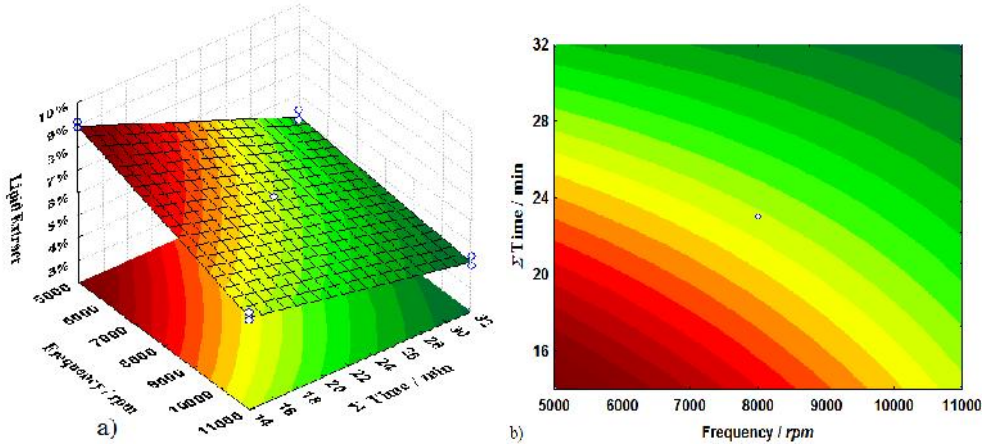


SOURCE: Author

The Pareto's chart (Figure 14) shows that time, frequency and their interaction have significant effects on extraction, because all the blocks pass the threshold. In addition, can be inferred that the variables of time and frequency have negative effects on the performance of the extraction of lipids from microalgae, being the time the factor that mostly negatively affects the response variable. Finally, it should be noted that the combination of the independent variables has a positive effect on the response variable studied.

The interaction between time and frequency of homogenization can be seen in Figure 15 where the surface and the level curve shows a region with the higher (bottom right side) and another with a lower percentage of lipid extract (upper left side). Finding that the percentage of lipid extract is maximized when the variables of time and shaking are found on the lowest level within the experimented region, i.e. 14 minutes and 5000 *rpm.*

Figure 15. Effect of frequency and shaking time on the percentage of lipid extract for *Amphiprora* sp. microalgae. a) Response surface plot b) contour diagram



SOURCE: Author

2.3.4.4. Extraction efficiency. The extraction efficiency is shown in Table 12. The *Nannochloropsis* sp. strain presented the lowest yield, *Closterium* sp., and *Botryococcus Braunii* approached a yield of 50% and best results were obtained using the strains *Amphiprora* sp. and *Guinardia* sp. This high performance was due to the rapid separation of the lipid extract and the solid in the filtration stage. Are also shown the differences in the efficiencies with the adjusted method and operating conditions reported in literature.

Table 12. Extraction efficiency for several strains of microalgae using SHE method: [a, b, c] base extraction method, [d]method adjusted

Strains	Total lipids (%)	Extracted Lipids (%)	Efficiency (%)
Nannochloropsis[a] sp.	11	1.50	13
Botryococcus braunii[b]	15	5.60	37
Closterium[c] sp.	22	9.10	41
Amphiprora[d] sp.	12	9.03	75
Guinardia[d] sp.	7	5.80	87

SOURCE: Author

2.4. CONCLUSIONS

Three methods of microalgae oil extraction by combining cell disruption and solvent based lipid removal and recuperation were designed and adjusted, towards the development of a topology of biorefinery, different alternatives for microalgal biomass rupture were evaluated, showing that for all cases, the incorporation of a cell disruption stage (chemical or mechanical) increases the lipid extraction efficiency, with chemical cell disruption, the recovery rate of lipids was proportional to the concentration of hydrochloric acid within the range established for the pretreatment of biomass. However, an acid concentration of 0.5 mol L^{-1} was the most suitable for the cell disruption process, reducing by 250 % w/w reagent consumption compared to the maximum concentration worked, without significantly affecting the extraction yield, organosolv pretreatment also showed high efficiency on the increase of lipid yield for extraction methods without homogenization.

Adjustment of continuous reflux solvent extraction also corroborates convenience of cell disruption, organosolv pretreatment (56.5%) was the most efficient in this case. Higher oil yield was reached using hexane as solvent and an operating time of eight hours, these conditions increased significantly the efficiency of the process (56.5% and 53.6% respectively). Furthermore, using the best experimental conditions, the extraction efficiency was over 50% for the algae strains *Amphiprora* sp., *Desmodesmus* sp. and *Tetraselmis* sp.

For solvent extraction with high speed homogenization best operating conditions were: Biomass/ solvent ratio 1:20, homogenization frequency 5000 rpm, homogenization total time 14 minutes and centrifugation time of 3400 rpm by 15 minutes. Moreover, the addition of water in the first part of stirring facilitated the filtration but decreased the percentage of oil extraction in a

range between 15-40%. Use of acid hydrolysis or organosolv pretreatment is not necessary because cell disruption is performed by the high speed homogenization process.

3. CHAPTER III. MULTIPARAMETER COMPARISON OF ADJUSTED OIL EXTRACTION METHODS IN LAB-SCALE[3]

3.1. INTRODUCTION

The progressive replacement of oil with biofuels will require certain changes in the current production of goods and services. For this reason, research about sustainability of biofuels production from renewable resources is increasing [122]. According to Chisti Y [123], energy production, goods and services are necessary, but they must be socially, economically and environmentally sustainable. Microalgae is an energy source that offers considerable amounts of fuel from small crop areas and lower production costs, which further helps in the mitigation of global warming; its culturing tolerates high concentrations of CO_2 and decreases the amount of nitrogen oxides released into the atmosphere. The most conventional biodiesel-from-microalgae production chain until now is composed by the stages of cultivation, harvesting of biomass, drying, lipid extraction and oil transesterification [124].

Despite of continuous and positive advances in algal research, biodiesel-from-microalgae production chain is not sustainable yet, in energy terms, comparison of energy demands for microalgal biodiesel production shows that energy required in all stages of production process is more than energy produced by third generation biodiesel [125], In this sense, results of studies related to bioprospecting, exploration and production of microalgae biomass

[3] This chapter is based on the paper "*Microalgae Based Biorefinery: evaluation of oil extraction methods in terms of efficiency, costs, toxicity and energy in lab-scale*" by Angel Darío González Delgado & Viatcheslav Kafarov, published in ION Journal Vol. 26 (1), 29-37. (2013).

made by research centers as the NREL In United States, the CISOT and CIEMAT in Spain [126], the CIDES and ICP in Colombia [127], among others, concludes that production of biodiesel from microalgae can be economically viable if total biomass components are used for obtaining biofuels and high value products and the concept of biorefinery is incorporated.

The extraction of carbohydrates, lipids, pigments, proteins and special substances from microalgae biomass is under research for obtaining several bioproducts [128] focusing on the use of multifunctional processes for simultaneous extraction separation and transformation of two or more desired products [129], or in optimization of operating conditions and routes for obtaining a desired specific metabolite, pigments extraction can be made by cell breaking, solvent extraction and centrifugation, and purification is made using microfiltration, drying or lyophilization [130], reducing sugars can be obtained by hydrolysis reaction with simultaneous cell wall disruption for oil extraction [131], proteins are extracted for use as fertilizer [132], animal feed supplement [133] and substrate for fermentation [134].

Several methodologies are under study in lab-scale for extracting and separating lipids from microalgae biomass, most methods are composed by the stages of cell wall disruption and lipid separation from biomass. For cell wall disruption, various thermal, chemical and physical methods have been evaluated. In previous chapter, coupled methods of cell disruption and oil extraction were designed and adjusted using autoclave, organosolv pretreatment and acid hydrolysis, McMillan, Watson, Ali and Jaafar [135], evaluated microwave, water bath, blender, ultrasonic and laser treatment, Vanthoor-Koopmans, Wijffels, Barbosa and Eppink [136] also exposes in their review other novel techniques of cell disruption. After this stage is necessary a further step of solvent addition for lipid recovery, several polar, non-polar and combination of solvents are being evaluated in microalgae oil extraction,

methodologies and results of adjustment of solvent based methods can be seen in detail in previous chapter of this book and the works of Fajardo et al. [137] and Halim, Danquah and Webley P [138]. More advanced methods are also been evaluated as enzymatic extraction [139], supercritical fluid extraction [140], wet extraction [141], Osmotic shock [142] and *in-situ* transesterification [143].

One of the goals pursued by researchers in this area, is to find a method for microalgae oil extraction which can be at the same time efficient, cheap, selective to lipids desired, reproducible and scalable, for achieve this goal, several studies must be developed in order to find the process that allows an effective oil extraction in terms of efficiency, purity of product desired, energy requirements, costs and environmental impacts. The main objective of this study, is the evaluation and comparison of five solvent-based microalgae oil extraction methods in lab-scale previously developed, incorporating additional criteria commonly used in literature (oil yield/extraction efficiency), these criteria are energy consumption during method performing, costs extraction in terms of materials, energy and equipment usage and toxicity of solvents selected for lipid extraction.

Although is well known by the authors the availability of robust methodologies for evaluation of each one of parameters discussed in this study as energy, exergy, and emergy analysis from the energetic point of view [144], techno-economic analysis with scenarios comparison and sensitivity analysis for evaluation of technologies from the economic point of view [145], and optimization of biorefineries taking into account economic and safety objectives [146], the scope of this research is to provide a big picture of the behavior of several oil extraction methods used on several microalgae strains in lab-scale under several criteria in order to provide some lights for further deeper study of techniques. As secondary contribution, morphological

response of bioprospected strains used for evaluation of oil extraction methods is also discussed such as some issues to consider for integration of technologies developed with other methods for extraction and separation of additional microalgae metabolites according to biorefinery concept.

3.2. METHODOLOGY

3.2.1. Microalgae Strains

Bioprospected microalgae strains were provided by Morrosquillo Corporation (Punta Bolivar, Colombia); biomass was cultivated in f/2 medium, harvested by flocculation, dried and refrigerated until use. Characterization of different strains was developed by the Colombian Petroleum Institute ICP-ECOPETROL. As is mentioned in abstract, microalgae strains used for this study were *Nannochloropsis* sp., *Guinardia* sp., *Closterium* sp., *Amphiprora* sp. and *Navicula* sp.

3.2.2. Oil extraction in lab-scale

Solvent-based oil extraction methods evaluated (solvent extraction with high speed homogenization, continuous reflux solvent extraction and ethanol-hexane method) were designed and adjusted by authors in previous chapter, finding the best operating conditions as the first stage of cell wall disruption as second stage of solvent oil extraction and lipid purification, for all methods cell disruption is intended to destroy the microalgae cell wall to facilitate the recovery of intracellular products and obtain greater amounts of lipids, all oil extraction experiments were made by triplicate, methods were performed as follows:

3.2.2.1. Solvent extraction assisted with high speed homogenization (SHE). This is a rapid and effective method, which mainly includes the stages of strong homogenization, centrifugation and filtration, for its performance, methanol, chloroform and biomass are mixed in a mass ratio of 6:12:1 under environmental conditions, methanol is a polar solvent that dissolves polar lipids, on the other hand, chloroform is a non-polar solvent which dissolves the neutral lipids present in the extraction and water is a polar solvent allows separate methanol/polar lipids phase of the chloroform/neutral lipids, the mixture is stirred and separated by filtration, obtaining a liquid phase with high percentage of lipids and a solid stream of biomass, liquid fluid is mixed with water in 4:1 ratio for phase separation, after that, hydrophilic/hydrophobic phases are separated using centrifugation for 15 minutes at 3400 rpm the upper phase methanol/water from the centrifuge tube was removed while lower phase biomass/lipids Chloroform, was filtered by gravity. Solvents are recovered by evaporation and condensation using a roto-evaporator. Finally, the lipid extract was allowed to volatilize to constant weight for its measurement, cell disruption in this method is achieved by mechanical action in homogenization stage.

3.2.2.2. Extraction with the mixture Ethanol/Hexane (EHE). This method is based in a lipid extraction method developed by Fajardo et al. [137], this procedure uses two solvents for extraction and subsequent purification of the extract. Ethanol is used in the first stage to recover the lipid content of microalgae; the crude oil obtained with ethanol contains unsaponifiable lipids, such as pigments, proteins, amino acids and other lipid and non-lipid contaminants. As a second step, the addition of water and hexane to the crude extract, obtained above, generates the formation of a biphasic system, in which lipids are transferred to the hexane phase, and the impurities are retained in the hydroalcoholic phase. This phase separation occurs due to the difference in solubility between solvents. It is performed by decanting and is

repeated five times by adding more water and hexane to the hydroalcoholic phase. The proportion water content has been optimized to displace the equilibrium distributions of lipids to the hexane phase, for cell disruption a solution with 5 g of biomass and 0.5 mol L^{-1} of hydrochloric acid was prepared and subjected to a stirring speed of 500 rpm for 120 minutes at room temperature, subsequently, vacuum filtration was performed where the pH was raised about 6 or 7 with the addition of distilled water, thereby obtaining hydrolysed biomass and water-soluble phase. Hydrolyzed biomass was dried to 105 ºC for 4 h.

3.2.2.3. Continuous reflux solvent extraction (CSE). This is a multiple-extraction procedure that consists in a first cell disruption stage in which 5 g of biomass are mixed with water, methanol and sulphuric acid in a 1:5:0.8:0.32 ratio, mixture is placed in a 25 L Autoclave by 4 h, water-soluble compounds in the cell were dissolved by the acid and formed a compound called solubilised mass, which is separated from the non-polar phase by vacuum filtering, followed by a neutralization of the biomass to stop cell degradation and drying at 105ºC during 4 hours, for solvent extraction, a typical Soxhlet extractor with 45/50 outer/upper and 24/40 lower/inner joint for 250 mL capacity was used, pre-treated dry biomass was put in a cartridge and solvent was heated to boiling point, then condensing it on the cartridge of biomass, giving way to the solid-liquid extraction of present lipids, the process described is repeated for 16 hours, during solvent extraction, the amount of biomass and the ratio biomass/solvent were kept constant, solvent used for this method was hexane. After extraction, extract-solvent mixture was filtered, distilled and the remnant solvent was evaporated. Total lipids were also quantified by gravimetric methods.

3.2.2.4. Hexane and Cyclohexane based extraction (HBE and CBE respectively). In the first stage of cell disruption, 5 g of microalgae biomass

are mixed with hydrochloric acid 0.5 mol L-1, mixture was stirred for 120 minutes at room temperature, after that, vacuum filtration was performed where the pH was raised about 6 or 7 with the addition of distilled water, finally, hydrolyzed biomass was dried to 105 ºC for 4 h, for solvent extraction, biomass was mixed with fresh hexane or cyclohexane in a 1:20 ratio and stirred at 500 rpm for 24 h in order to promote the solvent-biomass contact, finally, solvent-extract solution is separated from biomass by vacuum filtration and solvent is recovered by distillation.

3.2.3. Parameters for comparison of oil extraction methods

3.2.3.1. Lipid yield and lipid extraction efficiency. It was estimated the yields and efficiencies for each of the methods based on the gravimetric analysis done to each, oil yield in every test was calculated using the Equation 1, from amount of biomass used and oil obtained. To calculate lipid extraction effectiveness, the term Relative Extraction Ratio is introduced; this ratio is defined as the lipid yield reached using any extraction method evaluated related to lipid yield reached performing SHE method, which is used for total lipid determination, Equation 2 was used for calculation of Relative Extraction Ratio.

$$LipidYield = \frac{lipidextractweight}{totalbiomassweight} * 100 \qquad (1)$$

$$RelativeExtractionRatio\,(RER) = \frac{OilYield}{Totallipiddetermined} * 100\% \qquad (2)$$

Statistical comparison of lipid yield. Results of oil extraction for methods evaluated were compared in order to determine significant differences between methodologies performed, comparison was made for the five methods in one strain, and process was repeated for rest of strains evaluated,

statistical procedure used was the One-way Anova, which test differences among three or more sets of data, for the special case where two extraction methods are compared t-test is used and relation between Anova and t-test was made using the expression $F=t^2$. Confidence interval was set on 95%, in addition, values of variance and standard error were calculated for each method in each strain evaluated, consideration of equal variances was not assumed for statistical comparison, for statistical analysis was used the online application SISA (Simple Interactive Statistical Analysis) in options Oneway Anova and T-test [147].

3.2.3.2. Cost of extraction. An estimate of the value of application of each method in lab-scale was calculated using an economic gross evaluation taking into account the cost of solvents and volume used in each extraction method, cost of microalgae was not taking into account in order to provide an estimated non-dependent of biomass production costs, costs of utilities which includes electric energy, water, heating and cooling services were also calculated according to their prices in local conditions, a percentage of 10% corresponding to equipment depreciation and consumption of minor materials was assumed according to heuristic rules. Excepting the CSE method, cost decrease by solvent reutilization was not taken into account.

$$C_{met} = [\sum_{1}^{m} C_{solv} \cdot V_{solv} + \sum_{1}^{m} C_{utilites} \cdot U_{utilities}] * 1.1 \qquad (3)$$

3.2.3.3. Toxicity. As all microalgae oil extraction methods evaluated in this study are solvent-based, toxicity is considered as a very important aspect due to the implications of the use of these substances; toxicity was used as safety gross evaluation criteria. $LD_{50\%}$ is a measure of inherent toxicity of a solvent that is defined as the lethal concentration that would kill the half of the affected population. $LD_{50\%}$ was chosen as toxicity criteria because values are available in literature for solvents evaluated. Exists other toxicity values as

IDLH, AEGL and ERPG, however IDLH and AEGL were not used due to inconsistencies in their values reported in literature, ERPG was also discarded because in comparison to $LD_{50\%}$, is less applicable for solvents. In methods with solvent mixtures for extraction, the solvent with lower $LD_{50\%}$ was taken as reference. The method whit higher $DL_{50\%}$ was considered more tolerable in comparison to other lower values. In order to obtain a better data analysis, values were normalized to the same biomass amount (1 g of dry biomass) and extraction time (1 h).

3.2.3.4. Energy requirements. Energy requirements were calculated for each extraction method taking into account electric and/ or heating services required for performing. Values were estimated according to the electric power of the equipment used in each stage (homogenization, drying, vacuum separation, solvent recovery etc.) and time spent in extraction procedure which depends of each oil extraction method, power values were taken from equipment handbooks, internal power loses were not taken into account calculations were made using Equation 4, for detailed explanation of terms used in equations 1-4, please see nomenclature section.

$$E_{met} = \sum_{1}^{n} P_{eq} \cdot t_{et} \qquad (4)$$

3.2.3.5. Morphological response. Observation in optical microscope is performed to the biomass of the five strains at objective100x before and after every procedure in order to see its influence in the cell and its damage on the morphology of the same.

3.3. RESULTS AND DISCUSSION

3.3.1. Characterization of microalgae strains

According to the characterization of studied microalgae strains shown in Table 13, *Amphiprora* sp. presents the highest lipid percentage, followed by *Navicula* sp., *Nannochloropsis* sp. presents the highest composition of proteins and can be potentially used for food and feed, while *Guinardia* sp. is mostly composed by carbohydrates, cellulose and hemicelluloses, and could be used for reducing sugars production and transformation to third generation bioethanol. Profile more suitable for the development of a topology of biorefinery corresponds to *Amphiprora* sp. owing to their balanced composition of lipid and non-lipid components.

Table 13. Microalgae strains composition (modified from UIS-ICP-Morrosquillo [127])

	Nannochloropsis sp.	*Guinardia* sp.	*Closterium* sp.	*Amphiprora* sp.	*Navicula* sp.
Carbohydrates (%)	3	13	14	12	9
Lipids (%)	23	13	19	33	32
Proteins (%)	46	29	40	25	37
Cellulosic Material (%)	18	35	17	20	12
Ash (%)	10	10	10	10	10
Total	100	100	100	100	100

3.3.2. Multicriteria comparison of oil extraction methods in lab-scale.

3.3.2.1. Extraction Efficiency. As is shown in Table 14, extraction efficiency depends as extraction method performed as microalgae strain used, according to extraction results is clear that microalgae strain *Amphiprora* sp. presents the highest oil yield for all five methods evaluated, followed by *Navicula* sp. except when EHE method is performed, this behavior can be explained from the biologic point of view, owing to these two strains belong to the *Naviculales* order, which presents seams in their valvs, while the strain *Nannochloropsis sp.* whose cell wall is composed by several xylan layers making difficult chemical disruption and decreasing extraction efficiency. *Guinardia* sp. microalgae strain presents the highest reproducibility of third generation energy crops studied, this can be owed to a very low percentage of polar lipids and chlorophylls, which increases the standard deviations when selective and non-selective methods are compared, however, relative extraction ratio is lower than values obtained for *Amphiprora* sp., *Navicula* sp. and *Closterium* sp.

Table 14. Extraction efficiency results

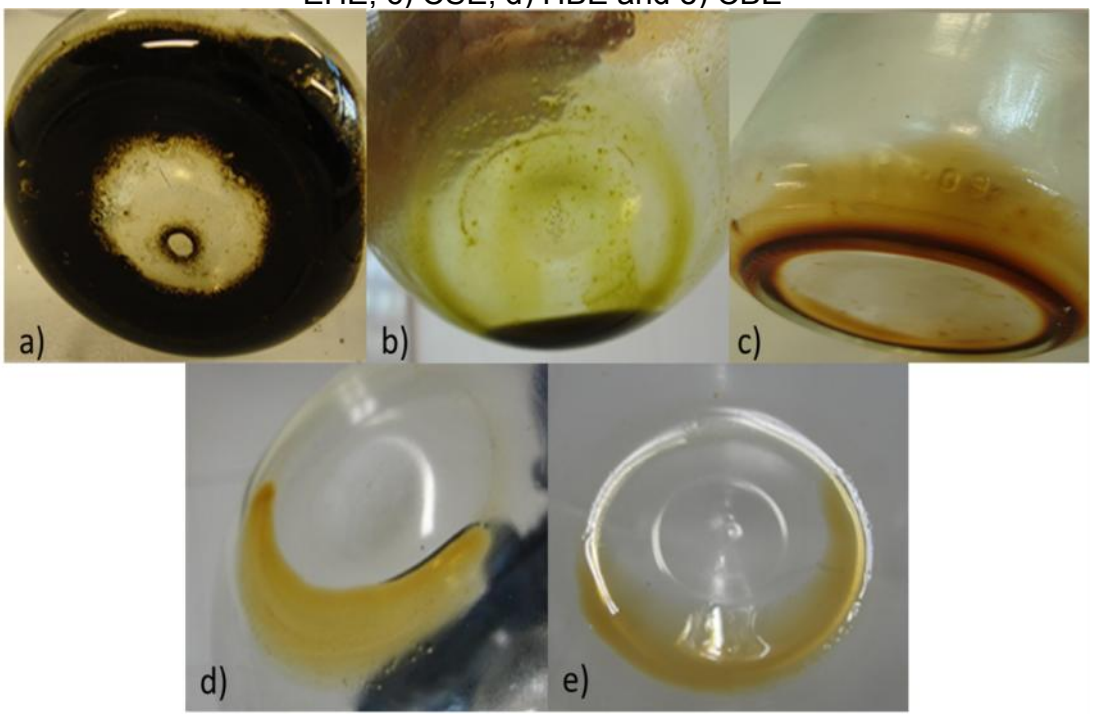
Microalgae strain	SHE		EHE		CSE		HBE		CBE	
	RER (%)	Stdev	RER (%)	Stdev	RER (%)	Stdev	RER (%)	Stdev	RER (%)	Stdev
Nannochloropsis sp.	100.0	1.71	4,87	0.13	10.65	0.37	16.75	7.87	15.15	1.72
Guinardia sp.	100.0	1.70	9.28	1.70	13.15	1.00	9.55	3.60	12.83	0.40
Closterium sp.	100.0	1.10	22.62	4.90	50.57	10.50	36.15	0.40	29.04	4.00
Amphiprora sp.	100.0	1.90	43.66	2.10	92.04	2.60	74.52	2.40	72.49	3.90
Navicula sp.	100.0	1.65	22.01	2.48	73.06	7.35	64.05	3.66	68.39	2.39

SOURCE: Author

By comparing Relative extraction ratio of methods evaluated in five strains can be seen that extraction method used as reference for calculations (SHE

method), presents the highest average extraction efficiency, derived by the combination of polar/non-polar solvents and high speed homogenization, which contributes to increase the amount of final product obtained. However, as is reported by Archanaa, Moise, and Suraishkumar G. [148], methods which uses methanol-chloroform as solvents can over-estimate the amount of biofuel-related lipids, because these methods also extracts other products as chlorophylls, in Figure 16 can be seen that SHE extract presents darker tone in comparison to other extracts, which shows the presence of non-lipid components, purity of extracted oil affects quality of final product desired from this microalgae metabolite (High value fatty acids or biodiesel).

Figure 16. Lipid extracts from microalgae obtained in lab-scale, a) SHE, b) EHE, c) CSE, d) HBE and e) CBE



SOURCE: Author

After Solvent extraction with high speed homogenization (SHE method), Continuous reflux solvent extraction method (CSE) presents the highest average relative extraction ratio, being potentially used for effective lipid extraction in lab scale, however, the scaling-up of this method can represent a process design challenge, owing to equipment, energy and solvent requirements. Batch methods as hexane and cyclohexane based extraction (HBE and CBE respectively) presents good extraction ratios in comparison to CSE method, with the advantage of an easier scaling-up, and lower solvent requirements, HBE extraction can be more attractive for a large scale microalgae processing owing to solvent cost, oil extraction using the ethanol-hexane mixture presents the lowest average standard deviation of methods evaluated which could be positive for ensure reproducibility of the oil extraction, however relative extraction ratio of this method does not overcome relative extraction ratio of any other method evaluated for the same strain.

Statistical comparison of methods. Table 15 shows the results of statistical comparison of oil extraction methods taking into account the extraction efficiency, results shows that although behaviour of oil extraction methods is affected by the strain evaluated which is coherent with the analysis made in previous section, however, it can be seen that in most of cases (strains) there is no significant differences between performing HBE and CBE methods, showing that not worth it to continue using both methods in lab-scale for future work, nevertheless, is also clear that selection criteria between HBE and CBE cannot be efficiency, for selecting the more convenient method, must be compared using additional criteria discussed in further sections of this work. It also can be seen that there is no significant differences between CSE and HBE for most of strains evaluated, so, other criteria must be taken into account for a more robust comparison of these two methods. On the other hand, EHE method presents significant differences in comparison to other C6-based extraction methods in all cases.

Table 15. Statistical comparison results

Strain	Method	Variance	Standard Error	95% of C.I.		Non-Significant differences
Nannochloropsis sp.	SHE	2.92	0.99	95.75	104.25	-
	EHE	0.02	0.08	4.55	5.19	3
	CSE	0.14	0.21	9.73	11.57	2
	HBE	61.94	4.54	2.80	36.30	1,2,3
	CBE	2.96	0.99	10.88	19.42	1
Guinardia sp.	SHE	2.89	0.98	95.78	104.22	-
	EHE	2.89	0.98	5.06	13.50	1,2
	CSE	1.00	0.57	10.67	15.63	1,3,4
	HBE	12.96	2.08	0.61	18.49	2,3,5

	CBE	0.16	0.23	11.84	13.82	4,5
Closterium sp.	SHE	1.21	0.64	97.27	102.73	-
	EHE	24.01	2.83	10.45	34.79	1
	CSE	110.25	6.06	24.49	76.65	2
	HBE	0.16	0.23	35.16	37.14	2
	CBE	16.00	2.31	19.10	38.98	1
Amphiprora sp.	SHE	3.61	1.10	95.28	104.72	-
	EHE	4.41	1.21	38.44	48.88	-
	CSE	6.76	1.50	85.58	98.50	-
	HBE	5.76	1.39	68.56	80.48	1
	CBE	15.21	2.25	62.80	82.18	1
Navicula sp.	SHE	2.72	0.95	95.90	104.10	-
	EHE	6.15	1.43	15.85	28.17	-
	CSE	54.02	4.24	54.80	91.32	1,2
	HBE	13.40	2.11	54.96	73.14	1,3
	CBE	5.71	1.38	62.45	74.33	2,3

SOURCE: Author

3.3.2.2. Costs of extraction. If extraction costs in lab-scale are compared, lowest value belongs to EHE method and followed by EHE method, these values are due to low solvents amount needed to perform these methods and low cost of ethanol and hexane in comparison to other organic solvents, while higher extraction costs belongs to CBE method, which is drastically increased by the costs of cyclohexane which is near to 13 times more expensive than hexane in local market.

3.3.2.3. Toxicity. Values of solvents used shows that SHE method is the most harmful of methods evaluated, owing to the use of highly toxic solvents as methanol and chloroform which is disadvantageous for a large-scale processing without appropriate safety-based process design, extraction methods which uses hexane as solvent (CSE and HBE) presents the lowest toxicity. If is analysed the toxicity parameter together with solvent recovery for studied methods, can be seen a disadvantage of performing this method frequently in lab-scale, by the release of high amounts of highly toxic solvents, requiring adequate facilities and protection, can be convenient to use SHE method once for an estimation of total lipid content of feedstock and used as reference. However, using an adequate large-scale process design which takes into account all safety aspects or appropriate assumptions, can be interesting the evaluation of this method. CSE presents higher solvent loses in comparison to HBE, however, in SCE case solvent is lost by continuous evaporation and condensation and for HBE, bulk of the solvent non-recovered is in mixture with algae meal after extraction, for this reason is recommendable a further drying of algae meal and condensation of vapours released for a more effective hexane recovery.

3.3.2.4. Energy Requirements. it can be seen that lower energy requirements corresponds to SHE method followed by HBE/CBE and highest energy requirements are presented by CSE method (Table 16), this difference can be explained by the heating and cooling requirements that Soxhlet extraction system needs, extraction methods with high energy requirements must be discarded for a large scale microalgae processing if the final use of microalgae components is energetic, EHE method presents high energy requirements and low efficiency as is shown in previous section. When solvent recovery is considered for evaluation of oil extraction methods, is understandable that energy requirements increases, because an additional energy input is necessary for condensing the solvent separated from the lipid

extract, and for separating solvent mixtures in methods where is required, in this scenario, method with higher energy requirements is EHE, for efficient first-step extraction with ethanol, recovered solvent must be separated from water added for phase separation, and hexane must be condensed after lipid extraction and separation.

Table 16. Comparison of oil extraction methods in lab-scale

Extraction method	Cost of extraction [USD/g biomass]		$LD_{50\%}$ [mg/kg]		Energy requirements [KW h]		Solvent recovered [%]
	Basic	Solvent recovery	Basic	Solvent recovery	Basic	Solvent recovery	
SHE	0.28	0.18	1194	1194	0.72	1.59	55
EHE	0.11	0.04	10600	10600	1.75	2.62	85
CSE	1.90	1.90	28710	28710	2.37	2.37	80
HBE	0.18	0.05	28710	28710	1.51	2.26	85
CBE	2.39	1.36	6200	6200	1.51	2.26	85

SOURCE: Author

Taking into account results obtained in Table 16, can be established that for a lab-scale microalgae oil extraction, method most convenient to perform is HBE, because its low energy consumption compared to other methods, low extraction costs and relatively low toxicity of solvent used, on the other hand, CBE method becomes non-convenient for oil extraction from microalgae due to its high cost of cyclohexane and high toxicity, in addition, lipid yield obtained with this method is similar to yields of HBE method.

3.3.2.5. Influence of solvent recovery on parameters evaluated. Solvent recovery plays an important role on selection of oil extraction methods for a large-scale processing and can change results obtained in lab-scale, is important to take into account that depending on the extraction method, bulk

of the solvent must be recovered from the algae meal and/or from the lipid extract, and there is an amount of solvent which cannot be recovered, this affects negatively the impacts of method performing from the safety point of view, and the cost of extraction by including the costs of solvent recovery and input of fresh solvent for replacement of the non-recovered solvent, from the energy point of view, must be taken into account the energy consumption of solvent evaporation and condensation for re-using.

In SHE method, chloroform must be separated as from lipid extract as from algae meal, owing to low boiling point of this solvent and the high speed of homogenization which produces an increase of temperature of the extraction system, chloroform loses are significant (around of 50%), and after extraction, algal meal also contains a significant amount of solvent which is not recovered affecting safety of process and economics by fresh solvent requirements and commercialization potential of algal meal or utilization of algal meal for obtaining other bioproducts under biorefinery concept.

For EHE method, algal meal contains only ethanol, because there is no contact between hexane and biomass, which allows higher possibilities of further processing of algae meal without significant co-product purification, if is desired to convert meal carbohydrates into reducing sugars, can be used a organosolv pretreatment which includes ethanol with an acid for hydrolysis reaction, in this sense, is more convenient the EHE method in comparison to SHE method, hexane is also easily recovered from hydrophobic phase and can be used again for extraction decreasing processing costs.

In CSE method, as the solvent is continuously evaporated and condensed during extraction for effective lipid recovery, this continuous reflux increases solvent loses during extraction process, and is more significant at long extraction times, issue that is characteristic of this method. On the other hand,

if the extraction process is stopped when the amount of solvent in contact with biomass is minimum. By the nature of the process, solvent separation from lipid extract can be performed in the same extraction system, which is a benefit in lab-scale, but difficult to achieve in large scale without additional equipment.

For the cases of HBE and CBE methods, separation of solvent from biomass is difficult with loses of biomass/solvent mixture during the process, however, this disadvantage can be avoided in large-scale with appropriate equipment, for CBE extraction, there is a higher impact derived of solvent loses from the safety point of view, despite amount of solvent recovered is similar to HBE extraction, lower $LD_{50\%}$ makes more dangerous the exposition to solvent vapours. Solvent loses in CBE also impacts strongly in operating costs of extraction owing to high cost of cyclohexane, in lipids-solvent separation for both methods, no significant hexane/cyclohexane loses are presented.

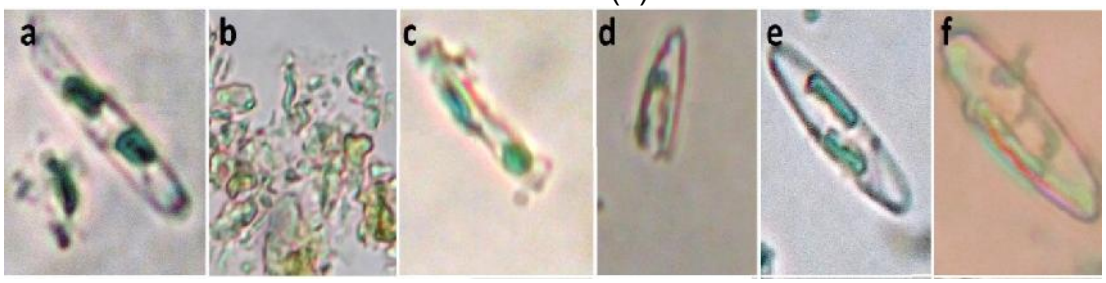
3.3.3. Morphological response by strain to oil extraction methods

3.2.6.1 Guinardia sp. Morphological comparison of a microalgae strain to all oil extraction methods performed was made using the strain *Guinardia* sp. (Figure 17), when this microalgae is submitted to SHE extraction the cell shape is strongly affected and broken, can be seen pieces of frustules, free chloroplasts and other fragments of totally destroyed cells (Figure 17b), cells after EHE method keeps still their frustules, the only significant change observe

d by optic microscopy is related to the shape of the strain, all cells individually observed keeps their two chloroplasts within the cell wall (Figure 17c), with performing of CBE extraction can be observed cell disruption in several cells and absence of lipid drops which were extracted by cyclohexane

in higher percentage than other methods (Figure 17d), microalgae exposed to HBE method showed a change in cell shape and cell disruption in high percentage evidenced by the presence of free chloroplasts, in come cells there was not disruption but inner metabolites looks disordered dislocated (Figure 17d), finally, when microalgae strain is submitted to CSE method there is a higher percentage of non-broken cells, however, this method presented the higher Relative efficiency, this behaviour can be explained because CSE method does not use mechanical or magnetic stirring, for this reason the possibility of cell rupture by mechanical action is lower, but solvent can remove lipid components going across the damaged cell wall (Figure 17f).

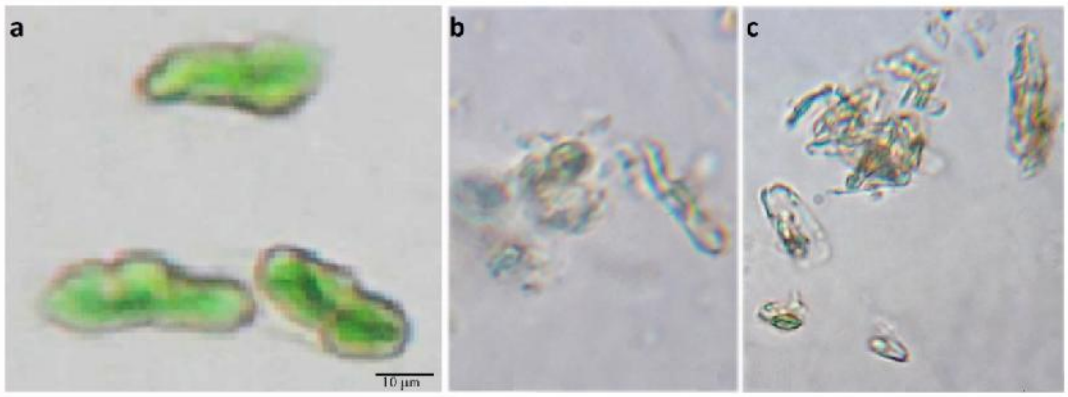
Figure 17. Morphological response of *Guinardia* sp. strain to SHE oil extraction method (b) EHE method (c), CBE method (d), HBE method (e) and CSE method (f) in lab-scale. Left-side image correspond to cells before oil extraction (a)



SOURCE: Author

3.3.3.1. *Amphiprora* sp. After observation of cells before extraction process can be seen that *Amphiprora* sp. strain presents an irregular shape which is not common in diatoms (Figure 18a), this phenomenon can be derived of previous stages of microalgae biomass production chain as drying, in which some cell wall components can be degraded because of high temperature used for this step.

Figure 18. Morphological response of *Amphiprora* sp. strain to SHE oil extraction method (b) and CSE (c) in lab-scale. Left image correspond to cells before oil extraction (a)



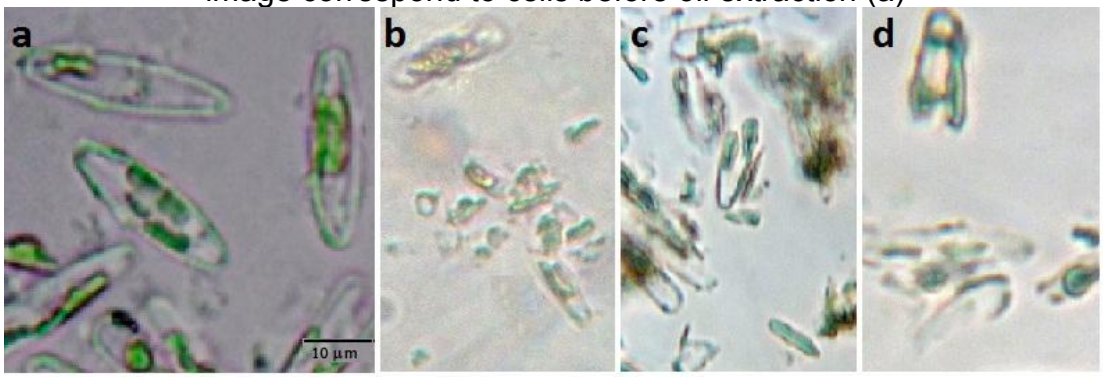
SOURCE: Author

After performing SHE extraction using this biomass (Figure 18b), can be observed significant changes in the morphology of the cell as the presence of chloroplast outside of the cell and changes in shape and colour of the cell, this changes are promoted by two main factors, mechanical destruction by high speed homogenization and effectiveness of solvents mixture used for microalgae compounds removal, however, degree of cell destruction confirms the low selectivity of SHE method for extraction of lipids usable in biodiesel production. When biomass is submitted to CSE method can be seen that microalgae cell wall is still present although is drastically deformed and damaged, is also shown that most of intracellular content including lipids was released, hexane could break through the degraded cell wall dissolving neutral lipids and other non-polar components (Figure 18c).

3.3.3.2. *Navicula* sp. For *Navicula* sp. microalgae biomass can be seen that morphology of the cell is not affected by previous drying step (Figure 19a), this is due to the thickness of the microalgae frustule, which protects the cell from external damage factors. After oil extraction using EHE method (Figure 19b), can be still found cells without damage and other with most of metabolites present within the cell, this morphological response helps to

explain the low efficiency of EHE method in comparison to other microalgae oil extraction methods evaluated, Figure 19c shows microalgae biomass after performing HBE method where can be seen a higher percentage of broken cell walls in comparison to EHE method, can be observed several chloroplast outside of the cell which means that metabolites were released, but were not dragged by the solvent, behaviour of microalgae biomass after CBE method performing was very similar (Figure 19d), this observation confirms the selectivity of non-polar solvent based extraction methods to microalgae lipids.

Figure 19. Morphological response of *Navicula* sp. strain to EHE oil extraction method (b) HBE method (c) and CBE method (d) in lab-scale. Left image correspond to cells before oil extraction (a)



SOURCE: Author

3.4. CONCLUSIONS

Extraction method showed different yields depending on microalgae strain evaluated, for all cases, variation of oil yield and oil extraction efficiency as function of microalgae strain used for evaluation is an important issue to consider, because a large scale extraction method must show high yields for several strains, this can depend on nature of microalgae strain and/or cultivation, harvesting and drying conditions, *Amphiprora* sp. presented the highest oil yield of strains evaluated for all five extraction methods, followed by *Navicula* sp., this can be explained because both strains belongs to the same order (Naviculales), with similar cell walls and compositions as is shown in Table 13. On the other

hand, *Nannochloropsis* sp. presented the lowest oil yield for all methods studied, which is not consistent with literature, inferring that a previous biomass processing stage could decrease and/or degrade neutral lipid percentage of strain. Taking into account biomass composition, morphologic response and oil yield, microalgae genera *Amphiprora* sp. emerges as a potential strain for the development of a topology of biorefinery.

SHE method shows the highest yield as result of combination of polar and non-polar solvents, as disadvantage presents the extraction of non-desirable lipids for biodiesel production, as sterols, pigments and other non-lipid metabolites, taking into account that, in lab-scale is convenient the utilization of this method for total lipid determination in non-characterized strains, however, overestimation of lipid percentage derived of extraction of other microalgae metabolites must be taken into account, in addition, SHE method presents the highest toxicity and lowest percentage of solvent recovery of methods evaluated, which makes expensive and risky the continuous utilization of this method even with solvent recovery strategies.

Statistical comparison showed that there is no significant differences between C6-based extraction methods (CSE, HBE and CBE) for most of strains studied, taking into account lipid extraction efficiency criteria, then, is convenient to choose only one of these methods for application in lab scale and evaluation as emerging technology in large scale and for further synthesis of a microalgae-based biorefinery topology. CSE method shows good results in terms of efficiency, low toxicity and higher yields than other methods evaluated, besides, selectivity of hexane to neutral lipids usable for biodiesel production promotes its inclusion in a microalgae based biorefinery. however, scaling-up of CSE could be not feasible in terms of energy requirements owing to energy input necessary for continue evaporation and condensation of solvent, HBE method also uses hexane and presents lower energy requirements than CSE for both scenarios

evaluated, also presents lower costs of extraction and energy requirements in solvent recovery scenario than CSE, derived of lower biomass/solvent ratio, and higher amount of solvent recovered. For CBE method in terms of technology implementation, the purchase of an expensive and more toxic solvent with similar yields and recovery percentage to hexane is not attractive in any scale. Taking into account all issues mentioned, HBE method is the most convenient for utilization in lab-scales under the criteria evaluated, also becomes as a promising alternative for scaling-up and further evaluation in a biorefinery superstructure. Solvent recovery must be a mandatory parameter for performing solvent-based oil extraction methods in lab-scale, with benefits in all aspects evaluated in this work, in addition is a fixed stage in large-scale sustainable production processes.

3.5. NOMENCLATURE

$C.I.$: Short name for Confidence Interval

C_{met}: Cost of application of certain method

C_{solv}: Cost of a specific solvent per volume units

E_{met}: Energy requirements of a given method

$LD_{50\%}$: Median Lethal Dose of a substance used as indicator of its acute toxicity

m: Number of solvents used performing a given method

m_e: Amount of extract obtained after carrying out certain method

m_0: Initial amount of biomass subjected to extraction of certain specie

m_p: Amount of biomass obtained after pre-treatment

n: Number of equipment used to perform a given method

P_{eq}: Nominal electric power of equipment

RER: Short name for Relative Extraction Ratio

$Stdev$: Short name for Standard deviation

t_{eq} : Time of use of equipment

V_{solv} : Volume of solvent used in a given method

w_i: Variable weighting value assigned to particular criteria

4. CHAPTER IV. STUDY OF FERMENTABLE SUGARS PRODUCTION FROM MICROALGAE AND LIPID EXTRACTION USING SEPARATED AND MULTIFUNCTIONAL PROCESSES: EXPERIMENTAL AND MODELLING[4]

4.1. INTRODUCTION

Microalgae have recently been rediscovered as promising candidates for biotechnological applications and efficient energy production systems. Due to the unique cellular structure of algae, they can collect energy more efficiently than land plants, the extraction of carbohydrates, lipids, pigments, proteins and special substances from microalgae biomass is under research for obtaining several bioproducts [149], an interesting alternative for obtaining these products is the use of multifunctional processes for simultaneous extraction separation and transformation of two or more desired products [150], the other alternative consists in the optimization of operating conditions and routes for obtaining a desired specific metabolite, extraction of pigments can be made by cell breaking, solvent extraction and centrifugation, or using supercritical fluids, and purification is made using microfiltration [151], reducing sugars can be obtained by hydrolysis reaction with simultaneous cell wall disruption for oil extraction [152], proteins are extracted for its use as fertilizer [153], animal feed supplement [154] and substrate for fermentation [155].

This chapter is focused on the evaluation of routes for obtaining valuable metabolites of microalgae *Amphiprora* sp. and *Navicula* sp. and comparison

[4]This chapter is based on the papers "*Design of a multifunctional reactor for third generation biofuels production*" by Angel Darío González Delgado & Viatcheslav Kafarov, published in Chemical Engineering Transactions Journal Vol. 21, 1297-1302 (2010), "*Microalgae Based Biorefinery: Evaluation of Several Routes for Joint Production of Biodiesel, Chlorophylls, Phycobiliproteins, Crude Oil and Reducing Sugars"* by Angel Darío González Delgado & Viatcheslav Kafarov, published in Chemical Engineering Transactions Journal Vol. 29, 607 – 612 (2012). And "*Evaluation of lipid and monosaccharide obtaining routes from microalgae biomass under the biorefinery concept*" by Angel Darío González Delgado, Laura Peñaranda, Karen Sepúlveda, Yury Alvarez and Viatcheslav Kafarov, published in ION Journal Vol. 24 (2), 13-22 (2011).

of methods and bioproducts using acid hydrolysis processes, solvent extraction, and organosolv pretreatment. In addition, the work shows the implementation of a multifunctional process, compiling the hydrolysis, extraction and transesterification in a system, comparing monosaccharides concentration, lipids and other obtainable bioproducts in relation to time, and defining kinetic parameters associated with that process.

4.2. MATERIALS AND METHODS

Microalgae biomass of *Amphiprora* sp. and *Navicula* sp. was provided by the Morrosquillo Corporation (Punta Bolivar, Colombia), harvested by flocculation and dried in an oven at 105°C for 8 hours.

4.2.1. Production of monosaccharides

Acid hydrolysis pretreatment and Organosolv pretreatment were used for both cellular wall disruption and reducing sugars production. For the first method described above, solutions with 10 g of dry biomass of the microalga *Amphiprora* sp. and 150 mL of 0.5 mol L^{-1} hydrochloric acid were prepared and independently subjected to a stirring speed of 500 rpm for 30, 60 and 120 minutes at room temperature. Subsequently, vacuum filtration was performed where the pH was adjusted near to 7 with the addition of distilled water, thereby obtaining two products, hydrolyzed biomass which is used for lipid extraction and water-soluble bioproducts in acid solution which contains monosaccharide desired. Hydrolyzed biomass was dried to 102 ºC for 4 hours, by this step remaining water was removed in the biomass and after that, hydrolyzed biomass was used for lipid extraction by CSE method. On the other hand, water-soluble bioproducts in acid solution were neutralized with sodium hydroxide (NaOH) and reducing sugars were measured.

For Organosolv pretreatment, an experimental design was made for evaluate the effect of acid concentration and time on the reducing sugars yield, with best operating conditions obtained, comparison is made, after this procedure, two phases were obtained, a solid phase with pretreated biomass and a liquid phase of pretreatment liquor which contains monosaccharides desired, pretreatment liquor was separated of pretreated biomass by vacuum filtration. After that, pretreated biomass was used of the same way as is written previous section. Neutralization of pretreatment liquor was made by adding calcium carbonate ($CaCO_3$) to a pH of about 5 or 6, finally, total reducing sugars were measured.

Measurement of total reducing sugars for both monosaccharide production alternatives evaluated was performed using the method of dinitrosalicylic acid (DNS) proposed by Miller [156]. For its quantification, base 10 dilutions were used for each of the samples taken by water-soluble bioproducts in acid solution and pretreatment liquor. Absorbance measurements were performed at 540 nm on a spectrophotometer MERCK Spectroquant®Pharo 300.

4.2.2. Lipid extraction

Biomass of microalgae *Amphiprora* sp. and *Navicula* sp. after monosaccharides production was subjected to lipid extraction by CSE method using hexane as solvent, taking repeated wash times of 16 hours. Subsequently, extract was filtrated in order to remove biomass residues or impurities, obtaining the solvent with lipids extracted from each strain. A portion of the solvent was removed by simple distillation and the other was allowed to volatilize until obtain the concentrated lipid extract, tests were not greater than 120 minutes and 16 hours due to high energy requirements and reagent consumption.

To calculate lipid extraction effectiveness, the term Relative Extraction Ratio is introduced; this ratio is defined as the lipid yield reached using any extraction method evaluated related to lipid yield reached performing SHE method, which is used for total lipid determination, Equations. (1) And (2) were used for calculation of lipid yield and Relative Extraction Ratio respectively.

$$Lipid\ yield\ (\%) = \frac{oil\ weight}{biomass\ weight} \times 100 \quad (1)$$

$$Relative\ Extraction\ Ratio\ (\%) = \frac{Lipid\ yield}{Total\ lipid\ determined} \times 100 \quad (2)$$

4.2.3. Multifunctional process

This process involved the implementation of joint treatment of acid hydrolysis or cellular disruption, oil extraction and in situ transesterification [157]. This scheme proposed by the author conducted two simultaneous systems, in which ethanol and methanol were evaluated as solvents/reagents. Biomass-alcohol ratio used was 1:6 and sulfuric acid was used as catalyst for transesterification in oil-acid ratio of 1:1. Reaction systems were continuously stirred at 500 rpm for 10 hours at 60 ºC, 1 mL samples were taken at different time intervals. Each sample was taken to centrifugation for 10 min to separate hydrolyzed and water-soluble biomass. Liquor was neutralized by the addition of 50 mL sodium hydroxide (NaOH) 1N, adjusting a pH near to 7. Subsequently, 1.5 mL of hexane and 0.5 mL of distilled water were added in order to obtain a three-phase system consisting of hexane phase, residual biomass and hydro-alcoholic layer. The addition of hexane and water to each sample, described above, allowed the separation of hydrophobic components

as lipids and alkyl esters of residual biomass and hydro-alcoholic phase where the contents reducing sugars and other polar components. Hexane phase was analyzed using Infrared Spectroscopy in order to detect products obtained using a Shimadzu FTIR-8400S (Fourier Transform Infrared Spectrophotometer) in the wavelength range of 400-4000 cm^{-1}. On the other hand, hydroalcoholic phase was treated using the DNS method. For remaining biomass in each system, lipid extraction was carried out in order to quantify non extracted and/or transesterified lipids.

4.2.3.1. Kinetic modeling of monosaccharide production. Kinetic parameters for transformation of cellulosic components of *Navicula* sp. in reducing sugars and degradation of these sugars were found based on the model presented by Téllez-Luis *et al*. [157] for polysaccharides hydrolysis, this model has been adapted for microalgae biomass successfully [150], and describes a first-order consecutive reaction with two irreversible steps, where *PM* refers to polysaccharides of the microalga, *RS* to reducing sugars and *DP*, degradation products (Equation 3).

Differential equations are also proposed for describe the changes in the concentration of polysaccharides, monosaccharides and degradation products, Equation 4 expresses the reaction rate of monomerization of polysaccharides, Equation 5 describes the rate of production of reducing sugars, where *C* is the concentration of polysaccharides from microalgae and *A* is the concentration of total reducing sugars. Is also presented the Arrhenius equation which relates the rate constant K_i as a function of temperature (Equation 6).

$$\frac{dC}{dt} = -K_1 [C] \quad (4)$$

$$\frac{dA}{dt} = K_1 [C] - K_2 [A] \quad (5)$$

$$K_i = C_{acid}^{n} A_i e^{-E_i/RT} \quad (6)$$

Using MATLAB software v. 7.10 and the Solver tool in Microsoft Excel, kinetic parameters of reaction systems were determined, with calculated kinetic data were modeled responses involved with total reducing sugar concentration by modifying operation conditions.

4.3. RESULTS AND DISCUSSION

According to the characterization of studied microalgae shown in Table 17, *Navicula* sp. presents higher percentages of proteins than *Amphiprora* sp., ash percentage for both strains were normalized to 10%. Values reported does not presents significant differences, and both microalgae strains belongs to the same order, for these reasons, microalgae biomass used can be considered comparable for the evaluation of routes.

Table 17. Metabolites characterization of *Amphiprora* sp. and *Navicula* sp. microalgae

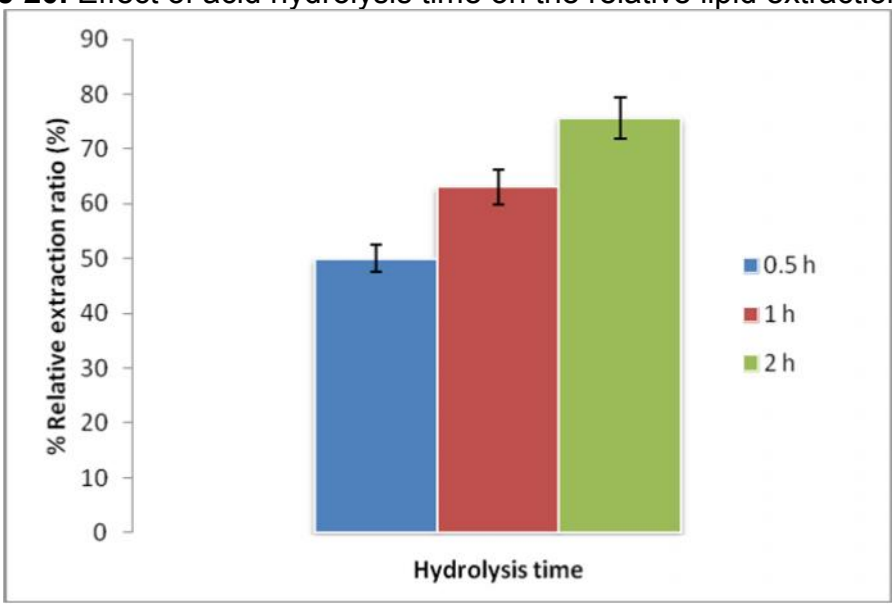
Microalgae Strain	Proteins (%)	Carbohydrates (%)	Cellulosic Material (%)	Lipids (%)	Ash (%)
Amphiprora sp.	25.0	12.0	20.0	33.0	10.0
Navicula sp.	37.0	9.0	12.0	32.0	10.0

SOURCE: Author

4.3.1. Evaluation of hydrolysis-solvent extraction route (HSE)

4.3.1.1. Effect of hydrolysis time. The increase in contact time of hydrochloric acid to the microalga biomass of *Amphiprora* sp. influenced the release of lipids after solvent extraction. When the cellular lysis time was 120 minutes, a Relative Extraction Ratio of 75.58 % was reached, followed by 63% and 50% when the pretreatment time was 60 and 30 minutes, respectively. Acid hydrolysis method facilitated lipid extraction by breaking the cellular walls and allows to the solvent easier access to the microalgae oil, which was reflected in the increase of Relative Extraction Ratio. According to results obtained, 120 minutes of hydrolysis reaction was chosen for subsequent treatments.

Figure 20. Effect of acid hydrolysis time on the relative lipid extraction ratio

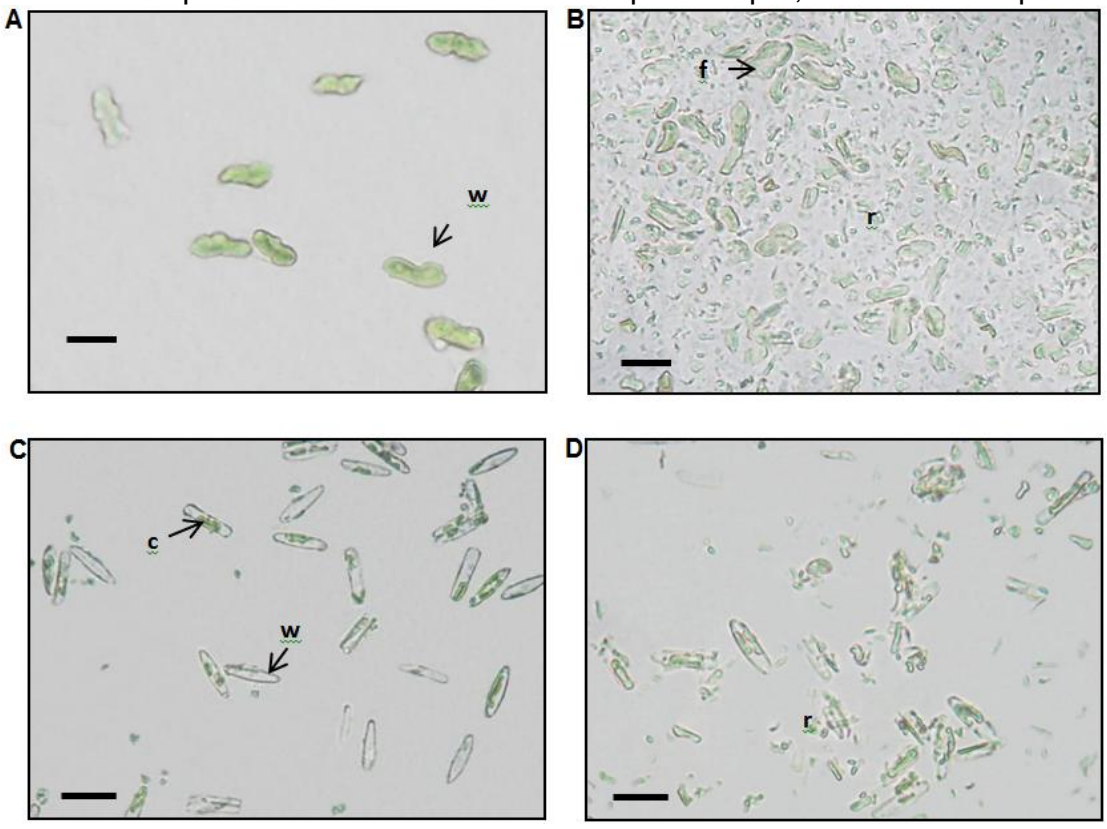


SOURCE: Author

Acid hydrolysis affects the morphology of microalgae *Amphiprora* sp. and *Navicula* sp., cellular wall of these microalgae are composed of hydrated silica and proteins. The rigidity of the frustules was affected by the action of acid pretreatment, so that it can be seen remnants of cellular wall and

cytoplasmic constituents (Figures 21b and 21d). *Amphiprora* sp. showed an increase in the volume of some cells, accompanied by the subsequent fragmentation of the microalgae and therefore the release of intracellular content (Figure 21b).

Figure 21. Comparison of microalgae. A. *Amphiprora* sp. dry biomass, before acid pretreatment. B. *Amphiprora* sp. biomass after 120 minutes of acid hydrolysis. C. *Navicula* sp. biomass before cellular lysis. D. *Navicula* sp. hydrolyzed biomass, after 120 minutes of reaction. w: Cellular wall; r: Remnants of frustules and organelles; **f**: Cellular fragmentation; c: Chromatophores. The scale in A and B equals 20 µm, C and D to 50 µm



SOURCE: Author

4.3.2. Evaluation of Organosolv pretreatment - solvent extraction route (OSE)

4.3.2.1. Effect of time and acid concentration. an experimental 2^2 central composite design was proposed, the levels are shown in Table 18, this kind of design was selected because scans a wide response area, the response variable is the yield of reducing sugars, the independent variables are both acid concentration and reaction time.

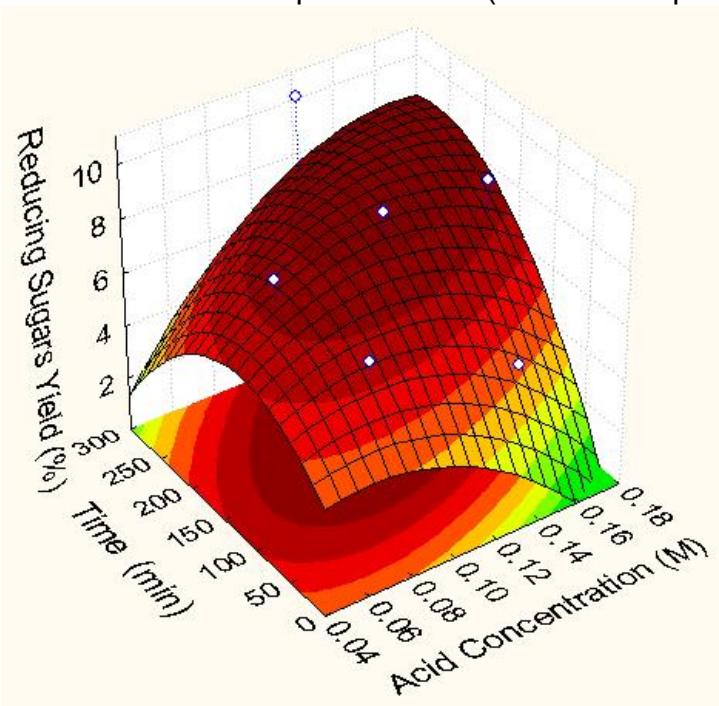
Table 18. Experimental 2^2 central composite design

	levels				
Factors	-1.41	-1	0	1	1.41
H_2SO_4 concentration (M)	0.066	0.080	0.115	0.150	0.164
Time (min)	22.721	60	150	240	277.279

SOURCE: Author

It is shown that yield of total reducing sugars increases with reaction time and acid concentration, with very short reaction times and high acid concentration there is lower reducing sugars yield, this is owing to the presence of hard to hydrolyze hemicelluloses which needs higher reaction times for its conversion in reducing sugars (Figure 22), a similar effect is seen at lower acid concentrations and high exposition times, this means that acid concentration contributes in a great way to microalgae biomass cellulosic material degradation, but the main effect is given for the exposition time, this can be explained because water without acid can be also as a cell disruptor agent, in a similar way as actuate in the vapor explosion technique used for the pretreatment of lignocellulosic material for second generation biofuels production.

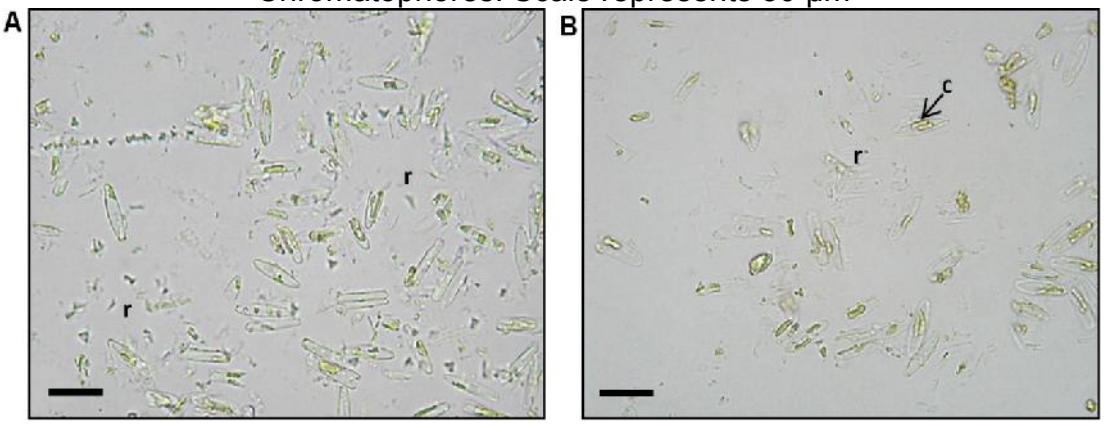
Figure 22. Reducing sugar yield for microalgae (solvent free basis) as a function of acid concentration and process time (reaction temperature: 121°C)



SOURCE: Author

Organosolv pretreatment applied to the microalga *Amphiprora* sp. increased the lipid extraction efficiency compared with acid hydrolysis. Through this procedure, Relative Extraction Ratio of 92.04 % was reached. The increase in the release of microalgae oils was given to the operating conditions of the method, as the increase in pressure, temperature and contact time of biomass with disrupting agents such as methanol and sulfuric acid, caused a greater degree of cellular lysis, organosolv pretreatment increases Relative Extraction Ratio compared to control, this pretreatment promotes an aggressive disruption on the cellular wall of *Amphiprora* sp. (Figure 23). The remains of microalgal organelles after solvent extraction confirm the effectiveness of this route.

Figure 23. Cellular structure of *Amphiprora* sp. A. After pretreatment Organosolv. B. after lipid extraction. r: Remnants of cellular structures; c: Chromatophores. Scale represents 50 µm



SOURCE: Author

4.3.3. Evaluation of multifunctional-system routes (MSE) and (MSM)

4.3.3.1. Modeling reducing sugars yield. Multifunctional system involves joint stages of acid hydrolysis or cellular disruption, lipid extraction and in situ transesterification. Cell wall breaking of microalga *Navicula* sp., releasing polysaccharides and lipids from walls and cytoplasm, operating conditions allows carrying out the hydrolysis and transesterification reactions. Triglyceride molecules released from the cellular disruption step reacts with ethanol or methanol, under the catalytic action of sulfuric acid, yielding reducing sugars, fatty acids esters and glycerin. In this multifunctional process, sulfuric acid acts as a catalyst for hydrolysis and in situ transesterification reactions. Equation (7) was obtained by mathematical development of Equations (4) to (6); it relates the concentration of total reducing sugars with constant speed and time. Numerical value in the equation is based on microalgae polysaccharide material reported by Ververis et al. [158].

$$C_{RS} = \frac{25{,}328\ K_1}{K_1 - K_2}\left(e^{-K_1 t} - e^{K_2 t}\right) \quad (7)$$

Table 19 shows the kinetic parameters obtained from multifunctional process route using ethanol (MSE) and methanol (MSM), *n* is an exponential factor obtained experimentally that power the acid concentration, *A* is a pre-exponential factor, *E* is the activation energy of the reaction, *K* represents the rate constant, $X_{E/M}$ the relationship between the experimental concentration of reducing sugars obtained respect to reducing sugars concentration modeled in time, $Y_{E/M}$ is the ratio of the logarithm of the experimental concentration of RS compared to the logarithm of the concentration of reducing sugars modeled in time.

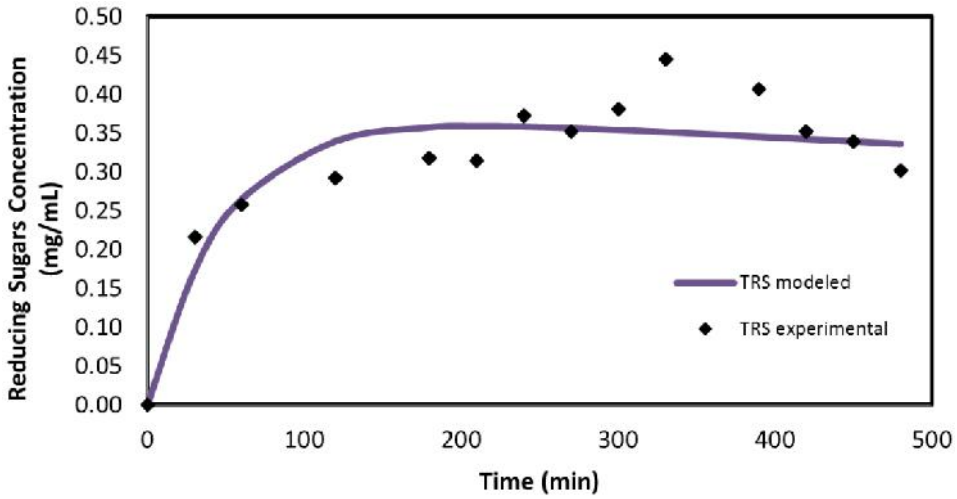
Table 19. Modeled kinetic parameters for the microalga *Amphiprora* sp. using ethanol and methanol

Reaction System	Product	n	A (min^{-1})	E (kJ/mol)	K (min^{-1})	$X_{E/M}$	$Y_{E/M}$
(MSE)	Reducing Sugars	0.16	0.05	15.52	$3.02*10^{-4}$	1.01	0.99
	Degradation Products	0.35	1.13	14.08	$2*10^{-2}$		
(MSM)	Reducing Sugars	0.16	0.03	15.52	$1.75*10^{-4}$	0.99	1.04
	Degradation Products	0.34	0.45	14.38	$6.98*10^{-3}$		

SOURCE: Author

4.3.3.2. Effect of solvent. Through the calculated kinetic parameters, concentration of reducing sugars was modeled. The behavior of the concentration of reducing sugars in each system shows a stabilizing trend (Figures 24 and 25). The difference in the reaction systems evaluates corresponds to the rate of sugars production from the polysaccharide chains contained in the cellular structure of the microalga *Amphiprora* sp.

Figure 24. Modeling of the production of total reducing sugars in time using MSE route

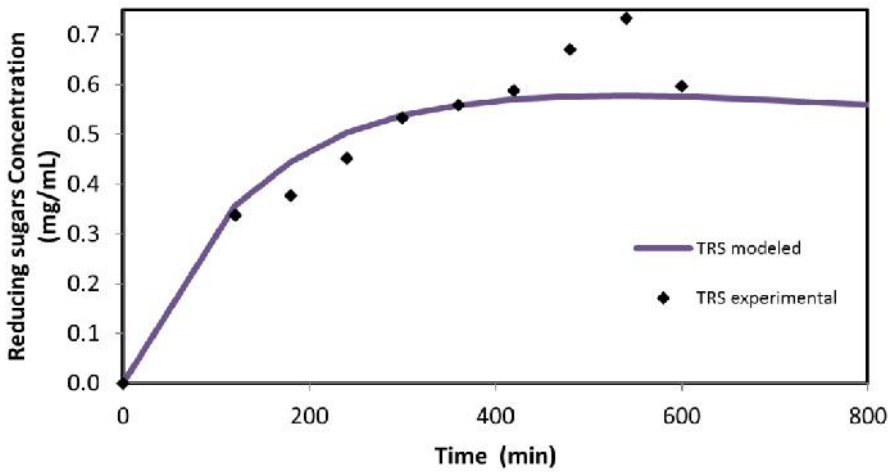


SOURCE: Author

According to the model established for the multifunctional system with ethanol, reducing sugars production reaches a plateau value after 90 minutes of reaction, after this time there was a minimal degradation of sugars. This behavior can be attributed to the role of sulfuric acid, it breaks the cellular walls releasing sugar molecules, but after some time of contact, these monomers are gradually degraded.

Behavior above described also appears in the MSM route, the difference lay in the total reducing sugar concentration reached in the process. After 420 minutes of reaction the highest concentration of reducing sugars was reached, after this time, concentration of sugars remains relatively constant. Subsequently, free reducing sugars are degraded by action of acid (Figure 25).

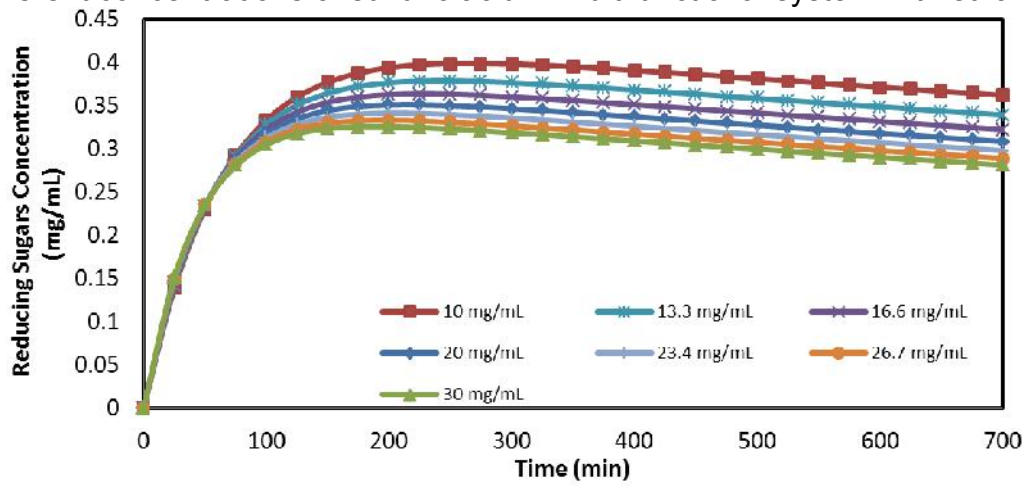
Figure 25. Modeling the production of total reducing sugars in time using MSM route



SOURCE: Author

3.3.2 Sensibility analysis of reducing sugars production and degradation. Effect of sulfuric acid concentration on the total reducing sugars production for MSE and MSM were modeled. The different concentrations of acid conducted a proportional cellular disruption for 75 minutes in the process, after this time moment generated differences in the concentration of reducing sugars, so that the sulfuric acid concentration corresponding to 10 mg/mL allows obtaining higher amounts of reducing sugars, 0.40 mg/mL for a time of 275 minutes of reaction. The increase in acid concentration produces a reduction in the concentration of RS, for example, to simulate the production of sugar with an acid concentration of 30 mg/mL, the peak of RS was 0.32 mg/mL in 175 minutes (Figure 26).

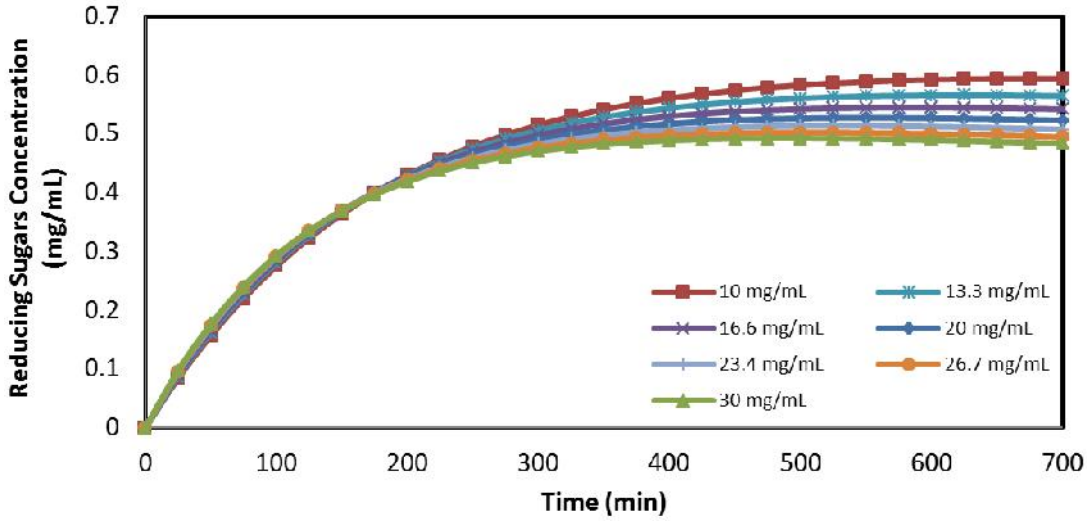
Figure 26. Modeling the concentration of total reducing sugars in time, using different concentrations of sulfuric acid in multifunctional system with ethanol



SOURCE: Author

For MSM system, different concentrations of sulfuric acid evaluated showed similar behavior to the concentration of reducing sugars during the first 175 minutes of process, where production of sugars was 0.40 mg/mL, approximately. After 675 minutes, it was the highest concentration of total reducing sugars, 0.59 mg/mL, which was achieved with an acid concentration of 10 mg/mL (Figure 27).

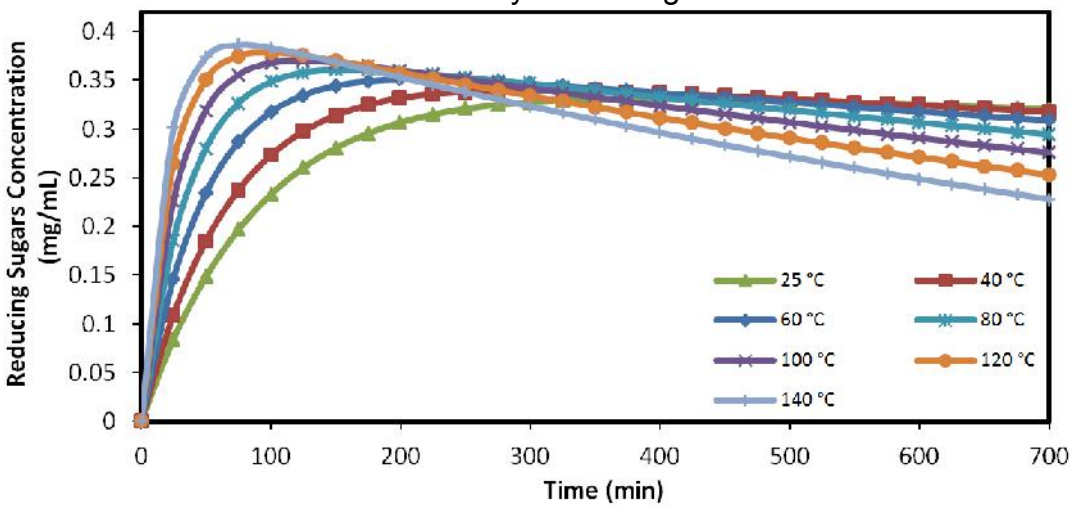
Figure 27. Modeling the concentration of total reducing sugars in time, using different concentrations of sulfuric acid in multifunctional system with methanol



SOURCE: Author

Effect of sulfuric acid concentration was similar in both scenarios; at lower concentrations production of total reducing sugars was increased. Alcohol used affects the concentration of reducing sugars and reaction time. Multifunctional system using methanol yielded higher reducing sugars concentrations for the same reaction time in comparison to the same system using ethanol. Influence of temperature on the concentration of total reducing sugars was also evaluated, in the ethanol-based system, higher temperatures triggered the concentration of sugars, reaching 0.39 mg/mL in 75 minutes of reaction (Figure 28).

Figure 28. Effect of temperature on the total reducing sugar production in the multifunctional system using ethanol



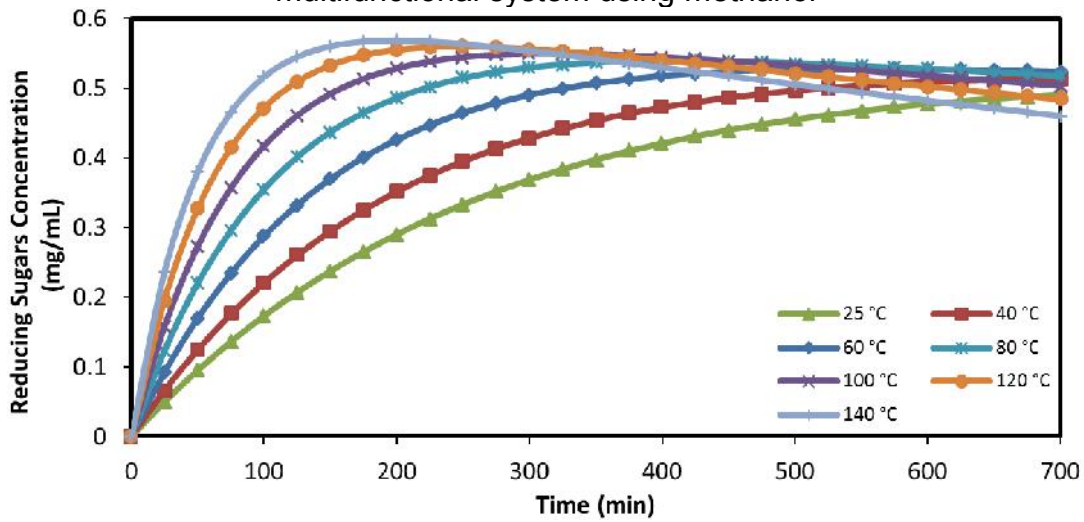
SOURCE: Author

Changes in temperature increases cellular lysis significantly, which was reflected in the rapid rate of reducing sugars production. However, this variable also contributes to their faster degradation. According to the modeling of multifunctional system with methanol, using a reaction temperature of 140 °C, concentration of reducing sugars reaches a value of 0.57 mg/mL for a reaction time of 200 minutes, after this point relevant, reducing sugars begins to degrade slowly. Behavior of reducing sugars production and degradation differs drastically if reaction systems are compared, showing that the type of alcohol used also affects the shape of the curves when effect of temperature is evaluated.

Multifunctional system model results shows that temperature effect is more significant than acid concentration effect (Figure 29), allowing to obtain higher reducing sugars amounts in less time, however, degradation products concentration is also increased at long times when temperature is increased, for this reason is recommended to stop the reaction when plateau value is reached, for all cases studied, is clear that use of methanol as solvent is more

convenient than the use of methanol for a multifunctional reaction system focused on reducing sugars production.

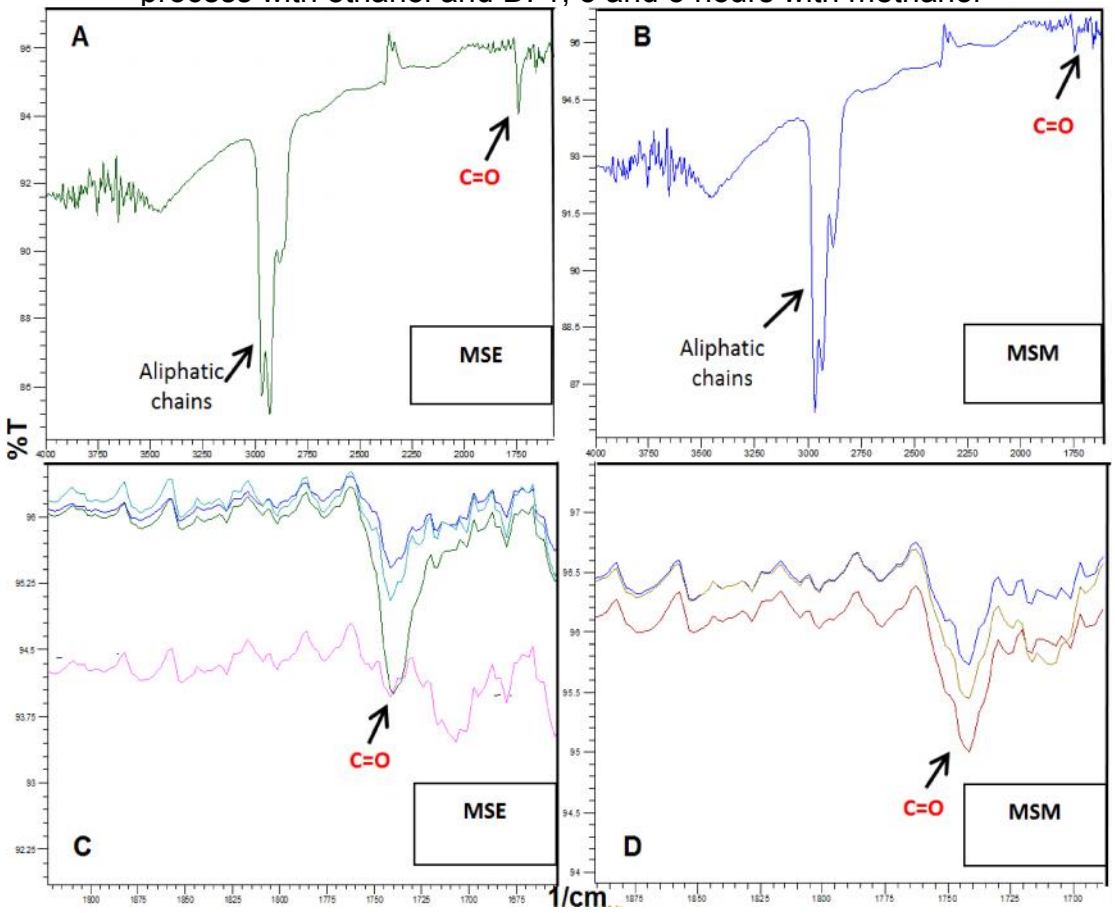
Figure 29. Effect of temperature on the total reducing sugar production in the multifunctional system using methanol



SOURCE: Author

4.3.3.3. Determination of the alkyl esters production in MSE and MSM routes. By measurements of mid-infrared transmittance, the presence of alkyl esters or biodiesel in the hexane phase of the samples was evaluated. The spectra obtained by spectroscopic Fourier transform infrared (FTIR) indicated an increase in the band area corresponding to the carbonyl bond (C=O) around of 1750 cm^{-1} and the strip forming of the aliphatic chains between 2800 and 3000 cm^{-1}, after 2 hours of reaction for the system with ethanol and 1 hour for the process with methanol (Figures 30a and b). Carbonyl peak, characteristic of esters increased with reaction time, attributed to the oil release and/or formation of alkyl-esters (Figures 30c and d). This process facilitated the direct conversion of microalgae biomass to alkyl esters, eliminating the step of solvent-based lipid extraction, which is necessary to obtain oil by the conventional method.

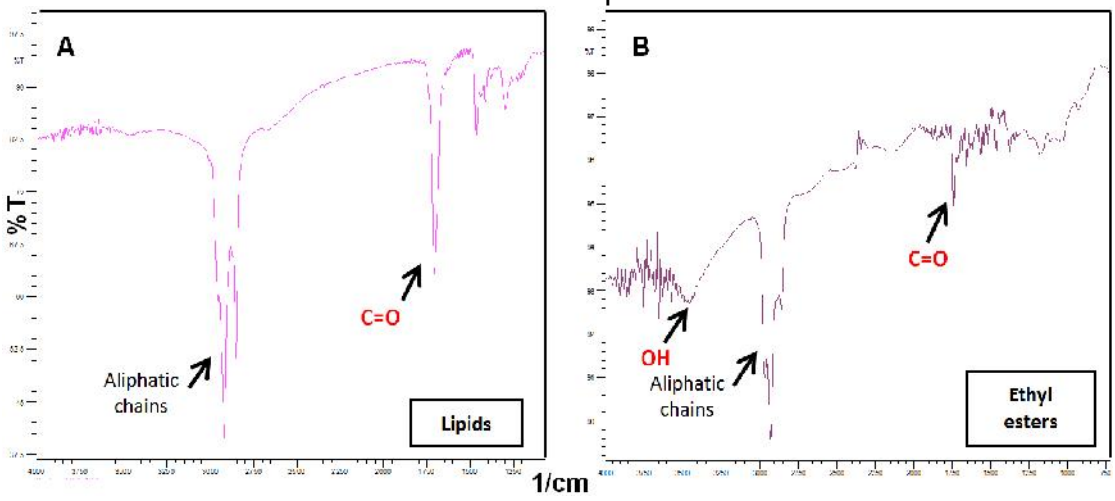
Figure 30. FTIR spectra of the hexane phase of the samples taken from the multifunctional process. A. Sample of 4 hours for the system with ethanol and B. methanol. C. Expansion of C=O bands for samples 2, 3 and 4 hours in the process with ethanol and D. 1, 3 and 5 hours with methanol



SOURCE: Author

Additionally, for ensure the presence of biodiesel after the multifunctional system implementation, two additional comparisons were made, FTIR spectra of hydrophobic phase from reaction system was compared to *Navicula* sp. oil FTIR spectra obtained by HSE route (Figure 31), the ester bond has been reported in both lipids and biodiesel [159, 160], specifically the carbonyl bond (C=O) was found to 1704 cm^{-1} in the sample of lipids and 1750 cm^{-1} in ethyl esters. The band of aliphatic chains was identified in 2918 cm^{-1} for oil and 2970 cm^{-1} for ethyl esters.

Figure 31. FTIR spectra of A. lipids obtained by acid hydrolysis - solvent extraction route and B. hexane phase of the sample of 10 hours reaction of multifunctional process



SOURCE: Author

For hydrophobic layer of MSE and MSM routes, was identified a peak at 3400 cm^{-1}, which corresponds to the OH bond, characteristic spectra of glycerin, according to Ooi et al., [161]. This was attributed in this case to the source of biodiesel, which comes from a multifunctional system where there was not a products purification process.

FTIR spectra peaks for lipids, alkyl esters and petrodiesel were also compared (Table 20). The ester bond of microalgae lipids comprising the region from 1654 to 1746 cm^{-1}, spectra of samples evaluated shows a peak 1704 cm^{-1}. The carbonyl group peak was reported at 1750 cm^{-1} in spectra of palm esters and lipids [162] and alkyl esters of *Amphiprora* sp. In petrodiesel, vibration was found only for aliphatic chains between 2800 and 3000 cm^{-1} [163]. This region is present in biodiesel as petroleum diesel and is due to the absorption of infrared bond olefin (CH).

Table 20. Spectrum characteristic regions of lipids, biodiesel and petrodiesel

Sample	Vibration	Microalgae	Palm	Petrodiesel
		Region (cm^{-1})		
Lipids	Aliphatic chains (CH_3 y CH_2)	2954-3025 2918*	2800-3000	Absent
	Carbonyl bond (C=O)	1654-1746 1704*	1750	
Biodiesel/fossil diesel	Aliphatic chains (CH_3 y CH_2)	2970*	2800-3000	2800-3000
	Carbonyl bond (C=O)	1750*	1750	
	Glycerol (bond OH)	3400		

* Regions identified in this study using FTIR.

SOURCE: Author

Effect this system on cellular structure of the microalga *Amphiprora* sp. allowed to obtain monosaccharides and biodiesel through extraction and in situ transesterification of lipids released in the process. Monosaccharides are abundant constituents of the polysaccharides in the cellular wall and cytoplasm of *Amphiprora* sp., main sugars present are glucose, galactose, mannose, ribose and other sugars in varying proportions. These sugars related to the structural polysaccharides of diatoms were released by the attack of acid catalyst (H_2SO_4), through hydrolysis of the glycosidic bond that allows the progressive formation of monomers.

4.3.3.4. Fatty acid composition of lipids extracted. Studies performed by other authors concluded that high percentages of C16 and C18 monounsaturated Fatty Acid Alkyl Esters are ideal constituents of biodiesel, owing to their behavior related to oxidative stability and crystallization [164], taking into account this topic, as *Navicula* sp. as. *Amphiprora* sp. microalgae

oil presents similar percentages of monounsaturated C18 and did not show presence of monounsaturated C16 (Table 21). Taking into account a desirable fatty acid for suitable biodiesel properties, which includes a high percentage of monounsaturated fatty acids and low percentages of saturated fatty acids, trienoic fatty acids and very long chain fatty acids [168], oil from *Amphiprora* sp. microalgae is more suitable for biodiesel production than *Navicula* sp. microalgae oil. However, taking into account selection criteria recommended by Moser & Vaughn [165], neither *Amphiprora* sp. nor *Navicula* sp. are suitable for a good quality biodiesel without adding an additive to the fuel produced.

Table 21. Characterization of oil extracted from *Amphiprora* sp. and *Navicula* sp. microalgae

Fatty acid	% Fatty acids	
	Amphiprora sp.	*Navicula* sp.
C14:0	9	18.1
C16:0	5.5	14.3
C18:1	5.9	4.3
C18:2n9,12t	31.7	11.9
C18:2	15.2	17.5
C6:0	1.8	0
C8:0	0.5	0
C11:0	0.1	1.3
C12:0	0.4	1.1
C13:0	1	4.3
C15:0	1.4	3.2
C18:1n9t	3.3	1
C18:3	4.6	2.4
C20:2n11,14c	3.7	3.8
C20:4	0.8	0.4
C22:0	0.8	1.8
C22:2	3.9	1.7
C23:0	0.4	1
Saturated fatty acids (%)	20.9	45.1
Monounsaturated fatty acids (%)	9.2	5.3
Polyunsaturated fatty acids (%)	59.9	37.7

SOURCE: Author

4.3.4. Comparison of routes evaluated

After a comparison of best operating conditions for all routes evaluated as reducing sugars production as microalgae oil extraction (Table 22), is shown that OSE route is the most adequate for an effective lipid extraction for microalgae *Amphiprora* sp., followed by MSE and MSM routes, these routes besides allows a direct transesterification of extracted oil, however, separation of products obtained is limited to hydrophilic/lipophilic components, purification of biodiesel, oil and fermentable sugars must be studied in further research, HSE and OSE routes presents lower extraction efficiencies and needs additional esterification/transesterification steps for microalgal biodiesel production, nevertheless, these routes are convenient if the goal is to separate high value fatty acids prior to glycerides transesterification, in addition, existing large-scale biofuels production infrastructure can be more adaptable to these kind of routes.

Table 22. Comparison of routes for obtaining reducing sugars and lipids from *Amphiprora* sp

Route	HSE	OSE	MSE	MSM
Reducing Sugars yield (mg/mL)	0.45	1.47	2.5	2.63
Relative Extraction Ratio (%)	75.58	92.04	89.01	82.32

SOURCE: Author

Comparison of routes taking into account reducing sugars production shows that multifunctional systems are more convenient from the efficiency point of view than cell disruption/oil extraction routes, obtaining up to five times more reducing sugars than HSE route, according to Ferrer et al. [166], concentration of reducing sugars in sugar cane bagasse, is in the range from 2.58 to 20.45 mg/mL, with an overall average of 10.53 mg/mL, although yield

of reducing sugars from microalgae is lower than bagasse, values obtained in this study are comparable to values obtained with other sources as bean dregs waste [167] and cashew apple bagasse [168]. In a microalgae-based topology of biorefinery is attractive the utilization of fermentable sugars for bioethanol production which can be used as reagent for transesterification and organosolv pretreatment, pigments and proteins extraction, or can be purified and valued as biorefinery product for commercialization, distribution and use.

4.4. CONCLUSIONS

Four routes for obtaining reducing sugars, and lipids from microalgae biomass were evaluated and compared. For acid hydrolysis-solvent extraction (HSE) route, as hydrolysis time as solvent extraction time has a positive effect on microalgae oil release, a Relative Extraction Ratio of 75.58 % and a reducing sugars yield of 0.45 mg/mL were reached using a hydrolysis time of 120 minutes and a solvent extraction time of 16 hours, having also a significant effect on microalgae cell morphology for both studied strains. Fatty acid composition of lipids extracted reveals that despite neither *Amphiprora* sp. nor *Navicula* sp. oil satisfies completely the parameters proposed by Moser and Vaughn for a good quality biodiesel, oil of *Amphiprora* sp. is more suitable for biodiesel production taking into account percentage of monounsaturated fatty acids, saturated fatty acids, trienoic fatty acids and very long chain fatty acids.

Implementation of Organosolv pretreatment-solvent extraction route (OSE) presented a lipid extraction efficiency of 92.04% and a reducing sugars yield of 1.47 mg/mL, both results were higher in comparison to HSE route, matching operating conditions for both routes can be concluded that as the presence of an organic solvent as the increase of temperature in cell

disruption stage produces higher process efficiencies, this pretreatment promotes an aggressive disruption on the cellular wall of the strain which is evidenced with the remnants of microalgal organelles after solvent extraction. Multifunctional-system routes using ethanol (MSE) and methanol (MSM) are schemes that allow the production of monosaccharides by the dissociation of cellular structure, oil extraction and transesterification of lipids released in the process. Kinetic parameters for reducing sugars production and degradation were and reaction in time was modeled, for both systems behavior is similar, but reducing sugars concentration peak is reached faster in MSE system. Sensitivity analysis of reactions modeled shows that lower concentration of sulfuric acid and higher temperatures increases the production of RS. The versatility of bioproducts and operating conditions through these routes are considered important for the development and scaling of biorefineries.

Alkyl esters production in MSE and MSM routes was confirmed by FTIR experiments by comparing microalgae crude oil, palm oil, petrodiesel and hydrophobic phase obtained after performing multifunctional system experiments ratifying the potential of this alternative for obtaining multiple products in the same volume unit, however, separation of extracted and/or transformed microalgae components must be studied and could decrease the viability of this route.

Comparison of evaluated routes shows that OSE is the most convenient of routes evaluated from the efficiency point of view for cell disruption and microalgae oil extraction in lab-scale, and multifunctional routes presents the highest reducing sugars yield which are comparable with bean dregs waste and cashew apple bagasse.

5. CHAPTER V. COMPUTER AIDED PROCESS ENGINNERING APPLIED TO EVALUATION OF MICROALGAE PROCESSING ALTERNATIVES[5]

5.1. INTRODUCTION

The creation and using of a process simulation model can provide several benefits for existing and emerging technologies evaluation as the ability to analyze the behavior of the process without the costs of pilot plant trials or test runs, saving man-hours and losses in production, also, with process simulation can be avoided safety problems that might occur when operational conditions changes in a process. This advantage of study the behavior of a system without building it can be used for evaluation and comparison of novel designs and technologies as those related to microalgae processing.

The first step in building a process simulation is usually establishing the chemical basis for the model. This consists of choosing the components that will be included in the mass balance [169], for the case of microalgae processing, it is necessary a robust modelling of microalgae strain and the inclusion of microalgae components that are not available in software library. Second step is the choose of an appropriate thermodynamic model which can predict some physical properties and phase equilibrium, after that, plant

[5] This chapter is based on the papers "*Evaluation of alternatives for microalgae oil extraction based on exergy analysis*" by Angel Darío González-Delgado, Yeimmy Peralta-Ruiz, and Viatcheslav Kafarov, published in Applied Energy Journal, Vol. 101, 226 – 236 (2013), "*Energy integration of bioethanol production process topology from microalgae biomass: evaluation of SSCF, SSF, Acid Hydrolysis and product purification alternatives*" by Angel Darío González-Delgado, Yenifer Pardo, Yeimmy Peralta-Ruiz, and Viatcheslav Kafarov, published in Chemical Engineering Transactions Journal, Vol. 35, 1069-1074 (2013), "*Environmental assessment of microalgae biodiesel production in Colombia: Comparison of three oil extraction systems*" by Angel Darío González-Delgado, Yenifer Pardo, Israel Herrera-Orozco and Viatcheslav Kafarov Published in CT&F Journal, Vol. 5(2), 85-100 (2013), and "*Simulation of bioethanol production process from residual microalgae biomass*", by Angel Darío González-Delgado, Yenifer Pardo, Yeimmy Peralta-Ruiz, and Viatcheslav Kafarov published in Computer Aided Chemical Engineering Journal, Vol. 30 (1), 1048 – 1052 (2013).

capacity is determined, suitable unit operations and reactors are included, and setting up input conditions such as flow rate, temperature, pressure, among others are established. Simulation is assembled, debugged, checked and corrected, and technical results obtained are interpreted, with technical information given by the simulation, emerging pathways can be compared, or information can be used as starting point for further evaluations under another criterion (energy, economics, safety, environmental etc.).

In this study, several cases of study are shown in order to demonstrate the different computer-based approaches for the evaluation, comparison and selection of microalgae processing alternatives which can be used. For the simulation of some process steps was used the industrial process software Aspen Plus, version 7.1, which has been widely used for studies related to simulation of existing and emerging technologies for biofuels and co-products production in large scale using several feedstocks [170-172].

5.2. MODELING MICROALGAE STRAIN FOR EVALUATION

This study starts with the robust modelling of a representative microalgae strain, which allow obtaining more realistic results in comparison to utilization of an empirical formula for microalgae, and gives a better characterization of process streams. Selection of microalgae for use in the production of biofuels and other valuable products, must have a number of features such as: high oil content for the case of biodiesel or high value fatty acids production, high biomass productivity in order to decrease the area needed for cultivation, resistance to the pollution from the environment and resistance to the invasion of other organisms as predators or in competition for food, low production costs, among others. Studies reported shows different oil percentage for different strains and for the same strain [173-175], for this reason, can be concluded that oil content in microalgae can vary depending of strain, and in the same strain depends on cultivation conditions (light,

nutrients, time of harvesting, changes in diet during growth, among others) Table 1 shows the oil productivity of microalgae. Percentage of other metabolites is also important to be taken into account for the development of a simulation for microalgae processing alternatives and for the development of a microalgae-based biorefinery.

Unfortunately, excepting the oil percentage, most of information mentioned above is not available for all existing microalgae strains, for this reason, the most important criteria for selecting the strain in this study will be the availability of detailed information related to microalgae composition besides oil percentage as amino-acid profile, fatty acids profile, percentage of triglycerides, percentage of cellulosic material.

Considering the information shown in Table 23; and the information available about physicochemical characterization of strains and cultivation behaviour; *Chlorella* sp. was taken as the representative genera for simulation and evaluation of processing alternatives. It is reported that this strain presents high growth rates and can produce large quantities of lipids [176]. For the simulation of microalgae *Chlorella* sp. biomass composition, normalization was made taking into account experimental information reported in literature and unpublished results obtained in lab-scale by Center for Sustainable Development in Industry and Energy.

Table 23. Lipid content of several species of marine and freshwater microalgae (modified from Mata, Martins and Caetano, 2010)

Strains of marine and freshwater microalgae	Total lipid content (% in dry weight biomass)
Ankistrodesmus sp.	24–31
Botryococcus braunii	25-75
Chaetoceros calcitrans	14.6-39.8
Chlorella emersonii	25-63

Chlorella protothecoides	14.6-57.8
Chlorella vulgaris	5-58
Chlorella sp.	10-48
Chlorococcum sp	19.3
Dunaliella sp.	17.5-67
Dunaliella tertiolecta	16.7-71.0
Haematococcus pluvialis	25
Isochrysis galbana	7-40
Nannochloris sp.	20-56
Nannochloropsis oculata	22.7-29.7
Nannochloropsis sp.	12-53
Neochloris oleobundans	29-65
Pavlovalutheri	35.5
Phaeodactylum tricornutum	18-57
Porphyridium cruentum	9-60.7
Scenedesmus obliquus	11-55
Spirulina maxima	4-9

Oil content of microalgae strain was fixed in 30% [6], which corresponds to 5.11% of free fatty acids and 94.89% corresponding to triglycerides [177], the same percentage distribution was taken for fatty acids present in triglyceride profile, based on the work associated to *Chlorella* sp. microalgae oil composition developed by Petkov & Garcia [178], and the simulation of Chlorella vulgaris microalgae oil for a esterification/transesterification process developed by Sanchez et al. [179], a robust model of microalgae oil was developed, containing 9 fatty acids and 9 triglycerides, as assumption, triglycerides present in microalgae oil were considered homogeneous by a lack of a representative triglyceride profile reported in literature and taking into account that in transesterification stage, these chains are broken obtaining a known fatty acids methyl esters profile [180], protein percentage was fixed in 40%, aminoacids present in significant percentage were also simulated [181], carbohydrates percentage was fixed in 25% divided in lignin, cellulose and

hemicellulose [182]. Table 24 shows the consolidated and normalized modelled composition of *Chlorella* sp. microalgae.

Table 24. Modelled composition of *Chlorella* sp. Microalgae

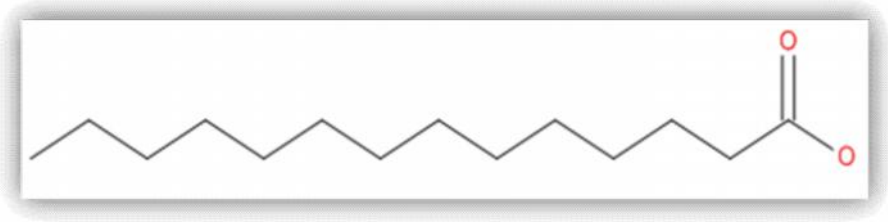
	Components	*Chlorella* sp. composition (%)
Free fatty acids		
	C14:00	0.14
	C16:00	0.38
	C16:01	0.03
	C16:02	0.15
	C16:03	0.14
	C18:00	0.01
	C18:01	0.08
	C18:02	0.31
	C18:03	0.29
	Total Free fatty acids	1.53
Triglycerides		
	TAG-C14:00	2.56
	TAG-C16:00	7.15
	TAG-C16:01	0.57
	TAG-C16:02	2.85
	TAG-C16:03	2.56
	TAG-C18:00	0.26
	TAG-C18:01	1.42
	TAG-C18:02	5.69
	TAG-C18:03	5.41
	Total Triglycerides	28.47

Amino acids		
	Aspartic acid	4.49
	Glutamic acid	5.47
	Glycine	4.35
	Alanine	5.40
	Valine	3.86
	Leucine	4.28
	Proline	5.05
	Lysine	7.15
	Total Amino acids	40.05
Carbohydrates		
	Cellulose	7.10
	Lignin	1.52
	Hemicellulose	16.30
	Total Carbohydrates	24.92
Water		5.03
Total microalgae		100.00

SOURCE: Author

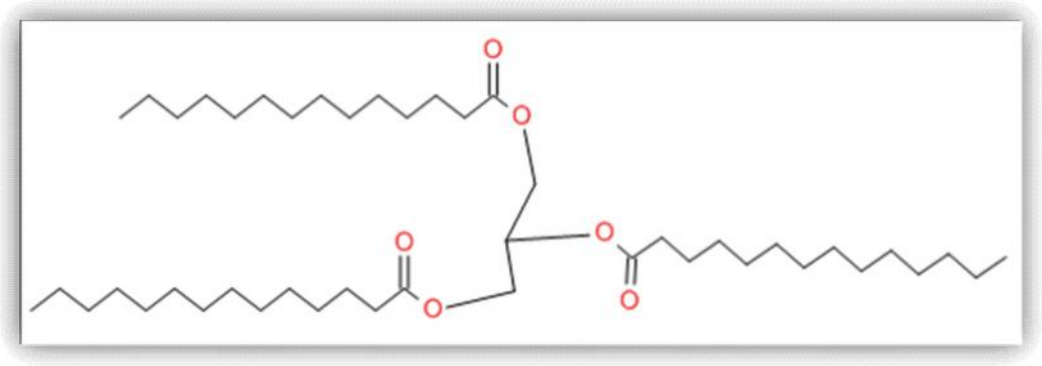
Chemical compounds available in the software database were selected and used, for compounds without availability in the software library, molecules were created using Symyx Draw, Figures 32 and 33 shows some structures developed; after that, the molecules created were exported to the process simulation software using the User Defined Compound Wizard tool, also some properties known as normal boiling point, molecular weight, acentric factor and critical properties were introduced.

Figure 32. Free Fatty Acid molecule C14: created in Symyx Draw



SOURCE: Author

Figure 33. Triglyceride molecule TAG14:00 created in Symyx Draw



SOURCE: Author

Unknown properties of molecules were estimated using UNIFAC (Universal Functional Group Activity Coefficient) model, Fragment-based method and the database Thermo Data Engine (TDE). Thermodynamic properties were calculated based on molecular structures of each compound, NRTL (Non-Random Two Liquids) and Soave-Redlich-Kwong thermodynamic model was used for process simulation, this model was chosen because of its good representativeness of polar-non polar mixtures. Binary interaction coefficients were calculated by the UNIFAC method.

5.3. CASES OF STUDY

5.3.1. Simulation and exergy analysis of microalgae oil extraction alternatives

Thermodynamic techniques like energy analysis, exergy analysis, emergy analysis, among others have been widely used for evaluation of industrial systems and thermal energy storage processes [183-186]. Energy analysis includes balances based on the first law of thermodynamics, and calculation of energy efficiencies for the steps studied. However, an energy balance neither offers information related with the energy degradation nor quantifies the usefulness or quality of the mass and energy streams of the system evaluated. Exergy analysis is presented as an alternative which overcomes the limitations of the first law of thermodynamics. Exergy analysis shows the sites of energy degradation in a process and can help to improve a unitary operation, a technology or a process [187]. In addition, exergy analysis allows to evaluate and select different alternatives to improve the design of a process, which makes it an appropriate tool for evaluation of novel technologies for advanced biofuels production.

The term exergy can be defined as the maximum theoretical useful work that could be obtained from a system that interacts only with the environment if this has not reached the thermodynamic equilibrium [188], taking into account that, the exergy of a system depends on the reference state selected, for this reason, a good choice of reference state must be made in order to avoid erroneous results.

For a general steady state, steady-flow process, four balance equations must be applied in order to find the work and heat interactions. These equations are the principle of mass/matter conservation given by Equarion (1), the first law of thermodynamics given by Equation (2), the second law of thermodynamics given by Equarion (3), and the global exergy balance given by Equation (4).

$$\sum_i(\dot{m_i})_{in} = \sum_i(\dot{m_i})_{out} \quad (1)$$

$$\sum_i(\dot{m}_i * h_i)_{in} - \sum_i(\dot{m}_i * h_i)_{out} + \dot{Q} - \dot{W} = 0 \quad (2)$$

$$\sum_i(\dot{m}_i * s_i)_{in} - \sum_i(\dot{m}_i * s_i)_{out} + \sum_i \frac{\dot{Q}_i}{T_i} = \dot{S}_{gen_i} \quad (3)$$

$$\dot{E}x_{mass,in} - \dot{E}x_{mass,out} + \dot{E}x_{heat} - \dot{E}x_{work} = \dot{E}x_{loss} \quad (4)$$

Mass exergy component expressed as is shown in Equation (5), is divided into four specific components: the physical exergy ($\dot{E}x_{phy}$) related to temperature, enthalpy and entropy given by Equation (6); chemical exergy ($\dot{E}x_{chem}$) related to the chemical exergy of each compound per mol (Ex^o_{ch}); potential exergy, $\dot{E}x_{pot}$ and kinetic exergy, $\dot{E}x_{kin}$. The calculation of the chemical exergy of each compound per mol (Ex^o_{ch}) is given by Equation (7), and is a function of the chemical exergy of each elemental compound ($Ex^o_{ch,elem}$), the number of atoms of each element contained into the stream (n_{elem}) and the Gibbs free energy of formation for the compound (ΔG^o_f) [189]. The chemical exergy of the process streams was evaluated by Eq. (8), where y_i is the molar fraction of the component i, $Ex^o_{ch,i}$ is chemical exergy of pure compound, T_o is reference temperature and R is the gas constant. Kinetic and potential exergy was neglected because its contribution to the total exergy balance is minimal. Regarding exergy balance, the exergy transfer by heat

flow at a temperature T ($\dot{E}x_{heat}$) and exergy by work flow ($\dot{E}x_{work}$) was evaluated by Equation (9) and (10) respectively [187].

$$\dot{E}x_{mass} = \dot{E}x_{phy} + \dot{E}x_{chem} + \dot{E}x_{pot} + \dot{E}x_{kin} \quad (5)$$

$$\dot{E}x_{phy} = \left(\dot{H} - \dot{H}_o\right) - T_o * (\dot{S} - \dot{S}_o) \quad (6)$$

$$Ex^o_{ch} = -\Delta G^o_f + \sum_i n_{elem} * Ex^o_{ch,i} \quad (7)$$

$$Ex_{ch,mx} = \sum_i y_i * Ex^o_{ch,i} + RT_o \sum_i y_i * \ln(y_i) \quad (8)$$

$$\dot{E}x_{heat} = \left(1 - \frac{T_o}{T}\right) * \dot{Q} \quad (9)$$

$$\dot{E}x_{work} = \dot{W} \quad (10)$$

For cases of study presented in subsections 3.1 and 3.2, oil production capacity was chosen taking into account the necessary oil amount to produce 100,000 tons/year of third generation biodiesel, which corresponds to an approximate lipid flow of 104,000 tons/year. The main processing units for the three oil extraction routes include distillation columns, heat exchangers, pumps, mixers, liquid-liquid and solid-liquid separators. Mixers were used for blending solvents with biomass. Heaters were utilized for heating and cooling streams and centrifugal pumps were used for moving liquid streams. Separation of liquid phases was carried out using decanters. Separation and recovery of solvents in all methods were made by the use of distillation towers with ten ideal stages with total condenser and kettle reboiler in order to recover around 95% of the solvents in the oil stream.

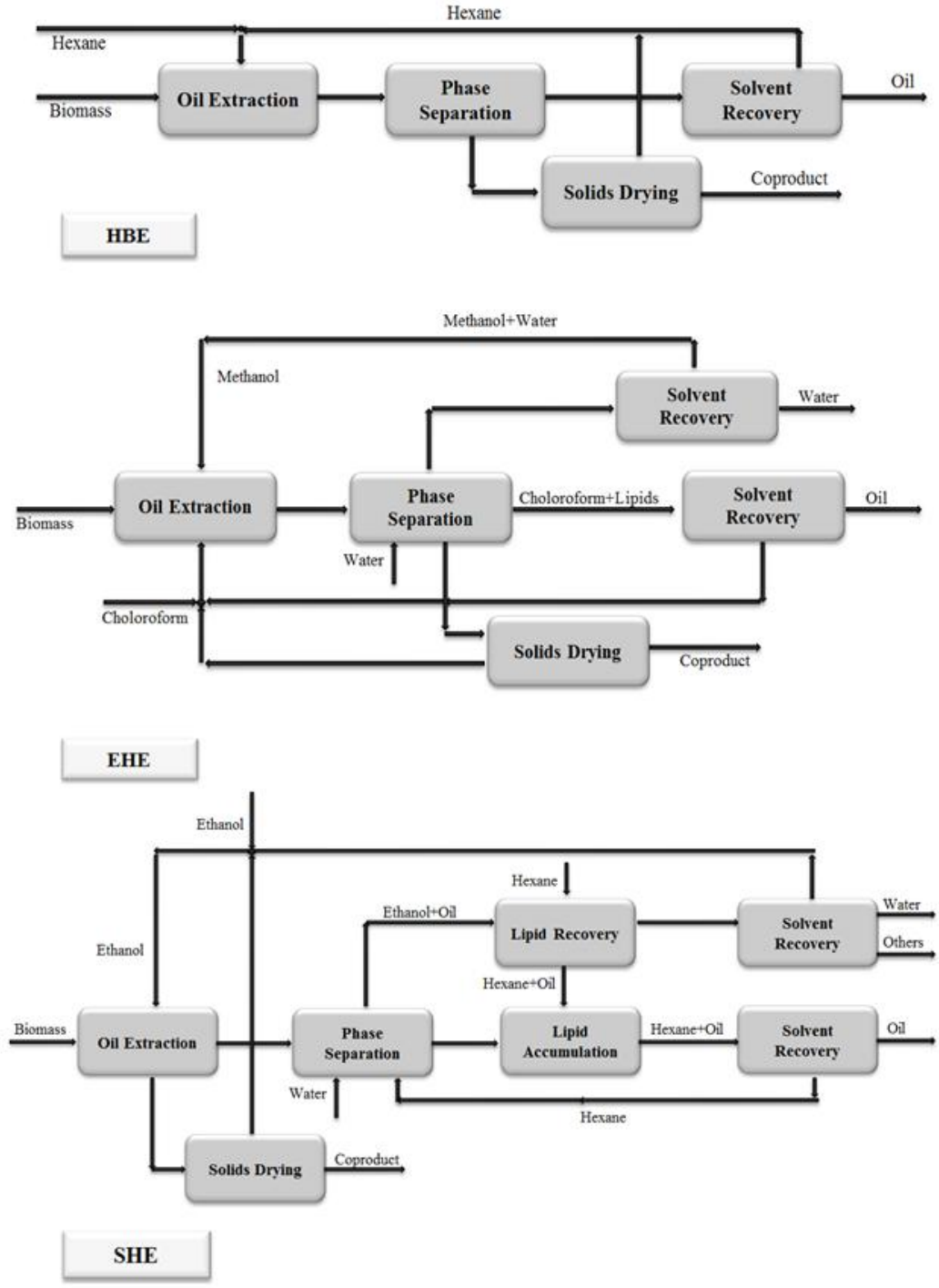
In order to avoid difficulties presented in lab scale related to purity of oil extracted given by selectivity of solvents which are pointed out previous chapters of this book, hydrocyclons and filters were used for homogenization

and separation of the microalgae solids from the oil. Continuous rotary vacuum filters were also included with the objective of removing most of the remaining solids, obtaining a separation efficiency of 97%. For the simulation of all routes, an oil extraction efficiency of 98% was assumed in order to obtain the maximum exergetic efficiency which is possible to reach.

The routes selected for the evaluation were hexane-based extraction (HBE), oil extraction using the mixture ethanol/hexane (EHE) and extraction with methanol-chloroform mixture (SHE). In simulation of HBE method, hexane is added to microalgae biomass under environmental conditions (298 K, 101.325 kPa) in a 20:1 mass ratio, after that, mixture is separated and filtered, obtaining a liquid stream rich in hexane and oil, and a stream of biomass rich in carbohydrates and proteins. Oil/hexane stream is separated through distillation and hexane is recirculated to the process, liquid components present in biomass stream after filtration are separated and purified in order to increase extraction efficiency, this method was chosen due to its easiness to perform and similarity with solvent-based processes for oil recovery from soybean.

In second route, crude microalgae oil was extracted by mixing ethanol with biomass in a mass ratio 4:1 at environmental conditions (298 K, 101.325 KPa), the mixture is stirred and goes to a separation process using a hydrocyclone in which solid and liquid phases are separated, residual biomass is once again sent to be mixed with ethanol in order to increase process efficiency, liquid water is added until an ethanol concentration of 40% is reached and hexane is also added in a 1:1 ratio with hydroalcoholic solution. The mixture is placed in a decanter where hydrophilic/hydrophobic phases are separated, hexane phase with selected lipids is distilled obtaining a product stream, hexane is recirculated and hydroalcoholic phase is also distillated recovering part of the ethanol for reuse in the process.

Figure 34. Schematic flowsheet of oil extraction methods evaluated using exergy analysis



SOURCE: Author

In simulation of SHE route, methanol, chloroform and biomass are mixed under environmental conditions in a mass ratio of 6:12:1, the mixture is homogenized and separated by filtration, obtaining a liquid stream with high percentage of lipids and a solid stream of biomass, the liquid phase is mixed with water in 4:1 ratio for a second phase separation. The hydrophilic and hydrophobic phases are split and each stream is distillated in order to obtain algae oil, methanol, chloroform and wastewater. Solvents are recycled. Although this method uses highly toxic solvents, it was included in this work due to its high efficiency of total lipid extraction in comparison to other methods in lab scale and the need of its evaluation from the energy point of view. Figure 34 shows the schematic flowsheet of oil extraction methods evaluated.

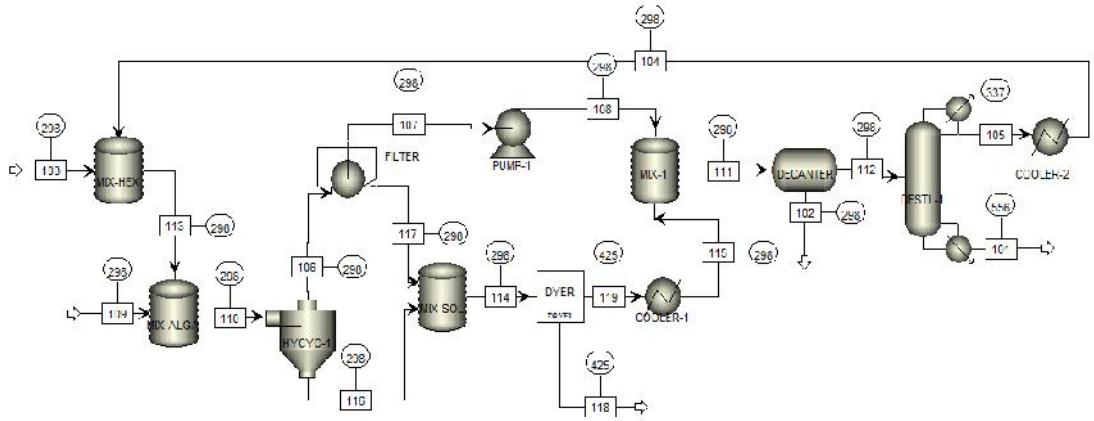
Estimation of energy consumption for each oil extraction method was made based on requirements of thermal energy for heat exchangers, reboilers and related operation units, obtained from each simulation performed. Global mass balance of all streams of the process was performed, and the thermodynamic properties needed to develop the exergy balance were obtained as is described in section 2 of this chapter, after that, chemical and physical exergies of streams involved in the routes were calculated. Each operation unit was independently balanced. Exergy was determined for each compound, mixture and utilities. As dead state conditions were taken environmental conditions (298 K, 101.325 KPa), exergetic efficiency of each oil extraction method was calculated using Equation 11.

$$\psi = 1 - \left(\frac{Exergy\ loss}{Exergy\ input}\right) \tag{11}$$

According to simulation of microalgae oil extraction methods four general stages are common in all methods: biomass-solvent mixing, oil extraction, solvent recovery (oil separation) and solids drying, simulation of hexane-

based extraction route (HBE) is shown in Figure 35. The system with four mixing units for fresh solvent input, solvent biomass mixing post-extraction solids mixing and liquid oil/hexane streams combination have to be incorporated. Solids streams after oil extraction are rich in carbohydrates and proteins as shown in Table 25, composition of this stream confirms the potential of coproduct as a feedstock for downstream processing, stream 101 which corresponds to microalgae oil extracted, presents a free fatty acid mole fraction of 0.14 which shows that lipids obtained can be used for a transesterification process without the need of previous free fatty acids esterification.

Figure 35. Simulation of hexane method for microalgae oil extraction (HBE)



SOURCE: Author

Table 25. Main process streams conditions for hexane based microalgae oil extraction (HBE)

Streams	101	106	109	111	112	114	116	117	118
Pressure (kPa.)	101.325	101.325	101.325	101.325	101.325	70.927	101.325	70.927	101.325
Temperature (K)	554.8	298	298	298	298	298	298	298	433
Mass Flow (kg/h)	12236.44	726857	36669	746488.20	745839	44461.65	43451.43	1010.22	23820.18
Component (mole fraction)									
Cellulose	0	0	0.06	0	0	0.04	0.04	0.05	0.08

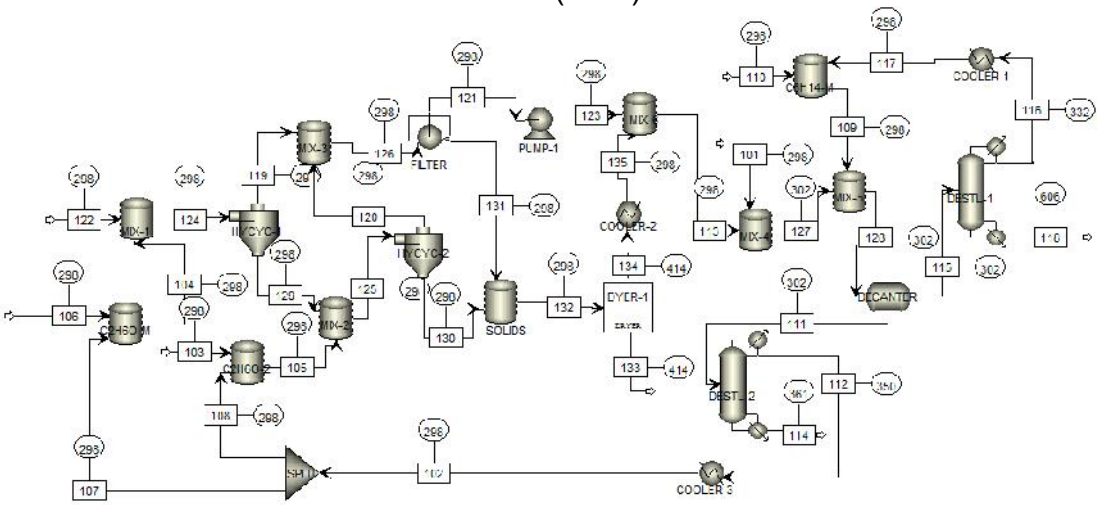
Lignin	0	0	0.01	0	0	0.01	0.01	0.01	0.02
Hemicellulose	0	0	0.17	0	0	0.1	0.1	0.14	0.21
Aspartic	0	0	0.05	0	0	0.03	0.03	0.04	0.06
Glutamic	0	0	0.05	0	0	0.03	0.03	0.04	0.06
Glycine	0	0	0.1	0	0	0.06	0.06	0.08	0.13
Alanine	0	0	0.1	0	0	0.06	0.06	0.08	0.13
Valine	0	0	0.05	0	0	0.03	0.03	0.04	0.07
Leucine	0	0	0.05	0	0	0.03	0.03	0.04	0.06
Proline	0	0	0.07	0	0	0.04	0.04	0.06	0.09
Lysine	0	0	0.08	0	0	0.04	0.04	0.06	0.1
C14H2-01	0.01	0	0	0	0	0	0	0	0
C16H3-01	0.04	0	0	0	0	0	0	0	0
C16H3-02	0	0	0	0	0	0	0	0	0
C16H3-03	0.01	0	0	0	0	0	0	0	0
C16H3-04	0.01	0	0	0	0	0	0	0	0
C18H3-01	0	0	0	0	0	0	0	0	0
C18H3-02	0.01	0	0	0	0	0	0	0	0
C18H3-03	0.03	0	0	0	0	0	0	0	0
C18H3-04	0.03	0	0	0	0	0	0	0	0
TGC14-01	0.09	0	0.01	0	0	0	0	0	0
TGC16-01	0.21	0	0.01	0	0	0	0	0	0
TGC16-02	0.02	0	0	0	0	0	0	0	0
TGC16-03	0.09	0	0.01	0	0	0	0	0	0
TGC16-04	0.08	0	0.01	0	0	0	0	0	0
TGC18-01	0.01	0	0	0	0	0	0	0	0
TGC18-02	0.04	0	0	0	0	0	0	0	0
TGC18-03	0.16	0	0.01	0	0	0	0	0	0
TGC18-04	0.15	0	0.01	0	0	0	0	0	0
H2O	0	0.01	0.14	0.01	0	0	0	0	0
C6H14	0.03	0.99	0	0.99	1	0.52	0.53	0.37	0

SOURCE: Author

Results of ethanol-hexane route (EHE) simulation (Figure 36) shows that more steps are needed for a continuous extraction process, as occurs in lab-scale; this means the installation of more equipment affecting the technical practicability of the route and the economy of the process. However the use of ethanol gives the possibility of several operation alternatives in a complete production chain, for example, the use of stream 112 which corresponds to ethanol output at 350 K from DESTL-2 can be used in transesterification

stage for ethyl esters production. As shown in Table 26, an effective lipid separation was reached and mass flow distribution in route stages is different to mass flow in HBE route, caused by the presence of additional steps.

Figure 36. Simulation of microalgae oil extraction using the mixture ethanol–hexane (EHE)



SOURCE: Author

Table 26. Main process streams conditions for microalgae oil extraction using the mixture ethanol-hexane (EHE)

Streams	115	118	119	122	124	125	128	129	130	132
Pressure (kPa.)	101.325	101.325	101.325	101.325	101.325	101.325	101.325	101.325	101.325	51.67
Temperature (K)	302	604.6	297.7	298	297.7	297.9	302.2	297.7	297.9	297.9
Mass Flow (kg/h)	653366.7	12212.38	177640.3	36662	210744.3	128752.5	1286204	33104.07	28405.48	31675.21
Component (mole fraction)										
Cellulose	0	0	0	0.06	0	0.01	0	0.04	0.03	0.04
Lignin	0	0	0	0.01	0	0	0	0.01	0.01	0.01
Hemicellulose	0	0	0	0.17	0.01	0.02	0	0.1	0.09	0.11

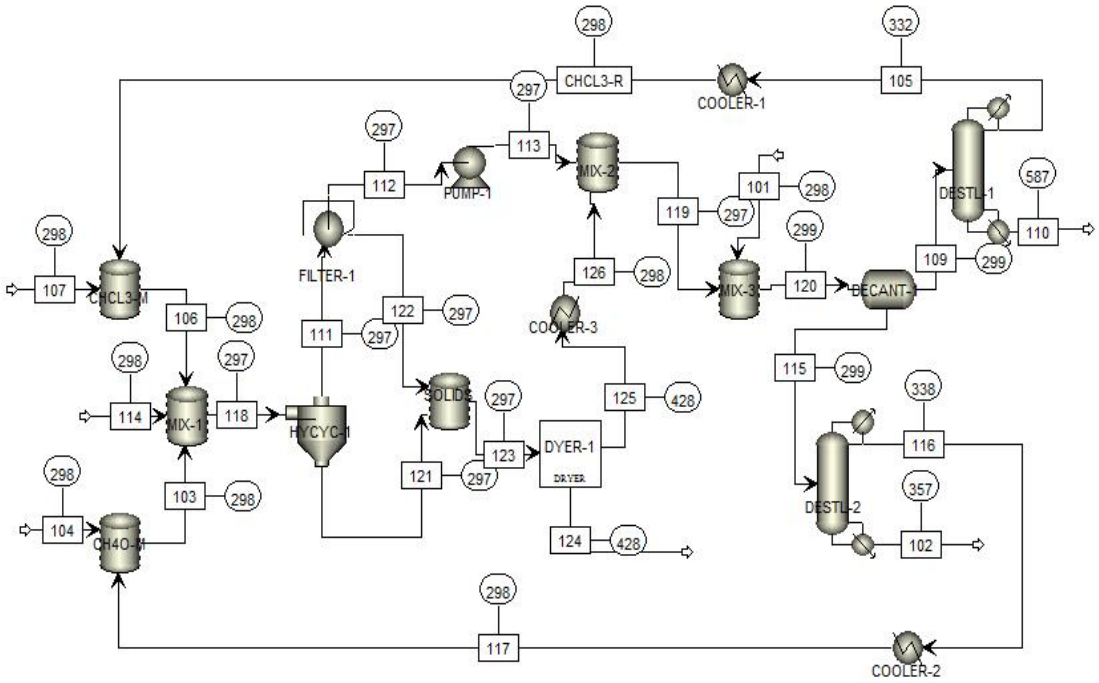
Aspartic	0	0	0	0.05	0	0	0	0.03	0.02	0.03
Glutamic	0	0	0	0.05	0	0	0	0.03	0.03	0.03
Glycine	0	0	0	0.1	0.01	0.01	0	0.06	0.05	0.07
Alanine	0	0	0	0.1	0.01	0.01	0	0.06	0.05	0.07
Valine	0	0	0	0.05	0	0	0	0.03	0.03	0.04
Leucine	0	0	0	0.05	0	0	0	0.03	0.03	0.03
Proline	0	0	0	0.07	0	0.01	0	0.04	0.04	0.05
Lysine	0	0	0	0.08	0	0.01	0	0.04	0.04	0.05
C14H2-01	0	0.01	0	0	0	0	0	0	0	0
C16H3-01	0	0.04	0	0	0	0	0	0	0	0
C16H3-02	0	0	0	0	0	0	0	0	0	0
C16H3-03	0	0.02	0	0	0	0	0	0	0	0
C16H3-04	0	0.01	0	0	0	0	0	0	0	0
C18H3-01	0	0	0	0	0	0	0	0	0	0
C18H3-02	0	0.01	0	0	0	0	0	0	0	0
C18H3-03	0	0.03	0	0	0	0	0	0	0	0
C18H3-04	0	0.03	0	0	0	0	0	0	0	0
TGC14-01	0	0.09	0	0.01	0	0	0	0	0	0
TGC16-01	0	0.22	0	0.01	0	0	0	0	0	0
TGC16-02	0	0.02	0	0	0	0	0	0	0	0
TGC16-03	0	0.09	0	0.01	0	0	0	0	0	0
TGC16-04	0	0.08	0	0.01	0	0	0	0	0	0
TGC18-01	0	0.01	0	0	0	0	0	0	0	0
TGC18-02	0	0.04	0	0	0	0	0	0	0	0
TGC18-03	0	0.16	0	0.01	0	0	0	0	0	0
TGC18-04	0	0.15	0	0.01	0	0	0	0	0	0
H2O	0	0	0.15	0.14	0.14	0.12	0.62	0.08	0.08	0.06
C6H14	0.97	0.01	0	0	0	0	0.21	0	0	0
C2H6OH	0.03	0	0.84	0	0.81	0.8	0.17	0.45	0.5	0.4

SOURCE: Author

The simulation of solvent-based oil extraction with homogenization using the mixture methanol-chloroform (SHE), involves the use of two solvents, these are added in the same process unit (MIX-1), for this reason, only one hydrocyclone is necessary for oil separation as is shown in Figure 37, Route needs less equipment in comparison to EHE route, but two separation units are also need for chloroform-lipids and methanol-water mixtures. Table 27 shows the main process streams conditions for SHE route, differences in

temperatures of streams are caused by the nature of solvents used in this route.

Figure 37. Simulation of microalgae oil extraction using the mixture methanol–chloroform (SHE)



SOURCE: Author

Table 27. Main process streams conditions for microalgae oil extraction using the mixture methanol-chloroform (SHE)

	109	110	111	113	114	118	120	123	124
Pressure (kPa.)	101.325	101.325	101.325	101.325	101.325	101.325	101.325	51.67	101.325
Temperature (K)	299	586.8	296.6	296.6	298	296.6	299.1	296.6	427.7
Mass Flow (kg/h)	447383.7	12212.6	651982.8	651298.4	36687	691686.7	810963.3	40388.27	23831.88
Component (mole Fraction)									
Cellulose	0	0	0	0	0.06	0	0	0.03	0.08
Lignin	0	0	0	0	0.01	0	0	0.01	0.02
	0	0	0	0	0.17	0	0	0.1	0.21

Hemicellulose									
Aspartic	0	0	0	0	0.05	0	0	0.03	0.06
Glutamic	0	0	0	0	0.05	0	0	0.03	0.06
Glycine	0	0	0	0	0.1	0	0	0.06	0.13
Alanine	0	0	0	0	0.1	0	0	0.06	0.13
Valine	0	0	0	0	0.05	0	0	0.03	0.07
Leucine	0	0	0	0	0.05	0	0	0.03	0.06
Proline	0	0	0	0	0.07	0	0	0.04	0.09
Lysine	0	0	0	0	0.08	0	0	0.04	0.1
C14H2-01	0	0.01	0	0	0	0	0	0	0
C16H3-01	0	0.04	0	0	0	0	0	0	0
C16H3-02	0	0	0	0	0	0	0	0	0
C16H3-03	0	0.02	0	0	0	0	0	0	0
C16H3-04	0	0.01	0	0	0	0	0	0	0
C18H3-01	0	0	0	0	0	0	0	0	0
C18H3-02	0	0.01	0	0	0	0	0	0	0
C18H3-03	0	0.03	0	0	0	0	0	0	0
C18H3-04	0	0.03	0	0	0	0	0	0	0
TGC14-01	0	0.09	0	0	0.01	0	0	0	0
TGC16-01	0	0.22	0	0	0.01	0	0	0	0
TGC16-02	0	0.02	0	0	0	0	0	0	0
TGC16-03	0	0.09	0	0	0.01	0	0	0	0
TGC16-04	0	0.08	0	0	0.01	0	0	0	0
TGC18-01	0	0.01	0	0	0	0	0	0	0
TGC18-02	0	0.04	0	0	0	0	0	0	0
TGC18-03	0	0.16	0	0	0.01	0	0	0	0
TGC18-04	0	0.15	0	0	0.01	0	0	0	0
H2O	0	0	0.01	0.01	0.14	0.01	0.43	0	0
CH4OH	0.01	0	0.65	0.65	0	0.64	0.37	0.36	0
CHCL3	0.98	0.01	0.34	0.34	0	0.33	0.2	0.19	0

SOURCE: Author

For exergy analysis of alternatives, the process of extracting oil from microalgae was divided into four general steps: biomass-solvent mixing, oil extraction, solids drying and solvent recovery. Taking into account simulation results and composition for each stream; physical and chemical exergies were estimated, Table 28 shows chemical exergies calculated for the main components of microalgae oil extraction, values calculated for known components as water or ethanol were validated with values reported in

literature, highest specific chemical exergies corresponds to triglycerides which are the product of interest for further biodiesel production. Exergy of heat flux was also considered and calculated.

Table 28. Specific chemical exergies of main components

Component	Specific chemical exergy (kJ/kmol)	Component	Specific chemical exergy (kJ/kmol)	Component	Specific chemical exergy (kJ/kmol)
		C14H2-01	8774870	TGC14-01	28019930
Cellulose	2834125	C16H3-01	10085570	TGC16-01	31948550
Lignin	5352170	C16H3-O2	9929620	TGC16-02	31480910
Hemicellulose	2313790	C16H3-03	9773740	TGC16-03	31013270
Aspartic	2079030	C16H3-04	9617860	TGC16-04	30545630
Glutamic	2475000	C18H3-01	11394270	TGC18-01	35877170
Glycine	1114980	C18H3-02	11174370	TGC18-02	52903360
Alanine	2000300	C18H3-03	11048870	TGC18-03	34941890
Valine	3307400	C18H3-04	10925044	TGC18-04	34474250
Leucine	3962170	C6H14-02	4114133.66	C2H6OH	1362935
Proline	3005530	H2O (Liquid)	770	CHCL3	458386
Lysine	3900890	H2O (Vapor)	9495	CH4OH	722115

SOURCE: Author

Table 29 shows power consumption of equipment in the three routes of extraction including hydrocyclons, filters and pumps, as shown, highest energy consumption corresponds to HBE route followed by SHE route, As occurs in lab-scale, where lipid extraction efficiency of SHE route is higher than HBE, energy consumption can be decreased for HBE route since lower extraction efficiencies implies lower lipid flow to be pumped and separated from solvent, while for SHE route, simulation shows a significant energy consumption which cannot be decreased if lab-scale extraction efficiency is set because of its capability for total lipid extraction and not for the specific lipids needed for biodiesel production, for this reason and from the energy

consumption point of view, SHE method is less convenient than other methods evaluated for a large-scale biomass processing.

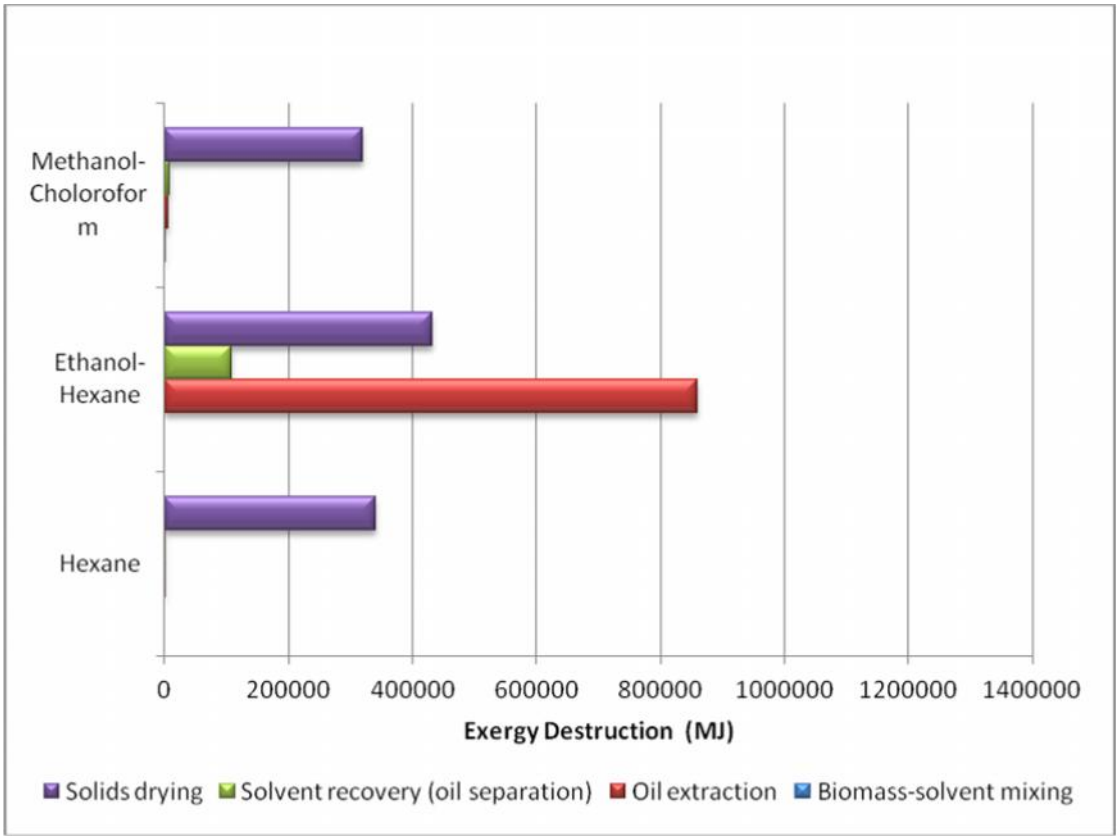
Table 29. Energy consumption of main equipment used in oil extraction routes evaluated

	Energy Consumption (kWh)			
	Hydrocyclone 1	Hydrocyclone 2	Filter	Pump
HBE	74.25	-	0.5	21
EHE	14.3	6.3	0.5	8.2
SHE	53.4	-	0.5	13.1

SOURCE: Author

For all analyzed systems, the highest exergy inputs are represented by the microalgae biomass, solvents used and utilities; among the greatest exergy outputs are the oil and residual biomass. In this process, the residual biomass was considered as a coproduct, due to its high content of carbohydrates and proteins it can be used for other applications such as bioethanol production or as a dietary supplement; taking this into account, exergy losses decreased substantially for waste streams. As shown in Figure 38, the highest exergy losses were occurred in the EHE route, specifically in the oil extraction stage, being significantly higher than HBE and SHE, this difference can be explained by the amount of separation units required which increases exergy of utilities, heat and work for the case of pumps increasing significantly exergy inputs of the route. The stage that shows more irreversibilities for all oil extraction routes studied is the solids drying, with a total exergy loss of 432,900 MJ for EHE; 321,041 MJ for SHE and 341,166 MJ for HBE; also it is shown that biomass-solvent mixing stage presents the lowest exergy losses for all the three routes with values of zero for EHE and HBE extraction routes.

Figure 38. Destroyed exergy by stage for microalgae oil extraction methods evaluated



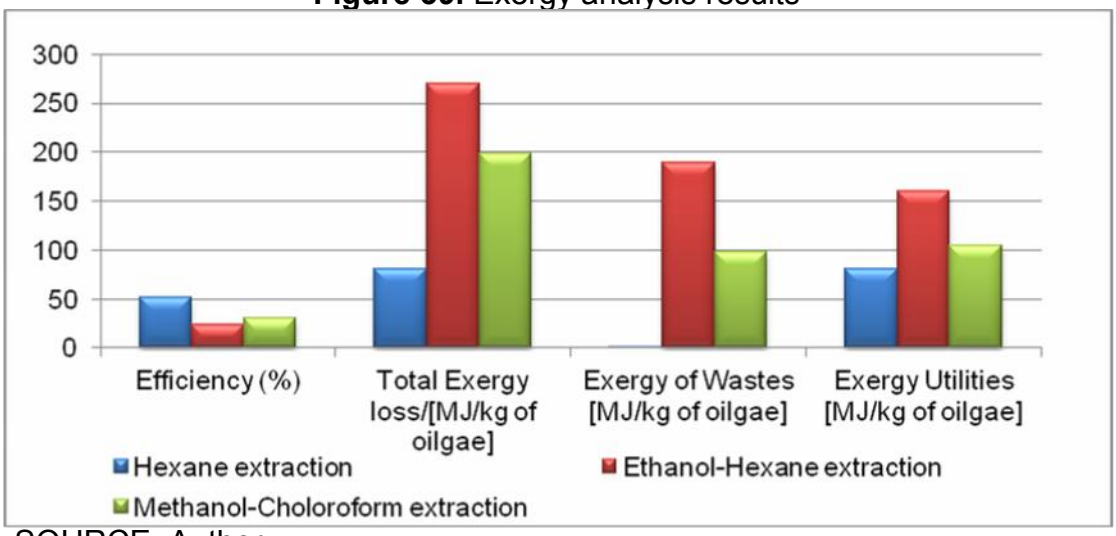
SOURCE: Author

EHE route presents the highest total exergy destruction of all routes evaluated; this high exergy loss can be explained by the additional equipment required to perform the route in a large scale process in comparison to the equipment used in other routes evaluated in this work.

Overall exergy efficiency for each process was also calculated; the total irreversibilities and the exergy of wastes by alternative, and these results are shown in Figure 39. The hexane-based extraction (HBE) shows the highest exergy efficiency, lower total irreversibilities, lower exergy losses for wastes, and lower utilities required, while the EHE route has the highest exergies in each of the categories quantified. Results show that for the three solvent-based routes studied, the extraction with hexane HBE is the most suitable for

the extraction of oil from microalgae in a large scale from the energy point of view, with a maximum exergetic efficiency of 51%, but they also demonstrate the need to reduce waste and the amount of utilities in all three routes through methodologies such as energy and process integration which could diminish significantly the overall irreversibilities of the processes.

Figure 39. Exergy analysis results



SOURCE: Author

Considerations made in this study related to oil extraction efficiency does not have a significant effect on exergy destruction and process irreversibilities calculated for three microalgae oil extraction routes evaluated, since mass flow of solvents used, which represents a significant exergy input, depends only of total biomass processed, and does not depend on the amount of oil separated from coproduct stream, likewise, exergy of wastes is not affected by this consideration because streams of water after solvents recovery for SHE and EHE routes remain constant. If extraction efficiency decreases, solids stream will increase its exergy due to the presence of oil not extracted, and exergy of oil stream will be lower, however overall exergy efficiency of the process will not change significantly. The second assumption related to the presence of homogeneous triglycerides in microalgae oil affects chemical

exergy of compounds and exergy of oil stream, issue which must be taken into account for transesterification process, but does not affect in a great way exergy destruction during oil extraction. Also, exergy of wastes is not affected by triglyceride profile because wasted streams are free of these components. Exergy losses are affected by hydrocyclons efficiency assumed, because if this value decreases, oil stream will need more equipment for product purification increasing exergy destruction and utilities required, exergy of wastes will be also affected by the presence of valuable biomass components, however, for this reason, exergy efficiency calculated is taken as the maximum which can be reached by the systems evaluated.

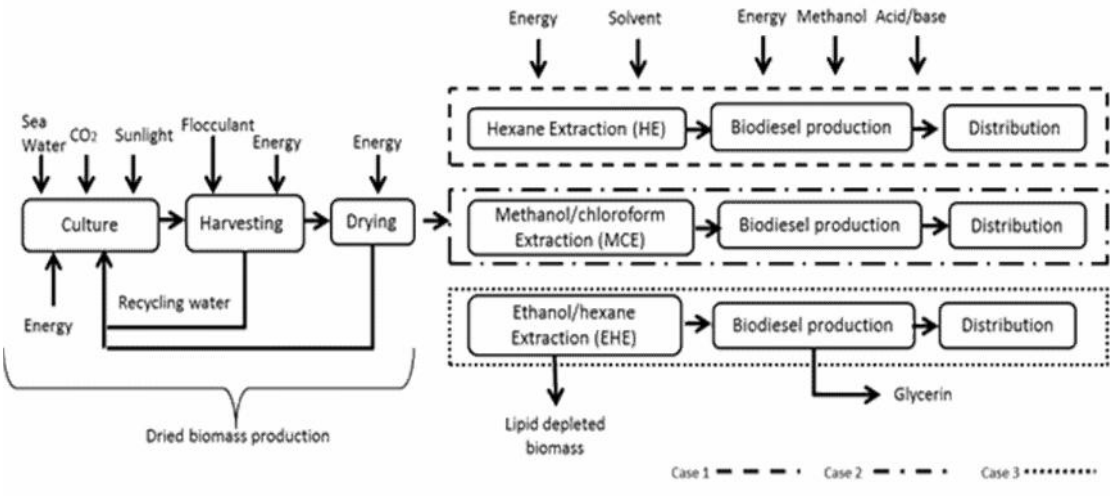
5.3.2. Environmental evaluation of microalgae oil extraction alternatives in a biodiesel-from-microalgae production chain.

Life Cycle Assessment (LCA) is a standardized method which allows the integral record, quantification and evaluation of the environmental damages connected with a product, a procedure, or a service in the context of a given question. The ISO 14040/44 methodology for LCA has been used for these purposes [190-192]. The life cycle concept is a cradle to grave systems approach for the study of feedstock, production and use. The concept revolves around the recognition of different stages of production starting from upstream use of energy for cultivation of the feedstock, followed by the different processing stages [193].

The overall goal of the study was to compare three scenarios of biodiesel production from microalgae dried biomass applying the LCA technique by means of the ‘‘cradle to grave” concept, the routes of oil extraction used are the same evaluated in subsection 3.1 (oil extraction using ethanol-hexane mixture, hexane based extraction and extraction using the mixture methanol-chloroform), taking as starting point the results obtained from simulation of routes shown in

Figures 35 to 37 and Tables 25 to 27, in addition, other stages of a hypothetical biodiesel-from-microalgae production chain were included (Figure 40).

Figure 40. Process chain for biodiesel production from dried biomass production to distribution



SOURCE: Author

The basis for comparison or the functional unit was defined as 1 kg of biodiesel. Temporal horizon was 100 years, due this is the time considered in the impact assessment methodology Environmental Product Declarations (EPD), location of the system was in the Colombian Caribe region; as a consequence climatic data to determine water loss by evaporation were based on statistics of the North Colombia. Besides, neither the construction nor the maintenance of the plant was taken into consideration. Likewise, economic and social factors were not included. Regarding the co-product allocation rules for the extraction and esterification-transesterification stages, the hierarchy proposed by the ISO 14040 standard was followed. Furthermore, following the criteria in the quality requirements of the inventory data and according to the rules of LCA, if there are no current flows available, the internationally recognized databases are used in such a way that the values used for these flows respond to controlled processes with regulations more restrictive than the Colombian, e.g., Renewable Energy Directive (Directive 2009/28/EC).

Assumptions regarding rates of growth, nutrient requirements, yields of lipids, microalgae composition and energy requirements for dry biomass production were based on the results from the literature. Owing to the lack of public information related to microalgae oil extraction in large scale; the analyzed process refers to an on extrapolation from lab-scale studies. The distances over which the raw materials and products would be transported were taken over the Caribe region in Colombia. In order to achieve this, the system was divided into three stages: extraction, biodiesel production and the final process included in this study is transportation and distribution of the biodiesel, Table 30 shows the mass and energy flows used in this study for the microalgae biomass production, and Table 31 shows the mass and energy flows for oil transformation into biodiesel using transesterification, all these data was taken from literature and normalized to the functional unit.

Table 30. Mass and energy flow for biomass production. Base 1 kg of biodiesel

Dried biomass production	Value	Units
Flow input CO_2	6.45	kg
Flow input Urea (N)	0.05	kg
Flow input sea water	62.65	kg
Flow input $Al_2(SO_4)_3$	0.190	kg
Heat consumption	92.71	MJ
Electricity consumption	7.32	kW·h
Flow out dried biomass	3.09	kg
Emissions to air		
Water	62.50	kg
N_2O	5.764E-07	kg
Emissions to water		
Salts, unspecified	83.59	kg
Final waste Residue		
Solid losses	3.50E-03	kg

SOURCE: Author

Life Cycle Inventory (LCI) phase involves data collection and modelling of the product system, as well as description and verification of data. This encompasses all data related to environmental and technical quantities for all

relevant unit processes within the study boundaries that compose the product system. In this sense, mass and energy balances for the different raw materials and processes biodiesel production from microalgae dried biomass, were performed over each stage.

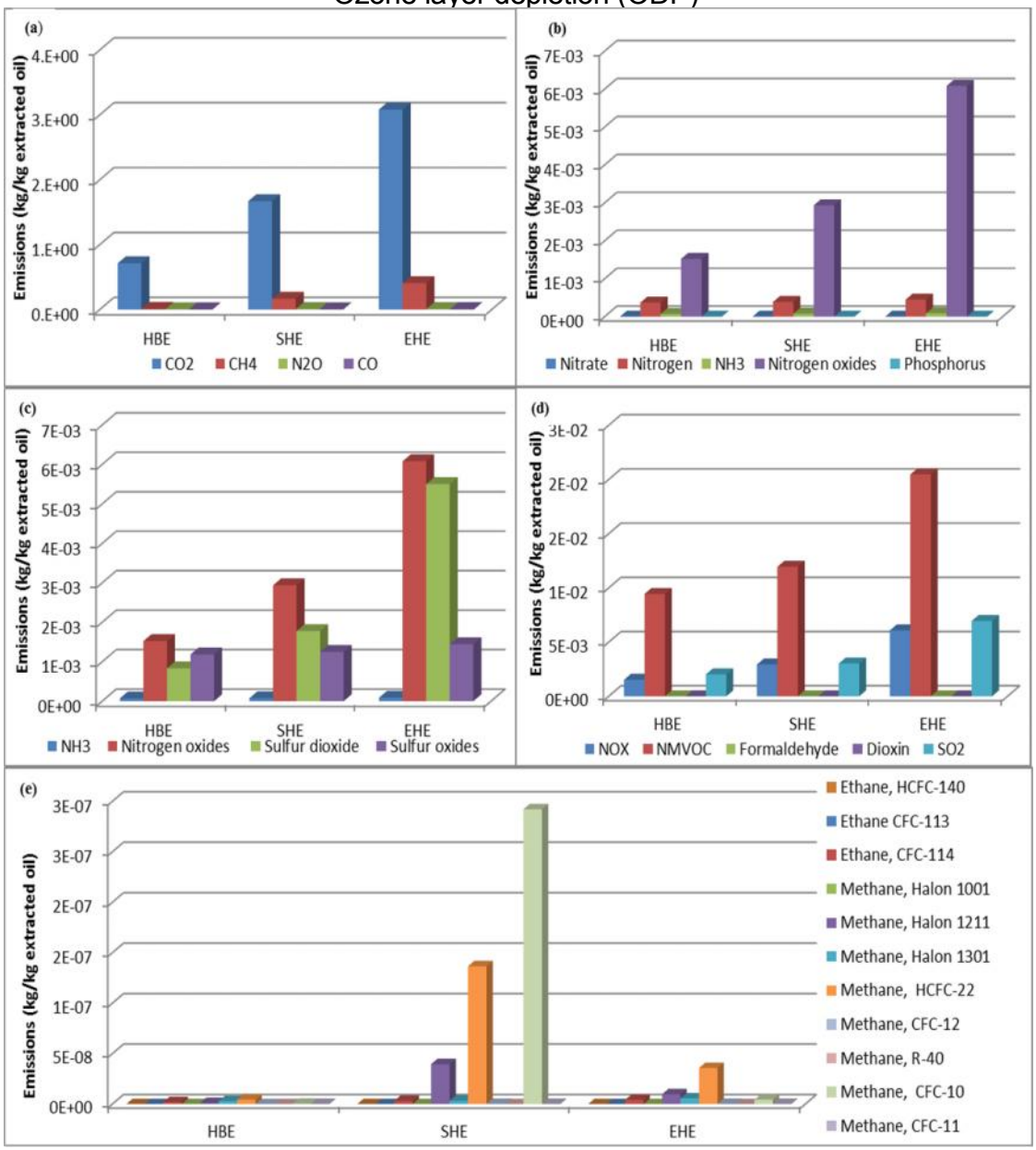
Table 31. Mass and energy flow for 1 kg of biodiesel

Biodiesel production	Value	Units
Flow input microalgae oil	1.028	Kg
Flow input methanol	0.15	Kg
Flow input *NaOH*	0.011	Kg
Flow input H_2SO_4	0.013	Kg
Flow input water	0.088	Kg
Heat consumption	1.87	MJ
Electricity consumption	0.003	kW·h
Flow out Glycerin (coproduct)	0.134	Kg
Emissions to water		
Waste water	0.152	Kg

SOURCE: Author

Inventory data for those energy and material inputs not available, were obtained from eco-profiles within SimaPro7.1 software [194] and the ECOINVENT database [195]. Each case study, involves the processes related to the production of raw materials (methanol, chloroform, hexane, ethanol are considered fossil origin), including the production process, from obtaining of raw materials to the final product manufacture and transportation. Similarly, the electricity and heat production include production and transportation. The energy consumption for each oil extraction method and oil esterification/transesterification stage were made based on the thermal energy requirements for heat exchangers, reboilers and dryers obtained from each simulation performed. Steam is used for the heating process. The natural gas consumed to provide the required steam energy was calculated based on data reported by *Unidad de Planeación Minero Energética of Colombia* [196].

Figure 41. Contribution to the inventory of emissions of the three oil extraction systems studied in each impact category. (a) Global-warming potential (GWP100). (b) Eutrophication. (c) Acidification. (d) Photochemical oxidation. (e) Ozone layer depletion (ODP)



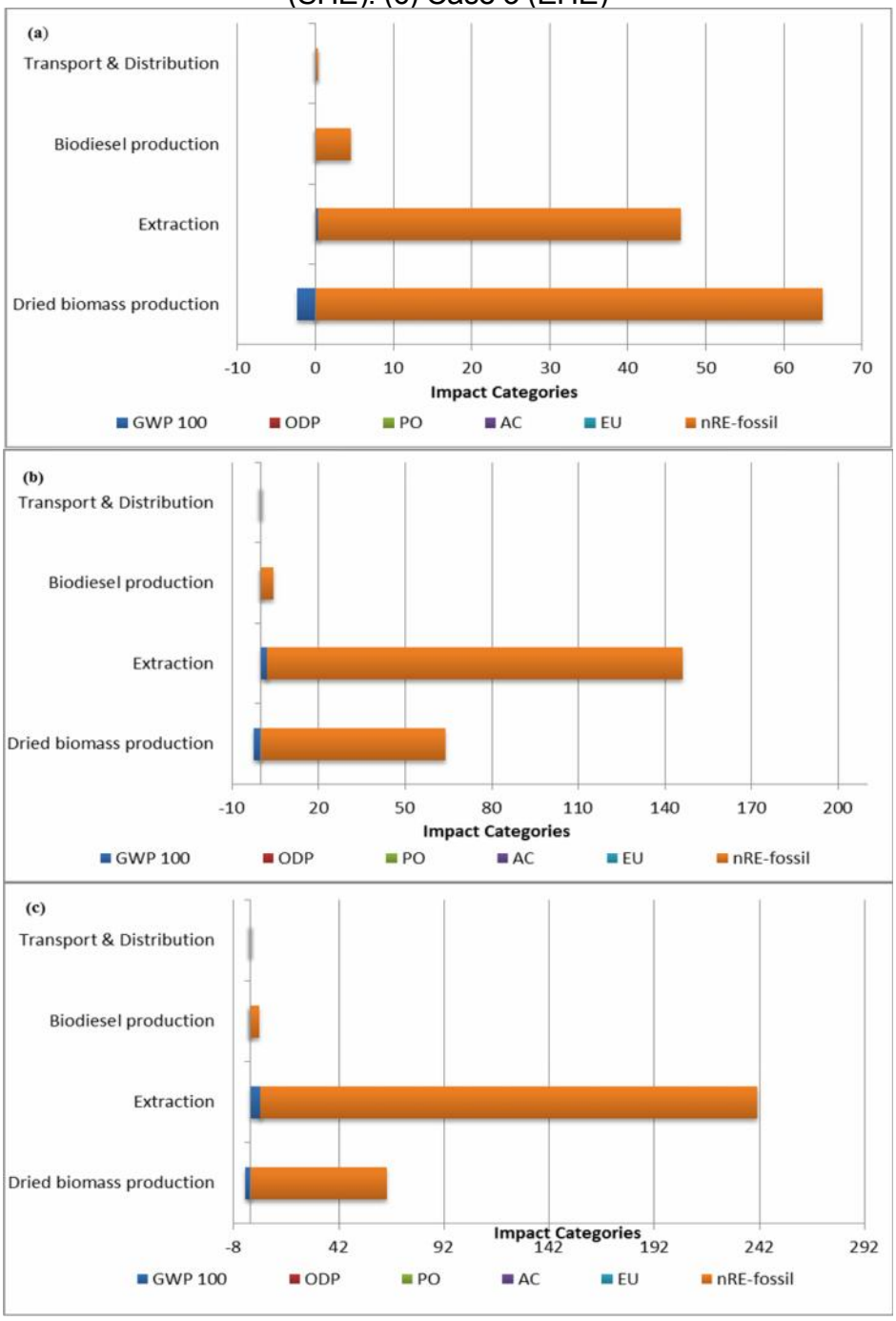
SOURCE: Author

The impact categories considered were: Global Warming Potential (GWP), acidification (AC), eutrophication (EU), photochemical oxidation (PO), ozone layer depletion (ODP) and non-renewable fossil (nRE-fossil). The emissions associated with the inventory results of the different extraction systems studied

are shown in the Figure 41. They were grouped by categories of impact, Figure 41a shows the emissions of CO_2, CH_4, N_2O, and CO included in the category of GWP, Figure 41b, c, d and e summarizes the most common substances in the categories of eutrophication, acidification, photochemical oxidation and ozone layer depletion.

According to Figure 41, case EHE-based biodiesel production presents the higher value for almost all studied emissions. CO_2 emissions (Figure 41a) are mainly due to use ethanol, which represent the 74.68% of contribution. These emissions are normally derived from fossil fuels. In this case, use of natural gas for oil extraction contributes with 6.28% and with 18.77% for dried biomass production. Furthermore, this scenario shows significant emissions of nitrogen oxides (Figure 41b, c), sulfur dioxide (Figure 41c) and non-methane volatile organic compounds, unspecified origin (NMVOC) (Figure 41d) by the ethanol, natural gas and dried biomass production. In contrast, from Figure 41e, it can be observed that the biggest pollutant emissions, corresponds to SHE-based biodiesel production, the emission are mainly due to methanol and chloroform use and comes from extraction system used. In this case, methane-Halon 1211 and methane- HCFC-22 represent 96.93% y 96.72% of the methanol used and methane CFC-10 represent 99.64% of the chloroform used.

Figure 42. Potential environmental impact for: (a) Case 1 (HBE). (b) Case 2 (SHE). (c) Case 3 (EHE)



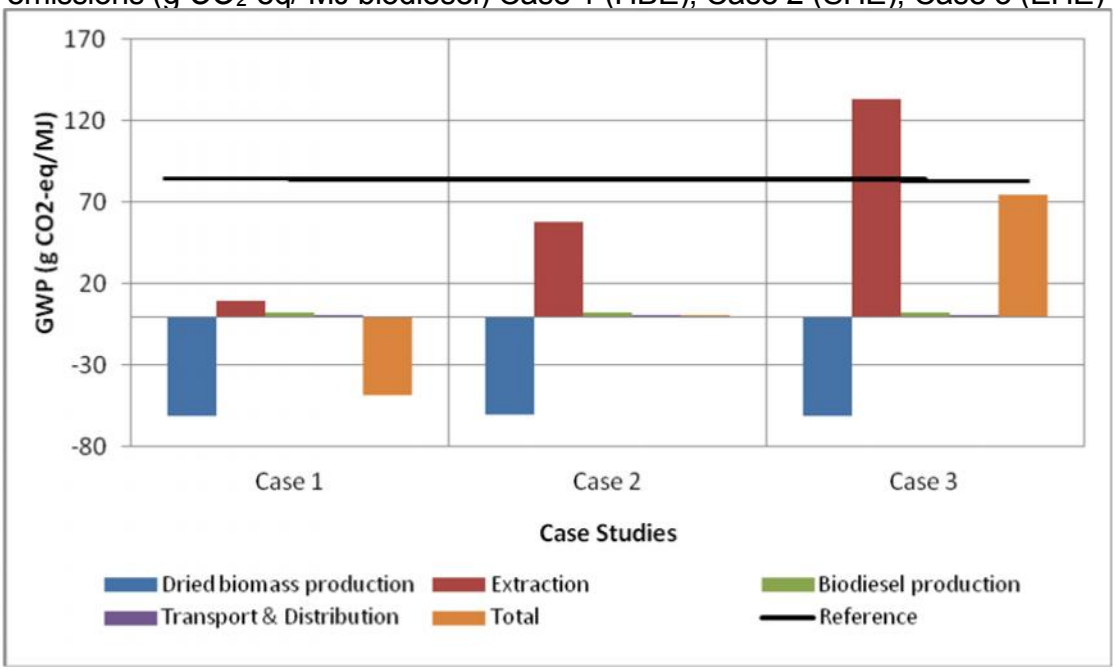
SOURCE: Author

The potential impacts evaluation of the stages involving the Life Cycle of the whole biodiesel production is shown in Figure 42. Figure 42a shows that for all alternatives studied, the most influential impact category is Non renewable

fossil, This can be mainly due to the significant consume of natural gas as fuel for heat generation. In HBE-based biodiesel production, the most important contributors are dried biomass production, which represents approximtely 55,9% and oil extraction stage within 40% of contribution.The greater consumption of heat in the process of dried biomass production is generated by the biomass drying stage prior to oil extracction stage.This behavior is contrasting for cases 2 and 3, where oil extraction process shows a higher contribution than dried biomass production. In case 2 (SHE), oil extraction contributes with 68% in front of 30% for dried biomass. In case 3 (EHE), this percent increases to 77%.

In order to assess the theoretical compliance with sustainability criteria proposed in European Directive 2009/28/EC, Non-renewable energy consumption and Global Warming Potential associated with the three cases of biodiesel production were compared with 1 MJ of conventional fossil diesel (Figure 43). Results indicate that HBE-based production chain has lower no-renewable energy consumption and GWP than the SHE-based and EHE-based production chains.

Figure 43. Results comparing three cases studies. Greenhouse gases emissions (g CO_2-eq/ MJ biodiesel) Case 1 (HBE), Case 2 (SHE), Case 3 (EHE)



SOURCE: Author

For all the scenarios, transesterification stage and distribution present unrelevants contributions. European Directive 2009/28/EC establishes a common framework for the promotion of energy from renewable sources. In this sense, Article 17 refers to the sustainability criteria for biofuels and bioliquids, highlighting that the GHG emission saving from the use of biofuels and bioliquids shall be at least 35%. For biofuels, for the purposes of the calculation referred to GHG savings, the fossil fuel comparator emissions shall be 83.8 g CO_2 eq/MJ. Figure 43 illustrates the GHG savings for three biofuels production cases using the previous default value for conventional diesel.

Results shows a significative reduction of GHG for HBE-based biodiesel production (156%), which can be explained due to immense capture of CO_2 during biomass production stage. This reduction decrease for SHE-based (99.46%) and EHE-based (14.68) respectivaly.

5.3.3. Comparison of alternatives for bioethanol production from defatted microalgae biomass.

Ethanol can be produced from microalgae biomass with high percentage of cellulosic material, Mature technologies for bioethanol production from biomass are based on sugars fermentation which are obtained from industrial processing of feedstocks with high percentage of sugars or cellulose, most of them are important for human and animal diet. Most of microalgae species contains some common components such cellulose, proteins, pectins as polyuronic acids, arabinans and glactans, hemicelluloses as xylans and arabinoglactans and other carbohydrates, most of the polymers located in the microalgal cell wall can be converted in monomers through an acid, alkaline or enzymatic reactions [197].

Bioethanol can be produced using the microalgae biomass before or after oil extraction. First option can be useful in cases where oil percentage in microalgae is not significant, or where cell wall disruption using hydrolysis produces a significant amount of reducing sugars, second option gives the interesting possibility of producing both biodiesel and ethanol from the same feedstock using the stream of defatted biomass obtained in simulations of microalgae oil extraction.

Energy integration is a technique for process design which looks for minimization of the energy consumption and maximization of the heat recovery. Analysis starts with the mass and energy balance for the process, simulation tools can be used for achieving this stage. After that, targets for energy Integration are identified and network is designed. Utility levels that are supplied to the process that is evaluated or designed, can be part of a centralized utility system. Energy integration provides a well-structured

methodology for energy saving in cooling and heating, from the basic mass and energy balance to the total utility system.

The microalgae genera modeled and used in this study was *Chlorella* sp., which was previously modelled, and technologies evaluated were Simultaneous Saccharification and Co-fermentation SSCF (route 1), in this pathway, a hydrolysis step reduces cellulose and hemicelluloses to hexoses and pentoses, which simultaneously are fermented using *Zimomonas mobilis* and *Saccharomyces cerevisiae*. It is reported that the production rate does not have a high impact on enzymatic hydrolysis because its concentrations are low, but presence of alcohol inhibits specific growth rate and accelerates cell degradation [198].

Second pathway evaluated was Simultaneous Saccharification and Fermentation SSF (route 2), which has been experimentally studied for bioethanol production from lignocellulosic material. This pathway performs the stage of hydrolysis coupled to fermentation stage, this variation allows to decrease the final product inhibition, however, is difficult to find the operating conditions for efficient performing of microorganisms involved in both stages. This technique is one of the most promising because only one reactor is used for hydrolysis and fermentation, improving the conversion of sugars to ethanol, the key of SSF process is the fast ethanol production from glucose [199].

Third route evaluated was Separate Saccharification and Fermentation using acid hydrolysis SHF (route 3), Acid hydrolysis was identified experimentally as convenient alternative for reducing sugars production from microalgae as is shown in previous chapters, although literature also reports high reducing sugars yields from microalgae using another alternatives for hydrolysis [200], sugars obtained are mainly glucose, xylose and cellubiose. In this pathway,

hydrolysis and fermentation steps occurs in different reactors optimizing operating conditions for each stage, best operating conditions found the author in unpublished research works and literature were used for simulation of pathway, for evaluation of this route, acid hydrolysis was chosen first stage of bioethanol production chain.

Table 32. Main components used in microalgal bioethanol production simulation

Component	Type	Formula
Water	Conventional	H2O
Cellulose	Solid	(C6H10O5)X
Hemicellulose	Solid	(C5H8O4)X
Xylose	Conventional	C5H10O5
Sulfuric Acid	Conventional	H2SO4
Cellulose	Solid	CHXNXOXSX
Cellubiose	Conventional	C12H22O11
Ethanol	Conventional	C2H6O
Carbon Dioxide	Conventional	CO2
Ammonia	Conventional	NH3
Oxygen	Conventional	O2
Acetic Acid	Conventional	C2H4O2
Lactic Acid	Conventional	C3H6O3
Glucose	Conventional	C6H12O6
Glycine	Conventional	C2H5NO2
Calcium Hydroxide	Solid	Ca(OH)2
Calcium Sulfate	Solid	CaSO4*2H2O

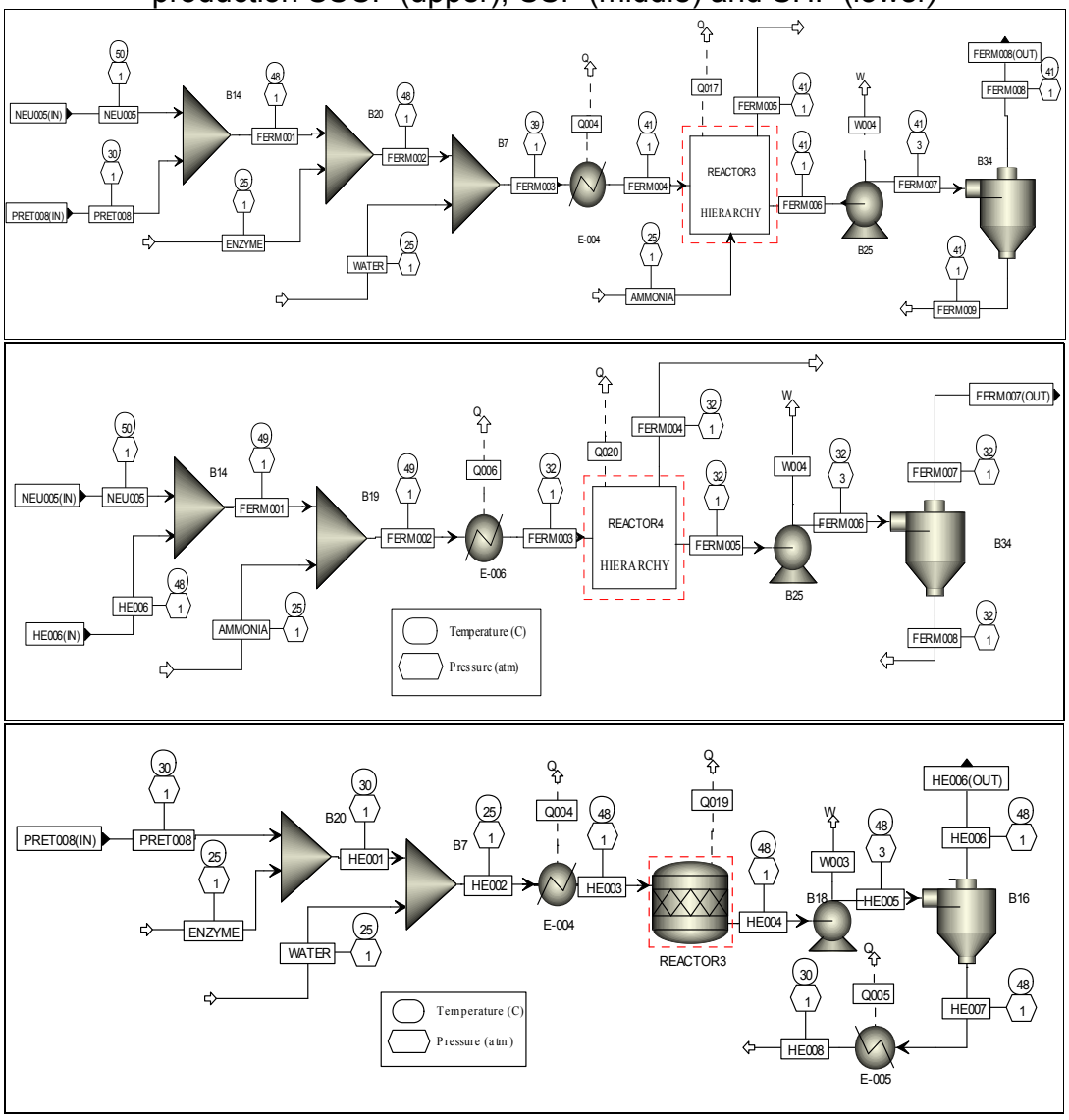
SOURCE: Author

Table 32 shows the main components used for simulation, in contrast with previous cases of study shown in subsections 3.1 and 3.2, for this case composition of microalgae was modified, in order to give the percentages of "defatted" biomass components, density of algae was 0.65 g/cm^3 and percentage of microalgae components was introduced as follows: cellulose (15.4%), hemicellulose (31%), proteins (27%), other carbohydrates (17.4%) and other components (9.2%).

Process was simulated in steady state and separation stages were simulated in equilibrium stage, for all simulations operation pressure was 101325 Pa. Figure 44 shows important stages of simulation of three routes evaluated for energy integration, at the top of figure is shown the fermentation stage of SSCF route, where cellulase and recombinant *Zymomonas mobilis* are used in REACTOR 3 (red lines) for hydrolysis and pentoses and hexoses fermentation in a multifunctional unit, reaction temperature was set on 41 °C.

At the middle of Figure 44 is shown the simulation of SSF fermentation, which does not require the addition of cellulase, however, hydrolysis must be performed in a separate unit, fermentation is performed in REACTOR 4 (red lines), and temperature was set on 32°C for efficient hexoses fermentation. At the bottom of Figure 44 is shown enzymatic hydrolysis stage for route 3, where reaction is performed in REACTOR3 (red lines) using cellulase at 48 °C.

Figure 44. Simulation of routes evaluated for microalgal bioethanol production SSCF (upper), SSF (middle) and SHF (lower)



SOURCE: Author

In the first stage of microalgal bioethanol purification, conventional distillation was used in order to increase ethanol concentration to 50%, followed by a rectification column were ethanol purity of 91.4% was reached, finally, extractive distillation with glycerol was used for obtain a final bioethanol concentration of 99.5%, this alternative was chosen by its low energy requirements in comparison to other technologies. Table 33 shows the specifications of output streams for fermentation steps in each route.

Simulation results of each technological alternative for microalgal bioethanol production shows that using a microalgae after lipid extraction flow of 34,000 kg/h, using route 1 (SSCF) is obtained a bioethanol flow of 8,028 kg/h, using route 1 (SSF) bioethanol flow is 6,840 kg/h and using route 3 (SHF) bioethanol flow was 6,290 kg/h, which corresponds to bioethanol yields of 23.6%, 20.1% and 18.5% respectively.

Table 33. Composition of product streams for routes evaluated in microalgal bioethanol simulation

Streams	Stages	
SSCF Route		
Mass flows (kg/s)	Fermentation	Bioethanol Separation
Total mass flow	56.66	2.26
Bioethanol flow	2.74	2.23
Water	47.52	0.03
Xylose	0.01	0.00
Hemicellulose	0.00	0.00
Cellulose	0.00	0.00
Glucose	0.01	0.00
Oxygen	0.02	0.00
Ammonia	3.60	0.00
Carbon Dioxide	2.60	0.00
Z. mobilis	0.66	0.00
S. cerevisiae	0.45	0.00
Temperature (K)	314.15	315.31
SSF Route		
Mass flows (kg/s)	Pentoses Fermentation	Hexoses Fermentation
Total mass flow	73.86	7.78
Bioethanol flow	0.76	0.33
Water	70.16	6.61
Xylose	0.07	0.00
Hemicellulose	0.00	0.00
Cellulose	0.00	0.00
Glucose	0.00	0.01
Oxygen	0.01	0.00
Ammonia	0.25	0.50
Carbon Dioxide	0.72	0.31
Z. mobilis	0.10	0.00
S. cerevisiae	0.00	0.41
Temperature (K)	308.15	308.15

SHF Route			
Mass flows (kg/s)	Hydrolysis	Pentoses Fermentation	Hexoses Fermentation
Total mass flow	92.69	62.69	31.56
Bioethanol flow	0.00	1.25	0.63
Water	83.49	58.63	25.13
Xylose	2.74	0.11	0.03
Hemicellulose	0.01	0.00	0.00
Cellulose	0.00	0.00	0.00
Glucose	1.34	0.00	0.02
Oxygen	0.00	0.01	0.00
Ammonia	0.00	1.35	1.35
Carbon Dioxide	0.00	1.18	0.60
Z. mobilis	0.00	0.07	0.00
S. cerevisiae	0.00	0.00	1.25
Temperature (K)	394.15	303.15	308.15

SOURCE: Author

According to simulation results, SSCF technology (route 1) shows the highest efficiency of microalgal ethanol production for the routes evaluated, in addition, acid hydrolysis shows lower efficiencies in terms of reducing sugars production in comparison to obtained data from enzymatic hydrolysis, this can be explained by the selectivity of enzymes in comparison to acid hydrolysis reaction which presents low efficiencies in cellulose hydrolysis.

Table 34. Comparison of microalgal bioethanol production routes using energy integration

	SSCF		SSF		SHF	
	Base Case	Energy Integration	Base Case	Energy Integration	Base Case	Energy Integration
Heat Exchangers	12	22	19	38	14	26
Total Area (m2)	34,700	26,285	49,059	9,950	35,505	9,078
Heating Service (GJ/h)	14.6	2.3	548	503	923	700
Cooling Service (GJ/h)	630	617.1	576	531	832	609

SOURCE: Author

Table 34 shows energy integration results for routes evaluated, SSF route requires 14,000 kW more in heating services than SSCF technology, this

difference is caused by the higher amount of separation units in SSF route and the need of additional stages of fermentation products purification. Taking into account energy requirements and bioethanol yield, SSCF technology is more convenient in a large-scale microalgal bioethanol production.

As the route with highest bioethanol yield and lower energy requirements, SSCF route was assessed in energy integration section using as molecular sieves as extractive distillation for bioethanol purification. Extractive distillation was compared to molecular sieves as alternatives for microalgal bioethanol purification from the energetic point of view (Table 35), difference between energy requirements were calculated in 12.4 GJ/h y 215.3 GJ/h for heating and cooling services respectively, being more convenient the use of molecular sieves for large scale microalgal bioethanol purification.

Table 35. Compositions of output streams for separation technologies evaluated

	Extractive distillation	Molecular sieves
Total Area (m^2)	34,700	18,285
Heating Service (GJ/h)	14.7	2.2
Cooling Service(GJ/h)	629.7	414.4

SOURCE: Author

5.4. CONCLUSIONS

Computer Aided Process Engineering gives to researchers the opportunity of evaluating novel methodologies developed in lab scale, in addition, several methodologies as exergy analysis, life cycle assessment and energy integration are also being used for the design, screening and comparison of these technologies based on the behavior of the process in large scale and the selection of promising pathways towards sustainable development.

In this study was shown the application of these tools for the selection of the more convenient microalgae biomass processing technologies towards the

development of a topology of microalgae-based biorefinery, and it was demonstrated using three cases of study that the combination of these tools can be successfully used as decision-making criteria for the depuration of process alternatives, in addition, relevant information about an emerging process can be obtained decreasing the uncertainties for further process synthesis. All cases of study presented were based on the simulation of different biomass extraction/transformation alternatives and the robust modelling of a representative microalgae strain, which allow obtaining more realistic results in comparison to utilization of an empirical formula for microalgae, and gives a better characterization of process streams.

For the first case of study, three solvent based routes for microalgae oil extraction were evaluated through exergy analysis, showing that microalgae oil extraction with hexane (HBE) is the most suitable of alternatives evaluated for a large scale from the energy point of view, obtaining by this route a maximum exergetic efficiency of 51%. With highest exergy destruction in solids drying stage, in addition, this route presents the lowest exergy losses related to wastes, their utilities are less than other routes studied and presents the lowest total irreversibilities (981,978 MJ). Although this extraction route requires more electricity than other routes evaluated, this consumption is minimum in comparison to other utilities as water and steam cooling. On the other hand, ethanol-hexane route (EHE) presented the lowest exergy efficiency (24%) of the routes evaluated, the highest exergetic losses of the extraction processes evaluated due to the necessity of more mixing and extraction units than other methods, increasing also energy losses related to heat transfer. Although SHE route is commonly used in lab-scale for a rapid total lipid determination on microalgae biomass, this route is not recommended for a large scale process because presents high total irreversibilities, exergy of wastes and exergy of utilities. Taking into account streams compositions, it is shown that residual biomass after oil extraction

must be used for downstream processing because of its high exergy, even higher than oil exergy, which shows the need of the utilization of this defatted biomass as feedstock for other processes.

Second case of study shows the environmental evaluation of three biodiesel-from microalgas production chains by varying the oil extraction method using the methodology of life cycle assessment (LCA), strain modelled, simulations and methods used were the same than case study 1, evaluation shown that consume of fossil fuel, in terms of natural gas for heat generation, it is the most important vector in impact quatification. In the best scenario (HBE-based production chain) contribute with more than 50%. For GHG emissions, comparing with European sustainability criteria showed hypothetical reduction in two of three scenarios. HBE-based production chain presents the most important reduction, near to 156%, respect to fossil reference. SHE-based production chain presents a reduction approximately to 99% and HBE-based production chain, presents the lowest reduction of the alternatives evaluated (14%). In terms of impacts, for all the scenarios, transesterication stage and distribution present unrelevants contributions. Regarding the differences between the three scenarios of biodiesel production analyzed, the HBE-based production chain presents an excellent environmental performance in all categories analyzed, except for ozone layer depletion (ODP).

In third case of study presented, three alternatives for microalgal bioethanol production from defatted biomass were evaluated from the energetic point of view, and energy integration methodology was applied to each alternative in order to improve the routes proposed. Technology of Simultaneous Saccharification and Co-fermentation SSCF (route 1) shows the highest bioethanol yield and lowest energy requirements after energy integration. Separated hydrolysis and fermentation SHF (route 3) presents the lowest efficiency. Finally, it could be established that the use of molecular sieves

technology for bioethanol dehydration in the last part of the process represents lower energy requirements respect to extractive distillation with glycerol.

6. CHAPTER VI. DEVELOPMENT OF A TOPOLOGY OF MICROALGAE-BASED BIOREFINERY: PROCESS SYNTHESIS AND OPTIMIZATION USING A COMBINED FORWARD-BACKWARD SCREENING AND SUPERSTRUCTURE APPROACH[6]

6.1. INTRODUCTION

As is described through this book, microalgae is emerging as a promising feedstock for biorefineries and many researchers are developing alternatives for microalgae metabolites separation, transformation and utilization at lab-scale and by the use of Computer Aided Process Engineering. Previously, it has been shown some contributions to the expansion of these emerging alternatives palette by the development of methods for oil extraction and reducing sugars production, and the reduction of alternatives to the most promising by the utilization of computer-aided tools as process simulation, exergy analysis and Life Cycle Asessment. However, despite the significant advances in alternative ways of microalgae biomass use and the expected maturation of technologies for third generation biofuels production, the range of alternatives for microalgae utilization as biorefinery feedstock is still wide.

Since there is an enormous number of possibilities of existing and emerging technologies for microalgae processing, it is important to have efficient synthesis and screening techniques. Specifically, a big-picture approach can yield useful insights that narrow the search space and utilize the appropriate level of details for conceptual design.

This chapter is aimed at the synthesis and screening of alternate pathways of the processing of microalgae. In addition to the convenient routes for

[6] This chapter is based on the paper "*Development of a topology of microalgae-based biorefinery: process synthesis and optimization using a combined forward-backward screening and superstructure approach*" by Angel Darío González Delgado, Viatcheslav Kafarov and Mahmoud El-Halwagi, under evaluation in Applied Energy Journal (2014).

microalgae processing, there are various emerging technologies under development for microalgae processing in each stage of theoretical biofuels-from-microalgae production chains. Novel process synthesis and optimization approaches can be used for finding the combination of alternatives that enable reaching a defined objective (e.g., maximum yield, maximum profit, minimum processing steps, minimum waste, minimum emissions, maximum feedstock flexibility, highest energy or exergy efficiency).

This work presents a combination of forward-backward screening and superstructure synthesis and optimizaton approach for topology synthesis and screening. Each topology should include the principal details of a flowchart that shows the sequence of processes needed to transform the raw materials into the desired products.

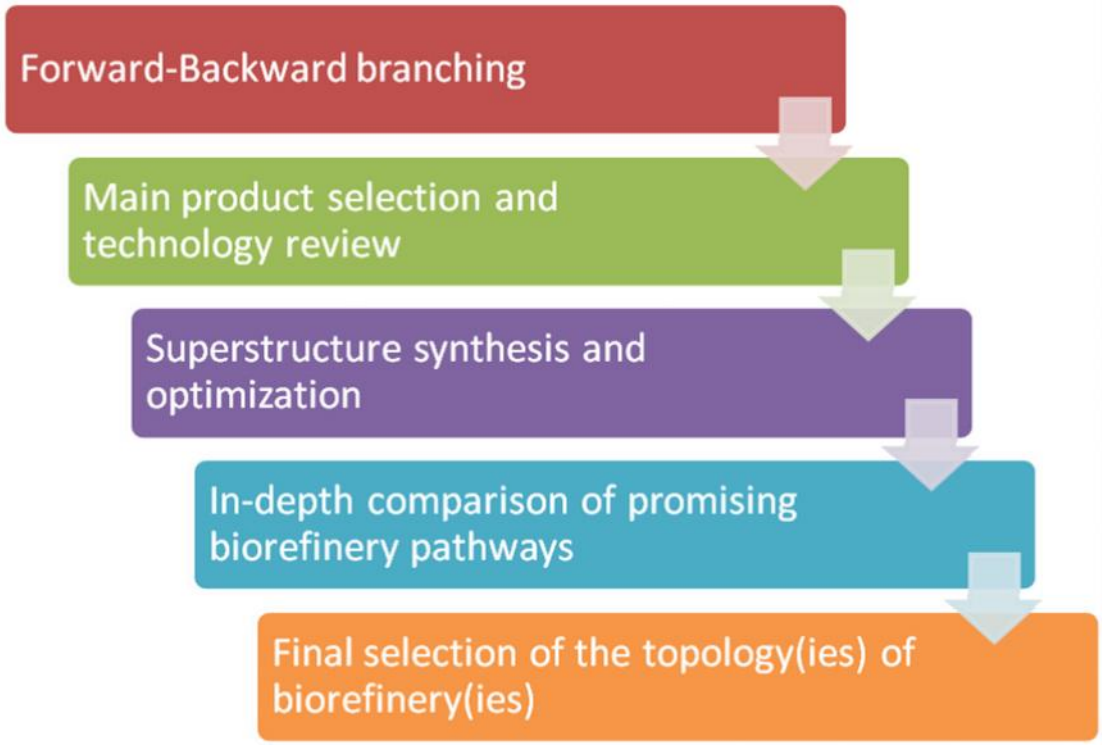
6.2. PROBLEM STATEMENT

Given a microalgae strain with certain composition, a set of potential products, and a set of existing and emerging technologies for extraction and transformation of a microalgae feedstock and/or intermediate/metabolites, it is desired to synthesize and screen topological pathways so as to meet certain desired objectives (e.g., maximum product yield, maximum profit, etc.).

6.3. DESCRIPTION OF THE PROCEDURE

The methodology for the synthesis and analysis of topological pathways for the processing of microalgae introduced is shown in Figure 45. This methodology uses a hierarchical approach that starts with top-level data and focuses attention and effort on the promising pathways, integrating various process synthesis and optimization concepts such as forward-backward branching, superstructure optimization, and in-depth analysis for high-priority pathways.

Figure 45. Methodology proposed for synthesis and analysis of topological pathways for the processing of microalgae



SOURCE: Author

6.3.1. Forward-Backward Branching.

The first step in the approach is an adaptation of the forward-backward branching approach developed by Pham and El-Halwagi (2012) [201]. The procedure for the development of the microalgae-based biorefinery starts with the forward pre-screening from the microalgae biomass to the products wich can be potentially obtained from a microalgae production system. These chemical species are divided into reaction products (which are obtained form

chemical and/or thermal processes such as hydrolysis, direct transesterification or pyrolysis), extraction products (which are metabolites separated from the microalgae biomass for direct use, purification or transformation such as lipids, carbohydrates, proteins and special substances), and *in-vivo* product (which are produced by the microorganisms in their biological reactions as photosyntesis and metabolic cycles; this group includes substances secreted to the culture media as alcanes, exopolysaccharydes, and other specific special substances).

Given the wide variety of microalgae strains with different compositions and specific substances obtainable form each strain as toxins, vitamins, fatty acids antioxidants, pigments, aminoacids, among others, products are clustered in broader groups. Next, a backward branching was made starting from the desired products that can be ultimately obtained in a microalgae biorefinery. Therefore, the backward branching identifies the chemical speciess needed to yield these products. Given the wide spectrum of potential products, pre-screening and selection of the products are carried out based on top-level information. Subsequently, matching of identical species in forward and backward trees is made, to generate a prospective pathway. To keep the level of complexity of the generated pathways, the maximum number of intermediates allowed in forward-backward branching was taken as one. After that, selection of the main product and chemical species involved in their production is carried out based on experimental and literature information.

6.3.2. Main product selection and technology review.

Once forward-backward matching is performed, a main product of microalgae-based biorefinery is chosen. In this work, a potentially obtainable biofuel is selected as the main product. Co-products and intermediates may

include chemical species. A comprehensive review and selection of existing and emerging technologies for obtaining chemical species has been carried out taking into account information found in literature and experimental results developed by authors, for each technology evaluated. Specifically, information about yield were gathered and classified into maximum theoretical yield and the practically achievable yield. The two terms are related via an efficiency factor which represents the fraction of the theoretical yield that can be obtained using a certain technology. Economic data were also collected or generated. For economic calculations of biomass processing technologies, fixed and operating costs were calculated. For this study, the microalgae cost was taken as $50 /tonne of biomass. In cases where cost information of the technology applied to microalgae biomass was not available, economic data were used for the same technologies involving similar chemical species as feedstocks. Cost factors: an α factor for fixed costs and a β factor for operating costs were also calculated for stages of biomass preparation and added to the cost of technology where necessary. The recovery period for the biorefinery was taken as ten years.

6.3.3. Superstructure synthesis and optimization.

A superstructure is based on layers for both chemical species and conversion operators (technologies for extracting intermediates/metabolites or transforming them into the main product and co-products). In order to limit the complexity of the problem, the maximum number of layers of the conversion technologies was taken as four. No limit was placed on the number of technologies per layer. When chemical specie crosses a technology layer without any modification, a blank technology is included. With all information of chemical species and conversion technologies involved in biofuel production from microalgae, the superstructure is constructed by creating alternating layers of chemical species and processing technologies with

separate indices. Production of the same chemical specie in different layers is treated separately. Processing costs in each technology for extraction and/or transformation of chemical species are given by the production capacity of each chemical specie. The basis for calculations was estimated using a biorefinery production capacity of 100,000 tonnes of microalgae biomass (dry base where necessary) per year, and values of chemical species input in further layers are a function of efficiency of processing technologies and percentage of specific feedstock (carbohydrates, lipids, proteins, etc.) in the biomass.

After superstructure is constructed, an optimization function is provided for the selection of production pathways. In this work, screening and optimization of the pathways are made based on technical and economic criteria, looking for the maximization of main product revenue, which is defined as annual sales, less annual production costs, less annual cost of feedstock. The result of this optimization is at least one promising alternative for obtaining the main product from microalgae biomass, with some residues and/or co-products.

6.3.4. In-depth comparison of promising biorefinery pathways.

After the superstructure optimization, the resulting processing alternatives are ranked according to the economic data. For the prioritized pathways, focus is next given to more detailed analysis in order to obtain a more accurate comparison of alternatives. Additional factors can be included such as CO_2 tax credit/subsidies and costs of residues treatment to comply with local environmental policies. Other comparison criteria can be taken into account outside of optimization function such as comparison of payback period of potential alternatives or anticipated fluctuations of the cost and availability of a feedstock over a certain time horizon. As a result of the more in-depth analysis, one or more suitable topologies of microalgae-based

biorefineries can be generated while accounting for various objectives. The limited number of promising alternatives can now be simulated in details with equipment sizing and the associated techno-economic analysis. This way, the detailed simulation, design, and economic analysis are reserved for the promising alternatives.

6.4. MATHEMATICAL FORMULATION

The superstructure contains a number (NP) of layers of chemical species designated under the index *i,* and (NP – 1) layers of processing technologies, designated by the index *k*. The first chemical-species layer (*i* = 1) is the Whole microalgae biomass, while the last chemical species layer (*i* = NP) represents the main product (biofuel). Chemical-species layers beetwen 1 and NP represent the intermediates, residues, and/or co-products involved in the biorefinery. A certain chemical specie, c, is produced in a layer *k* from one technology and can be used as feedstock for other technology in layer *k*+1. In addition, the optimization formulation includes the following constraints:

The performance model for metabolites extraction and/or transformation g_i in layer *k* (referred as $\psi_{g_i,k}$) relates the flowrates of the different chemical species entering and leaving the conversion operator, i.e.

$$(F^{out}_{g_i,k,1},...,F^{out}_{g_i,k,c},...,F^{out}_{g_i,k.NC}) = \psi_{g_i,i}\,(F^{in}_{g_i,k,1},...,F^{in}_{g_i,k,c},...,F^{in}_{g_i,k.NC},d_{g_i},O_{g_i}) \qquad \forall g_i,\ \forall k \quad (1)$$

where $F^{out}_{g_i,k,c}$ and $F^{in}_{g_i,k,c}$ are the flowrates in tonnes per year of chemical species *c* leaving and entering transformation technology g_i in layer *k*. The design and operating variables of each technology g_i are denoted by d_{g_i} and O_{g_i}, respectively.

In the cases where chemical reaction is necessary for obtaining a chemical specie, the flowrates of the chemical species *c* in layers *k* + 1 and *k* (designated respectively by $F_{c,i+1}$ and $F_{c,i}$) are related by the rates of formation or depletion via chemical reaction over all the conversion operators in that layer, i.e.

$$F_{c,k+1} = F_{c,k} + \sum_{g_i} r_{g_i,c,k} \qquad \forall g_i\ ,\ \forall k \tag{2}$$

where $r_{g_i,c,i}$ is the rate of production/consumption of chemical specie *c* in conversion operator g_i and is given a positive sign for production and a negative sign for consumption.

Mass balance of the chemical specie *c* from chemical-species layer *i* to the extraction/transformation technology in layer *k* is given as follows:

$$F_{c,i} = \sum_{g_i} F^{in}_{g_i,c,k} \qquad \forall c\ ,\ \forall k \tag{3}$$

The flowrate of each chemical specie leaving the extraction and/or transformation operator g_i is calculated through a given yield ($y_{g_i,i,c}$) times the flowrate of a limiting component (the index of the limiting component in reaction cases is c= $c^{\lim}_{g_i}$ and its inlet flowrate is $F^{in}_{g_i,c^{\lim}_{g_i},i}$), and times the efficiency of the technology performed, in extraction cases, the yield is the maximum amount of microalgae specific metabolite present in microalgae strain i.e.,

$$F^{out}_{g_i,c,i} = y_{g_i,c,i} F^{in}_{g_i,c^{\lim}_{g_i},i} X_{g_i,k} \qquad \forall g_i\ ,\ \forall c\ ,\ \forall i\ ,\forall k \tag{4}$$

For including the economic criteria into the optimization, the term total annualized cost (TAC) is introduced, and is defined as the summation of annualized fixed costs (*AFC*) and annual operating costs (*AOC*) (e.g., El-Halwagi, 2012 [202]).

$$TAC = AFC + AOC \tag{5}$$

The total annualized cost of extraction/transformation technology g_i in layer k, $TAC_{g_i,k}$, is given through the function $\Omega_{g_i,k}$ as follows:

$$TAC_{g_i,k} = \Omega_{g_i,k}\left(F^{in}_{g_i,k,1},...,F^{in}_{g_i,k,c},...,F^{in}_{g_i,k.NC},d_{g_i},O_{g_i}\right) \qquad \forall g_i,\ \forall k \tag{6}$$

The annualized fixed costs $\left(AFC_{g_i,k}\right)$ of technology evaluated g_i in layer k is given by a cost factor ($\alpha_{g_i,k}$) times the flowrate of the limiting component entering the transformation technology, or the flowrate of the feedstock containing the chemical specie to extract, capacity differences between data reported in literature and this work were adjusted using the seven tenths factor rule, i.e.

$$AFC_{g_i,k} = \alpha_{g_i,k}\left(F^{in}_{g_i,c^{\lim}_{g_i},k}\right)^{0.7} \qquad \forall g_i,\ \forall k \tag{7}$$

The annual operating costs $\left(AOC_{g_i,k}\right)$ of technology evaluated g_i in layer k is given by a cost factor ($\beta_{g_i,k}$) times the flowrate of the limiting component entering the transformation technology, or the flowrate of the feedstock containing the chemical specie to extract, i.e.

$$AOC_{g_i,k} = \beta_{g_i,k} F^{in}_{g_i,c^{\lim}_{g_i},k} \qquad \forall g_i\ ,\ \forall k \tag{8}$$

The objective function involves the maximization of revenue derived by the selling of final product which is defined as the value of the product less the cost of microalgae biomass and the TAC of the chemical species processing, i.e.,

$$\text{Maximize } C^{\text{Product}} F_{p,NP} - \sum_k \sum_{g_i} TAC_{g_i} - C^{\text{Biomass}} F^{\text{Biomass}} \tag{9}$$

Where C^{Product} is the selling price of the product (e.g., \$/ton), C^{Biomass} is the cost of the feedstock (e.g., \$/ton) and F^{Biomass} is flowrate of the feedstock.

After the superstructure optimization, additional issues must be considered for selecting the biorefinery topology. One of these issues is the cost of co-products obtained using the promising pathway without any further processing. Therefore, the objective function is modified as follows:

$$\text{Maximize } C^{\text{Product}} F_{p,NP} + \sum_k \sum_m C_{m,k}{}^{\text{Co-Product}} F_{m,k}{}^{\text{Co-Product}} - \sum_k \sum_{g_i} TAC_{g_i} - C^{\text{Biomass}} F^{\text{Biomass}} \tag{10}$$

Other issues to consider may include economic indicators such as the payback period (PP) of the process, which can be calculated as follows (e.g., El-Halwagi (2012) [202]):

$$PP = \frac{\text{FixedCapitalInvestment}}{(\text{AnnualSales} - \text{TotalAnnualizedCost}) \times (1 - \text{TaxRate}) + \text{AnnualDepreciation}} \tag{11}$$

An environmental indicator which can be related to the economic indicators and calculated if necessary is the tax credit for CO_2 capture. In this work, it was assumed that the growth of 1 tonne of microalgae biomass corresponds

to the consumption of 1.8 tonne of CO_2, as some technologies in the promising pathway can release carbon dioxide. This value is discounted from the total CO_2 generated and the net value is multiplied by the tax credit for CO_2 [203].

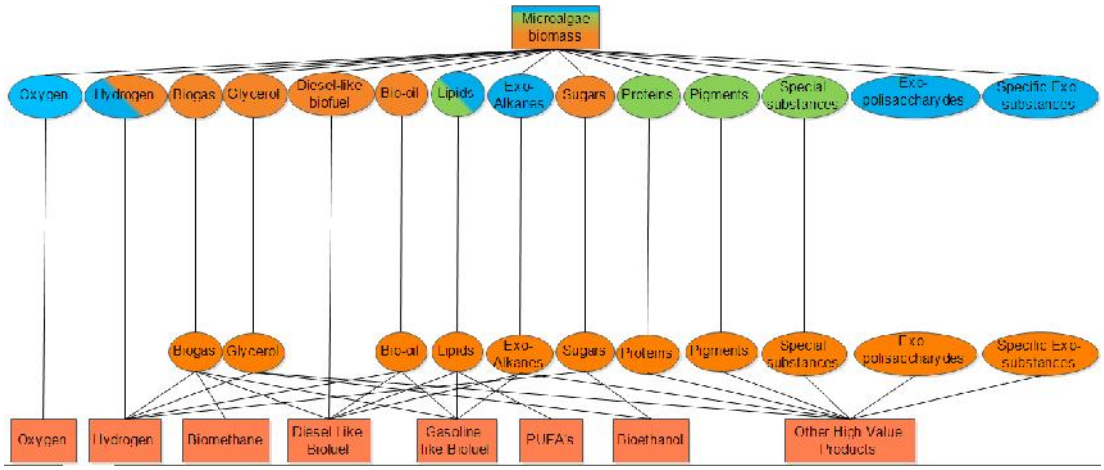
6.5. RESULTS

Figure 46 shows the results of matching between the products which can be obtained from microalgas biomass and those that are desirable in a topology of biorefinery. Intermediates which do not constitute a pathway between the feedstock and the products are not shown. Some components were lumped as certain intermediates or products. For example biogas was used to represent a mixture of CH_4 and other compounds (e.g., CO, H_2, CO_2, N_2, O_2). Polyunsaturated Fatty Acids for food, feed and cosmetics, or other lipid-based high value products as dielectric fluids.

Certain products may be obtained via a single pathway (e.g., polyunsaturated fatty acids “PUFAs”). Other products may be produced via several intermediates and pathways. An example of such products is diesel-like biofuel. Carbon monoxide and hydrogen present in biogas may be used to produce liquid hydrocarbon fuels (including diesel-like product) using the Fischer-Tropsch reaction. Additionally, direct transesterification of biomass (wet or dry) without lipid extraction can produce diesel-like biofuel. This biofuel can be also obtained by upgrading of bio-oil, a complex mixture obtained from thermal treatments of biomass (including microalgae). The most studied alternative in lab-scale research for biodiesel from microalgae is by transformation of the microalgal oil using esterification, transesterification or hydrotreatment technologies. A novel alternative recently rediscovered is the direct secretion of alkanes in diesel range by microalgae strains during cultivation, which is a promising way for obtaining *in-vivo* biodiesel, as other

alkanes are also secreted by microalgae, can be also obtained hydrocarbons in gasoline range, lipid secretion is also taken into account for obtaining oil usable for biodiesel production.

Figure 46. Matching results after forward-backward branching for the development of a microalgae-based biorefinery. Dark-grey nodes represents reaction products, light-grey nodes represents extraction products and white nodes represents *in-vivo* products



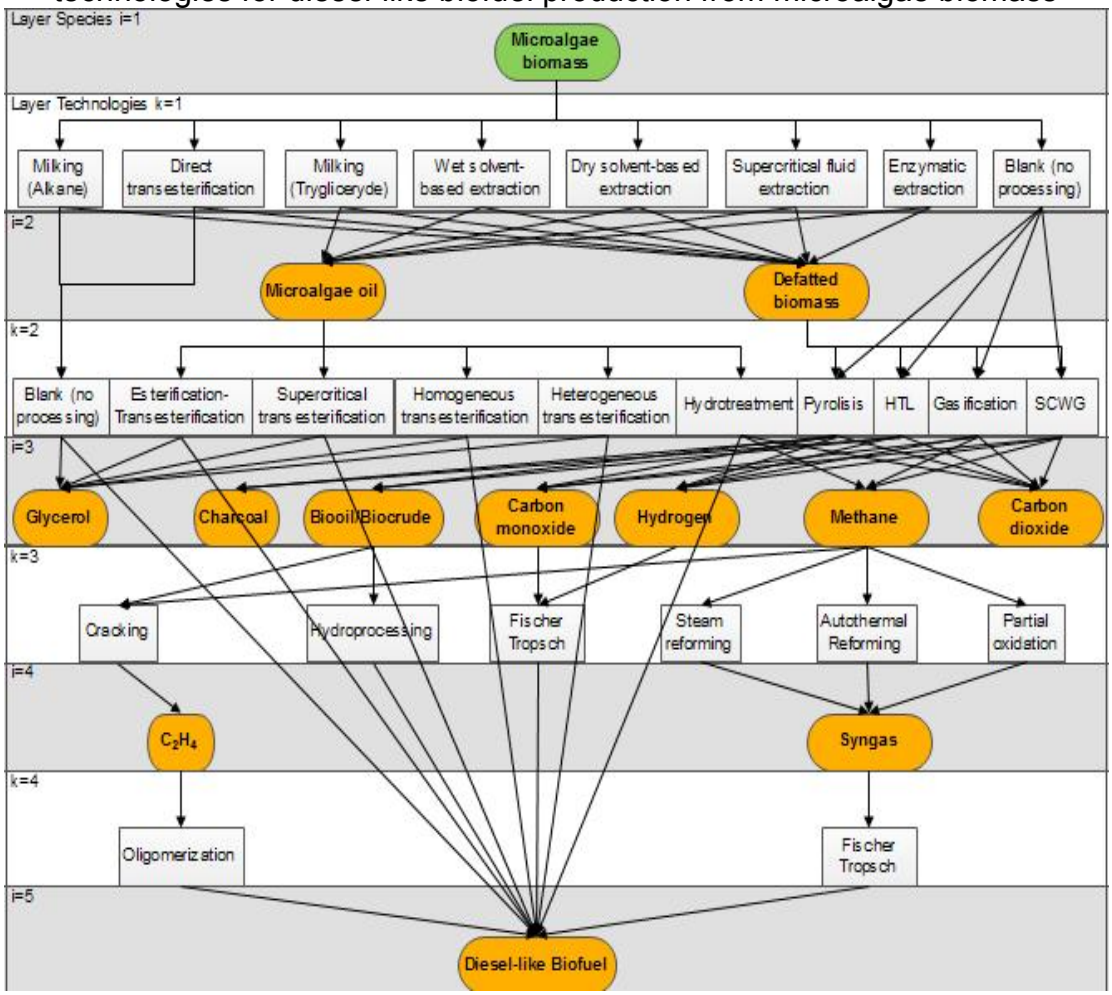
SOURCE: Author

Depending on the used strain of microalgae, other high value products can be obtained in a topology of a biorefinery. These products are very specific of each specie and are present in low percentages in comparison to bulk biomass. However, their high commercial value can make their production even more economically viable that the production of lower value substances such as biofuels. High value substances can be also extracted, transformed or secreted by specific strains in the case of exo-polysaccharydes or exo-proteins. This group includes recombinant proteins, biotoxins, vitamins, antioxidants, acids, fibers, biomarkers, chlorophylls, phycobiliproteins, carotenoids among others.

As shown in Figure 46, the biorefinery products with more intermediate matches after forward-backward branching are hydrogen and diesel-like biofuel, both of them involve matched intermediates, however, diesel-like biofuel branches presents more shared intermediates with other products compared with hydrogen branches, which is desirable taking into account the biorefinery concept. Acccording to these results, the main product selected in this study for the development of the topology of microalgae-based biorefinery is the diesel-like biofuel. Furthermore, substantial data are available oil extraction yield and oil transformation into biodiesel, thermal treatment of microalgae biomass, and some novel results of economic evaluations.

With microalgae biomass as feedstock and diesel-like biofuel as main product, a superstructure with chemical species and extraction/transformation technologies is constructed (Figure 47). Eight technologies are located in layer k=1. Direct secretion of alkanes and direct secretion of oil during microalgae cultivation, where molecules are released to the culture media [204], represent technologies that avoid the costs of biomass processing but require axenic culture conditions thereby incresing the cost of cultivation [205]. Direct transesterification of microalgae biomass, in which biomass is treated with an alcohol and an acid for simultaneous cell disruption, lipid release and lipid transesterification, can be performed in a multifunctional unit for simultaneous reduction of sugars production [206], or in separate units wherea non-polar solvent is also used for phase separation [207]. This technology can be performed using wet or dry microalgae biomass [208]. In this study, direct transesterification was analyzed using wet biomass.

Figure 47. Superstructure of chemical species and extraction/transformation technologies for diesel-like biofuel production from microalgae biomass



SOURCE: Author

Solvent-based extraction of microalgae oil can be performed using several solvents and mixtures and can be assisted by other techniques such as ultrasound, microwaves or high speed homogenization, using different

extraction times, and biomass-to-solvent ratios and temperatures. Several oil extraction methods have been designed and adjusted by manipulating these variables [209], and these methods have been compared using several microalgae strains in terms of toxicity, cost, energy, and efficiency in lab-scale [210], and simulated for comparison in large-scale from the energy [211] and environmental points of view [212]. For the superstructure evaluation, solvent-based oil extraction methods were classified into dry extraction [211] and wet extraction [213]. Other evaluated technologies included supercritical fluid extraction [214] and Enzymatic extraction [215].

Depending on each technology in layer k=1, products obtained in layer i=2 can be microalgae oil, alkanes, defatted biomass, or the whole microalgae biomass if there is no processing in layer k=1. For microalgae oil, evaluated technologies for biodiesel production included homogeneous transesterification [216], heterogeneous transesterification [217], supercritical transesterification [218], a combined esterification-transesterification process [219], and oil hydrotreatment [220]. For defatted or complete biomass processing, the considered technologies included pyrolysis [221], hydrothermal liquefaction (HTL) [222], gasification [223] and supercritical water gasification (SCWG) [224].

The main products obtained in layer i=3 are diesel-like biofuel, syngas, bio-oil and methane. Other products such as charcoal and carbon dioxide were obtained, but not taken into account for further processing. Glycerol is a co-product obtained after microalgae oil transesterification and can be converted into hydrogen using technologies such as dark fermentation, photofermentation, steam reforming, pyrolysis or gasification. This hydrogen (along with carbon monoxide) can be converted into diesel-like biofuel using gas to liquid technologies. On the other hand, glycerol can be converted into bioethanol using fermentation technologies and this bioethanol can be

converted in diesel-like using dehydration followed by oligomerization. These routes were not analyzed since the total number of conversion steps necessary for biofuel production exceeds the maximum allowed in this superstructure optimization Bio-oil from chemical species in stage i=3 can be upgraded to diesel-like biofuel using hydroprocessing or cracking and oligomerization. Methane can be converted to syngas using steam reforming, autothermal reforming or partial oxidation. The syngas is converted to diesel using Fischer-Tropsch synthesis. The superstructure terminated with chemical species in layer i=5 with diesel-like biofuel as the main product. Table 36 summarizes the data for the superstructure optimization.

Table 36. Technical data for superstructure optimization

Feedstock	Technology	Product	Harvesting	Drying	Yield (Ton product/ Ton feedstock)	Technology efficiency	Adapted from
Microalgae biomass	Alkane secretion	Diesel-like	No	No	0.32	0.95	[205]
Microalgae biomass	Direct transesterification	Diesel-like	Yes	No	0.32	0.57	[208]
Microalgae biomass	Wet extraction	Microalgae oil	Yes	No	0.32	0.79	[213]
Microalgae biomass	Enzymatic degradation	Microalgae oil	Yes	No	0.32	0.58	[225]
Microalgae biomass	Oil secretion	Microalgae oil	No	No	0.32	0.95	[226]
Microalgae biomass	Dry extraction	Microalgae oil	Yes	Yes	0.32	0.90	[227]
Microalgae biomass	Supercritical extraction	Microalgae oil	Yes	Yes	0.32	0.94	[218]
Vegetabl	Esterification-	Diesel-	No	No	1.00	0.97	[219]

e oil	transesterification	like					
Vegetable oil	Hydrotreatment	Diesel-like	No	No	0.85	0.99	[220]
Vegetable oil	Transesterification (heterogeneous)	Diesel-like	No	No	1.00	0.94	[217]
Vegetable oil	Transesterification (homogeneous)	Diesel-like	No	No	1.00	0.90	[216]
Vegetable oil	Supercritical transesterification	Diesel-like	No	No	1.00	0.93	[218]
Microalgae biomass	SCW Gasification	Methane	Yes	No	0.23	0.84	[224]
Microalgae biomass	SCW Gasification	Syngas	Yes	No	0.25	0.84	[224]
Microalgae biomass	Gasification	Syngas	Yes	Yes	1.00	0.52	[223]
Microalgae biomass	Gasification	Methane	Yes	Yes	1.00	0.25	[223]
Microalgae biomass	Pyrolysis	Bio-Oil	Yes	Yes	1.00	0.58	[221]
Microalgae biomass	Pyrolysis	Syngas	Yes	Yes	1.00	0.02	[221]
Microalgae biomass	HTL	Biocrude	Yes	No	0.72	0.88	[222]
syngas	Fischer-Tropsch	Diesel-like	No	No	0.60	0.78	[228]
Methane	Steam reforming	Syngas	No	No	2.12	0.85	[229]
Methane	Autothermal reforming	Syngas	No	No	2.04	0.75	[229]
Methane	Partial oxidation	Syngas	No	No	1.33	0.75	[229]
Methane	Cracking	Ethylene	No	No	0.58	0.93	[230]

Biocrude	Hydroprocessing	Diesel-like	No	No	1.00	0.60	[231]
C_2H_4	Oligomerization	Diesel-like	No	No	1.00	0.26	[232]

SOURCE: Author

Depending on the strain and cultivation conditions, microalgae biomass may have different compositions. In this study, a glyceride percentage of 32% was selected for the superstructure evaluation. This percentage can be found in strains such as *Chlorella* sp., *Dunaliella* sp., *Chaetoceros Calcitrans*, *Nannochloropsis* sp. [233, 234], *Navicula* sp. or *Amphiprora* sp. [209], Table 37 shows the composition of microalgae modeled for this study in terms of lipids, carbohydrates, proteins and special substances. The table also shows the cost parameters of microalgae biomass pretreatment as well as other parameters such as the cost of feedstock and product and processing capacity of the biorefinery.

Table 37. Microalgae composition modelled and economic parameters for case study

Parameter	Unit	Value
Microalgae composition		
Carbohydrates	%	36
Lipids (TG)	%	32
Lipids (HVFA)	%	8
Proteins	%	20
Special substances	%	4
Selling price of main product	$/ton	900
Processing capacity	ton biomass/yr	100,000
Cost of feedstock	$/ton	50
Microalgae harvesting		
Annualized fixed cost parameter for different capacity	$*yr$^{-0.3}$*ton$^{-0.7}$	97
Annualized operating cost parameter for different capacity	$/ton	1.92
Microalgae drying		
Annualized fixed cost parameter for different	$*yr$^{-0.3}$*ton$^{-0.7}$	348

capacity		
Annualized operating cost parameter for different capacity	$/ton	200

SOURCE: Author

Table 38 shows the results of the economic evaluation for each technology in layer k of the superstructure. Technologies such as oil or alkane secretion feature high values of α, caused by the special cultivation conditions required for obtaining the hydrocarbons related to the need to avoid the presence of undesirable microorganisms into the culture media which can consume the released products for their growth. The β values for oil and alkane secretion (which are related to the separation of desired compounds) are lower in comparison to other technologies present in superstructure. The α parameter is also high in enzymatic degradation of microalgae cell wall for oil extraction owing to the high cost of enzymes which cannot be re-used more than four times. A lower α value was found for homogeneous transesterification because this technology is a mature and well-known process used for biodiesel production from several vegetable oils and is available in commercial scale.

Table 38. Cost parameters for microalgae-to-diesel superstructure optimization from an economic point of view

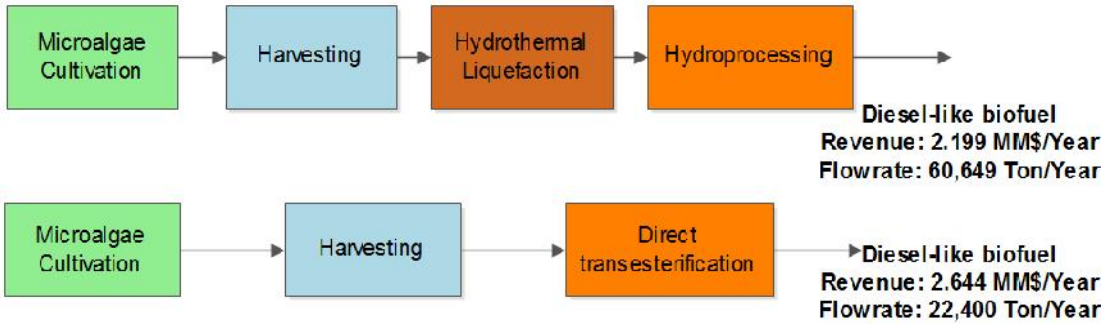
Process	Product	α ($*yr$^{-0.3}$*ton$^{-0.7}$)	β ($/ton feed)	Adapted from
Alkane secretion	Diesel-like	22130.40	173.99	[205,227]
Direct transesterification	Diesel-like	930.54	439.63	[216]
Wet extraction	Microalgae oil	798.44	194.32	[226]
Enzymatic degradation	Microalgae oil	20015.76	242.64	[235,236]
Oil secretion	Microalgae oil	22130.40	164.92	[205,227]
Solvent extraction	Microalgae oil	858.20	309.82	[216]
Supercritical extraction	Microalgae oil	2311.58	479.92	[237]
Esterification/transesterification	Diesel-like	738.14	353.00	[238]
Hydrotreatment	Diesel-like	595.94	199.00	[226,227]
Transesterification (heterogeneous)	Diesel-like	721.77	211.87	[239]

Transesterification (homogeneous)	Diesel-like	369.07	154.55	[240]
Supercritical transesterification	Diesel-like	545.17	80.03	[241]
SCW Gasification	Methane	3493.25	462.32	[242]
SCW Gasification	Syngas	3493.25	462.32	[242]
Gasification	Syngas	2905.01	423.10	[230]
Gasification	Methane	2905.01	423.10	[230]
Pyrolysis	Bio-Oil	1505.26	265.50	[230]
Pyrolysis	Syngas	1505.26	265.50	[230]
HTL	Biocrude	2798.42	227.10	[230]
Fischer-Tropsch	Diesel-like	2409.82	150.10	[230]
Steam reforming	Syngas	2593.16	623.40	[243]
Autothermal reforming	Syngas	1880.04	592.23	[243,244]
Partial oxidation	Syngas	2333.84	529.89	[243,244]
Cracking	C2H4	7584.73	35.87	[230]
Hydroprocessing	Diesel-like	595.94	199.00	[227]
Oligomerization	Diesel-like	706.23	55.79	[230]

SOURCE: Author

After superstructure optimization (see Annex B), only two alternatives show a positive economic balance with close results under this criterion (Figure 48). The first route starts with biomass harvesting for concentration of microalgae to 20% in slurry. After that, the mixture is fed to a hydrothermal liquefaction process which gives an aqueous phase and an organic phase, known as bio-crude or bio-oil. Char and gas are also obtained as co-products. Biocrude is upgraded to liquid biofuels using hydroprocessing technologies, taking alkanes in diesel range as main product. The product flowrate in this route is 60,649 tonnes of diesel-like biofuel per year. The second route with positive economic balance under conditions studied in this paper includes the stages of microalgae harvesting and further direct transesterification of lipids into biomass using a mixture of alcohol, acid and organic solvent for product separation. The co-products obtained using this route are glycerol and algae meal. The product flowrate for the second route corresponds to 22,400 tonnes of diesel-like biofuel per year.

Figure 48. Optimal pathways for diesel-like biofuel production from microalgae biomass after superstructure optimization for maximum annual revenue



SOURCE: Author

The superstructure optimization results show that promising routes for microalgae processing do not include a drying stage. This confirms the need to avoid the drying stage in a microalgae based biorefinery where the main product is biodiesel. Thermal routes where drying of biomass is necessary for transformation as gasification or pyrolysis are not competitive with direct transesterification with dry biomass. Another observation to consider is the need of processing the whole biomass in bio-oil based pathway, no matter which oil extraction method is used. If the process is performed using defatted biomass, the topology loses its profitability. The optimization results also show the need for improving the technologies for microalgae oil extraction. Gasification-based routes for diesel-like production from microalgae biomass did not offer promising results from the economic point of view under the conditions evaluated.

According to the proposed methodology, the next step corresponds to a more in-depth comparison of the two promising pathways. The transesterification-based route features a lower number of conversion steps for obtaining the main product, which is advantageous in terms of equipment necessary for intermediates processing. On the other hand, the hydrothermal liquefaction “HTL”-based pathway gives a higher amount of product than the

transesterification-based pathway. The co-products obtained without further biomass processing; in the transesterification based pathway have potential use as feedstock for bioethanol or biohydrogen production, or for obtaining high value products. However, this algae meal contains a high amount of water and residues of alcohol, acid and organic solvent. This makes it difficult to find a proper use for this meal without a purification process and decreasing their commercial value. Crude glycerol is also obtained but suffers from the same purity problem as the algae meal. Furthermore, commercial plants of biodiesel production from other feedstocks also produce large quantities of crude glycerol as co-product. This excessive supply lowers the value of crude glycerol. Consequently, only defatted biomass was taken as the co-product in this pathway.

On the other hand, HTL-based pathway gives (without further processing) the following a main co-products: CO_2, which does not command a meaningful value, charcoal, aqueous HTL co-product, which is a substance rich in nitrogen and has been used as a nutrient source for microalgae cultivation in low concentrations, biogasoline from the biocrude upgrading and other hydrocarbons. The primary co-product of value is gasoline. Other comparison criteria were included as the tax credit for CO_2 capture, in which HTL is more advantageous than transesterification because this process does not release carbon dioxide during biomass processing. Other economic parameters were included for comparison of the pathways such as break-even point and payback period of the alternatives. Table 39 shows the results of more in-depth comparison of microalgae biorefineries and the new objective values obtained after modification of optimization function, annual revenue for HTL-based pathway overcomes significantly profitability of transesterification pathway. Besides, the lower tax credit income, this increase is given mainly by the cost of gasoline which is obtained without including any additional process to the biorefinery. The payback period is lower for the

transesterification-based microalgae biorefinery owing to the lower fixed capital investment required for this pathway, but is not significantly lower than HTL-based biorefinery which is still attractive.

Table 39. In-depth Comparison of promising pathways

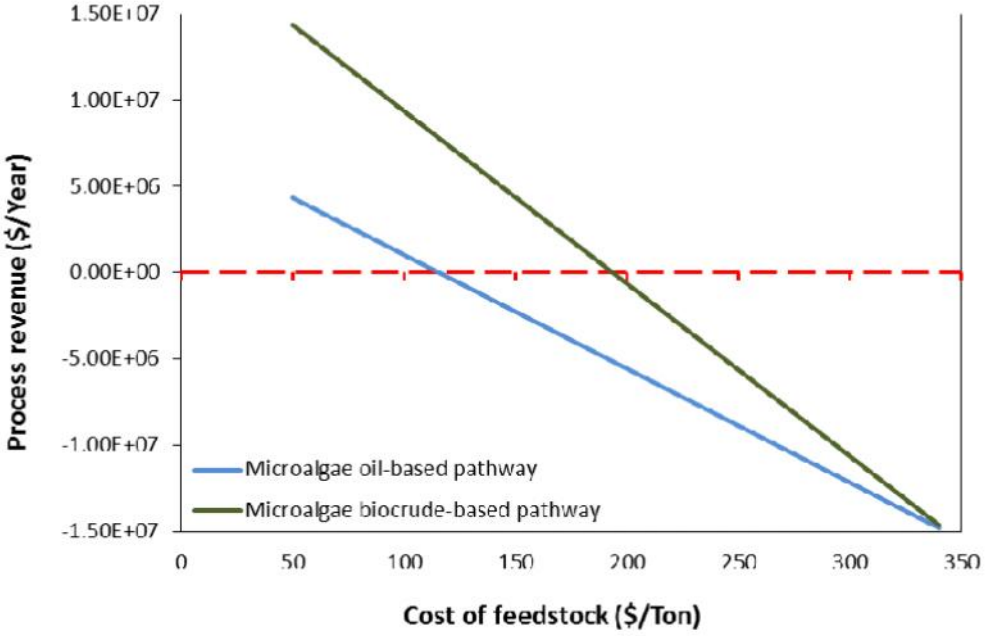
Parameter	Transesterification-based pathway	HTL-based pathway
Original objective value	2.645 MM $/year	2.199MM $/year
Co-products (without further processing)	Defatted biomass, glycerol	Aqueous HTL co-product, gas (CO_2), charcoal, gasoline, hydrocarbons
Cost of co-product	0.5*Cost of feedstock (only defatted biomass)	1137 $/ton (only gasoline)
Tax credit for CO_2 capture	1.950 MM $/year	1.755 MM $/year
Payback Period	4.9 years	6.6 years
Break-even point	$115 $/ton of biomass	$200 /ton of biomass
New objective value	6.295 MM $/year	16.124 MM $/year

SOURCE: Author

Cost of the feedstock is an important issue to consider for the development of integrated biorefineries. Additionally, the price stability over time is important. In microalgae biotechnology, current cost of biomass production is decreasing owing to the recent advances in microalgae cultivation technology. Nonetheless, after full development of microalgae production systems and stabilization of prices, it is predictable that an increase in microalgae production costs is very likely to occur because of supply and demand issues. Table 39 shows that the maximum allowed value of feedstock for transesterification pathway is lower than value for HTL-based biorefinery, which gives a higher flexibility in term of feedstock cost to this alternative. In addition, Figure 49 shows a break-even sensitivity analysis for two promising alternatives obtained after the superstructure optimization. In this case, it can

be seen that the revenue of transesterification based pathway shows a lower sensitivity to the cost of microalgae biomass, which is positive in the scenario where cost of feedstock is likely to have price instability.

Figure 49. Break-even sensitivity analysis of promising topologies of microalgae-based biorefineries after superstructure optimization

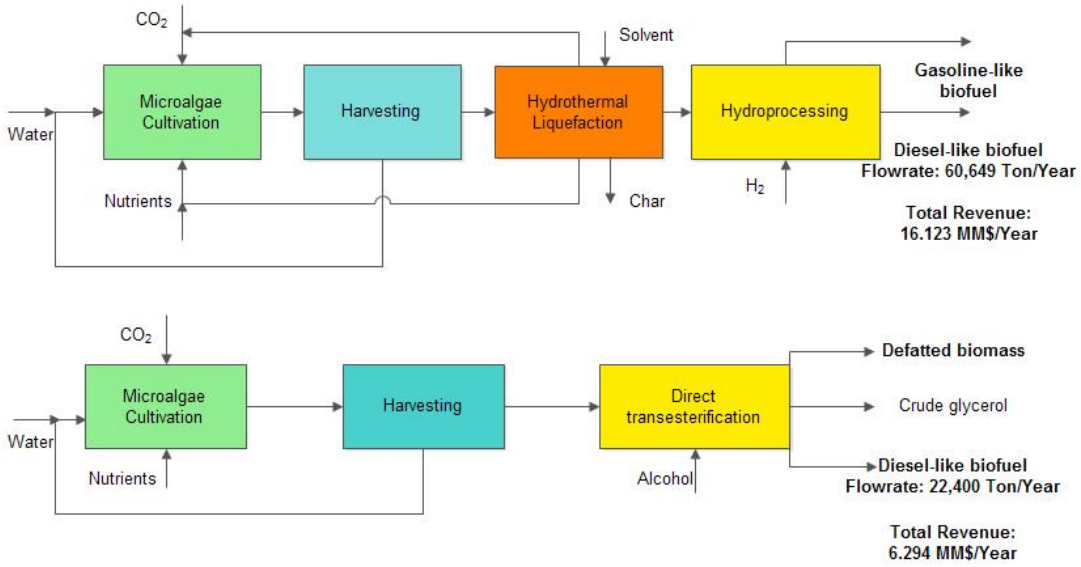


SOURCE: Author

A schematic representation of optimal pathways obtained from the superstructure after application of biorefinery concept can be seen in Figure 50. For HTL-based microalgae biorefinery, three process streams can be used for microalgae cultivation, the aqueous phase obtained after liquefaction as nutrients source for biomass growth, the CO_2 generated during thermal process as carbon source for microalgae, and the culture media separated from biomass during harvesting process. The main products obtained in the biorefinery are gasoline- and diesel-like biofuels. The second topology uses the water separated in the harvesting stage for cultivation. All these recycles can contribute to decreasing the costs of the microalgae production and to

resource conservation. These are important issues to consider in the development of sustainable processes.

Figure 50. Solutions to superstructure maximization taking into account additional economic parameters and applying the biorefinery concept



SOURCE: Author

6.6. CONCLUSIONS

A methodology for the synthesis and screening microalgae processing pathways has been proposed. The methodology is based on several integrated approaches including forward-backward branching, selection of main product, superstructure optimization, application of biorefinery concept, and multicriteria comparison of optimized alternatives. Two promising topologies of microalgae-based biorefineries were obtained: transesterification and hydrothermal liquefaction. More in-depth analysis was carried out to include additional screening criteria such as greenhouse gas emissions, resource conservation, impact of price instability, and break-even point analysis.

7. GENERAL CONCLUSIONS OF THE RESEARCH

Development of a new process inevitably requires collection of data from laboratories and pilot plants and simulations, in this work was shown a set of contributions for the development of a topology of microalgae-based biorefinery, starting from experimentation as primary technical information source, the computer aided process engineering for the evaluation of the increasing number microalgae processing alternatives and the development and utilization of a combined forward-backward screening and superstructure approach for the synthesis and optimization of the process, it was demonstrate that all three research areas were complementary and useful for obtaining final result.

Results obtained in lab scale and from process simulation, exergy analysis and environmental assessment confirms the Hexane Based Extraction method as the most convenient for lipid extraction in comparison to other methods evaluated, however, during superstructure optimization it was shown that under current state of technology development, biodiesel production methods based on oil extraction are not feasible from the economic point of view, alternatives less developed for microalgae as HTL or direct transesterification shows better results. In addition, despite the utilization of dried biomass in experimental part of this research, superstructure optimization shows that extraction and/or transformation methods involving drying of biomass as gasification, dry solvent-based extraction, supercritical extraction or pyrolysis are not recommendable from the economic point of view and must be avoided, it was proven that drying stage increases production costs and can degrade other products of interest; for reasons exposed above, research must be focused in wet extraction/transformation methods.

The big-picture approach developed and used in the final part of this book allowed to obtain from the enormous number of possibilities of existing and emerging technologies for microalgae processing, two feasible topologies of biorefineries with positive objective values (annual revenue) taking as main product diesel-like biofuel were developed, with expected maturation of technologies for microalgae processing, methodology proposed is still useful owing to their hierarchical characteristics, and also can be re-focused to other main product, or objective function (e.g., maximum yield, maximum profit, minimum processing steps, minimum waste, minimum emissions, maximum feedstock flexibility, highest energy or exergy efficiency).

Future work in experimental part must be focused in wet extraction methods in order to decrease costs derived from drying and keep the quality of other desirable metabolites, such as the improvement of methods for obtaining in-vivo products as milking, alkane secretion and exo-substances secretion, selective extraction of a specific substance inside a big fraction of similar metabolites (e.g. selective extraction of a specific fatty acid without extracting other lipids). In Computer Aided Process Engineering area, is recommendable the detailed study of feasible topologies developed in this study using process analysis and simulation, and the improvement of developed processes using process integration methodologies, it could be also interesting the optimization of a microalgae strain composition for their use in a given set of technologies for transformation and comparison of results with available composition of known microalgae strains.

8. SCIENTIFIC NOVELTY OF RESEARCH

The number of published scientific articles is a measure of the impact of the activity of a scientist or research group, and hence its importance. Publications are science indicators accepted and used by the United Nations Educational, Scientific, and Cultural Organization (UNESCO)[i], and the Colombian administrative Department of Science, Technology and Innovation (COLCIENCIAS)[ii].

Product of this research, it has been published 13 scientific articles and 8 conference papers since 2009 to 2013, taking into account only scientific papers, 8 of them were published in Journals indexed in the international bibliographic database Scopus, and 8 of them were published in journals indexed and homologated by the Colombian administrative department of science, technology and innovation COLCIENCIAS (Table 40).

Table 40. Scientific papers generated in this research and category of journals

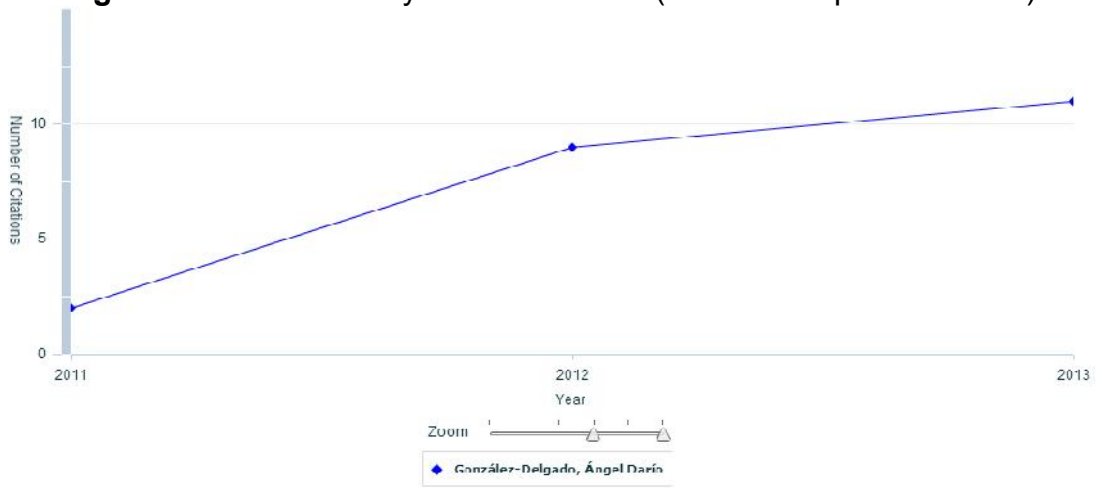
Paper	Journal	Indexed in Scopus	Indexed in COLCIENCIAS (category)
Obtaining high value products in a biorefinery topology using microalgae	CT&F – Ciencia, Tecnología y Futuro, 5(3), In press.	Yes	Yes (A1)
Evaluation of alternatives for microalgae oil extraction based on exergy analysis	Applied Energy, Vol. 101, 226 – 236.	Yes	Yes (A1)
Environmental assessment of microalgae biodiesel production in Colombia: Comparison of	CT&F – Ciencia, Tecnología y Futuro. Vol.	Yes	Yes (A1)

three oil extraction systems	5(2), 85-100.		
Microalgae based biorefinery: Issues to consider.	CT&F - Ciencia, Tecnología y Futuro, Vol. 4 (4), 47 – 60.	Yes	Yes (A1)
Microalgae Based Biorefinery: evaluation of oil extraction methods in terms of efficiency, costs, toxicity and energy in lab-scale.	ION, Vol. 26 (1), 29-37.	No	Yes (A2)
Evaluation of lipid and monosaccharide obtaining routes of microalgae biomass under the biorefinery concept	ION, Vol. 24 (2), 13-22.	No	Yes (A2)
Design and adjustment of coupled microalgae oil extraction methods for the development of a topology of biorefinery.	Prospectiva, ISSN 1692-8261, Vol. 10 (1) 113-123.	No	Yes (B)
Development of a methodology of microalgae oil extraction in the biodiesel from microalgae production chain	Prospectiva, ISSN 1692-8261, Vol. 7 (2) p.53 – 60	No	Yes (B)
Energy Integration of Bioethanol Production Process Topology from Microalgae Biomass: Evaluation of SSCF, SSF, Acid Hydrolysis and Product Purification Alternatives.	Chemical Engineering Transactions, Vol. 35, 1069-1074.	Yes	No
Microalgae Based Biorefinery: Evaluation of Several Routes for Joint Production of Biodiesel, Chlorophylls, Phycobiliproteins, Crude Oil and Reducing Sugars.	Chemical Engineering Transactions, Vol. 29, 607 - 612.	Yes	No

Simulation of bioethanol production process from residual microalgae biomass.	Computer Aided Chemical Engineering, Vol. 30 (1), 1048 - 1052.	Yes	No
Computer aided evaluation of eco-efficiency of solvent-based algae oil extraction processes for biodiesel production.	Computer Aided Chemical Engineering, Vol. 30 (1), 86 - 90.	Yes	No
Design of a multifunctional reactor for third generation biofuels production.	Chemical Engineering Transactions, ISSN 1974-9791, Vol. 21, 1297-1302.	Yes	No

Citation analysis displays the number of times other scientists cited a particular author, according to information shown in Scopus bibliographic database, works of the author has been referenced 28 times [3], if self-citations are excluded, it is shown that works of the author has been cited 22 times by other researchers (Figure 51).

Figure 51. Citation analysis of the author (source: Scopus database)



The *h-index* is an index that attempts to measure both the productivity and impact of the published work of a scientist, one scientist has an *h-index* of x, if

has x papers cited at least x times each one, for the case of the author, the *h-index* is equal to 3 [iii].

Besides the scientific papers, it has been also developed several products as 39 presentations in national and international scientific events with book of abstracts, 9 presentations in national and international scientific events without book of abstracts and 4 presentations in events with periodic proceedings. Detailed list of products obtained is shown below:

PUBLICATIONS IN CONGRESS PROCEEDINGS.

1. **González-Delgado, A. D.,** Peralta-Ruíz, Y., Pardo, Y., & Kafarov, V. 2013. Energy integration of bioethanol production process topology from microalgae biomass: Evaluation of SSCF, SSF, acid hydrolysis and product purification alternatives. Conference Proceedings. 13th Conference on Process Integration, Modeling and Optimisation for Energy Saving and Pollution Reduction PRES 2013. ISBN 978-88-95608-26-6, S.N. 1069.

2. **González-Delgado, A. D.,** Kafarov, V., 2012. Design, adjustment and comparison of several coupled methods for oil extraction of third generation energy crops for biodiesel production. Conference Proceedings. 20th International Congress of Chemical and Process Engineering CHISA 2012. ISBN 978-80-905035-1-9, S. N. 0539.

3. **González-Delgado, A. D.,** Molano, C., Álvarez, D., Kafarov, V., 2012. Microalgae based biorefinery: multi-parameter comparison of routes for microalgae oil extraction. Conference Proceedings. 20th International Congress of Chemical and Process Engineering CHISA 2012. ISBN 978-80-905035-1-9, S. N. 0542.

4. **González-Delgado, A. D.,** Peralta, Y., Pardo, Y., Kafarov, V., 2012. Exergetic life-cycle assessment (ELCA) of several extraction/transesterification routes for third generation biofuels production. Conference Proceedings. 20th International Congress of Chemical and Process Engineering CHISA 2012. ISBN 978-80-905035-1-9, S. N. 0594.

5. **González-Delgado, A. D.,** Peralta, Y., Pardo, Y., Kafarov, V., 2012. Microalgae based biorefinery: use of biomass after oil extraction for third generation bioethanol production. Conference Proceedings. 20th International Congress of Chemical and Process Engineering CHISA 2012. ISBN 978-80-905035-1-9, S. N. 0557.

6. **González-Delgado, A. D.,** Kafarov, V., 2012. Microalgae based biorefinery: Evaluation of several routes for joint production of biodiesel, crude oil and reducing sugars. Conference Proceedings. 15th Conference on Process Integration, Modeling and Optimisation for Energy Saving and Pollution Reduction PRES 2012. ISBN 978-80-905035-1-9, S. N. 1303.

7. **González-Delgado, A. D.,** Peralta, Y., Pardo, Y., Kafarov, V., 2012. Simulation and sensitivity analysis of variables affecting microalgae oil extraction. Conference Proceedings. 15th Conference on Process Integration, Modeling and Optimisation for Energy Saving and Pollution Reduction PRES 2012. ISBN 978-80-905035-1-9, S. N. 1307.

8. **González-Delgado, A. D.,** Kafarov, V., 2012. Avances en el desarrollo de una topología de biorefinería para la obtención de biocombustibles y productos de alto valor agregado a partir de biomasa de microalgas. Book of abstracts. V International Congress of Biofuels Science and Technology CIBSCOL 2012. ISBN: 978-958-46-0616-7.

9. Peralta, Y., Pardo, Y., **González-Delgado, A. D.,** Kafarov, V., 2012. Exergy and environmental analysis of oil extraction Methods for sustainable microalgal biodiesel Production. Book of abstracts. V International Congress of Biofuels Science and Technology CIBSCOL 2012. ISBN: 978-958-46-0616-7.

10. Pardo, Y., Peralta, Y., **González-Delgado, A. D.,** Kafarov, V., 2012. Evaluation of sustainability of solvent extraction process for algae biodiesel production. Book of abstracts. V International Congress of Biofuels Science and Technology CIBSCOL 2012. ISBN: 978-958-46-0616-7.

11. García, L., Amaya, E., **González-Delgado, A. D.,** Kafarov, V., 2012. Aprovechamiento de biomasa de la microalga amphiprora sp. Para la obtención de pigmentos, ficobiliproteínas y lípidos bajo el concepto de biorefinería. Book of abstracts. V International Congress of Biofuels Science and Technology CIBSCOL 2012. ISBN: 978-958-46-0616-7.

12. **González, A. D.,** Kafarov, V., 2011. Design and adjustment of coupled methods of microalgae oil extraction for third generation biofuels production in a topology of biorefinery. Conference Proceedings of the III International Congress of Materials, Energy and Environment. ISBN 978-958-8524-58-0.

13. **González, A. D.,** Kafarov, V., 2011. Microalgae based biorefinery: Morphological and statistical comparison of microalgae lab-scale oil extraction methods for three Colombian strains. Conference Proceedings of the III International Congress of Materials, Energy and Environment. ISBN 978-958-8524-58-0.

14. Peralta Y. Y., Pardo, Y., **González, A. D.,** Kafarov, V., 2011. Comparación de tres rutas de extracción de aceite de microalga mediante evaluación exegética y de impactos ambientales. Conference Proceedings of the III International Congress of Materials, Energy and Environment. ISBN 978-958-8524-58-0.

15. Alvarez, Y., **González, A. D.,** Kafarov, V., Evaluation of several routes for obtaining fuels, lipids and monosaccharides from third generation energy crops under biorefinery concept. 6th Dubrovnik Conference on Sustainable Development of Energy Water and Environment Systems. Dubrovnik, Croatia. ISBN: 978-953-7738-12-9. P.166.

16. Molano-Bayona, D., Álvarez-Suarez, D., **González-Delgado, A. D.,** Kafarov, V. 2011. Estudio comparativo de métodos de disrupción celular/extracción para la obtención de aceite de microalgas de bioprospección nacional. Book of abstracts. VI Congreso Colombiano de Botánica ISBN: 978-958-99009-1-8, p. 607.

17. Álvarez, Y., **González, A. D.,** Kafarov, V. 2011. Definición y comparación de rutas de uso de biomasa de las microalgas Amphiprora sp. y Navicula sp. para la obtención de biocombustibles y productos de alto valor agregado. Book of abstracts. VI Congreso Colombiano de Botánica ISBN: 978-958-99009-1-8, p. 605.

18. García, L., Amaya, E., **González, A. D.,** Kafarov, V. 2011. Obtención de Pigmentos y Purificación de Aceite Crudo en Cultivos Energéticos de Tercera Generación Bajo el Concepto de Biorefinería. Book of abstracts. XII Seminario Internacional del Medio Ambiente y Desarrollo Sostenible SIMADS 2011.

19. Álvarez, Y., **González, A. D.,** Kafarov, V. 2011. Ajuste y comparación de métodos de disrupción celular de microalgas para su utilización en biorefinería. Book of abstracts. XII Seminario Internacional del Medio Ambiente y Desarrollo Sostenible SIMADS 2011.

20. Álvarez, Y., **González, A. D.,** Kafarov, V. 2011. Definición y comparación de rutas de transformación de biomasa de las microalgas Amphiprora sp. y Navicula sp. bajo el concepto de biorefinería. Book of abstracts. XII Seminario Internacional del Medio Ambiente y Desarrollo Sostenible SIMADS 2011.

21. Peñaranda, L. A., Sepulveda, K., **González, A. D.,** Kafarov, V. Estudio de rutas de aprovechamiento de biomasa de la microalga Navicula sp. para la producción de biocombustibles. Book of abstracts. XII Seminario Internacional del Medio Ambiente y Desarrollo Sostenible SIMADS 2011.

22. García, J., Miranda, J., **González, A. D.,** Kafarov, V. 2010. Comparison of microalgae oil extraction methods for biodiesel production. Book of abstracts. IV International Congress of Biofuels Science and Technology CIBSCOL 2010. ISBN: 978-958-44-8185-6, p. 81.

23. González, S., Galindo, L., **González, A. D.,** Kafarov, V. 2010. Evaluación de rutas para la obtención de extractos lipidicos de microalgas bioprospectadas en colombia combinando métodos de disrupción celular y extracción con metanol- cloroformo. Book of abstracts. IV International Congress of Biofuels Science and Technology CIBSCOL 2010. ISBN: 978-958-44-8185-6, p. 16.

24. **González, A. D.,** Kafarov, V. 2010. Desarrollo combinado de disrupcion celular, extraccion y transesterificacion de metabolitos de microalgas

para la produccion de biodiesel. Book of abstracts. IV International Congress of Biofuels Science and Technology CIBSCOL 2010. ISBN: 978-958-44-8185-6, p. 65.

25. **González, A. D.,** Kafarov, V. 2010. Extraccion de lipidos de microalgas a partir de pretratamientos fisico-químicos para la produccion de biodiesel. Book of abstracts. IV International Congress of Biofuels Science and Technology CIBSCOL 2010. ISBN: 978-958-44-8185-6, p. 63.

26. Córdoba, L. S., López, L. M., **González, A. D.,** Kafarov, V. 2010. Obtención de aceite de microalgas nativas a escala de laboratorio mediante la combinación de disrupción celular y método de extracción soxhlet para la producción de biodiesel. Book of abstracts. IV International Congress of Biofuels Science and Technology CIBSCOL 2010. ISBN: 978-958-44-8185-6, p. 61.

27. Peñaranda, L. A., Sepulveda, K., Alvarez, Y. E., **González, A. D.,** Kafarov, V. 2010. Evaluación de rutas de obtención de lípidos y monosacáridos de biomasa de microalgas bajo el concepto de biorefinería. Book of abstracts. IV International Congress of Biofuels Science and Technology CIBSCOL 2010. ISBN: 978-958-44-8185-6, p. 79.

28. **González, A. D.,** Kafarov, V. 2010. Design of a multifunctional reactor for third generation biofuels production. Conference Proceedings. 13th Conference on Process Integration, Modeling and Optimisation for Energy Saving and Pollution Reduction. ISBN 978-88-95608-05-1, p.1297 - 1302.

29. **González, A. D.,** Kafarov, V., Guzmán, A. 2010. Reactor modelling for third generation biofuels production. Conference Proceedings. XIX International Conference on Chemical Reactors CHEMREACTOR-19. ISBN 978-88-95608-05-1, p.1297 - 1302.

30. **González, A. D.,** Kafarov, V., Guzmán, A. 2010. Comparison of microalgae oil extraction methods for third generation biofuel production. Conference Proceedings. 19th International Congress of Chemical and Process Engineering CHISA 2010. ISBN 978-80-02-02210-7, S. N. 1449.

31. **González, A. D.,** Anaya, J., Kafarov, V. 2010. Obtención de lípidos a partir del pretratamiento fisico-químico de biomasa de microalgas para la producción de biodiesel. Book of abstracts. XXIX Latin American Chemistry Congress – CLAQ 2010. ISBN 978-958-99607-0-7, S. N. BQB 23.

32. **González, A. D.,** Amaya, A., Sarmiento R., Kafarov, V. 2010. Desarrollo de una metodología para la extracción de aceite de microalgas empleando disrupción celular térmica y química. Book of abstracts. XXIX Latin American Chemistry Congress – CLAQ 2010. ISBN 978-958-99607-0-7, S. N. FQT 64.

33. **González, A. D.,** León, X., Silva, M., Kafarov, V. 2010. Aplicación de análisis y síntesis de procesos a la producción de biodiesel a partir de microalgas. Book of abstracts. XXIX Latin American Chemistry Congress – CLAQ 2010. ISBN 978-958-99607-0-7, S. N. MYE 08.

34. **González, A. D.,** Anaya, J., Kafarov, V. 2010. Obtención de lípidos a partir del pretratamiento fisico-químico de la biomasa de Nannochloropsis sp. para la producción de biodiesel. Book of abstracts. XXIX Latin

American Chemistry Congress – CLAQ 2010. ISBN 978-958-99607-0-7, S. N. MYE 111.

35. Córdoba, L. S., López, L. M., **González, A. D.,** Kafarov, V 2010. Diseño de una metodología para la extracción de aceite de microalgas nativas mediante el método soxhlet. Book of abstracts. XXIX Latin American Chemistry Congress – CLAQ 2010. ISBN 978-958-99607-0-7, S. N. MYE 113.

36. **González, A. D.,** Kafarov, V., 2010. Diseño de un reactor multifuncional para la producción de biocombustibles de tercera generación. Book of abstracts. XXIX Latin American Chemistry Congress – CLAQ 2010. ISBN 978-958-99607-0-7, S. N. MYE 55.

37. González, S., Galindo, L., **González, A. D.,** Kafarov V., 2010. Adaptación del método Bligh & Dyer a la extracción de lípidos de microalgas nativas para la producción de biodiesel. Book of abstracts. XXIX Latin American Chemistry Congress – CLAQ 2010. ISBN 978-958-99607-0-7, S. N. MYE 18.

38. **González, A. D.,** Kafarov, V., Guzmán, A., 2009. Producción de biocombustibles de tercera generación: extracción de aceite de microalgas para la producción de biodiesel. Conference Proceedings. IV Symposium of Applied Chemistry SIQUIA 2009. ISBN 978-958-8593-12-8.

39. **González, A. D.,** Kafarov, V., Guzmán, A., 2009. Desarrollo de métodos de extracción de aceite en la cadena de producción de biodiesel a partir de microalgas. Conference Proceedings II International Congress of Materials, Energy and Environment. ISBN 978-958-8524-19-1.

PUBLICATIONS IN PERIODIC PROCEEDINGS

1. Amaya, E. L., García, L. M., **González, A. D.,** Kafarov, V. 2011. Pigment extraction and crude oil purification of microalgae biomass for biodiesel production (In Spanish). Proceedings of the XXVI Colombian Congress of Chemical Engineering, ISSN 1692-925X.

2. Rueda, J., **González, A. D.,** Kafarov, V. 2011. Morphological response to cell disruption and five oil extraction methods of five national bioprospecting microalgae (In Spanish). Proceedings of the XXVI Colombian Congress of Chemical Engineering, ISSN 1692-925X.

3. Peñaranda, L. A., **González, A. D.,** Kafarov, V. 2011 Evaluation and comparison of routes for obtaining monosaccharides and lipids at lab scale from Navicula sp. (In Spanish). Proceedings of the XXVI Colombian Congress of Chemical Engineering, ISSN 1692-925X.

4. Alvarez, D., Molano, C., **González, A. D.,** Kafarov, V. 2011 Third generation biofuels production: Multicriteria comparison of microalgae oil extraction methods (In Spanish). Proceedings of the XXVI Colombian Congress of Chemical Engineering, ISSN 1692-925X.

PRESENTATIONS IN EVENTS WITHOUT BOOK OF ABSTRACTS

Colombian Engineering Meeting. Cali, Colombia, May 29-31, 2013.

- Peralta-Ruiz Y, **González-Delgado A**, Kafarov V., Desarrollo sostenible mediante producción de biocombustibles de tercera generación: evaluaciones técnicas, energéticas y ambientales.

22nd International Symposium on Chemical Reaction Engineering (ISCRE 22), Maastricht, the Netherlands, September 2-5, 2012.

- Pardo, Y., Peralta Y. Y., **González, A. D.,** Kafarov, V., Comparison of reaction systems of microalgal bioethanol production: SSF, SSCF and acid hydrolysis.

- Pardo, Y., Peralta Y. Y., **González, A. D.,** Kafarov, V., Exergy and environmental analysis of oil extraction methods for sustainable microalgal biodiesel production.

8th European Congress of Chemical Engineering and the 1st European Congress of Applied Biotechnology, Berlin, Germany. September 25-29, 2011.

- **González, A. D.,** Kafarov, V., Evaluation of Lab-scale Routes for Obtaining Sugars, Lipids and Biodiesel from Microalgae

- Pardo, Y., Peralta Y. Y., **González, A. D.,** Kafarov, V., Exergetic Life cycle Assessment (ELCA) of different solvent based microalgae oil extraction methods for biodiesel production.

- Garcia, J., Miranda, J., **González, A. D.,** Kafarov, V. Statistical comparison of lab-scale microalgae cell disruption/oil extraction methods for biodiesel production.

- Peñaranda, L. A., Sepulveda, K., Alvarez, Y. E., **González, A. D.,** Kafarov, V. Evaluation and comparison of biorefinery lab scale processes for Navicula sp. microalgae.

13th Conference on Process Integration, Modeling and Optimisation for Energy Saving and Pollution Reduction PRES 2010. Prague, Czech Republic. 28 August – 1 September 2010.

- **González, A. D.,** Kafarov, V. Design of a multifunctional reactor for third generation biofuels production.

  19th International Congress of Chemical and Process Engineering CHISA 2010. Prague, Czech Republic. 28 August – 1 September 2010.

- **González, A. D.,** Kafarov, V., Guzmán, A. Comparison of microalgae oil extraction methods for third generation biofuel production.

[i] UNESCO Institute for Statistics (2010). Measuring R&D: Challenges Faced by Developing Countries. ICAO, Montreal.

[ii] Salazar, M. (2011). Indicadores de ciencia y tecnología, Colombia 2011. Bogotá: Observatorio Colombiano de Ciencia y Tecnología.

[iii] SCOPUS. Author Detailed information='Angel Darío González-Delgado' (Web site). Dec 10th, 2013. Available in: http://www.scopus.com.ezproxy.unal.edu.co/authid/detail.url?origin=AuthorEval&authorId=55621117300. Accessed on December 10th, 2013.

REFERENCES

[1] Amores, M. J., Mele, F. D., Jiménez, L., & Castells, F. (2013). Life cycle assessment of fuel ethanol from sugarcane in Argentina. International Journal of Life Cycle Assessment, 1-14.

[2] Salazar-Ordóñez, M., Pérez-Hernández, P. P., & Martín-Lozano, J. M. (2013). Sugar beet for bioethanol production: An approach based on environmental agricultural outputs. Energy Policy, 55, 662-668.

[3] Koga, N., Kajiyama, T., Senda, K., Iketani, S., Tamiya, S., & Tsuda, S. (2013). Energy efficiency of potato production practices for bioethanol feedstock in northern japan. European Journal of Agronomy, 44, 1-8.

[4] Singh, V. (2012). Effect of corn quality on bioethanol production. Biocatalysis and Agricultural Biotechnology, 1(4), 353-355.

[5] Basavaraj, G., Parthasarathy Rao, P., Basu, K., Reddy, C. R., Kumar, A. A., Srinivasa Rao, P., & Reddy, B. V. S. (2013). Assessing viability of bio-ethanol production from sweet sorghum in india. Energy Policy, 56, 501-508.

[6] Tang, S., Zhao, H., Song, Z., & Olubajo, O. (2013). Glymes as benign co-solvents for CaO-catalyzed transesterification of soybean oil to biodiesel. Bioresource Technology, 139, 107-112.

[7] Jokiniemi, T., & Ahokas, J. (2013). A review of production and use of first generation biodiesel in agriculture. Agronomy Research, 11(1), 239-248.

[8] Martinez, D., & Kafarov, V. (2012). Computer aided estimation of sustainability of biodiesel production from palm oil, Computer Aided Chemical Engineering, 30, 222-226.

[9] Menten, F., Chèze, B., Patouillard, L., & Bouvart, F. (2013). A review of LCA greenhouse gas emissions results for advanced biofuels: The use of meta-regression analysis. Renewable and Sustainable Energy Reviews, 26, 108-134.

[10] Chu, Q., Yang, D., Li, X., Ma, B., Yu, S., & Yong, Q. (2013). An integrated process to enhance ethanol production from steam-exploded corn stover. Fuel, 107, 823-827.

[11] Ojeda, K., Sánchez, E., & Kafarov, V. (2011). Sustainable ethanol production from lignocellulosic biomass - application of exergy analysis. Energy, 36(4), 2119-2128.

[12] Menezes, E. G. T., Do Carmo, J. R., Menezes, A. G. T., Alves, J. G. L. F., Pimenta, C. J., & Queiroz, F. (2013). Use of different extracts of coffee pulp for the production of bioethanol. Applied Biochemistry and Biotechnology, 169(2), 673-687.

[13] Kim, S., & Kim, C. H. (2013). Bioethanol production using the sequential acid/alkali-pretreated empty palm fruit bunch fiber. Renewable Energy, 54, 150-155.

[14] Quintero, J. A., Moncada, J., & Cardona, C. A. (2013). Techno-economic analysis of bioethanol production from lignocellulosic residues in Colombia: A process simulation approach. Bioresource Technology, 139, 300-307

[15] Chang, F., Hanna, M. A., Zhang, D. -., Li, H., Zhou, Q., Song, B. -., & Yang, S. (2013). Production of biodiesel from non-edible herbaceous vegetable oil: Xanthium sibiricum patr. Bioresource Technology, 140, 435-438.

[16] Ong, H. C., Silitonga, A. S., Masjuki, H. H., Mahlia, T. M. I., Chong, W. T., & Boosroh, M. H. (2013). Production and comparative fuel properties of biodiesel from non-edible oils: Jatropha curcas, sterculia foetida and ceiba pentandra. *Energy Conversion and Management, 73*, 245-255.

[17] Dias, J. M., Araújo, J. M., Costa, J. F., Alvim-Ferraz, M. C. M., & Almeida, M. F. (2013). Biodiesel production from raw castor oil. Energy, 53, 58-66.

[18] Nigam, P. S., & Singh, A. (2011). Production of liquid biofuels from renewable resources. Progress in Energy and Combustion Science, 37(1), 52-68.

[19] Amaro, H., Guedes, C. & Malcata X. (2011). Advances and perspectives in using microalgae to produce biodiesel, Applied. Energy, 88(10), 3402-3410.

[20] UIS-ICP-Morrosquillo. Bioprospecting of Colombian microalgae for biodiesel production. Final Report, Bucaramanga; 2011.

[21] Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology Advances, 25(3), 294-306.

[22] Yuan, Z., Wang, Z., Takala, J., Hiltunen, E., Qin, L., Xu, Z., Zhu, L. (2013). Scale-up potential of cultivating chlorella zofingiensis in piggery wastewater for biodiesel production. Bioresource Technology, 137, 318-325.

[23] Chen, C. -., Chang, J. -., Chang, H. -., Chen, T. -., Wu, J. -., & Lee, W. -. (2013). Enhancing microalgal oil/lipid production from chlorella sorokiniana CY1 using deep-sea water supplemented cultivation medium. Biochemical Engineering Journal, 77, 74-81.

[24] Spolaore, P., Joannis-Cassan, C., Duran, E., & Isambert, A. (2006). Commercial applications of microalgae. Journal of Bioscience and Bioengineering, 101(2), 87-96.

[25] Fábregas, J., Otero, A., Maseda, A., & Domínguez, A. (2001). Two-stage cultures for the production of astaxanthin from haematococcus pluvialis. Journal of Biotechnology, 89(1), 65-71.

[26] Shi, X. -., Zhang, X. -., & Chen, F. (2000). Heterotrophic production of biomass and lutein by chlorella protothecoides on various nitrogen sources. Enzyme and Microbial Technology, 27(3-5), 312-318.

[27] Wu, S. -., Yu, S. -., & Lin, L. -. (2005). Effect of culture conditions on docosahexaenoic acid production by schizochytrium sp. S31. Process Biochemistry, 40(9), 3103-3108.

[28] Liang, Y., Sarkany, N., Cui, Y., Yesuf, J., Trushenski, J., & Blackburn, J. W. (2010). Use of sweet sorghum juice for lipid production by schizochytrium limacinum SR21. Bioresource Technology, 101(10), 3623-3627.

[29] Putt, R., Singh, M., Chinnasamy, S., & Das, K. C. (2011). An efficient system for carbonation of high-rate algae pond water to enhance CO2 mass transfer. Bioresource Technology, 102(3), 3240-3245.

[30] Benemann, J.R. & Oswald, W.J. (1993). Systems and economic analysis of microalgae ponds for conversion of carbon dioxide to biomass. Final Report: Grant No. DE-FG22-93PC93204. Pittsburgh Energy Technology Center, Pittsburgh, PA, US Department of Energy.

[31] Doucha, J., & Lívanský, K. (2006). Productivity, CO2/O2 exchange and hydraulics in outdoor open high density microalgal (chlorella sp.) photobioreactors operated in a middle and southern european climate. Journal of Applied Phycology, 18(6), 811-826.

[32] Molina Grima, E., Acién Fernández, F. G., García Camacho, F., Camacho Rubio, F., & Chisti, Y. (2000). Scale-up of tubular photobioreactors. Journal of Applied Phycology, 12(3-5), 355-368.

[33] Vunjak-Novakovic, G., Kim, Y., Wu, X., Berzin, I. & Merchuk, J. C. (2005). Air-lift bioreactors for algal growth on flue gas: mathematical modeling and pilot-plant studies. Industrial & Engineering Chemistry Research, 44, 6154–6163.

[34] Kaewpintong, K., Shotipruk, A., Powtongsook, S., & Pavasant, P. (2007). Photoautotrophic high-density cultivation of vegetative cells of haematococcus pluvialis in airlift bioreactor. Bioresource Technology, 98(2), 288-295.

[35] Jorquera, O., Kiperstok, A., Sales, E. A., Embiruçu, M., & Ghirardi, M. L. (2010). Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors. Bioresource Technology, 101(4), 1406-1413.

[36] Harith, Z. T., Yusoff, F. M., Mohamed, M. S., Din, M. S. M. & Ariff, A. B. (2009). Effect of different flocculants on the flocculation performance of microalgae, Chaetoceros calcitrans, cells. African Journal of Biotechnology, 8, 5971–5978.

[37] Vandamme, D., Foubert, I., Meesschaert, B., & Muylaert, K. (2010). Flocculation of microalgae using cationic starch. Journal of Applied Phycology, 22(4), 525-530.

[38] Knuckey, R. M., Brown, M. R., Robert, R., & Frampton, D. M. F. (2006). Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering, 35(3), 300-313.

[39] Poelman, E., De Pauw, N., & Jeurissen, B. (1997). Potential of electrolytic flocculation for recovery of micro-algae. Resources, Conservation and Recycling, 19(1), 1-10.

[40] Ehimen, E. A., Sun, Z. F., & Carrington, C. G. (2010). Variables affecting the in situ transesterification of microalgae lipids. Fuel, 89(3), 677-684.

[41] Richmond, A. (2004). Handbook of microalgal culture: biotechnology and applied phycology. Ames, Iowa, Unites States: Blackwell Science.

[42] Folch, J., Lees, M. & Stanley, G. H. S. (1957). A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry, 226(1), 497-509.

[43] Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37 (8), 911-917.

[44] Molina-Grima, E., Robles-Medina, A., Giménez-Giménez, A., Sánchez-Pérez, J., García-Camacho, F. & García-Sánchez, J. (1994). Comparison between extraction of lipids and fatty acids from microalgal biomass, Journal of the American Oil Chemists' Society, 71(9), 955-959.

[45] Manirakiza, P., Covaci, A., & Schepens, P. (2001). Comparative study on total lipid determination using soxhlet, roese-gottlieb, bligh & dyer, and modified bligh & dyer extraction methods. Journal of Food Composition and Analysis, 14(1), 93-100.

[46] Ramluckan, K., Moodley, K. G., & Bux, F. (2014). An evaluation of the efficacy of using selected solvents for the extraction of lipids from algal biomass by the soxhlet extraction method. Fuel, 116, 103-108.

[47] Li, Y. Q., Horsman, M., Wang, B., Wu, N. & Lan, C. Q. (2008). Effects of nitrogen sources on cell growth and lipid accumulation of green alga Neochloris oleobundans. Applied Microbiology and Biotechnology, 81(4), 629–636.

[48] Anderson, J. & Sorek, B. (2008). Microalgae: the fuel of tomorrow. Ninth Annual Freshman Conference. Pittsburgh, Pennsylvania, United States. 9112:C7

[49] Lee, J-Y., Yoo, C., Jun, S-Y., Ahn, C-Y. & Oh, H-M.(2010). Comparison of several methods for effective lipid extraction from microalgae. Bioresource Technology, 101(1), 575–577.

[50] Pernet, F. & Tremblay, R. (2003). Effect of ultrasonication and grinding on the determination of lipid class content of microalgae harvested on filters. Lipids, 38(11), 1191–1195.

[51] Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M. & Cintas, P. (2008). Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrasonics Sonochemistry, 15(5), 898-902.

[52] King, J. (2000). Advances in critical fluid technology for food processing. Food Science & Technology Today, 14(4), 186–191.

[53] Chester, T. L. & Pinkston, J. D. (2004). Supercritical fluid and unified chromatography, Analytical Chemistry, 76(16), 4606–4613.

[54] Cheung, P. C. K. (1999). Temperature and pressure effects on supercritical carbon dioxide extraction of n_3 fatty acids from red seaweed. Food Chemistry, 65(3), 399–403.

[55] Mendes, R. L., Fernandes, H. L., Coelho, J. P., Reis, E. C., Cabral, J. M. S. & Novais, J. M. (1995). Supercritical CO2 extraction of carotenoids and other lipids from Chlorella vulgaris. Food Chemistry, 53(1), 99–103.

[56] Mendes, R. L., Nobre, B. P., Cardoso, M. T., Pereira, A. P. & Palabra, A. F. (2003). Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorganica Chimica Acta, 356(1), 328–334.

[57] Herrero M., Cifuentes A. & Ibáñez E. (2006). Sub- and supercritical fluid extraction of functional ingredients from different natural sources: Plants, food-by-products, algae and microalgae: A review. Food Chemistry, 98 (1), 136-148.

[58] Herrero, M., Ibáñez, E., Señoráns, F. J. & Cifuentes, A. (2003). Accelerated solvent extracts from Spirulina platensis Microalgae: determination of their antioxidant activity and analysis by Micellar Electrokinetic Chromatography. Journal of Chromatography, 1047(2), 195–203.

[59] Minowa, T., Yokoyama, S., Kishimoto, M. & Okakurat, T. (1995). Oil production from algal cells of Dunaliella tertiolecta by direct thermochemical liquefaction. Fuel, 74(12), 1735–1738.

[60] Ward, O. P. & Singh, A. (2005). Omega-3/6 fatty acids: Alternative sources of production. Process Biochemistry, 40 (12), 3627-3652.

[61] Robles-Medina, A., Molina Grima, E., Giménez Giménez, A. & Ibáñez González, M. J. (1998), Downstream processing of algal polyunsaturated fatty acids. Biotechnology Advances, 16 (3), 517-580.

[62] Lee, S. J., Yoon, B. -., & Oh, H. -. (1998). Rapid method for the determination of lipid from the green alga botryococcus braunii. Biotechnology Techniques, 12(7), 553-556.

[63] Henriques, M., Silva, A. & Rocha, J. (2007). Extraction and quantification of pigments from a marine: A simple and reproducible method. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 1(1), 586-593.

[64] Gong, B. (2002). Components of Chlorella pyrenoidosa, Gong Bih Enterprise Limited Company. [scan de certificado de caracterización]. [Accesed Diciembre 07, 2013]. Avaliable in <http://www.gongbih.com/webc/html/chlorella/ch402.htm.>

[65] Mendes-Pinto, M., Raposo, M., Bowen, J., Young, A. & Morais, R. (2001). Evaluation of different cell disruption processes on encysted cells of Haematococcus pluvialis: effects on astaxanthin recovery and implications for bio-availability. Journal of Applied Phycology, 13(1), 19-24.

[66] Li, X., Xu, H., & Wu, Q. (2007). Large-scale biodiesel production from microalga chlorella protothecoides through heterotrophic cultivation in bioreactors. Biotechnology and Bioengineering, 98(4), 764-771.

[67] Mata T, Martins A, Caetano N. (2010). Microalgae for biodiesel production and other applications: a review. Renewable & Sustainable Energy Reviews, 14(1), 217–232.

[68] Peralta, Y., Sanchez, E., & Kafarov, V. (2010). Exergy analysis for third generation biofuel production from microalgae biomass. Chemical Engineering Transactions, 21, 1363-1368.

[69] Ueda R, Hirayama S, Sugata K, Nakayama H. (1996). Process for the production of ethanol from microalgae. US Patent No. 5,578,472.

[70] Choi, S. P., Nguyen, M. T., & Sim, S. J. (2010). Enzymatic pretreatment of chlamydomonas reinhardtii biomass for ethanol production. Bioresource Technology, 101(14), 5330-5336.

[71] González, A. D. & Kafarov, V. (2010). Design of a multifunctional reactor for third generation biofuels production. Chemical Engineering Transactions, 21(1), 1297-1302.

[72] Peñaranda, L.A., Sepúlveda, K.J., Álvarez, Y.E., González-Delgado, A.D., & Kafarov, V. (2011). Evaluation of lipid and monosaccharide obtaining routes of microalgae biomass under the biorefinery concept. Ion, 24(2), 13-22.

[73] Anh, N. T. N., Van Hoa, N., Van Stappen, G., & Sorgeloos, P. (2009). Effect of different supplemental feeds on proximate composition and artemia biomass production in salt ponds. Aquaculture, 286(3-4), 217-225.

[74] Harun, R. & Danquah M. (2011).Enzymatic hydrolysis of microalgal biomass for bioethanol production. Chemical Engineering Journal, 168(3), 1079-1084.

[75] González-Delgado, A. D., & Kafarov, V. (2012). Microalgae based biorefinery: Evaluation of several routes for joint production of biodiesel, chlorophylls, phycobiliproteins, crude oil and reducing sugars. Chemical Engineering Transactions, 29, 607-612.

[76] Shigechi, H., Koh, J., Fujita, Y., Matsumoto, T., Bito, Y., Ueda, M., Kondo, A. (2004). Direct production of ethanol from raw corn starch via fermentation by use of a novel surface-engineered yeast strain codisplaying glucoamylase and α-amylase. Applied and Environmental Microbiology, 70(8), 5037-5040.

[77] Babich, I. V., van der Hulst, M., Lefferts, L., Moulijn, J. A., O'Connor, P., & Seshan, K. (2011). Catalytic pyrolysis of microalgae to high-quality liquid bio-fuels. Biomass and Bioenergy, 35(7), 3199-3207.

[78] Yang, C., Jia, L., Chen, C., Liu, G., & Fang, W. (2011). Bio-oil from hydro-liquefaction of dunaliella salina over Ni/REHY catalyst. Bioresource Technology, 102(6), 4580-4584.

[79] Yang, Y. F., Feng, C. P., Inamori, Y., & Maekawa, T. (2004). Analysis of energy conversion characteristics in liquefaction of algae. Resources, Conservation and Recycling, 43(1), 21-33.

[80] Suali, E., & Sarbatly, R. (2012). Conversion of microalgae to biofuel. Renewable and Sustainable Energy Reviews, 16(6), 4316-4342.

[81] Ramzan, N., Ashraf, A., Naveed, S., & Malik, A. (2011). Simulation of hybrid biomass gasification using aspen plus: A comparative performance analysis for food, municipal solid and poultry waste. Biomass and Bioenergy, 35(9), 3962-3969.

[82] Chakinala, A. G., Brilman, D. W. F., van Swaaij, W. P. M., & Kersten, S. R. A. (2010). Catalytic and Non-catalytic Supercritical Water Gasification of Microalgae and Glycerol. Industrial & Engineering Chemistry Research, 49, 1113–1122.

[83] Chin, L. Y. & Engel, A. J. (1981). Hydrocarbon feedstocks from algae hydrogenation. Biotechnology and bioengineering symposium;11, 171–186.

[84] Wyman, C. E. (2001). Economics of a biorefinery for coproduction of succinic acid, ethanol, and electricity. Abstracts of Papers of the American Chemical Society.221 P. U119-U119 . Part 1 Meeting Abstract: 72-BIOT.

[85] International Energy Agency (2012). Technology Roadmaps-Bioenergy for Heat and Power. [Report in PDF format]. [Accessed: July, 9, 2013].

http://www.iea.org/publications/freepublications/publication/2012_Bioenergy_Roadmap_2nd_Edition_WEB.pdf.

[86] Cherubini, F. (2010).The biorefinery concept: using biomass instead of oil for producing energy and chemicals. Energy Conversion and Management, 51(7), 1412–1421.

[87] Taylor, G. (2008). Biofuels and biorefinery concept. Energy Policy 36(12), 4406–4409.

[88] Douglas, J.M. (1988). Conceptual Design of Chemical Processes. New York, McGraw Hill.

[89] Rudd, D.F., Watson, C.C. (1968). Strategy of process engineering. New York, Wiley.

[90] Yuan, Z., Chen, B., & Gani, R. (2013). Applications of process synthesis: Moving from conventional chemical processes towards biorefinery processes. Computers and Chemical Engineering, 49, 217-229.

[91] Bedenik, N. I., Pahor, B., & Kravanja, Z. (2004). An integrated strategy for the hierarchical multilevel MINLP synthesis of overall process flowsheets using the combined synthesis/analysis approach. Computers and Chemical Engineering, 28(5), 693-706.

[92] El-Halwagi, M. (2012). Sustanaible desing throught process integration: Fundamentals and applicatios to industrial pollution prevention, resourse conservation, and profitability enhancement. Oxford UK butterworth-Heinemann/Elsevier.

[93] Stuart, P. and M. M. El-Halwagi (Editors), Integrated Biorefineries: Design, Analysis, and Optimization, Taylor and Francis/CRC (2013).

[94] Alvarado-Morales, M., Terra, J., Gernaey, K. V., Woodley, J. M., & Gani, R. (2009). Biorefining: Computer aided tools for sustainable design and analysis of bioethanol production. Chemical Engineering Research and Design, 87(9), 1171-1183.

[95] Tay, D. H. S., Ng, D. K. S., Kheireddine, H., & El-Halwagi, M. M. (2011). Synthesis of an integrated biorefinery via the C-H-O ternary diagram. Clean Technologies and Environmental policy, 13, 567–579.

[96] Pham, V., & El-Halwagi, M. (2012). Process synthesis and optimization of biorefinery configurations. AIChE Journal, 58(4), 1212-1221.

[97] Sammons, N. E., Jr., Yuan, W., Eden, M. R., Aksoy, B. & Cullinan, H. T. (2008). Optimal biorefinery resource utilization by combining process and economic modeling, Chemical Engineering Research and Design, 86, 800–808.

[98] Ng, D. K. S., Pham, V., El-Halwagi, M. M., Jiménez-Gutiérrez, A., & Spriggs, H. D. (2009). A hierarchical approach to the synthesis and analysis of integrated biorefineries. In Proceeding of seventh international conference on foundations of computer-aided process design 2009 (pp. 425–432).

[99] Tan, R. R., Ballacillo, J. -. B., Aviso, K. B., & Culaba, A. B. (2009). A fuzzy multiple-objective approach to the optimization of bioenergy system footprints. Chemical Engineering Research and Design, 87(9), 1162-1170.

[100] Ng, D. K. S. (2010). Automated targeting for synthesis of an integrated biorefinery. Chemical Engineering Journal, 162(1), 67–74.

[101] Martin, M. & Grossmann, I. E. Superstructure optimization of lignocellulosic bioethanol plants. In Pierucci S, Ferraris BG, editors: 20th European symposium on computer aided process engineering, Amsterdam, Elsevier Science BV, vol 28, pp 943–948, 2010.

[102] Ojeda, K. A., Sánchez, E. L., Suarez, J., Avila, O., Quintero, V., El-Halwagi, M., & Kafarov, V. (2011). Application of computer-aided process engineering and exergy analysis to evaluate different routes of biofuels production from lignocellulosic biomass. Industrial and Engineering Chemistry Research, 50(5), 2768-2772.

[103] Kokossis, A. C., Yang, A., Tsakalova, M., & Lin, T. -. (2010). A systems platform for the optimal synthesis of biomass based manufacturing systems. In Pierucci S, Ferraris BG, editors: 20th European Symposium on Computer Aided Process Engineering, vol 28, pp 1105–1110.

[104] Bao, B., Ng, D. K. S., Tay, D. H. S., Jiménez-Gutiérrez, A., & El-Halwagi, M. M. (2011). A shortcut method for the preliminary synthesis of process-technology pathways: An optimization approach and application for the conceptual design of integrated biorefineries. Computers and Chemical Engineering, 35(8), 1374-1383.

[105] Tay, D. H. S., Ng, R. T. L., & Ng, D. K. S. (2012). Modular optimization approach for process synthesis and integration of an integrated biorefinery. Computer Aided Chemical Engineering, 31, 1045-1049

[106] Čuček, L., Martín, M., Grossmann, I. E., & Kravanja, Z. (2013). Multi-period synthesis of a biorefinery's supply networks

[107] Pardo-Cárdenas, Y., Herrera-Orozco, I., González-Delgado, A. & Kafarov, V. (2013). Environmental assessment of microalgae biodiesel production in Colombia: Comparison of three oil extraction systems. CT&F – Ciencia, Tecnología y Futuro, 5(2), 85-100.

[108] Pokoo-Aikins, G., Nadim, A., El-Halwagi, M. M., & Mahalec, V. (2010). Design and analysis of biodiesel production from algae grown through carbon sequestration. Clean Technologies and Environmental Policy, 12(3), 239-254.

[109] Ofori-Boateng, C., Keat, T. L., & Jitkang, L. (2012). Feasibility study of microalgal and jatropha biodiesel production plants: Exergy analysis approach. Applied Thermal Engineering, 36(1), 141-151.

[110] Sánchez, E., Ojeda, K., El-Halwagi, M., & Kafarov, V. (2011). Biodiesel from microalgae oil production in two sequential esterification/transesterification reactors: Pinch analysis of heat integration. Chemical Engineering Journal, 176-177, 211-216.

[111] Davis, R., Aden, A., & Pienkos, P. T. (2011). Techno-economic analysis of autotrophic microalgae for fuel production. Applied Energy, 88(10), 3524-3531.

[112] Delrue, F., Setier, P., Sahut, C., Cournac, L., Roubaud, A., Peltier, G., & Froment, A. -. (2012). An economic, sustainability, and energetic model of biodiesel production from microalgae. Bioresource Technology, 111, 191-200.

[113] Chisti, Y. (2007). Biodiesel from microalgae. Biotechnology Advances, 25(3), 294-306.

[114] González-Delgado, A. D, & Kafarov, V. (2011). Microalgae based biorefinery: Issues to consider. CTyF - Ciencia, Tecnologia y Futuro, 4(4), 5-22.

[115] González, A. D., Kafarov, V., Guzman, A. (2009). Development of a methodology of microalgae oil extraction in the biodiesel from microalgae production chain, Prospectiva, 7(2), 53 – 60.

[116] Bligh, E. G. & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian journal of biochemistry and physiology, 37 (8), 911-917.

[117] Fajardo, A. R., Cerdán, L. E., Medina, A. R., Fernández, F. G. A., Moreno, P. A. G., & Grima, E. M. (2007). Lipid extraction from the microalga *phaeodactylum tricornutum*. European Journal of Lipid Science and Technology, 109(2), 120-126.

[118] Halim, R., Gladman, B., Danquah, M. K., & Webley, P. A. (2011). Oil extraction from microalgae for biodiesel production. Bioresource Technology, 102(1), 178-185.

[119] Peñaranda, L.A., Sepúlveda, K.J., Álvarez, Y.E., González-Delgado, A.D., & Kafarov, V. (2011). Evaluation of lipid and monosaccharide obtaining routes of microalgae biomass under the biorefinery concept. Ion, 24(2), 13-22.

[120] González, A. D. & Kafarov, V. (2010). Design of a multifunctional reactor for third generation biofuels production. Chemical Engineering Transactions, 21(1), 1297-1302.

[121] Cravotto, G., Boffa, L., Mantegna, S., Perego, P., Avogadro, M. & Cintas, P. (2008). Improved extraction of vegetable oils under high-intensity ultrasound and/or microwaves. Ultrasonics Sonochemistry, 15(5), 898-902.

[122] Nigam, P. S., & Singh, A. (2011). Production of liquid biofuels from renewable resources. Progress in Energy and Combustion Science, 37(1), 52-68.

[123] Chisti, Y. (2010). A bioeconomy vision of sustainability. Biofuels, Bioproducts and Biorefining, 4(4), 359-361.

[124] Lam, M. K., & Lee, K. T. (2012). Microalgae biofuels: A critical review of issues, problems and the way forward. Biotechnology Advances, 30(3), 673-690.

[125] Khoo, H. H., Sharratt, P. N., Das, P., Balasubramanian, R. K., Naraharisetti, P. K., & Shaik, S. (2011). Life cycle energy and CO2 analysis of microalgae-to-biodiesel: Preliminary results and comparisons. Bioresource Technology, 102(10), 5800-5807.

[126] Oltra, C. (2011). Stakeholder perceptions of biofuels from microalgae. Energy Policy, 39(3), 1774-1781.

[127] UIS-ICP-Morrosquillo. Bioprospecting of Colombian microalgae for biodiesel production. Final Report, Bucaramanga;2011.

[128] Singh, J., & Gu, S. (2010). Commercialization potential of microalgae for biofuels production. Renewable and Sustainable Energy Reviews, 14(9), 2596-2610.

[129] González, A. D. & Kafarov, V. (2010). Design of a multifunctional reactor for third generation biofuels production. Chemical Engineering Transactions, 21(1), 1297-1302.

[130] Henriques, M., Silva, A. & Rocha, J. (2007). Extraction and quantification of pigments from a marine: A simple and reproducible method. Communicating Current Research and Educational Topics and Trends in Applied Microbiology, 1(1), 586-593.

[131] Peñaranda, L.A., Sepúlveda, K.J., Álvarez, Y.E., González-Delgado, A.D., & Kafarov, V. (2011). Evaluation of lipid and monosaccharide obtaining routes of microalgae biomass under the biorefinery concept. Ion, 24(2), 13-22.

[132] Wilkie, A. C., & Mulbry, W. W. (2002). Recovery of dairy manure nutrients by benthic freshwater algae. Bioresource Technology, 84(1), 81-91.

[133] Harun, R., Singh, M., Forde, G. M., & Danquah, M. K. (2010). Bioprocess engineering of microalgae to produce a variety of consumer products. Renewable and Sustainable Energy Reviews, 14(3), 1037-1047.

[134] Mussgnug, J. H., Klassen, V., Schlüter, A., & Kruse, O. (2010). Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. Journal of Biotechnology, 150(1), 51-56.

[135] McMillan, J. R., Watson, I. A., Ali, M., & Jaafar, W. (2013). Evaluation and comparison of algal cell disruption methods: Microwave, waterbath, blender, ultrasonic and laser treatment. Applied Energy, 103, 128-134.

[136] Vanthoor-Koopmans, M., Wijffels, R. H., Barbosa, M. J., & Eppink, M. H. M. (2013). Biorefinery of microalgae for food and fuel. Bioresource Technology, 135, 142-149.

[137] Fajardo, A. R., Cerdán, L. E., Medina, A. R., Fernández, F. G. A., Moreno, P. A. G., & Grima, E. M. (2007). Lipid extraction from the microalga phaeodactylum tricornutum. European Journal of Lipid Science and Technology, 109(2), 120-126.

[138] Halim, R., Danquah, M. K., & Webley, P. A. (2012). Extraction of oil from microalgae for biodiesel production: A review. Biotechnology Advances, 30(3), 709-732.

[139] Sari, Y. W., Bruins, M. E., & Sanders, J. P. M. (2013). Enzyme assisted protein extraction from rapeseed, soybean, and microalgae meals. Industrial Crops and Products, 43(1), 78-83.

[140] Halim, R., Gladman, B., Danquah, M. K., & Webley, P. A. (2011). Oil extraction from microalgae for biodiesel production. Bioresource Technology, 102(1), 178-185.

[141] Liu, C. -., Zheng, S., Xu, L., Wang, F., & Guo, C. (2013). Algal oil extraction from wet biomass of botryococcus braunii by 1,2-dimethoxyethane. Applied Energy, 102, 971-974.

[142] Yoo, G., Park, W. -., Kim, C. W., Choi, Y. -., & Yang, J. -. (2012). Direct lipid extraction from wet chlamydomonas reinhardtii biomass using osmotic shock. Bioresource Technology, 123, 717-722.

[143] Ehimen, E., Sun, Z., & Carrington, G. (2012). Renewability analysis of microalgae biodiesel using different transesterification processes. International Journal of Energy, Environment and Economics, 20(2), 135-156.

[144] Peralta-Ruiz, Y., González-Delgado, A. D., & Kafarov, V. (2013). Evaluation of alternatives for microalgae oil extraction based on exergy analysis. Applied Energy, 101, 226-236.

[145] Brandenberger, M., Matzenberger, J., Vogel, F., & Ludwig, C. (2013). Producing synthetic natural gas from microalgae via supercritical water gasification: A techno-economic sensitivity analysis. Biomass and Bioenergy, 51, 26-34.

[146] El-Halwagi, A. M., Rosas, C., Ponce-Ortega, J. M., Jiménez-Gutiérrez, A., Mannan, M. S., & El-Halwagi, M. M. (2013). Multiobjective optimization of biorefineries with economic and safety objectives. AIChE Journal, 59(7), 2427-2434.

[147] Uitenbroek DG. SISA Binomial. Southampton: D.G. Uitenbroek 1997. Retrieved July 02, 2013, from the World Wide Web: http://www.quantitativeskills.com/sisa/distributions/binomial.htm.

[148] Archanaa, S., Moise, S., & Suraishkumar, G. (2012). Chlorophyll interference in microalgal lipid quantification through the Bligh and Dyer method. Biomass and Bioenergy, 46, 805-808.

[149] Singh J, Gu S. Commercialization potential of microalgae for biofuels production. Renew Sustain Energy Rev 2010;14: 2596–610.

[150] González A, Kafarov V. Design of a multifunctional reactor for third generation biofuels production. Chem Eng Transact 2010;21: 1297-302.

[151] Henriques M, Silva A, Rocha J. Extraction and quantification of pigments from a marine: A simple and reproducible method. Commun Curr Res Educ Top Trends Appl Microbiol 2007;1: 586-93.

[152] Peñaranda LA, Sepúlveda KJ, Álvarez, YE, González-Delgado AD, Kafarov V. Evaluation of lipid and monosaccharide obtaining routes of microalgae biomass under the biorefinery concept. Ion 2011;24(2): 13-22.

[153] Wilkie A, Mulbry W. Recovery of dairy manure nutrients by benthic freshwater algae. Bioresour Technol 2002;84(1): 81-91.

[154] Harun R, Singh M, Forde G, Danquah M. Bioprocess engineering of microalgae to produce a variety of consumer products. Renew Sust Energ Rev 2010;14:1037–47.

[155] Mussgnug J, Klassen V, Schlüter A, Kruse O. Microalgae as substrates for fermentative biogas production in a combined biorefinery concept. J Biotechnol 2010;150(1):51-6.

[156] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. Anal Chem 1959;31(3):426–8.

[157] Téllez-Luis SJ, Ramírez JA, Vázquez M. Mathematical modelling of hemicellulosic sugar production from sorghum straw. J Food Eng 2002;52: 285–91.

[158] Ververis C, Georghiou K, Danielidis D, Hatzinikolaou DG, Santas P, Santas R, Corleti V. Cellulose, hemicelluloses, lignin and ash content of some organic materials and their suitability for use as paper pulp supplements. Biores. Technol 2007;98: 296–301.

[159] Laurens LM, Wolfrum EJ. Feasibility of spectroscopic characterization of algal lipids: chemometric correlation of NIR and FTIR spectra with exogenous lipids in algal biomass. Bioenerg. Res., 2011;4:22-35.

[160] Aliske MA, Zagonel GF, Costa BJ, Veiga W, Saul CK, Measurement of biodiesel concentration in a diesel oil mixture. Fuel 2007;86:1461-4.

[161] Ooi TL, Yong KC, Dzulkefly K, Wan-Yunus WMZ, Hazimah AH. Crude glycerin recovery from glycerol residue waste from a palm kernel oil methyl ester plant. J Oil Palm Res 2001;13:16-22.

[162] Martínez OM, Sánchez FJ, Suárez OY. Production of ethyl esters from RBD palm oil. Eng Res 2007;27:34-43.

[163] Mahamuni N, Adewuyi Y. Fourier transform infrared spectroscopy (FTIR) method to monitor soy biodiesel and soybean oil in transesterification reactions, petrodiesel-biodiesel blends, and blend adulteration with soy oil. Energ Fuel 2009;23: 3773–82.

[164] Knothe G. Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters. Fuel Process Technol 2005; 86:1059-70.

[165] Moser BR, Vaughn SF. Efficacy of fatty acid profile as a tool for screening feedstocks for biodiesel production. Biomass Bioenerg 2012;37: 31-41.

[166] Ferrer JR, Páez G, Arenas-de-Moreno L, Chandler C, Mármol Z, Sandoval L. Kinetics of the acid hydrolysis of sugar cane bagasse pith. Rev Fac Agron 2002;19: 23-33.

[167] Zhu G, Zhu X, Fan Q, Wan X. Production of reducing sugars from bean dregs waste by hydrolysis in subcritical water. J Anal Appl Pyrol 2011;90(2): 182-6.

[168] Kuila A, Singh A, Mukhopadhyay M, Banerjee R. Process optimization for aqueous extraction of reducing sugar from cashew apple bagasse: A potential, low cost substrate. LWT-Food Sci Technol 2011;44(1): 62-6.

[169] Sinnott, R. K., & Towler, G. (2013). Chemical engineering design.

[170] Silva Lora EE, Escobar Palacio JC, Rocha MH, Grillo Renó ML, Venturini OJ, Almazán del Olmo O. Issues to consider, existing tools and constraints in biofuels sustainability assessments. Energy 2011;36:2097–110.

[171] Morales M, Verelst H, Mesa L, González E. Simulation of furfural production process for revamping with ethanol technology from lignocellulosic residuals. Chem Eng Trans 2010;21:967–72.

[172] Santori G, Di Nicola G, Moglie M, Polonara F. A review analyzing the industrial biodiesel production practice starting from vegetable oil refining. Appl Energy 2012;92:109–32.

[173] Chisti, Y., Biodiesel from microalgae, Biotechnology Advances, Vol. 25, pp 294–306, 2007.

[174] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 2010;14:217–32.

[175] González-Delgado, A.D., Kafarov, V., Microalgae based biorefinery: Issues to consider. *CT&F - Ciencia, Tecnología y Futuro*, Vol. 4, No 4, pp 5-22, 2011.

[176] Phukan MM, Chutia RS, Konwar BK, Kataki R. Microalgae Chlorella as a potential bio-energy feedstock. Appl Energy 2011;88:3307–12.

[177] Ehimen EA, Sun ZF, Carrington CG. Variables affecting the in situ transesterification of microalgae lipids. Fuel 2010;89:677–84.

[178] Petkov G, Garcia G. Which are fatty acids of the green alga Chlorella? Biochem Syst Ecol 2007;35:281–5.

[179] Sánchez E, Ojeda K, El-Halwagi M, and Kafarov V. Biodiesel from microalgae oil production in two sequential esterification/transesterification reactors: pinch analysis of heat integration. Chem Eng J 2011;176:211–6.

[180] Kay RA. Microalgae as food and supplement. Crit Rev Food Sci Nutr 1991;30:555–73.

[181] Fowden L. The composition of the bulk proteins of Chlorella. Department of Botany. University College. London; 1951. p. 355–8.

[182] Ververis C, Georghiou K, Danielidis D, Hatzinikolaou DG, Santas P, Santas R. Cellulose–hemicellulose, lignin and ash content of some organic materials and their suitability for use as paper supplements. Bioresour Technol 2007;98:296–301.

[183] Feng X, Wang L, Min S. Industrial emergy evaluation for hydrogen production systems from biomass and natural gas. Appl Energy 2009;86:1767-73.

[184] Irimescu A, Lelea D. Thermodynamic analysis of gas turbine powered cogeneration systems. J Sci Ind Res 2010;69:548-53.

[185] Dixon G, Abdel-Salam T, Kauffmann P. Evaluation of the effectiveness of an energy efficiency program for new home construction in eastern North Carolina. Energy 2010;35:1491-6.

[186] Sorguven E, Ozilgen M. Thermodynamic assessment of algal biodiesel utilization. Renew Energ 2010;9:1956–66.

[187] Jaimes W, Acevedo P, Kafarov V. Exergy analysis of palm oil biodiesel production. Chem Eng Transact 2010;21:1345-50.

[188] Sciubba E, Wall G. A brief commented history of exergy from the beginnings to 2004. Int J Thermodyn 2007;10:1–26.

[189] Szargut J. Chemical exergies of the elements. Appl Energy 1989;32:269–86.

[190] ISO 14040. (2006). Environmental management-life cycle assessment-principles and framework. First edition, *International Organization for Standarization*, Switzerland.

[191] ISO 14044. (2006). Environmental management-life cycle assessment-requirements and guidelines, First edition, *International Organization for Standarization*, Switzerland.

[192] Stichnothe, H.& Schuchardt, F. (2010). Comparison of different treatment options for palm oil production waste on a life cycle basis. *Int. J. Life Cycle Ass.*, 15 (9), 907–915.

[193] Baliga, R. & Powers, S. (2010). Sustainable algae biodiesel production in cold climates. *Int. J. Chem. Eng.*, 2010: 1-13.

[194] Goedkoop, M., De Schryver, A. and Oele, M., 2008. Introduction to LCA with SimaPro 7. Netherlands: Pré Consultants.

[195] Frischknecht, R., Jungbluth, N., Althaus, H., Doka, G., Heck, T., Hellweg, S., Hischier, R., Nemeck, T., Rebitzer, G., Spielmann, M. & Wernet, G. (2007). *Overview and methodology*. Dübendorf: Rolf and Niels.

[196] Campos, A. J., Carmona, G. & López, F. D. (2010). Casos URE exitosos en Colombia: Reducción de los costos energéticos en sistemas de generación de vapor. *Informe Técnico*, Unidad de Planeación Minero Energética (UPME), Instituto Colombiano para el Desarrollo de la Ciencia y la Tecnología “Francisco José de Caldas” (COLCIENCIAS).

[197] Chong Fu C., Chieh Hung T., Yi Chen J., Hung Su C., Teng Wu W., 2010. Hydrolysis of microalgae cell walls for production of reducing sugar and lipid extraction, Bioresource Technology, 101, 8750-8754.

[198] Spatari S., Bagley D., MacLean H., 2010. Life cycle evaluation of emerging lignocellulosic ethanol conversion technologies, Bioresource Technology, 101, 654–667.

[199] Ojeda K., Sánchez E., El-Halwagi M., Kafarov V., 2011. Exergy analysis and process integration of bioethanol production from acid pre-treated biomass: Comparison of SHF, SSF and SSCF pathways, Chemical Engineering Journal, 176, 195-201.

[200] Galatro D., Verruschi E., 2012. Simulation of a hydrogen production process from algae, Chemical Engineering Transactions, 27, 373-378, DOI: 10.3303/CET1227063.

[201] Pham, V., & El-Halwagi, M. (2012). Process synthesis and optimization of biorefinery configurations. *AIChE Journal, 58*(4), 1212-1221.

[202] El-Halwagi, M. (2012). Sustanaible desing throught process integration: Fundamentals and applicatios to industrial pollution prevention, resourse conservation, and profitability enhancement. Oxford UK butterworth-Heinemann/Elsevier.

[203] Internal Revenue Service. Credit for Carbon Dioxide Sequestration 2013 Section 45Q Inflation Adjustment Factor (Document online). June 3th, 2013. Available in: http://www.irs.gov/irb/2013-23_IRB/ar07.html. Accessed on July 2th 2013.

[204] Sakamoto, K., Baba, M., Suzuki, I., Watanabe, M. M., & Shiraiwa, Y. (2012). Optimization of light for growth, photosynthesis, and hydrocarbon production by the colonial microalga Botryococcus braunii BOT-22.*Bioresource Technology, 110*, 474-479.

[205] Delrue, F., Li-Beisson, Y., Setier, P. -., Sahut, C., Roubaud, A., Froment, A. & Peltier, G. (2013). Comparison of various microalgae liquid biofuel production pathways based on energetic, economic and environmental criteria. *Bioresource Technology, 136*, 205-212.

[206] Peñaranda, L.A., Sepúlveda, K.J., Álvarez, Y.E., González-Delgado, A.D., & Kafarov, V. (2011). Evaluation of lipid and monosaccharide obtaining routes of microalgae biomass under the biorefinery concept. *Ion,* 24(2), 13-22.

[207] Ehimen, E. A., Sun, Z. F., & Carrington, C. G. (2010). Variables affecting the in situ transesterification of microalgae lipids. *Fuel, 89*(3), 677-684.

[208] Wahlen, B. D., Willis, R. M., & Seefeldt, L. C. (2011). Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. *Bioresource Technology,102*(3), 2724-2730.

[209] González-Delgado AD, Kafarov V. (2012). Design and adjustment of coupled microalgae oil extraction methods for the development of a topology of biorefinery. *Prospectiva,* 10(1), 113-123.

[210] González-Delgado, A.D., & Kafarov, V. (2013) Microalgae based biorefinery: evaluation of oil extraction methods in terms of efficiency, costs, toxicity and energy in lab-scale. *Ion*, 25(2),27-39.

[211] Peralta-Ruiz, Y., González-Delgado, A. -., & Kafarov, V. (2013). Evaluation of alternatives for microalgae oil extraction based on exergy analysis. *Applied Energy, 101*, 226-236.

[212] Pardo-Cárdenas, Y., Herrera-Orozco, I., González-Delgado, A. & Kafarov, V. (2013). Environmental assessment of microalgae biodiesel production in Colombia: Comparison of three oil extraction systems. *CT&F – Ciencia, Tecnología y Futuro*, 5(2), 85-100.

[213] Sathish, A., & Sims, R. C. (2012). Biodiesel from mixed culture algae via a wet lipid extraction procedure. *Bioresource Technology, 118*, 643-647.

[214] Crampon, C., Mouahid, A., Toudji, S. -. A., Lépine, O., & Badens, E. (2013). Influence of pretreatment on supercritical CO2 extraction from Nannochloropsis oculata. *Journal of Supercritical Fluids*. In press.

[215] Kim, J., Yoo, G., Lee, H., Lim, J., Kim, K., Kim, C. W., & Yang, J. -. (2013). Methods of downstream processing for the production of biodiesel from microalgae. *Biotechnology Advances. In press.*

[216] Apostolakou, A. A., Kookos, I. K., Marazioti, C., & Angelopoulos, K. C. (2009). Techno-economic analysis of a biodiesel production process from vegetable oils. Fuel Processing Technology, 90(7-8), 1023-1031.

[217] Kim, H. -., Kang, B. -., Kim, M. -., Park, Y. M., Kim, D. -., Lee, J. -., & Lee, K. -. (2004). Transesterification of vegetable oil to biodiesel using heterogeneous base catalyst. Catalysis Today, 93-95, 315-320.

[218] Marchetti, J. M., & Errazu, A. F. (2008). Technoeconomic study of supercritical biodiesel production plant. Energy Conversion and Management, 49(8), 2160-2164.

[219] Sánchez, E., Ojeda, K., El-Halwagi, M., & Kafarov, V. (2011). Biodiesel from microalgae oil production in two sequential esterification/transesterification reactors: Pinch analysis of heat integration. Chemical Engineering Journal, 176-177, 211-216.

[220] Furimsky, E. (2013). Hydroprocessing challenges in biofuels production. Catalysis Today, In press.

[221] Grierson, S., Strezov, V., Ellem, G., Mcgregor, R., & Herbertson, J. (2009). Thermal characterisation of microalgae under slow pyrolysis conditions. Journal of Analytical and Applied Pyrolysis, 85(1-2), 118-123.

[222] Yang C, Jia L, Chen C, Liu G, Fang W. Bio-oil from hydro-liquefaction of Dunaliella salina over Ni/REHY catalyst. Bioresour Technol 2011;102:4580–4.

[223] Suali, E., & Sarbatly, R. (2012). Conversion of microalgae to biofuel. Renewable and Sustainable Energy Reviews, 16(6), 4316-4342.

[224] Chakinala, A. G., Brilman, D. W. F., van Swaaij, W. P. M., & Kersten, S. R. A. (2010). Catalytic and Non-catalytic Supercritical Water Gasification of Microalgae and Glycerol. Ind. Eng. Chem. Res., 49, 1113–1122.

[225] Kim, S., & Kim, C. H. (2013). Bioethanol production using the sequential acid/alkali-pretreated empty palm fruit bunch fiber. *Renewable Energy, 54*, 150-155.

[226] Delrue, F., Setier, P. -., Sahut, C., Cournac, L., Roubaud, A., Peltier, G., & Froment, A. -. (2012). An economic, sustainability, and energetic model of biodiesel production from microalgae. Bioresource Technology, 111, 191-200.

[227] Davis, R., Aden, A., & Pienkos, P. T. (2011). Techno-economic analysis of autotrophic microalgae for fuel production. *Applied Energy, 88*(10), 3524-3531.

[228] Tijmensen, M. J. A., Faaij, A. P. C., Hamelinck, C. N., & Van Hardeveld, M. R. M. (2002). Exploration of the possibilities for production of Fischer Tropsch liquids and power via biomass gasification. Biomass and Bioenergy, 23(2), 129-152.

[229] Bičáková, O., & Straka, P. (2012). Production of hydrogen from renewable resources and its effectiveness. International Journal of Hydrogen Energy, 37(16), 11563-11578.

[230] Bao, B., Ng, D. K. S., Tay, D. H. S., Jiménez-Gutiérrez, A., & El-Halwagi, M. M. (2011). A shortcut method for the preliminary synthesis of process-technology pathways: An optimization approach and application for the conceptual design of integrated biorefineries. Computers and Chemical Engineering, 35(8), 1374-1383.

[231] Holladay, J. (2012, November 27) Thermochemical Conversion Processes to

Aviation Fuels. Available in http://www1.eere.energy.gov/biomass/pdfs /holladay_caafi_workshop.pdf. Accessed on July 2th 2013.

[232] Santa, A. (2009). Oligomerization of light olefins for the production of diesel using zeolite catalysts [in Spanish]. M. Sc. Thesis. University of Antioquia, Medellin, Colombia.

[233] Mata TM, Martins AA, Caetano NS. (2010). Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev, 14(1), 217–232.

[234] González-Delgado AD, Kafarov V. (2011). Microalgae based biorefinery: Issues to consider. CT&F Cien Tecnol Futuro, 4(4), 5-21.

[235] Ramirez (2009), Enzymatic corn wet milling: engineering process and cost model, Biotechnology for biofuels, january 2009.

[236] Woiciechowski, A.L., S. Nitsche, A. Pandey and C. Ricardo: Acid and enzymatic hydrolysis to recover reducing sugars from cassava bagasse: An economic study. Braz. Arch. Biol. Technol., 45, 393-400 (2002).

[237] Fiori, L. (2010). Supercritical extraction of grape seed oil at industrial-scale: Plant and process design, modeling, economic feasibility. Chemical Engineering and Processing: Process Intensification, 49(8), 866-872.

[238] Wisconsin Biorefining Development Initiative. BIOREFINING PROCESS. Available in http://www.biorefine.org/proc/ester.pdf. Accessed on June 23th 2013.

[239] Labib, T.M., Hawash, S.I., El-Khatib, K.M., Sharaky, A.M., El Diwani, G.I., Abdel Kader, E. (2013). Kinetic study and techno-economic indicators for base catalyzed
transesterification of Jatropha oil. Egyptian Journal of Petroleum 22, 9-16.

[240] You, Y. -., Shie, J. -., Chang, C. -., Huang, S. -., Pai, C. -., Yu, Y. -., & Chang, C. H. (2008). Economic cost analysis of biodiesel production: Case in soybean oil. Energy and Fuels, 22(1), 182-189.

[241] Marchetti, J. M., & Errazu, A. F. (2008). Technoeconomic study of supercritical biodiesel production plant. Energy Conversion and Management, 49(8), 2160-2164.

[242] Gasafi, E., Reinecke, M. -., Kruse, A., & Schebek, L. (2008). Economic analysis of sewage sludge gasification in supercritical water for hydrogen production. Biomass and Bioenergy, 32(12), 1085-1096.

[243] Thomas, M. Economics of steam methane reformation and coal gasification for Hydrogen production. Available in http://ufdcimages.uflib.ufl.edu/UF/E0/02/11/34/00001/vergis_m.pdf. Accessed on June 23th 2013.

[244] Petroleum Energy Center. Synthetic Gas Production Technology by Catalytic Partial Oxidation of Natural Gas. Available in http://www.pecj.or.jp/japanese/report/ reserch/report-pdf/H11_1999/99surv7-e.pdf. Accessed on June 15th 2013.

ANNEX A. OBTAINING CHLOROPHYLL IN A MICROALGAE-BASED BIOREFINERY PROCESS

INTRODUCTION

At present, the continuous use of petroleum-based fuels as a source of energy is unsustainable, given its non-renewable origin that is directly related to the development of crude oil by progressively depleting the reserves available, leading to an increase in fuel prices around the world, among other consequences [1]. From the environmental standpoint, there is a negative impact perceived as a result of the mass use of fossil fuels in terms of CO_2 emissions, which cause global warming along with other compounds [2]. These environmental and economic factors have led to the consideration of non-conventional alternatives for the production of fuels to meet requirements as clean, renewable sources of energy.

Biofuel production is globally perceived as a viable option because it covers three strategic objectives: energy security, economic prosperity and environmental stability, which is why specialists around the world have focused on the study of this energy source. Biofuels can be obtained from renewable resources such as biomass, thus preventing the net contribution of greenhouse gases into the atmosphere. This way, the CO_2 resulting from combustion can be used in the formation of new biomass.

Microalgae biomass is among the raw materials currently being studied with the greatest interest for biofuel production because it has been demonstrated that it can provide a wide variety of compounds of biotechnological interest. Microalgae biomass is usually made up of 20 - 30% usable lipids for biodiesel production, 20 - 55% carbohydrates for the production of ethanol, 40-50% protein, which can be used as a nitrogen source in various applications and

the rest is made up of other special substances that are typical of each strain of microalgae [3].

Up to now, however, the linear chains of biofuel production using microalgae cannot process large-scale biomass due to technical and economic factors. In order not to miss out on the huge potential of this raw material Gonzalez-Delgado and Kafarov [4], suggested alternative routes for the integral use of microalgae biomass, including the valorization of residual biomass from the extraction of oil for biodiesel production by obtaining other high-value products, as well as the incorporation of the biorefinery concept in microalgae processing.

This paper proposes the incorporation of a stage to extract certain high-value products using microalgae such as chlorophylls in the conceptual designs of third-generation biodiesel and bioethanol production chains, by studying the variables that affect the separation of these components for a strain of national bioprospecting. It also examines the operating conditions for better extraction efficiency and the subsequent execution of the optimized procedure at different stages of the biofuel production chain based on microalgae, with a triple benefit as a direct impact:

- Separation of unwanted microalgae components at the microalgae oil extraction stages and the subsequent transformation thereof into biodiesel.

- Production of high value co-products using unwanted substances from the useful metabolites for the production of biofuels.

- Approximation of the use of microalgae biomass to the biorefinery concept, conceiving the integration of a process of total biomass use, thus

improving the efficiency of the variables of the different processes to obtain products of interest such as biofuels.

THEORETICAL FRAMEWORK

Microalgae were the first organisms with the capacity to carry out photosynthesis and one of the agents in the creation of the earth's current atmosphere. These organisms are key elements in planetary balance, because they determine to a large extent the dynamics of carbon dioxide on earth and they are the basis of the food chain in the oceans [5]. These microorganisms can grow quickly and live in harsh conditions thanks to their cell structure. They convert sunlight, water, inorganic nutrients and carbon dioxide into biomass, efficiently producing lipids, carbohydrates and proteins in different proportions. It is estimated that there are more than 50,000 species of microalgae, but only about 30,000 have been analyzed [6].

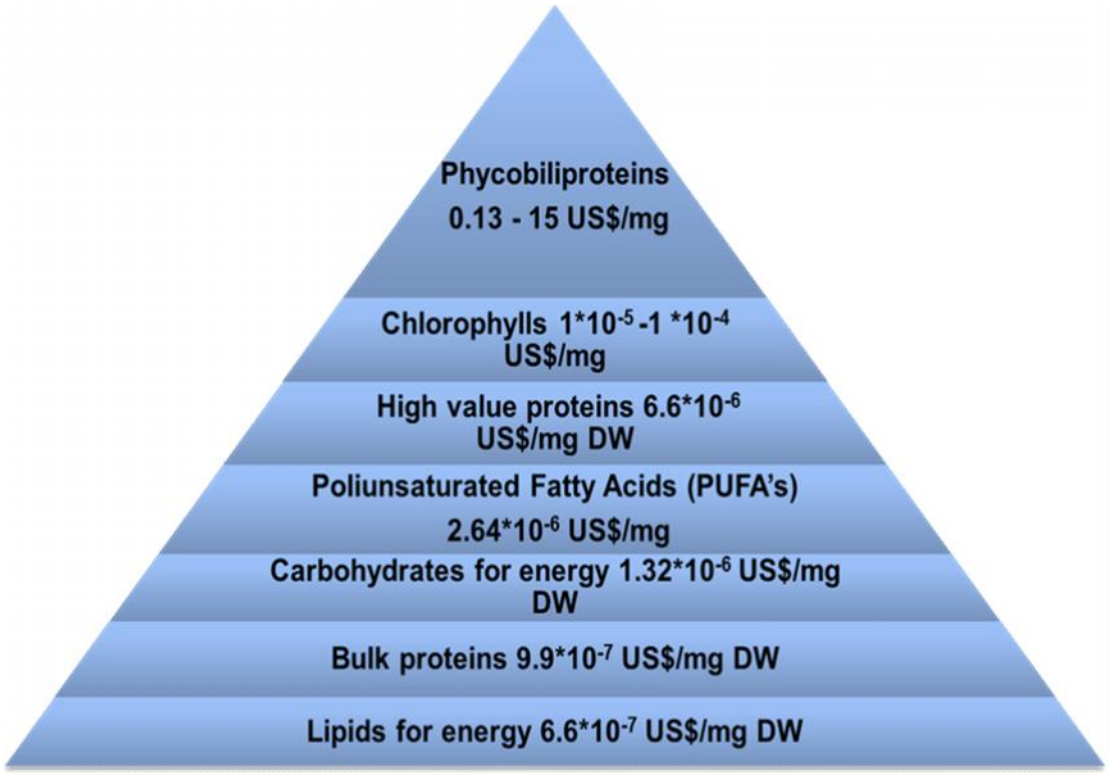
Microalgae biomass stands out as a promising source of energy, as it has been demonstrated that it can provide various products that are usable in energy systems such as methane produced by the anaerobic digestion of biomass, biodiesel from oil, biohydrogen and bioethanol [7]. Over the last five years, research on microalgae for biofuel production has been focused on biodiesel due to its high oil productivity per area unit [8], which means less land is required for cultivation and it does not compete directly with food crops. Various authors have contributed to the optimization of the stages in growing microalgae, in open ponds [9], as well as in photobioreactors [10] biomass harvesting [11], oil extraction [12,13] and transesterification for conversion into biodiesel [14] or hydrotreatment for transformation into Green-diesel. We have also developed approaches to large-scale processing of microalgae using software for the entire biodiesel production chain using

microalgae [15] and for particular stages comparing existing and emerging technologies for extracting oil [16] and converting it into biodiesel.

Among the microalgae's different metabolites, there are various products of high commercial value such as chlorophylls, which are essential in many everyday products. They also have medicinal and therapeutic applications [17]. However, these components are not used in biofuel production chains, and since the methods used in the processes of cell wall destruction and extraction are not selective, other metabolites (often unwanted) are obtained, thus affecting the purity of liquors rich in reducing sugars and triglycerides used in fermentation processes and transesterification respectively, and causing interference in the quantification of total lipids at the laboratory scale [18].

The biorefinery concept is based on processing biomass in a sustainable manner to obtain energy, biofuels and high value products through processes and equipment for biomass transformation. Microalgae are cataloged as promising candidates in biorefinery processes due to their varied composition and biotechnology potential, seeking the total use of their biomass [4], obtaining not only a lipid extract for biodiesel production, but also valuable by-products, whose higher commercial value can contribute to the viability of the chain of biofuel production from microalgae. Figure 52 illustrates the valorization of products produced from microalgae biomass.

Figure 52. Cost comparison of the various components of microalgae biomass (modified from Ramirez & Olvera, [20]; Wijffels, Barbosa & Eppink, [21])



EXPERIMENTAL DEVELOPMENT

Biomass of *Amphiprora* sp., was provided by Corporación Instituto de Morrosquillo (Punta Bolivar, Colombia), which was grown for 15 days in an F/2 medium. Two types of solvent were evaluated for chlorophyll and lipid extraction: polar (ethanol, methanol) and apolar (hexane, cyclohexane); in each experiment, 3 g of dry biomass were used in order to homogenize it. The biomass was macerated using a ceramic mortar. After that, the polar solvent was added as a biomass/solvent ratio of 1:10 g/mL. Then, the biomass-solvent mixture was stirred at 350 rpm and ambient temperature for 24 hours. After that, the mixture was filtered by vacuum and was washed with the solvent used in each experiment (ethanol or methanol) until colorless cells were obtained. The pigments from the liquid phase were quantified. To

purify the extract, 6 mL of apolar solvent and 4 mL of distilled water were added to the liquid phase in order to separate the phases. They were taken through a separating funnel and the lipophilic phase was extracted. This process was repeated four more times to ensure the effective separation of oil from microalgae. Finally, solvent was separated by volatilization in order to obtain the lipid extract to make the respective calculations.

Once the solvent mixture was obtained for the extraction of pigments and lipid extract, a central composite design was made for a confidence interval of 95% using STATISTICA 7.0 to evaluate the effect of the variables of temperature (35, 45 and 55ºC), time (2, 4 and 6 h) and biomass/solvent ratio (1/30, 1/60 and 1/90 g/mL) in the stirring stage of the chlorophyll-a and crude oil extraction. A replica was made of each combination suggested by the experimental design in order to reinforce the results by comparing the data obtained between the different levels and data from the same combination. Table 41 shows the values and levels of the variables studied.

Table 41. Experimental design of the variables studied

Factors	Levels				
	-1.68	-1	0	1	1.68
Biomass/Solvent Ratio (g/ml)	1/10	1/30	1/60	1/90	1/110
Temperature (°C)	28.3	35.0	45.0	55.0	61.7
Time (h)	0.65	2	4	6	7.35

The pigments were quantified by measuring the extracts from the hydroalcoholic phase following vacuum filtration in a UV-visible spectrophotometer (Spectroquant Pharo 300 Merck) at 665 and 650 nm. Using *Equation 1*, where Chl-a is the concentration of *chlorophyll-a* in mg/L. The data were standardized in order to rule out erroneous results by diluting the components at a larger volume of solvent.

$$\text{Chla}\,[mg/L] = (16.5 * A_{665}) - (8.3 * A_{650}) \quad (1)$$

For lipid extraction, the residual biomass was carried out in a Soxhlet extractor with 250 mL of the apolar solvent selected. The lipids were extracted from the biomass by heating through 18 hours. The apolar solvent is later recovered by simple distillation, and the lipid extract is subjected to volatilization up to constant weight. Chlorophyll and lipid extract percentages were calculated using *Equation 2*.

$$\%\text{ext} = \frac{\text{Mcomp.}}{\text{Mbiomass}} * 100\% \quad (2)$$

RESULTS

Selection of Extraction Solvents and Purification of High-Value Products

The results in Figure 53 show that the best solvent for extraction of desired high value products was ethanol, because it increased the amount of chlorophyll extracted compared to methanol. This result confirms the efficiency of said solvent for chlorophyll extraction in microalgae of the *Naviculales* order. In addition, ethanol has advantages to be taken into account for biorefinery development, such as low toxicity compared to methanol and the possibility of production from the fermentation of cellulosic material.

Figure 53. Evaluation of two polar solvents (ethanol and methanol) to separate chlorophyll-a from the microalgae *Amphiprora* sp

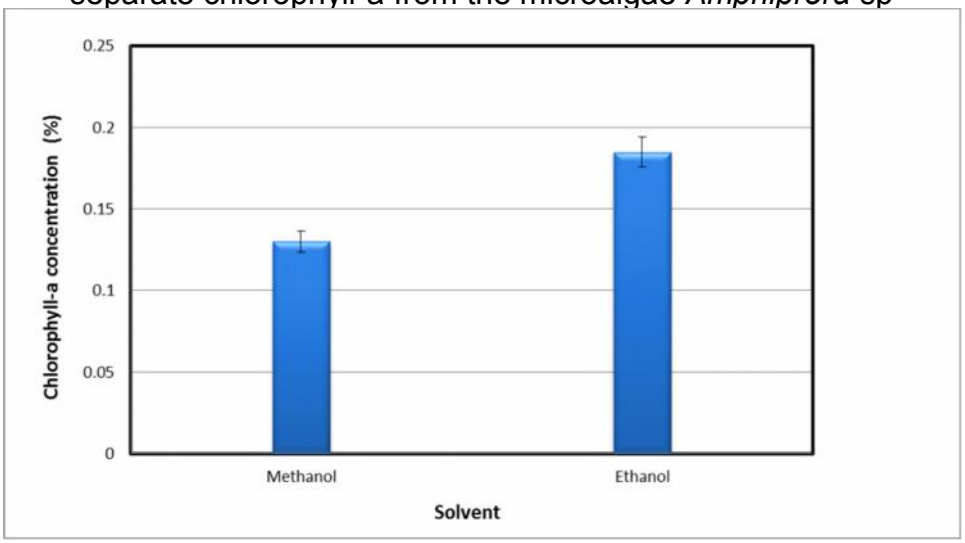
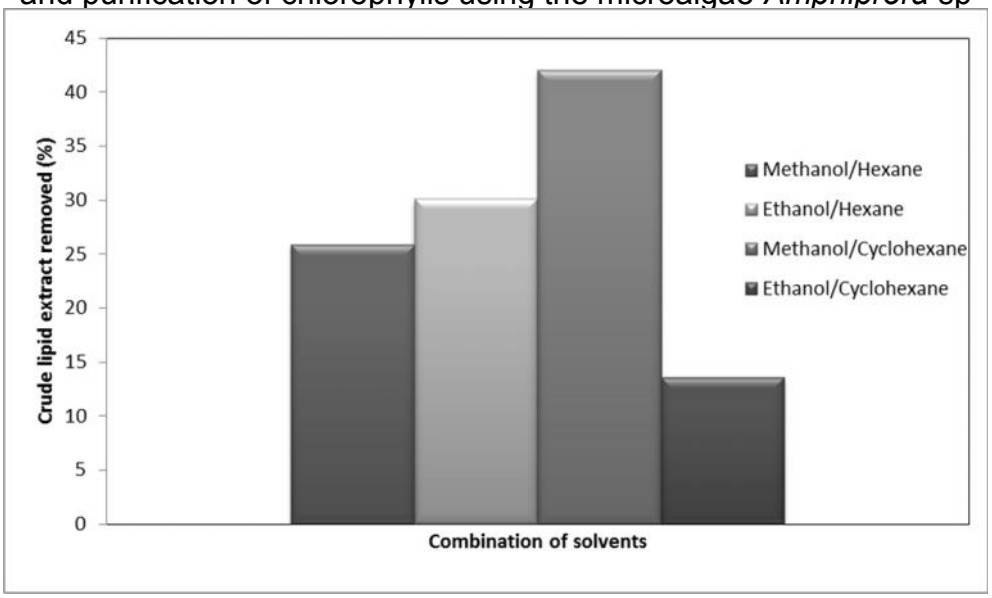


Figure 54 shows the results of purification of crude extract from chlorophyll by adding a neutral solvent that is similar to other metabolites that are present, for extraction with methanol, as well as with ethanol. It is noted that by using a mixture of cyclohexane/methanol a larger quantity of crude extract is removed. This means that the use of methanol on chlorophyll extraction drags a larger quantity of apolar metabolites, which are considered impurities of the product to be obtained, and they, in turn, are effectively removed with cyclohexane. It can also be seen in Figure 54 that the lowest percentage of crude extract removal is from the ethanol/cyclohexane mixture. This is due to the fact that, although ethanol and cyclohexane have low miscibility at the temperature of extraction of the high value products, there is little difference between the polarities of the solvents, which makes it difficult for the components from the hydrophilic phase to migrate to the hydrophobic phase. For the cases in which hexane was used as a purification solvent of the crude extract, the differences between removal percentages were not significant. This may be due to the fact that hexane has a low miscibility with ethanol as well as with methanol.

Figure 54. Comparison of polar and apolar solvent mixtures for the extraction and purification of chlorophylls using the microalgae *Amphiprora* sp



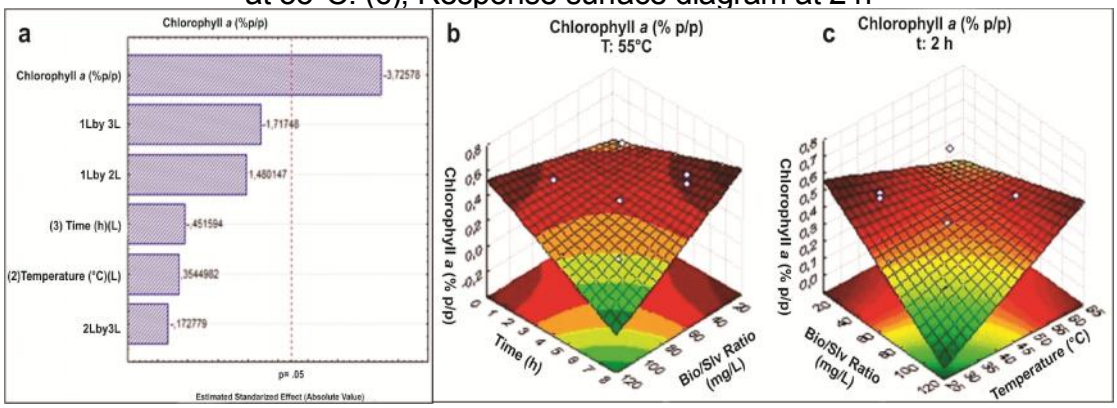
Based on the results obtained in this section, it can be said that for the extraction and purification of high value products such as chlorophylls, a mixture of ethanol/hexane should be used because the polar solvent generates more products of interest compared to methanol and, considering the biorefinery concept, this solvent is potentially obtainable by transforming microalgae components such as cellulose and hemicellulose. Furthermore, adding hexane increases the removal of hydrophobic components from the crude extract regardless of the type of polar solvent used. In addition, from the economic standpoint, it is a less expensive solvent than cyclohexane, which favors process economy.

Influence of Different Variables in the Extraction of Chlorophyll-a

Figure 55 shows the effect of the variables studied on the extraction of chlorophyll-a using the ethanol-hexane solvent mixture. The response surface diagrams show that the performance of the extraction decreases significantly upon increase of extraction time and quantity of solvent used for a constant

temperature. This may be due to degradation of the high value component studied. The diagram of the response surface for a constant time (Figure 55c) also shows the decrease in the chlorophyll-a percentage obtained at high biomass/solvent rations. This degradation may be due to factors such as changes in pH due to the presence of flocculant, presence of water in the extraction solvent or favorable operating conditions for the generation of phaeophytins and pyrophaeophytins due to consecutive reaction at low temperatures.

Figure 55. Effect of the variables of time, temperature, biomass/solvent ration and the combination thereof on the extraction of chlorophyll-a from the microalgae *Amphiprora sp*. (a), Pareto diagram. (b), Response surface diagram at 55°C. (c), Response surface diagram at 2 h



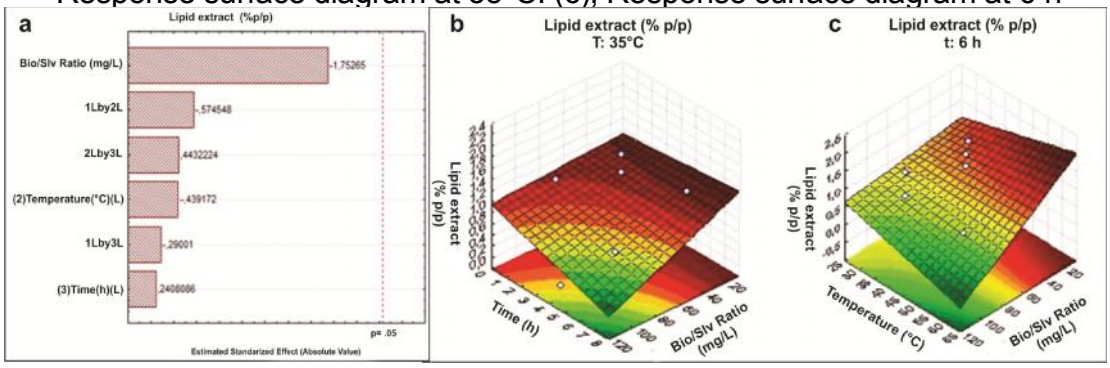
The Pareto diagram (Figure 55a) shows that the only significant variable in the extraction of chlorophyll-a from the microalgae *Amphiprora* sp. is the biomass/solvent ratio, and its effect is inversely proportional, as well as the effect of the extraction time variable. It is important to point out that interaction between extraction time and biomass/solvent ratio has a positive effect on extraction.

Influence of the Stage of Extraction of Products with High Added Value on the Efficiency of Oil Extraction for Biodiesel Production

According to the results illustrated in Figure 56, the inclusion of a prior stage to obtain high value products affects the efficiency of crude oil extraction from microalgae at a later stage, and the magnitude of this effect changes based on the different variables studied at the chlorophyll extraction stage. The effect is almost null when metabolites are extracted at high biomass/solvent ratios over prolonged times (Figure 56b) and event tends to be negative at high biomass/solvent ratios and high temperatures (Figure 56c).

The increase in the quantity of lipids obtained when chlorophylls are extracted at low biomass/solvent ratios and high temperatures may be due to the fact that the operating conditions that allow increased chlorophyll extraction weaken the cell wall of the microalgae by decomposing the cellulosic material present therein, thus allowing increased contact between the hexane and oil in the following stage of lipid extraction, facilitating the obtainment of crude oil. However, analysis of the magnitude of variations in extraction efficiency shows that they do not exceed 2% upon increase and 0.3% upon decrease, which is low compared to extraction efficiencies obtained at the laboratory scale for this strain at 92%. The Pareto diagram shows that none of the variables studied to obtain products with high added value significantly affect lipid extraction (Figure 56c).

Figure 56. Effect of the variables of time, temperature, biomass/solvent ratio and the combination thereof evaluated in the extraction of chlorophylls and phycobiliproteins, in the increase in the efficiency of oil extraction from the microalgae *Amphiprora* sp. for biodiesel production. (a), Pareto diagram. (b), Response surface diagram at 55°C. (c), Response surface diagram at 6 h



Selection of Operating Conditions for the Extraction of Microalgal Metabolites

The results of the central composite design show that extraction percentages are low compared to those obtained with other strains such as *S. platensis* for the case of phycobiliproteins [19]. This behavior may be attributed to factors such as the nature of the strain, which does not produce large quantities of chlorophylls, the quantity of ash from the biomass harvesting stage and the preliminary microalgae drying stage, which can degrade the metabolites of interest.

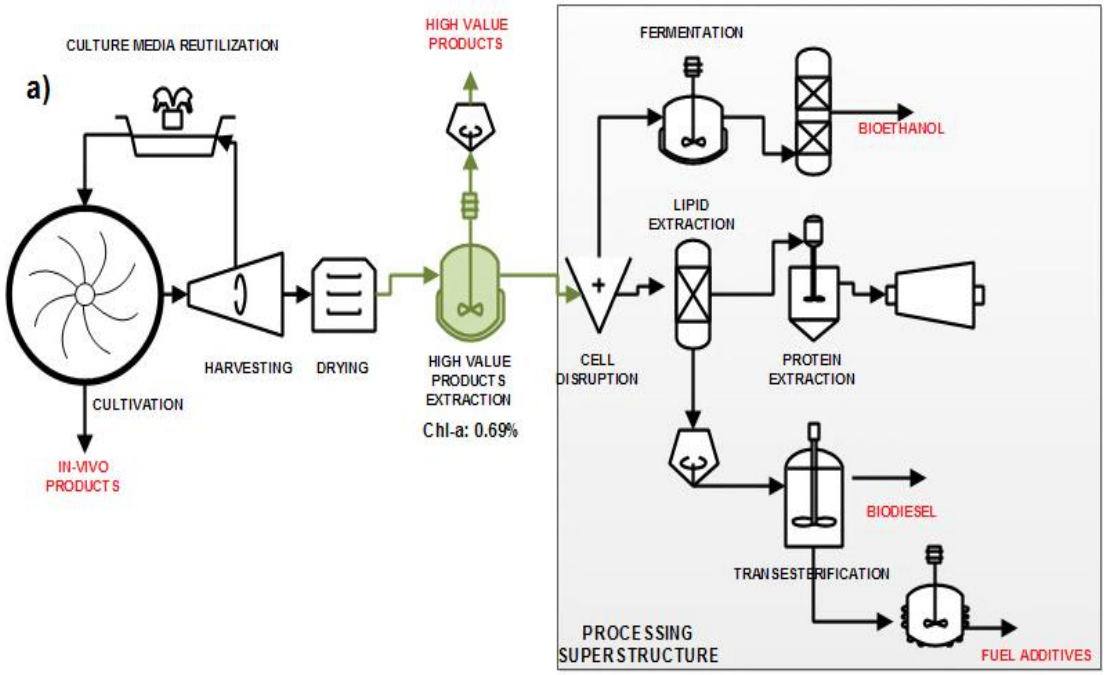
Table 42. Results of the different pigments and lipids extracted

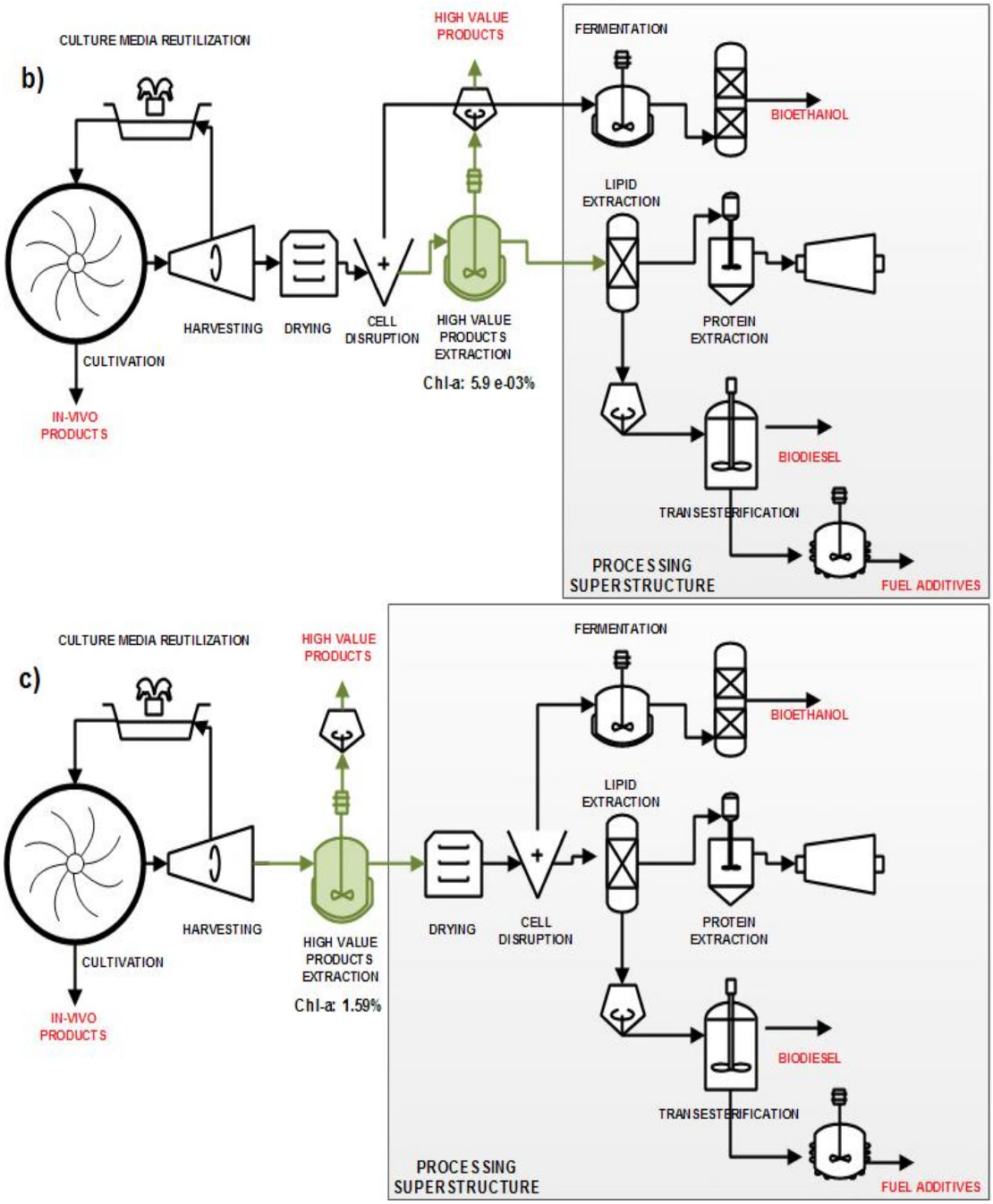
Test	Block	T (°C)	t (h)	Biomass/Solvent Ratio (g/ml)	Percentages Extracted (%)	
					Chl-a	Ext. Lipid
1	1	35	6	90	0.17	0.74
2	1	55	6	30	0.51	0.85
3	1	55	2	90	0.50	0.12
4	1	45	4	60	0.26	0.12
5	1	35	2	30	0.46	0.75

6	2	45	4	60	0.24	1.54
7	2	35	2	90	0.17	1.41
8	2	55	6	90	0.18	0.72
9	2	35	6	30	0.44	1.21
10	2	55	2	30	0.26	0.85
11	3	45	4	110	0.16	0.04
12	3	45	4	60	0.32	1.86
13	3	45	4	10	0.69	2.13
14	3	45	7.35	60	0.21	0.60
15	3	28.3	4	60	0.27	0.35
16	3	45	0.65	60	0.26	0.49
17	3	61.7	4	60	0.22	0.66

Location of the Stage of Extraction of Products with High Added Value in a Microalgae-Based Biorefinery

Figure 57. Chlorophyll extraction efficiencies when implementing the stage to extract high value products at different stages of a microalgae-based biorefinery configuration, a) prior to the cell disruption stage, (b) prior to the oil extraction stage, and (c) prior to the biomass drying stage





Based on the low percentages of chlorophyll obtained using microalgae biomass from the drying stage, we decided to test extraction with optimal operating conditions obtained at the end of the microalgae harvesting stage and following the cell disruption stage in order to compare the percentages of metabolites obtained. Figure 57 illustrates the three alternatives for the location of the

chlorophyll extraction stage with the respective extraction efficiencies, which can be after biomass drying and before cell disruption (Figure 57 a), after cell wall disruption but before lipid extraction (Figure 57 b), or after harvesting and before drying (Figure 57 c). It is clear that extraction efficiencies are higher when using moist biomass from the harvesting stage (Figure 57 c), which shows that both biomass flocculation and drying affect the content and extractability of the components, due to the fact that biomass drying temperature is 105°C, which is higher than chlorophyll degradation temperature. Water content in post-harvesting biomass does not affect the extraction efficiency of the products as significantly as the drying process, so the results show it is better to incorporate this stage prior to the microalgae drying stage.

CONCLUSIONS

The microalgae *Amphiprora* sp. is a promising strain for the development of a microalgae-based biorefinery due to the presence of special high-value substances in the composition thereof. The following are the best chlorophyll and lipid extraction conditions for biodiesel production using dry biomass of the microalgae Amphiprora sp., a temperature of 45ºC, a time of 4 h and a biomass/solvent ratio of 1/10 g/mL.

The statistical analysis of the results showed that the biomass/solvent ratio is the only significant variable for the extraction of chlorophyll-a, and less significant (but more influential than the other variables: temperature and time) in the extraction of crude oil.

Incorporating a chlorophyll extraction stage in a large-scale biorefinery process using microalgae is only convenient from the process efficiency standpoint when executed before the biomass drying stage, because the elimination of moisture from the strains to increase the efficiency of

fermentable sugar and oil extraction degrades the primary and secondary pigments of high commercial value.

NOMENCLATURE

Chla: Chlorophyll concentration in milligrams per liter.
A_{650}: Absorbance of a sample at 650 nm, dimensionless.
A_{665}: Absorbance of a sample at 665 nm, dimensionless.
%ext: Percentage of metabolite extracted in gram over gram.
Mccmp: Mass of the component removed in grams.
Mbicmass: Mass of the microalgae sample used in grams.

REFERENCES

[1] Tverberg, G. E. (2012). Oil supply limits and the continuing financial crisis. *Energy*. 37(1), 27–34.

[2] EPA. (2011). Inventory of US greenhouse gas emissions and sinks: 1990–2009 (April 2011), US EPA #430-R-11-005.

[3] Amaro, H., Guedes, C. & Malcata X. (2011). Advances and perspectives in using microalgae to produce biodiesel, *Appl. Energy*, 88(10), 3402-3410.

[4] González-Delgado, A. D. & Kafarov V. (2011). Microalgae based biorefinery: Issues to consider. *CT&F – Ciencia Tecnología y Futuro,* 4(4), 5-21.

[5] Richmond, A. (2004). Handbook of microalgal culture: Biotechnology and applied phycology. Ames: Blackwell Science.

[6] Mata, T. M., Martins, A. A. & Caetano, N. S. (2010). Microalgae for biodiesel

production and other applications: a review. *Renew. Sustain. Energy. Rev.,* 14(1), 217-232.

[7] Pragya, N., Pandey, K. K. & Sahoo, P. K. (2013). A review on harvesting, oil extraction and biofuels production technologies from microalgae. *Renew. Sustain. Energy. Rev*., 24: 159-171.

[8] Chisti, Y. (2007). Biodiesel from microalgae. *Biot. Adv*., 25(3), 294-306.

[9] Handler, R. M., Canter, C. E., Kalnes, T. N., Lupton, F. S., Kholiqov, O., Shonnard, D. R. & Blowers, P. (2012). Evaluation of environmental impacts from microalgae cultivation in open-air raceway ponds: Analysis of the prior literature and investigation of wide variance in predicted impacts. *Algal Research*, 1(1), 83-92.

[10] Pegallapati, A. K., Arudchelvam, Y. & Nirmalakhandan, N. (2012). Energy-efficient photobioreactor configuration for algal biomass production. *Bioresource Technol*., 126(1), 266-273.

[11] Garzon-Sanabria, A. J., Davis, R. T. & Nikolov, Z. (2012). Harvesting *Nannochloris oculata* by organic electrolyte flocculation: Effect of initial cell density, ionic strength, coagulant dogase, and media pH. *Bioresource Technol.*, 118(1), 418-424.

[12] González-Delgado, A. D. & Kafarov, V. (2012). Design and adjustment of coupled microalgae oil extraction methods for the development of a topology of biorefinery. *Prospectiva*, 10(1), 113-123.

[13] Halim, R., Danquah, M. K. & Webley, P. A. (2012). Extraction of oil from microalgae for biodiesel production: A review. *Biot. Adv*., 30(3), 709-732.

[14] Ehimen, E. A., Sun, Z. F. & Carrington, C.G. (2010). Variables affecting the *in situ* transesterification of microalgae lipids. *Fuel,* 89(3), 677-684.

[15] Ofori-Boateng, C., Teong, L. K. & JitKang, L. (2012). Feasibility study of microalgal and jatropha biodiesel production plants: exergy analysis approach. *Appl. Therm. Eng.,* 36(1), 141–151.

[16] Peralta-Ruiz, Y., González-Delgado, A. D. & Kafarov, V. (2013). Evaluation of alternatives for microalgae oil extraction based on exergy analysis, *Appl. Energ.,* 101: 226-236.

[17] Schumann, R., Häubner, N., Klausch, S. & Karsten, U. (2005). Chlorophyll extraction methods for the quantification of green microalgae colonizing building facades. *Int. Biodeter. Biodegr.,* 555(3), 213-222.

[18] Archanaa, S., Moise, S., & Suraishkumar, G. (2012). Chlorophyll interference in microalgal lipid quantification through the Bligh and Dyer method. *Biomass Bioenergy*, 46: 805-808.

[19] Silveira, S. T., Burkert, J. F. M., Costa, J. A. V., Burkert, C. A. V. & Kalil, S. J. (2007). Optimization of phycocyanin extraction from *Spirulina platensis* using factorial design. *Bioresource Technol.*, 98(8), 1629-1634.

[20] Ramirez, L. & Olvera, R. (2006). Uso tradicional y actual de *Spirulina* sp. (*Arthrospira* sp.). *Interciencia*. 31(9), 657-663.

[21] Wijffels, R., Barbosa, M. & Eppink, M. (2010). Microalgae for the production of bulk chemicals and biofuels. *Biofuels, Bioprod. Biorefin.*, 4(3), 287-295.

ANNEX B. LINGO CODES FOR SUPERSTRUCTURE OPTIMIZATION

SOURCE: Author

```
SETS:
Tech /1..2/;
Layer/1/:Fout;
Pair(layer,Tech)/1,1 1,2/:
Alfadir,Betadir,Eftechdir,Tyieldir,TAC,AFC,AOC,Ffeed,Fpro
d,X;
ENDSETS
DATA:
Tyieldir, Eftechdir, Alfadir, Betadir,Ffeedin, Costbio,
Costalgae= @OLE('C:\Users\adgonzalezd\My
Documents\superstructure biodiesel.xlsx');
ENDDATA

!one tech per layer is chosen;
@for(layer(i):
@sum(Pair(i,j):X(i,j))=1);


!Product Flowrate for each tehcnology is calculated;
@FOR(pair(i,j)|i#EQ#1:
Ffeed(i,j) = Ffeedin;
Fprod(i,j)= Ffeed(i,j)*Tyieldir(i,j)*Eftechdir(i,j));


@for(layer(i)|i#EQ#1:
Fout(i) = @sum(pair(i,j):(fprod(i,j)*X(i,j))));


!cost of each technology is calculated;
@FOR(pair(i,j):
AFC(i,j) = (Alfadir(i,j))*(Ffeed(i,j))^0.7;
AOC(i,j) = (Betadir(i,j))*Ffeed(i,j);
TAC(i,j) = AFC(i,j) + AOC(i,j));


!Optimization;
Max = costbio*Fout(1) - @sum(pair(i,j):(TAC(i,j)*X(i,j)))
- Costalgae*Ffeedin;


@for (Pair(i,j):@Bin(X(i,j)));
```

```
end
Max = costbio*Fout(1) - @sum(pair(i,j):(TAC(i,j)*X(i,j)))
- Costalgae*Ffeedin +0.5*Costalgae*0.68*Ffeedin;
!Technologies evaluated:
    Index Name
_____________________________________
1     Alcane secretion
2     Direct transesterification wet biomass
;


SETS:
Tech /1..11/;
Layer/1..3/:Fout;
Pair(layer,Tech)/1,1 1,2 1,3 1,4 1,5 1,6 1,7 2,8 2,9 3,10
3,11/:
Alfadef,Betadef,Eftechdef,Tyielddef,TAC,AFC,AOC,Ffeed,Fpr
od,X;
ENDSETS
DATA:
Tyielddef, Eftechdef, Alfadef, Betadef,Ffeedin, Costbio,
costalgae= @OLE('C:\Users\adgonzalezd\My
Documents\superstructure biodiesel.xlsx');
ENDDATA

!one tech per layer is chosen;
@for(layer(i):
@sum(Pair(i,j):X(i,j))=1);


!Product Flowrate for each tehcnology is calculated;
@FOR(pair(i,j)|i#EQ#1:
Ffeed(i,j) = Ffeedin;
Fprod(i,j)= Ffeed(i,j)*Tyielddef(i,j)*Eftechdef(i,j));

@for(layer(i)|i#EQ#1:
Fout(i) = @sum(pair(i,j):(fprod(i,j)*X(i,j))));

!cost of each technology is calculated;
@FOR(pair(i,j):
AFC(i,j) = (Alfadef(i,j))*(Ffeed(i,j))^0.7;
AOC(i,j) = (Betadef(i,j))*Ffeed(i,j);
TAC(i,j) = AFC(i,j) + AOC(i,j));
```

```
@FOR(pair(i,j)|i#GE#2:
Ffeed(i,j) = Fout(i-1);
Fprod(i,j)= Fout(i-1)*Tyielddef(i,j)*Eftechdef(i,j));

@for(layer(i)|i#GE#2:
Fout(i) = @sum(pair(i,j):(fprod(i,j)*X(i,j))));


!Optimization;
Max = (Costbio*Fout(3) -
@sum(pair(i,j):(TAC(i,j)*X(i,j))) - Costalgae*Ffeedin);


@for (Pair(i,j):@Bin(X(i,j)));

end


Max = (Costbio*Fout(3) -
@sum(pair(i,j):(TAC(i,j)*X(i,j))) - Costalgae*Ffeedin +
(1137*Fout(3)*0.15/0.85));


!Technologies evaluated:
```

	Index Name
1	Blank
2	Alkane secretion
3	Direct transesterification wet biomass
4	Wet extraction
5	Oil secretion
6	Solvent extraction
7	Supercritical extraction
8	Pyrolysis
9	HTL
10	Cracking
11	Hydroprocessing
12	Oligomerization

```
SETS:
Tech /1..9/;
Layer/1..2/:Fout;
```

```
Pair(layer,Tech)/1,1 1,2 1,3 1,4 1,5 2,6 2,7 2,8 2,9/:
Alfaoil,Betaoil,Eftechoil,Tyieldoil,TAC,AFC,AOC,Ffeed,Fpr
od,X;
ENDSETS
DATA:
Tyieldoil, Eftechoil, Alfaoil, Betaoil,Ffeedin, Costbio,
costalgae= @OLE('C:\Users\adgonzalezd\My
Documents\superstructure biodiesel.xlsx');
ENDDATA

!one tech per layer is chosen;
@for(layer(i):
@sum(Pair(i,j):X(i,j))=1);


!Product Flowrate for each tehcnology is calculated;
@FOR(pair(i,j)|i#EQ#1:
Ffeed(i,j) = Ffeedin;
Fprod(i,j)= Ffeed(i,j)*Tyieldoil(i,j)*Eftechoil(i,j));


@for(layer(i)|i#EQ#1:
Fout(i) = @sum(pair(i,j):(fprod(i,j)*X(i,j))));

@FOR(pair(i,j)|i#GE#2:
Ffeed(i,j) = Fout(i-1);
Fprod(i,j)= Fout(i-1)*Tyieldoil(i,j)*Eftechoil(i,j));

@for(layer(i)|i#GE#2:
Fout(i) = @sum(pair(i,j):(fprod(i,j)*X(i,j))));

!cost of each technology is calculated;
@FOR(pair(i,j):
AFC(i,j) = (Alfaoil(i,j))*(Ffeed(i,j))^0.7;
AOC(i,j) = (Betaoil(i,j))*Ffeed(i,j);
TAC(i,j) = AFC(i,j) + AOC(i,j));


!Optimization;
Max = Costbio*Fout(2) - @sum(pair(i,j):(TAC(i,j)*X(i,j)))
- Costalgae*Ffeedin;


@for (Pair(i,j):@Bin(X(i,j)));
```

```
end

!Technologies evaluated:
    Index Name

1     Wet extraction
2     Enzymatic degradation
3     Oil secretion
4     Solvent extraction
5     Supercritical extraction
6     Esterification/transesterification
7     Hydroprocessing
8     Transesterification (heterogeneous)
9     Transesterification (acid)
;
SETS:
Tech /1..10/;
Layer/1..2/:Fout;
Pair(layer,Tech)/1,1 1,2 1,3 1,4 1,5 1,6 1,7 2,8 2,9
2,10/:
Alfasyn,Betasyn,Eftechsyn,Tyieldsyn,TAC,AFC,AOC,Ffeed,Fpr
od,X;
ENDSETS
DATA:
Tyieldsyn, Eftechsyn, Alfasyn, Betasyn,Ffeedin, Costbio,
costalgae= @OLE('C:\Users\adgonzalezd\My
Documents\superstructure biodiesel.xlsx');
ENDDATA

!one tech per layer is chosen;
@for(layer(i):
@sum(Pair(i,j):X(i,j))=1);


!Product Flowrate for each tehcnology is calculated;
@FOR(pair(i,j)|i#EQ#1:
Ffeed(i,j) = Ffeedin;
Fprod(i,j)= Ffeed(i,j)*Tyieldsyn(i,j)*Eftechsyn(i,j));

@for(layer(i)|i#EQ#1:
Fout(i) = @sum(pair(i,j):(fprod(i,j)*X(i,j))));

!cost of each technology is calculated;
@FOR(pair(i,j):
```

```
AFC(i,j) = (Alfasyn(i,j))*(Ffeed(i,j))^0.7;
AOC(i,j) = (Betasyn(i,j))*Ffeed(i,j);
TAC(i,j) = AFC(i,j) + AOC(i,j));


@FOR(pair(i,j)|i#GE#2:
Ffeed(i,j) = Fout(i-1);
Fprod(i,j)= Fout(i-1)*Tyieldsyn(i,j)*Eftechsyn(i,j));

@for(layer(i)|i#GE#2:
Fout(i) = @sum(pair(i,j):(fprod(i,j)*X(i,j))));


!Optimization;
Max = (Costbio*Fout(2) -
@sum(pair(i,j):(TAC(i,j)*X(i,j))) - Costalgae*Ffeedin) ;


@for (Pair(i,j):@Bin(X(i,j)));

end

!Technologies evaluated:
    Index Name
1   Blank
2   Alcane secretion
3   Direct transesterification wet biomass
4   Wet extraction
5   Oil secretion
6   Solvent extraction
7   Supercritical extraction
8   SCW Gasification
9   Gasification
10  Pyrolysis
```