MICROALGAE DOWNSTREAM PROCESSING AND ECONOMICAL APPROACHES OF BIODIESEL PRODUCTON PROCESSES

Sergio Daniel Rios

Dipòsit Legal: T 540-2014

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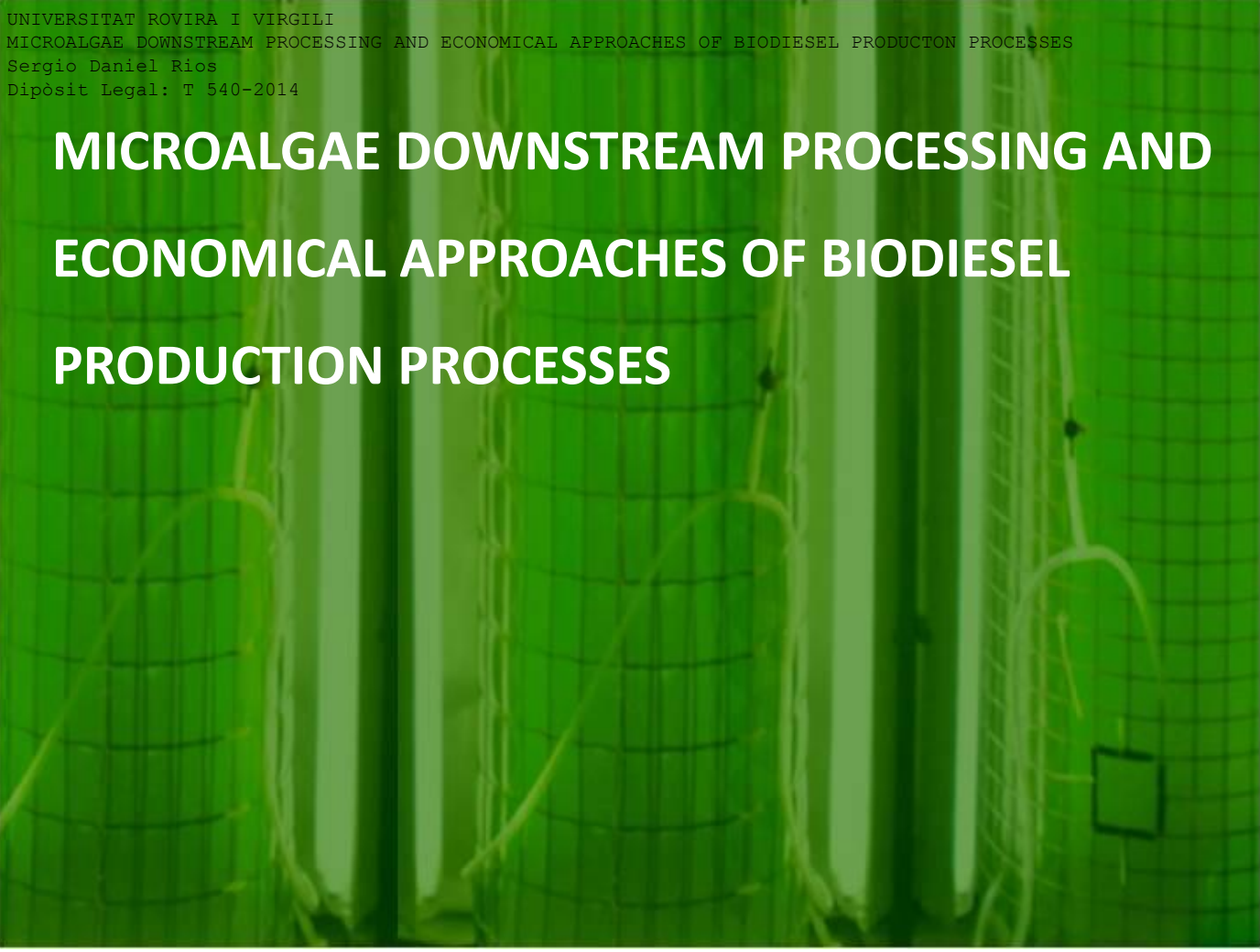

Sergio Daniel Ríos

Programa de Doctorat en Enginyeria Química, Ambiental i de Processos

Tarragona 2012

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Microalgae downstream processing and economical approaches of biodiesel production processes

Doctoral thesis presented by

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Los abajos firmantes

HACEN CONSTAR que el presente trabajo, que lleva por titulo

"Microalgae downstream processing and economical approaches of biodiesel production processes."

y que presenta *Sergio Daniel Ríos* para optar al grado de Doctor por la Universidad Rovira i Virgili, ha sido realizado en esta bajo nuestra dirección, y que todos los resultados presentados y análisis correspondientes son fruto de la investigación llevada a cabo por el mencionado candidato

para que conste, tengan en conocimiento y a los efectos que correspondan, firman este certificado,

Prof. Dr. Xavier Farriol Roigés
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A la memoria de mi madre

AGRADECIMIENTOS

Sinceramente creo que los agradecimientos son un deber hacia las personas o entidades que han apoyado o financiado este trabajo de una u otra manera, en este apartado destacan los directores a los que he de dar las gracias por darme la oportunidad de desarrollar mi trabajo al Dr. Xavier Farriol, el Dr. Joan Salvadó y el Dr. Carles Torras.

Quiero agradecer tanto a los miembros del tribunal, como a los que evaluarán el manuscrito para emitir el informe.

A la Universitat Rovira i Virgili que ha financiado los primero años de trabajo, y especialmente al Departament d'Enginyeria Química y finalmente el Institut de Recerca de Energia de Catalunya (IREC).

He de agradecer a todas las instituciones que de una u otra manera han financiado equipos, reactivos, análisis, etc.… y debería preguntar a Pepa cual es cual, que es ella quien lleva las cuentas…Pepa, gracias por hacer ese trabajo y llevar luz allí donde existen penumbras…o iluminar hacia donde es necesario.

Este podría ser el documento que indica la finalización de una etapa, o el comienzo de otra, de todas formas el que ha estado a mi lado, y sin importar la distancia física o emocional sabe bien lo agradecido que estoy con él/ella/eso/esa por lo que creo que personalizar esto es de muy mal gusto; así que vayan mis agradecimientos totales y globales al que de una u otra manera ha estado, no sólo al que ha apoyando, sino también al que no lo ha dificultado, es loable tanto lo uno como lo otro.

Como está escrito más arriba es el final y principio de una etapa por lo que no sólo agradezco al pasado sino a la persona que en un futuro estará o seguirá estando, creo que los mejores tiempos son los que faltan por venir.

Sobre todo siento la necesidad de dedicar este documento mi vida y mi trabajo a la memoria de mi madre, que ha sido mi inspiración durante todos estos años de vida (y esta vez hablo de sentimiento y no de obligación). Para ella toda mi gratitud.

No quiero olvidar a mi familia, la de siempre, la que ha estado en las duras y maduras, la que ha llegado hace poco e inyecta esperanza, la familia de sangre y la política… correcta e incorrecta.

Gracias

1.- Introduction

The generation, transport and consumption of conventional sources of energy, particularly in the transport sector, have raised many concerns about air pollution, environmental degradation, and petroleum consumption. In this sense, the development of alternative technologies is acquiring importance, and many efforts are being done in the gradual replacement of fossil fuels. Within the renewable sources of energy, the production of liquid biofuels from organic feedstocks sources represents a feasible alternative that avoids important modifications of vehicle engines, maintaining most of the infrastructures for the supply chain and can replace partially the petroleum-based fuels.

Biofuels have many advantages over fossil fuels: as well as being more environmentally friendly and a renewable source of resources, every region in the world has a raw material that can be used, to a greater or lesser extent, to produce some sort of biofuel, thus providing work for the population and improving the economies of the countries that so far have been dependent on petroleum imports.

Biodiesel is a renewable fuel obtained from vegetable oils or animal fats. Its properties are similar to that of fossil fuels, allowing its use for diesel engines, both pure and blended with others diesel fuels.

In order to avoid food competition, microalgae oil has been choose as a firm alternative to replace the total diesel used for transport purposes. For these purposes, microalgae should be produced in open pond or photobioreactors, and then it must be concentrated to finally take the intracellular valuables molecules such as lipids, proteins or carbohydrates.

The objectives of this chapter are shows the general background and state of the art of different alternatives to culturing and harvesting microalgae to obtain biomass and the subsequent step of transforming the biomass in liquid fuel.

This thesis is focused in the development and study of different pathways of the downstream process of biodiesel production from microalgae in order to make the process economically feasible or being a first approach for features improvements.

1.1.- Environmental Issues

According to the Energy Information Administration (EIA) in the International Energy Outlook Projection 2011 ((EIA) 2011), Over the next 25 years, demand for liquid fuels will increase more rapidly in the transportation sector than in any other end-use sector.
On the other hand, the British Petroleum (BP) report 2011 ((BP) 2011) establishes that the proven reserves of petroleum will be exhausted in less than 45 years at the current rate of consumption unless more reserves are found. However, petroleum consumption has increased in the last decade and, as a result of the economic crisis and with petroleum at $100 the barrel, petroleum companies have decreased their investment in new explorations so reserves are expected to continue decreasing
Both the aforementioned sources of energy information agree that petroleum production in 2010 was around 82 million barrels a day. The EIA envisages that petroleum and non petroleum liquid fuel consumption will increase to 106.6 million barrels a day by 2030. This increase will largely take place in non-OPEC countries (emerging economies such as China and India) and the transportation sector will account for nearly 80% of the total liquid oil consumption.
Of all the petroleum refined in 2011, about 68% was used for transport purposes (57 million barrels of middle and light distillates a day).
The combustion of these petroleum derivatives leads to a considerable build up of CO_2 in the atmosphere. CO_2 is one of the main gases associated with the greenhouse effect (together with NO_x, O_3 and VPM), which most countries in the world, except for those that have not signed the Kyoto Protocol of 1997, are trying to revert.
The liquid fuels used by means of transport were responsible of more than 30% of the CO_2 emissions in the world in 2010, some 9 billion metric tons ((EIA) 2011).
Petroleum is not distributed evenly around the world and, in fact, all reserves are centralized in just a few countries (more than half in the Middle East).
This means that all the countries with no reserves are sensitive to changes in these countries. In 2011, for example, the upheaval in Libya, Egypt and other countries increased the price of petroleum by more than 30% in a few months, a very good reason

for decentralizing liquid fuels by providing alternative raw materials. All regions in the world have their own raw materials that can be used, to a greater or lesser extent, as precursors to produce some sort of biofuel and, in the process, provide work for the population and improve their import-dependent economies.

1.1.1 Renewable energy for the transport sector

To mitigate the problem of environmental pollution and the exhaustion of petroleum supplies, it has been suggested that biofuels such as biodiesel and bioethanol can be used. In general, these fuels are carbon neutral since the raw materials on which they are based are photosynthetic organisms such as the plants from which biodiesel oil is extracted (rapeseed, soya, sunflower, palm, etc.) or fermented bioalcohols (corn, sugar cane, etc.).

The paths for producing biodiesel and bioalcohols from different raw materials are well documented (Rejinders, 2006; Demirbas, 2007; Frondel and Peters, 2007; Narayanan et al., 2007; Palligarnai, 2008; Demirbas, 2009). Some authors discuss and compare the advantages of each fuel (Hill et al., 2006; Reijinders, 2006; Agarwal, 2007; Granada et al., 2007; Demirbas, 2008). Others integrate the two technologies (Gutierrez et al., 2008).

A new report by the International Energy Agency (IEA) 2010, says that "most biofuel technologies could cost the same, or even less, than fossil fuels". The report, called Biofuels for Transport, notes that around US$13 trillion of investment funds are needed to make sure that the market for biofuels as a sustainable and renewable energy source is effectively developed.

The EIA (2011) ((EIA) 2011) reports that the production of liquid biofuels (biodiesel + bioethanol) in 2010 was 1.85 million barrels a day, where biodiesel is around 3% of the total biofuel production .

Biofuels have many advantages over fossil fuels: as well as being more environmentally friendly and a renewable source of resources, every region in the world has a raw material that can be used, to a greater or lesser extent, to produce some sort of biofuel, thus providing work for the population and improving the economies of the numerous countries that have so far been dependent on petroleum imports.

1.1.2 First and second generation biofuel

First- and second-generation biofuels like ethanol and biodiesel have a number of inherent limitations that make them less than ideal as a long-term replacement for petroleum. The primary feedstocks for first-generation ethanol (corn and sugarcane) and biodiesel (rapeseed, soybeans, and palm) are all food-based crops. These fuels can be used in unmodified engines above small blends but are not applicable to the jet fuel market.

The ethical question of use those crops that are edible or compete directly for land necessary to produce food to produce biofuel made that several groups (Green Peace, Intermon Oxfam, etc) claim that the growing production of biofuels is responsible for the increasing food and feed prices, and the destruction of forests.

According to the Gallagher review on the indirect effects of biofuels production (2008), the rise in biofuel culture directly affects feedstock because it destroys habitats (particularly in Africa and South America) and impacts on the local environment (air, water and soil), and also involves a range of social issues including poor working conditions, loss of indigenous people's land rights and rising food prices. It also affects food security for the poor or displaces agricultural production to uncultivated areas with the resulting impact on biodiversity, greenhouse gas (GHGs) savings, among other things.

Second generation biofuels are fuels that can be manufactured from various types of biomass not using such as food crops. Second generation biofuel production is more sustainable and has a lower impact on food production although they compete for scarce cropland, fresh water, and fertilizers (i.e. second generation ethanol is produced from cellulose rather than sugar made from corn or sugar cane).

While first- and second-generation biofuels account for more than 99% of current global biofuel production, a number of important technologies are on the brink of commercialization that produce "drop-in" fuels with the same chemical characteristics of petroleum.

If canola is planted to produce all the biodiesel needed to replace the amount of petroleum produced annually; a cultivated surface area of 3.7 thousand million hectares would be necessary (more than twice the current estimated land use for cropland) (Chisti, 2007; Schneider, 2008) because canola can produce 5 petroleum equivalent barrels per hectare and year. Although palm oil productivity is five times greater than

canola, the special weather conditions (tropical) required, makes it a less feasible option than canola.

1.1.3 Third generation biofuel

Third generation biofuel are considered to be a viable alternative that can avoid major drawbacks associated with first and second generation biofuel. Third generation biofuel are derived from microalgae and/or seaweeds to produce bioethanol and/or biodiesel and from green microalgae and microbes to produce hydrogen and/or other biofuels.
Microalgae seem to be able to totally replace fossil fuels because their productivity is extremely high: some strains are capable of reproducing more than three times a day (Xiaoling and Quingyu, 2006; Chisti, 2007). The total wild biomass of some microalgae contains more than 50% lipids (Borkowitka 2005; Donghi et al., 2008; Rosenberg et al., 2008; Xin et al., 2008; Packer, 2009) while the biomass of some other species that have been genetically modified or have undergone some sort of stress during growth may have a lipid content that exceeds 80% (Spolaore et al., 2006; Chisti, 2007; Packer, 2009).

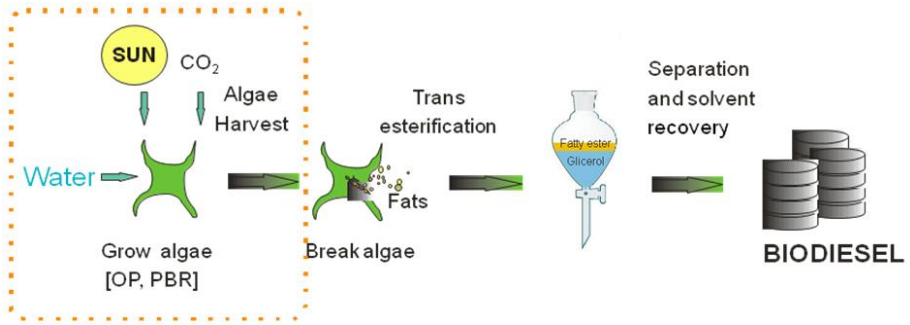


Figure 1 Third generation biodiesel production steps from microalgae

From the economic point of view, microalgae may be described as microorganisms with an ability to “harvest the sun” and hence transform its radiant energy into valuable products, with the use of (theoretically) inexpensive natural resources such CO_2 and H_2O (figure 1) (Carvalho et al., 2006).
Microalgae are a photosynthetic organism, which means that they may be useful for decreasing the CO_2 in the atmosphere, because they use it as a substrate together with solar energy (Spolaore et al., 2006).

To satisfy the annual petroleum requirements for 2007, (Chisti 2007) assumes a scenario with a microalgae production of 127 T $ha^{-1}year^{-1}$ in open ponds (OPs) and 263 T $ha^{-1}year^{-1}$ in photobioreactors (PBRs). This would involve 121,000 hectares of OPs and just over 58,000 hectares of PBRs (the surface area of Singapore), if the lipid content of both is 80% of the total biomass produced. Photobioreactors have already produced up to 150 T $ha^{-1}year^{-1}$ biomass (Carlsson et al., 2007).

1.2.- Microalgae

1.2.1 Microalgae history

The era of microalgae started with (Beijerinck 1890) who cultivated the first unialgal cultures with the green algae species "*Chlorella vulgaris*". In 1919, Warburg used microalgae cultures to investigate the physiology of plants. The tough economic conditions worldwide during and after World War II led to increased interest in harvesting microalgae because they reproduced so efficiently and were an inexpensive source of protein. In the preliminary years of microalgae research, culturing and harvesting were examined to determine whether they could be a feasible replacement for animal protein. Nowadays, however, studies have been focusing on microalgae as an alternative feedstock to fossil fuels and/or a solution to global warming—GHG effect—as CO_2 sink.

Algae are not only sources of food for humans and animals, but are also the sources of a wide range of chemical compounds such as the phycocolloids used in industry, food technology and as pharmaceuticals. In the last few decades the emphasis has moved from 'wild' harvests to the farming and controlled cultivation and to the production of valuable new products. Continued technical innovation and market demand will result in further major advances and an expansion of the commercially available species and products. Genetic engineering methods are also beginning to be used for strain improvement, and algal genes are being used for the improvement of other plants such as crop plants.

Commercial large-scale culture of microalgae commenced in the early 1960's in Japan with the culture of *Chlorella* followed in the early 1970's with the establishment of a *Spirulina* harvesting and culturing facility in Lake Texcoco, Mexico by Sosa Texcoco S.A. (Borowitzka, et al, 2005)

In 1977 Dai Nippon Ink and Chemicals Inc. established a commercial *Spirulina* plant in Thailand, and by 1980 there were 46 large-scale factories in Asia producing more than 1000 kg of microalgae (mainly *Chlorella*) per month (Kawaguchi, 1980) and in 1996 about 2000t of *Chlorella* were traded in Japan alone. Other Spirulina plants were established in the USA (*e.g.* Micro-bio in California and Cyanotech in Hawaii). (Spolaore et al 2006). Commercial production of *Dunaliella salina*, as a source of ß-carotene, became the third major microalgae industry when production facilities were established by Western Biotechnology Ltd. and Betatene Ltd. (now Cognis Nutrition & Health) in Australia in 1986. These were soon followed by other commercial plants in Israel and the USA. As well as these algae, the large-scale production of cyanobacteria (blue-green algae) commenced in India at about the same time. More recently several plants producing *Haematococcus pluvialis* as a source of astaxanthin have been established in the USA and India. Thus in a short period of about 30 years the industry of microalgal biotechnology has grown and diversified significantly (Becker, W. 2004)
Since the 1970s the National Renewable Energy Laboratory (NREL) has identified thousands of species of microalgae in an attempt to find a substitute for petroleum (Sheenan et al., 1998). However, they put this task to one side when petroleum prices fell.

1.2.2 Biorefinery concept

Microalgae are useful not only because of their high content of lipids, but also because of their high content of other types of compound (vitamins, proteins, fatty acids, pigments, etc.) which are of considerable added value for the nutraceutical industry, pharmaceuticals, cosmetics, the human and animal food industry, etc. (Mendola, 2003; Molina Grima et al., 2003; Wiffels, 2007; Lamers et al., 2008; Granado-Lorencio et al., 2009; Leary et al., 2009). They can also be used for treating waste waters (Vilchez et al., 1997).
According to US Energy Department “Biorefineries are similar to petroleum refineries in concept; however, biorefineries use biological matter (as opposed to petroleum or other fossil sources) to produce transportation fuels, chemicals, and heat and power biorefinery” (Figure 2).

Many authors suggested this possible solution to address the problem of costs associated with different stages of processing and production of different products from microalgae (Chisti, 2007; Powell and Hill, 2009; Rupprecht, 2009; Tran et al., 2010)

There can be several combinations of feedstocks or combination of technologies to produce different products that mainly focus on producing biofuels and sub-products including chemicals (or other materials), heat and power.

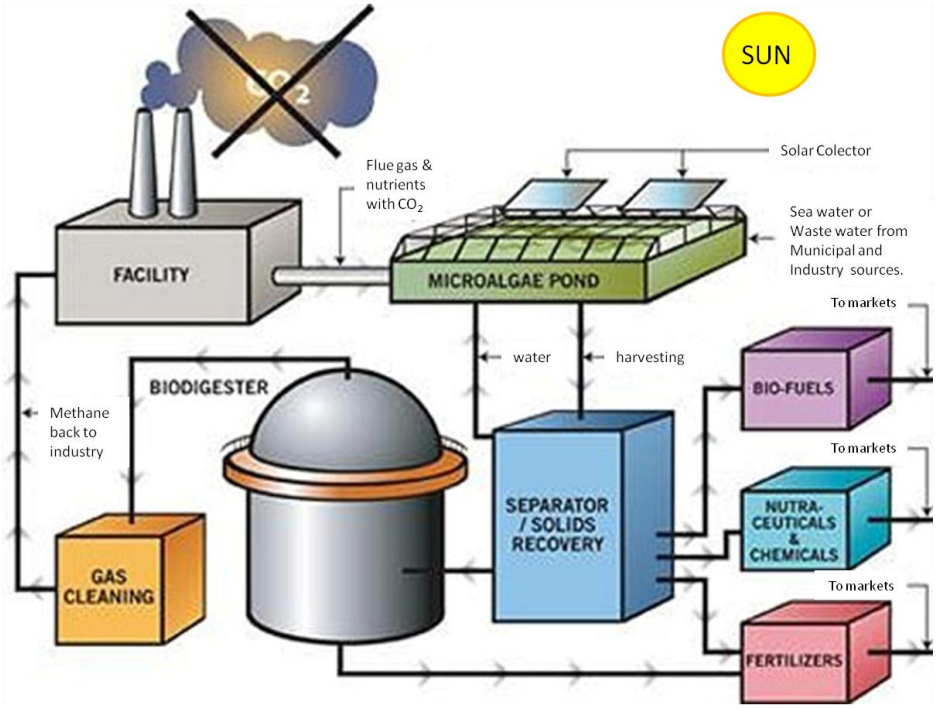


Figure 2: Possible integrated biorefinery from microalgae as a raw material. Modified from http://www.news.cnet.com

Microalgae have a very high photosynthetic efficiency. In order to enhance optimum microalgal growth, light, water quality, pH, CO_2, temperature and nutrient availability are the most important parameters. However, the decision about which microalgae culture systems to implement on a commercial scale should also take into account such additional factors as latitude, local weather and water resources.

To take advantage of the ability of microalgae to store energy in the chemical bonds of the molecules produced some barriers still have to be overcome. These barriers can be found in the following main process steps:

- In microalgae culture, the growing speed and final solid concentration in the broth have to be improved. Likewise, CO_2 fixation (and O_2 release) also needs to be improved.
- Low-energy technologies have to be improved so that they can be applied to microalgae concentration.

1.2.3 Microalgae culturing

Various techniques can be used to cultivate microalgae: for example, open ponds-OPs (natural or manmade) or photobioreactors-PBRs.

1.2.3.1 Open ponds

Natural OPs are lakes or basins with natural borders and natural microalgae species. Man made OPs is constructed in various designs that vary in size, shape, construction material, agitation type or inclination.

Richmond (2004) identified the following major designs of ponds that operated on a large scale:

- inclined systems, in which mixing is done by pumping and gravity flow;
- circular ponds, in which the water is agitated by a rotating arm; and
- raceway ponds, which are constructed as an endless loop, and in which the culture is circulated by paddle wheels.

For the production of microalgae, OPs have the advantages that they are easy to scale up, and practical to construct and operate with sunlight. Their disadvantages are that it is hard to control CO_2 use and culture conditions, the risk of contamination is high and only a limited number of microalgae species can grow properly in them.

In this kind of reactor the microalgae species have to resist seasonal (summer / winter) and daily (day/night) temperature differences, and not be contaminated by protozoa and/or other dominant algae. For commercial production, then, *Chlorella sp.* and *Spirulina sp.* were chosen because of their high growth rates in suitable nutrient combinations and pH ranges. On the other hand, although *Isochrysis* species are not highly suited to be grown in OPs, they were also selected because of their high production of long chain polyunsaturated fatty acids (PUFAs) (Richmond, 2004a).

For example the Myanmar Spirulina Factory (Yangon, Myanmar) sells tablets, chips, pasta and liquid extract, and Cyanotech (a plant in Kona, Hawaii, USA) produces products ranging from pure powder to packed bottles under the name Spirulina pacifica. *Chlorella* is produced by more than 70 companies; Taiwan Chlorella Manufacturing and Co. (Taipei, Taiwan) is the largest producer (400T of dried biomass per year) (Spolaore et al., 2006).

Figure 3 Algae open pond farm for biofuel and jet fuel production (http://www.petrosuninc.com)

An Arizona Energy Company is betting big on algae, PetroSun Biofuels has opened a commercial algae-to-biofuels farm on the Texas Gulf Coast near scenic Harlingen Texas. The farm is a 1,100 acre network of saltwater ponds, 20 acres of which will be dedicated to researching and developing an environmental jet fuel (figure 3).

All commercial applications of microalgae have been summarized by Spolaore et al. (2006) but it should be borne in mind that not all microalgae studies are developed to have a commercial use.

According to the articles reviewed above, microalgae are produced exclusively between spring and autumn, so it is difficult to discuss the biomass productivity of microalgae in annual terms because all data are given in g/(m^2day); different species, the different locations of OPs and, therefore, different light intensities do not allow proper comparisons to be performed. Some authors (Baussiba et al., 1988; Doucha & Livansky, 1995; Doucha & Livansky, 2000; Jimenez et al., 2003a & 2003b) do not

mention water evaporation in OPs and did nothing to prevent it. Neither do they mention the effects of rain. The only exception is Hase et al. (2000) who investigated productivity on a bench scale under greenhouse conditions.

In the articles reviewed the biomass growth rates are only given for certain sunny months. If production of microalgae had been examined continuously for a complete year, the average biomass rates would probably have been lower than the results presented.

From the commercial point of view, the parameters that need to be checked when growing microalgae are the biomass production rate and the percentage of fatty acids. With the exception of (Baussima et al. 1988), who drew attention to the lipid formation rate of *Isochrysis galbana* and looked at microalgae as a source of PUFA rich food, no data could be found about the fatty acid content of species even though it is a very important parameter.

1.2.3.2 Photobioreactors

It has already been mentioned that open ponds present such problems as contamination, difficult culture control, limited production periods and low productivity. Photobioreactors (PBR), however, are designed for industrial scale, closed-system culturing of microalgae with large surface-volume ratios. Although setting up PBRs on a commercial scale requires more capital and knowledge to operate than OPs, solar energy use and biomass productivity can be higher. In addition, the lower risk of contamination allows producers to grow a much wider variety of species of microalgae in PBRs. If the microalgae are to be grown in these closed systems, the efficiency of light and the quantity of CO_2 are deemed to be the limiting factors.

The main categories of PBRs are according to (Richmond, 2004b);

- flat or tubular;
- horizontal, inclined, vertical or spiral; and
- manifold or serpentine.

PBRs are also classified according to their operation modes (Richmond, 2004b):

- air or pump mixed;
- single phase reactors that are filled with media, and gas exchange takes place in a separate gas exchanger; and

- Two-phase reactors in which both gas and liquid are present and continuous gas mass transfer takes place in the reactor itself.

Of all the design categories, tubular PBRs are believed to be one of the most efficient reactors for microalgae culturing on an industrial scale. They are easy to construct, protect the growth medium from contaminants, provide high solar energy utilization operate at high biomass concentrations and prevent water loss due to vaporization.

In spite of these advantages, temperatures are high especially in summer, which limits the growth of the photosynthetic microorganism. As mentioned in (Ugwu et al., 2002), inefficient mass transfer rates in tubular PBRs might be another disadvantage of scaling up.

Figure 4 Tubular Photobiorreactors for microalgae production (Pilot Plant-Repsol, Tarragona)

The tubular PBRs studied in the literature are of a variety of design concepts: for example, tubular (Torzillo et.al., 1986), horizontal tubular (Tredici and Zittelli, 1998; Sanchez Miron et. al., 1999), inclined tubular (with/without static mixers) (Ugwu et. al., 2002; 2005), helical tubular (Scragg et. al., 2002; 2003; Watanabe and Saiki, 1997), airlift driven-external loop tubular (Acien Fernandez et al., 2001) etc.

Vertical and horizontal tubular PBRs offer variations in particular paths - light regimes, gas-liquid hydrodynamics and mass transfer behaviour- and some of them are interrelated as hydrodynamics and light regimes (Sanchez Miron et al., 1998*).*

Mixing the culture in PBRs with air or static mixers usually has a positive effect on the growth of microalgae, which increases productivity. However, from the economic point

of view, providing PBRs with high aeration rates or mixers for mixing the culture might lead to high operational costs in the commercial processes.

Ugwu et al. (2002) investigated the mass transfer characteristics of bioreactors equipped with static mixers of different types and spacing distances to determine how these mixers affected inclined tubular PBRs. (Ugwu et al. 2005) showed that mixers efficiently circulated microalgae cells between the illuminated and dark sections of the tubes. It was concluded that the mixers increased biomass productivity and decreased the photoinhibitory effect of light during outdoor culturing. On the other hand, in contrast with achieving higher productivity, mixers could cause shear stress on cells as high aeration rates (Mazzuca Sobczuk et al., 2006; Kaewpintong et al., 2007; Ugwu et al., 2008).

Fernandez et al. (2000) have drawn our attention to airlift-driven external-loop tubular PBRs, which are designed to satisfy the demands for external irradiance-dependent cell growth, oxygen accumulation in the solar loop, oxygen removal in the airlift device and the hydrodynamics of the airlift system, which determine the flow characteristics of the solar receiver.

(Molina Grima et al. 1999; 2001) also analyzed the design of tubular reactors and discussed light regimes, mass transfer properties and scale-up strategies.

Flat–plate PBRs are constructed very simply. If the ratio of illuminated area to culture volume is high, productivity rates can also be high. Because the penetration of light is one of the factors that affect the growth of microorganisms, the plate must be constructed taking into account the latitude where the PBRs are going to be built. Biomass productivity can be affected not only by the configuration of the plate, but also by the plate dimensions. (Hu et al. 1996) pointed out that panel reactors seem to be more promising than horizontal reactors, because their variable plate orientations (with a reactor angle of 90^{o}C or less and different lengths of the reactor light path) aimed for maximum solar energy use throughout the year.

In flat-plate PBRs, mass transfer rates that include the transfer of CO_2 from gas to growth medium are affected by the dimensions of plates. Nevertheless, it must be remembered that the mass transfer rate of CO_2 can be the most important and limiting factor on growing of microalgae through the path of photosynthesis when the other limiting factors are appropriate.

According to (Zhang et al. 2002), although the relationship between dimension and mass transfer has been studied in bubble type bioreactors (Schugerl and Lucke, 1977),

and stirred-tank type bioreactors (Asenjo and Merchuk, 1994), less work has been performed on the relationship between the dimensions and mass transfer of a vertical flat-plate PBR (VFPP).

Reviewed data shows that three types of measures are used to compare the performance of algal cultures:

- volumetric productivity: biomass productivity per unit volume of reactor per unit time.
- areal productivity: biomass productivity per unit ground area of reactor per unit time.
- productivity per illuminated surface area: biomass productivity per unit of illuminated surface area of reactor per unit time.

PBRs do not have the same surface area-illumination ratio or illuminated surface-volume ratio, so it is difficult to compare biomass efficiencies because the yield concepts are different. As yet, there is no standardized way of presenting the reactor performance.

In horizontal systems such as tubular reactors, (Tredici et al. 1997) concluded that the parameter required to compare the biomass productivities of microalgae is areal productivity. They recognized, however, that this parameter would be of little use in vertical or highly inclined systems because a considerable amount of solar radiation is not related to the land area that the PBR occupies. In these systems the photosynthetic efficiency (PE) of the cultures, defined as the fraction of light energy that is converted into chemical energy during photoautotrophic growth, together with their volumetric productivity would be preferred for evaluating productivities.

The PE and the productivity of microalgal cultures in outdoor PBRs are affected by the light regimes inside the reactor, which depend not only on the algal concentration, and the dimensions and the type of reactor, but also on the incoming light, which in turn depends on latitude and weather conditions.

In addition to PE, to describe the impact of climatic conditions on biomass productivity, (Zhang et al. 2001) used irradiation utilization efficiency (IUE), which they defined as the efficiency of solar irradiation conversion to biomass energy to describe the impact of climatic conditions on the biomass productivity.

Not only costless sunlight or unproblematic artificial light, but also novel techniques are undertaken into account to produce microalgae in PBRs to utilize solar irradiation completely, whereas approximately the 50% of it (Lehr and Posten, 2009) could be used

for photosynthesis. Supplying light to the reactor for producing microalgae with fiber optic cables, solar collectors, etc. is not a new idea (Feurmann and Gordon , 1999; An and Kim, 2000; Gordon, 2002; Bayless et al., 2003; Suh and Lee, 2003; Ono and Cuello, 2004; Chen et al., 2006; Chen et al., 2008; Lehr and Posten, 2009) but has not been applied even in pilot scale. Two of the recent articles focusing on this concept are published by (Zijffers et al. 2008a&b) which the goals of their studies were designing a PBR that benefits solar energy efficiently to use for producing biomass at such intensities.

Although there is little risk of contamination from other species in PBRs, *Chlorella*, *Spirulina* and *Synechocystis sp.* are again the species that are most commonly chosen because they provide commercial products efficiently and much is known about how they behave.

Reviewed bibliography shows that microalgae were produced not only to generate commercial products but also to capture CO_2.

Despite being a very important parameter, the fatty acid content of the species produced in PBRs has only been provided by (Torzillo et al. 1986 and Scragg et al. 2002), also volumetric productivities of photobioreactors might be higher than those of open ponds but the areal productivities were nearly within the same range.

1.2.3.3 Microalgae culturing summary:

To sum up, positive and negatives points of each way to produce microalgae are easy to understand from the previous comments.

From one point of view (private companies) open ponds are the chosen technology for biomass from microalgae production because it's the most studied, cheapest and easy to scale up. But seems to be that this technology can not improve the productivity per area, the limiting factors (light irradiation, temperature, water loosing, contamination, aeration, used culture) are difficult to improve even with genetic engineering.

Scientist community, on the other side, believe that PBR, is a relative new technology and can be improved a lot, specially because it have a lot of variable to drive and its possible to find a compromise between these and the final product needed, also because is possible to do a combination of techniques and can work not only on seasonal periods of time with very high productivity.

1.2.4 Microalgae concentration

Of the various biotechnological aspects involved in the production of photosynthetic microbial biomass, the separation of the cells from the culture solution is particularly important for determining the cost and quality of the product.

It is estimated that at least 20-30% of the total cost of producing biomass must be attributed to the process of recovery (Gudin & Thepenier, 1986; Pushparaj et al., 1993; Lee et al., 1998; Oh et al., 2001; Kim et al., 2005). This is mainly due to the size of the microalgae (0.5 –30 µm), the slight difference in their densities and the liquid in which they are contained, and the low concentrations in which they are found in the culture (around 1kg/m^3), which means that large amounts of water have to be processed.

To overcome these difficulties, at least two separation methods need to be combined. In-depth research is needed for each species if the best combination of separation methods is to be determined. The objectives, then, are to increase the concentration of microalgae and reduce the volumes (or flows) to be handled.

Various general guidelines can be followed to determine which combination of methods is best for obtaining biomass that is ready to use and best suited to the desired final product.

1.2.4.1 Sedimentation, flocculation sedimentation/flotation

Natural sedimentation can be the first step in concentration (pre-concentration). Some reports confirm that when CO_2 ceases to make a contribution to the culturing media, sedimentation occurs because of the weight of the microalgae species in a few hours (Olaizola, 2003; Heasman et al., 2001; Shelef et al., 1984). This sedimentation might be due to the consumption of reserve sources such as lipids, which are necessary if biodiesel has to be produced, and a series of fatty acids with high value aggregates such as EPA, ß-carotene, etc (Chisti, 2007; Molina Grima et al., 2003). It is then necessary to determine of the extent to which the intracellular content varies over a period of time without the contribution of the substrate being withdrawn.

When natural sedimentation is not fast enough, flocculants can help to concentrate the particles more easily in suspension. (Knuckey et al. 2006) defined flocculation as the coalescence of finely divided suspended solids into larger loosely packed conglomerates, a process used widely in industry to remove suspended solids, e.g. for

clarification of drinking water. In general, they say, the first stage of flocculation is the aggregation of suspended solids into larger particles resulting from the interaction of the flocculants with the surface charge of the suspended solids. The second stage involves the coalescing of aggregates into large flocs that settle out of suspension.

Flocculation is a more suitable method for harvesting microalgae than centrifugation (which is common on a lab scale but not so common on an industrial scale because of its high cost) and filtration (which can be used for large microalgae).

The flocculation mechanism depends on cell and flocculant charges. Microalgae cells have negative charges on the surface of cell walls (at natural water pH) which prevent cells from aggregating in suspension, so they should be neutralized by using flocculants. The efficiency of flocculation is affected by the concentration, the ionic strength, the zeta potential (ξ) – which is also called the electrokinetic potential (Somasundaran, 2006) –, the pH of the culture solution, the dosage of the flocculants and co-flocculants, the pH adjustment before or after the flocculants are added, and the mixing time and speed. The degree of salinity also influences the effectiveness of flocculants. If the ionic strength is high, flocculants cannot make proper bridges between cells and this leads to low efficiency.

Metal salts (aluminium sulphate, ferric chloride, ferric sulphate, etc) are largely preferred in flocculation processes, because they lead to high harvesting efficiencies.

The application of polymers in flocculation is by no means new. Ionic polymers are also called polyelectrolytes and characterised by their ionic properties. Compared with metal salts, cationic polyelectrolytes are less efficient at recovering microalgae (Pushparaj et al., 1993). Many studies have found that optimum flocculation occurs at polyelectrolyte dosages around the dosage that just neutralizes the particle charge or gives a zeta potential close to zero (Kleimann et al., 2005; Bolto and Gregory, 2007).

Even though metal salts and polyelectrolytes are efficient at harvesting microalgae, they can also cause toxicity in the final product or the rest of the solution.

Nevertheless, natural polymers such as chitosan, cationic starches or bentonite, which are used in the agro food industry to clarify wines, have cationic properties. Chitosan (Muzzarelli, 1977), a polymer that is obtained by deacetylating chitin using the KOH process (Bough et al., 1978), is the most prominent of the natural cationic polymers. It is known to be non-toxic and non-hazardous to human health and also enables the medium to be recycled after the process has been completed.

The downstream process after flocculation could be the parameter that determines which flocculants is most efficient and more environmentally friendly for the process as a whole.

1.2.4.2 Filtration

Another way of separating solids from liquids is to use a filter or membrane. Filtration mainly separates solids from liquid by retaining a fraction of those particles (>90%) that are larger than the diameter of the pores of the permeable membrane (cut-off), which has different pressures on both sides of the membrane.

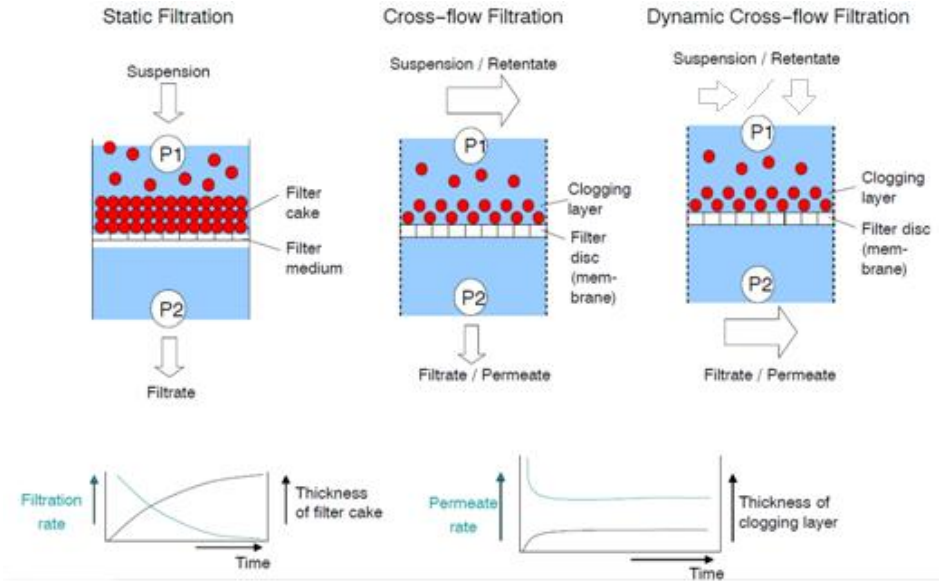


Figure 5 Principles of filtration techniques (modified from http://www.andritz.com)

Different filtration techniques are, static filtration (large cells), tangential or cross flow filtration where the shear stress over the membrane is performed by the pumping and finally the dynamic cross flow filtration where the membrane or a device next to the membranes are responsible to performed the shear stress by the movement (Figure 5).
At figure 5 it can see the different filtration techniques, where P1>P2 (transmembrane pressure TMP) and with the arrows showing how the currents are working, in our work, dynamic filtration have both currents permeate and retentate in a continuous movement.
To enhance flux capacity and optimal module operation, a variety of approaches can be used, focusing on the membrane (type parameters, including material, pore size, etc.),

the module (hydrodynamic parameters) or the feed slurry (physical parameters) (Belfort, et al. 1994)

Hydrodynamic parameters, such as velocity distribution, shear stress over the membrane, etc., can be modified by designing the membrane module or changing the operation conditions

Chemical changes can be made to membranes to improve particle repulsion and physical changes (such as particle size distribution using flocculation, aggregation, etc.) can be made to the feed solution to reduce membrane fowling.

Since some energy is needed for the slurry to flow and to give it a certain pressure, it is important that a sedimentation/flocculation step be performed before; to lower the volume to be processed if that is possible.

1.2.4.2.1 Static filtration:

Static filtration is a process in which the filtered slurry remains static, then the filter cake continues to grow thicker as filtration continues. Under static conditions, no cake erosion occurs. In theory, the filtrate volume increases as the square root of elapsed time. (Heasman et al. 2000) pointed out that very little importance has been given to the separation of microalgae through filtration. They also noted that filter papers and discs made from cellulose triacetate, felt filter socks and depth filters perform poorly when the entire solution is permeated.

This is performed through applying filtration as a step of direct purification, thereby proving that this method is sensitive to the variety of functions of algae. (Henderson et al. 2008) not only describe different properties of the microalgae species (morphology, mobility, surface load, cellular density, etc.) and their influence on separation methods, they also discuss the results of eliminating algae in the process of making water drinkable They also study the possibility of using filtration as a final step after flocculation and showed that 95% of microalgae could be separated by means of filters made from activated carbon, sand and gravel.

Although these filter types were not conceived for producing biomass, one specific case is reported in which, after filtering with diatomaceous clay, the cake is extracted with a small fraction of the filter (a scraping) and processed by direct extraction with organic solvent of ß-carotene of a filtered cake of *Dunaliella* (Ruane, et al.; 1977). These kinds of filters are not very efficient at obtaining ready-to-use biomass (Dodd, et al.; 1979).

(Shelef et al. 1984) described a battery of pressure and vacuum filters, highlighted its technical and design-related characteristics, and presented the pros and cons of each filter. In addition (Molina Grima et al. 2003) presented comparative tables based on a series of experiments in which (Mohn 1980) tested five different types of vacuum filters (a non-precoated vacuum drum filter, a vacuum drum filter precoated with potato starch, a suction filter, a belt filter and filter thickener) for *Coelastrum* where the initial biomass concentration was 0.1% and the final concentrations ranged from 5% to 37%.

Of the five filters tested in Mohn's experiment, two were found to be inadequate: the non-precoated vacuum drum filter, because of its high energy requirements, and the filter thickener, because of its low efficiency (after 15 minutes of operation, the filter clogged up). Depending on the species of microalgae being tested, the other three filters were found to be capable of good results.

In the same study, Mohn also tested five different types of pressure filters (a chamber filter press, belt press, pressure suction filter, cylindrical sieve and filter basket) for harvesting *Coelastrum*. The initial biomass concentration was 0.1% and the solids in the final concentrates ranged from 5% to 27%. The belt press was not recommended because the cake obtained was not dense enough unless flocculation was applied beforehand.

As well as these classical filtration processes, other types have also been studied by Mhon: for example, magnetic separation, deep-bed filtration, cartridge filters, vibrating screen filter and cross-flow ultra filtration. The conclusion they arrived is that they are not practical or the cost is too high for them to be used in industrial microalgae separation (Mohn, 1980). It must be highlighted that those studied were performed 3 decades ago, and from that time, the membrane and filtration technology have improved significantly.

1.2.4.2.2 Membrane cross-flow filtration:

The purpose of cross-flow filtration is to prevent membrane pore plugging, fowling, concentration polarization, etc. by creating a turbulent flow that is tangential to the membrane and causes good mixing. Filtration types can be classified according to the particle cut-off size as describe at table 1

The cost of the overall process of membrane cross flow filtration is mainly due to the energy needed to apply the force to obtain an inter-membrane pressure difference which

can be achieved by means of gravity, vacuum, pressure or centrifugal force and the fact that the used membranes have to be replaced periodically.

Table 1: Some characteristics and application of different membrane filtration domains

Filtration-domain	Cut-off (KDa)	Size (µm)	Application
Nano-filtration	0.1-1	0.001-0.008	Sucrose, chlorophyll
Ultra-filtration	0.8-1000	0.005-0.12	Virus, vitamins, proteins
Micro-filtration	>750	0.10-30	Bacteria, red blood cells

Microalgae are between 0.5 and 50 µm, so they are filtered using micro- filtration although some authors claim that ultra-filtration can avoid problems related to the characteristics of the substrate as it is explained the next.

Biological feeds are normally difficult to filter because the cake is really compressible. They can be highly fouling precursors especially because they are commonly a mix of organic matter of different sizes, shapes and compressibility. Also, concentration polarization phenomena may arise because the surface charge of the cells affects the interaction between the cell, the exogenous matter and the membrane surface. The filterability also depends on cell viability or when the cells are harvested. Low cell viability means that a large number of cells are dead or fragile and the resulting lysing releases matter into the medium, which can easily plug the membrane pores.

(Danquah et al. 2009) applied tangential flow filtration to a growth medium containing a *Tetraselmis suecica* / *Clorococum sp*. culture by means of a membrane with a cut-off diameter of 22µm. The authors carried out tests to determine the ideal transmembrane pressure (30psi) at which permeate is clean (i.e. free of microalgae) so that energy consumption could be optimized and the permeation rate could be maximum.

(Rossignol et al. 1999) tested micro- and ultra-filtration with flat membranes (polyacrylonitrile (PAN), polyvinylidene difluoride (PVDF), polyethersulfone (PES)) as a continuation of two different microalgae types (*Haslea ostrearia* and *Skeletonema costatum*) and evaluated the concentration obtained by varying parameters such as flow, trans-membrane pressure, concentration and suspension characteristics.

They conclude that the process is plausible for the type of species used, and point out that the best membrane for separation is PAN 40KDa cut off (ultra-filtration) at low trans-membrane pressure and tangential speeds of 0.2m/s.

As part of the MELiSSA project, (Rossi et al. 2004) tested 11 commercial organic membranes for the continuous harvesting of the cyanobacterium *Arthrospira platensis*

in order to obtain biomass for human consumption. The authors evaluated several parameters that influence permeation flux in both nano- and microfiltration: namely cut-off, pore size and membrane material (hydrophobic, hydrophilic or neutral). They also studied the phenomenon of membrane blocking.

Like the authors mentioned above, Rossi et al. found that the PAN 40 kDa cut-off membrane showed the best permeation and was the cleanest one after use. They evaluated a large scale process with longer times and more pump cycles, finding that these can injured the cells and it shout taken in account for industrially scale up for purposes of biodiesel or food.

(Rossi et al. 2008) pointed out the incidence of shear stress caused in the *Spirulina* by the pump, valves and the tangential filtration process in general, with the secretion of exo-polysaccharides (EPS) and their influence in membrane fouling. This fouling consists of a gel which blocks some of the membrane pores and reduces the flow of permeate to 15% compared to the filtration of fresh slurry working in conditions of critical and limited flow. These factors have to be considered when designing and evaluating this process type.

(Petrusevski et al. 1994) tested continuous tangential flow filtration for the purification of drinking water. They used a 0.45 μm pore-size membrane that gave good results, recovering 80-90% of the dispersed microalgae. The process did not affect the viability or growth of the algae, but it did reduce their motility by damaging the flagella.

(Jaouen et al. 1998) studied the extent to which the pump injured the microalgae during tangential filtration, and the viability and loss of mobility of the species T*etraselmis suecica*. Particular attention was paid to the relation between microalgae injury and the flow rate and number of pumping cycles in a closed system.

Further study is required of filter types, filtration methods and their incidence on microalgae species before it is determined which filtration process is best suited to the separation of microalgae for biodiesel.

An increase in microalgae concentration factor has a direct effect on membrane fouling. It leads to a considerable amount of energy and wasted time, which compromises the viability of the process.

In order to flush the foulants from the 50kDa PVC surface membrane (Zhang, Hu et al. 2010) proposed back wash with compressed air antifouling strategies. They also developed a mathematical model from the process and reported a volumetric reduction

factor (VRF) of 150 in 559 min. with an average flow rate of (45.5 L/m^2h), a surface membrane area of 0.12 m^2 and a trans-membrane pressure of (34 kPa)

They evaluated the cost of reaching the VRF in these conditions but their approach does not seem to me to be very reliable. If the initial flux remained constant ($90L/hm^2$) the VRF could not be 150. They may not have taken into account the membrane module price only the membrane price. Moreover the TMP they use (34.5 kPa) is very low for an ultra-filtration process according to the bibliography it should be between 100-500 kPa (Mengual 1989)

1.2.4.2.3 Membrane dynamic cross flow filtration:

Antifouling strategies are critically important for sustainable biomass concentration by filtration, and permeate flux must be high in continuous processes on an industrial scale. Large velocities and pressure gradients not only require too much energy from the pump, but also decrease the trans-membrane pressure (TMP), which means that the membrane process is not optimal.

The economic viability of microfiltration depends on the flux and the capacity of the module. The higher the flux, the faster the feed can be processed, which leads to higher productivities.

Table 2: Plateau permeability for several studies done with different membranes and algae species

Membrane	Microalgae species	Plateau permeability (L/h/m2/bar)	References.
Milipore 0.45 um	*Scenedesmus, Monoraphidium sp-. Navicula sp.*, etc	**20**	Petruevski et al (1995)
Polyacrylonitrile 40 kDa	*Haslea ostrearia*	**30**	Rossignol et al (1999)
Polyacrylonitrile 40 kDa	*Skeletonema costatum* (freeze dryed)	**60**	Rossignol et al (1999)
Polyacrylonitrile 40 kDa	*Arthrospita platensis*	**16.5**	Rossi et al (2004)
PVC UF 50kDa	*Scenedesmus cuadricauda*	**150**	Zhang et al (2010)
Ceramic 2um (DCF)	*N gaditana, P tricornutum* (Pre-treated)	**600**	Rios et al (2012)

To reduce the fouling effect, dynamic filtration can be used to maximize the velocity gradient over the membrane (shear stress) and turbulence. Dynamic cross flow filtration (DCF) reduces fouling phenomena and concentration polarization, which can substantially increase the permeate flux (Jp). Also, unlike in cross flow filtration, the inlet flow does not need to be much higher than this Jp. There are several types of dynamic filtration: for example, rotational system membranes or disks, vibratory systems, etc (Jaffrin 2008).

Figure 6 shows two representatives dynamic filtration systems a rotational and a vibrational one. Dynamic membrane filtration is gaining importance in a wide variety of applications such as chemicals, biotechnology, pharmaceuticals, etc.

(Ochirkhuyag, Mori et al. 2008) briefly described how dynamic cross flow filtration can be used with microalgae. They used a 0.1m^2 surface mono-disk, with 1µm filter paper over sintered polyethylene plates and a trans-membrane pressure (TMP) of 0.2 MPa. They obtained a permeate flux of 25L/h/m^2 at 400RPM.

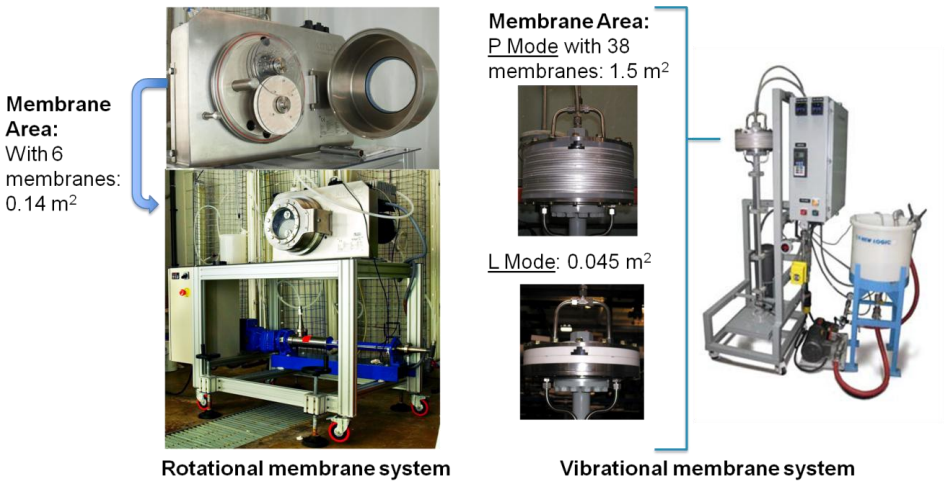


Figure 6 Dynamic membrane modules rotational (Krauss Maffei Process Technology) model Dynamic Crossflow Filter DCF 152/0,14) and vibrational system VSEP from new logic research inc. (property of IREC laboratories)

(Frappart, Massé et al. 2011) compared the performances and influence of hydrodynamic factors in tangential and dynamic ultra-filtration for microalgae separation. These authors reported about two times improvement on fluxes of dynamic filtration compared with tangential filtration.

Table 2 shows results from several recent permeabilities studies of microalgae tangential flow filtration (TFF) that choose ultra-filtration instead of microfiltration are at least one order of magnitude less than this study (Petruševski et al, 1995, Rossignol, et al 1999, Rossi, et al 2004).

Permeability at the plateau from the study made by (Zhang, et al 2010) presented the same order of magnitude but still 70% smaller than the great performance reached with the pre-concentrated slurry and the 600L/h/m^2/bar of permeability from (Ríos et al, 2012).These high concentrations and permeabilities are necessary for scale-up.

Table 2 show that, the use of microfiltration with different antifouling strategies is a promising method for concentrating microalgae because it performance according to permeability is 4 times higher than the best case of cross flow filtration, although is hard to compare because conditions between studies are different.

1.2.4.3 Centrifugation

Centrifugation is a process that increases the gravitational force on particles and, therefore, the rate of sedimentation. It is clearly the fastest of the various separation processes for this application, but it also requires the most energy.

Most microalgae can be harvested by means of centrifugation, although the time and intensity of the centrifugal force depends on the species in question and the relationship between its properties and those of the growth medium, since the method is based on the difference in density between the two substances.

Centrifugation can therefore be used to obtain a biomass that is free of flocculants and other chemical additives.

Three basic parameters must be taken into account when selecting this separation system: first, energy savings, which is especially relevant if the final aim is to obtain biodiesel; second, separation efficiency, as greater efficiencies require greater centrifugal force; and third, the degree of cell damage that the product may suffer under very intense centrifugal force, which may lead to the loss of intracellular content.

Although it is the most widely used method for this type of production, much of the information available on centrifugation dates back more than 20 years (Benemann et al., 1980; Mohn, 1980; Shelef et al., 1984; Chisti and Moo-Young, 1991).

More recent publications on the topic include (Heasman et al. 2000), who demonstrated the relationship between the efficiency of the concentration and the centrifugal force applied. Specifically, the authors found that 13000 x g yields >95% efficiency, 6000 x g yields 60% efficiency, and 1300 x g yields 40% efficiency, producing a final biomass to be used as food for oysters. The authors also highlighted that the viability of the cells was a function of the centrifugal force applied.

In the second part of the report, (Heasman et al. 2001) described a series of experiments conducted with nine different types of microalgae produced for animal consumption. Three types of centrifuges were used in the experiments: bucket centrifuge (800 x g), cream separator (3000 x g) and super centrifuge (13000 x g). The authors found that the super centrifuge provided the greatest separation efficiency, although the results depended considerably on the species and the energy required for each process.

On the basis of the work by (Mohn 1980), (Molina Grima et al. 2003), like (Shelef et al. 1984), drew up a comparative table of centrifugal systems for the harvesting of particular microalgae species. They provide figures of energy consumption and the percentage of separated solids as well as information about the use of hydrocyclones (static wall centrifuges), the separation efficiency of which is understandably low except when algae grow and form aggregates (*Coelastrum*).

(Shelef et al. 1984) described various types of centrifuge, discussed their design characteristics and their practicality, and stated whether or not they are recommended for concentrating microalgae.

(Morist et al. 2001) used a semi-continuous centrifuge with fixed revolutions of 10,000rpm but vary in the flow, the intermittent discharge time and the duration of discharge. They analysed the different parameters of slurry at both the entrance and exit of the centrifuge. By dissolving certain quantities of protein in the medium, they concluded that the longer the cells are exposed to this type of force, the higher the shear stress will be.

On the other hand, the percentage of water recovery is higher than 95% and the solid concentration factor varies among 20-30 for high flow-rates and high intermittent solids discharge time.

(Benemann 1996) cited cost estimation from Barclay 1987 who compared three, one step microalgae harvesting method: centrifugation, polyelectrolite flocculation and cross flow filtration, updating the price by inflation factor. The cost per ton on that year (1987) was ($1000, $450 and $500)/ton respectively for 4ha facility and producing 370mT/y of algal biomass and if all overhead, labour and other cost are apportioned.

For update the data to these time it should multiplicate those values by a factor of 2.12 according to CPI (consumer price index) from the United State department of labour (http://www.bls.gov/data/inflation_calculator.htm).

As it can see the centrifugation method is two time more expensive than others two method if it compared in only one step but it can improve drastically using these

method as a second step harvesting method starting with a initial concentration of 1-5% solids to produce an algae paste of 15-20% solids and according to (Benemann 1996) it cost of these second stage centrifugation is between $15-20/ton of biomass produced
Centrifugation process might be most suited to those cases in which the products are of high added value or cannot have any chemical additive for example, as that applied in the pharmaceutical industry, cosmetics, and so on. It might also be suited to the last phase of concentration, starting off from the damp biomass obtained through pre-concentration or natural sedimentation, depending on the algae type.

1.2.4.4 Concentration Summary

To sum up all the factors presented above, the following aspects should be considered if a process for producing biomass that can be used as biodiesel is to be viable:
Firstly, the chosen microalgae must have high fatty-acid content, and be capable of natural sedimentation in a few hours without losing any of these fatty acids. Secondly, once the slurry that has been separated from the damp biomass by sedimentation or flotation with or without flocculants (depending on the final use of the biomass), it is necessary to find a compromise for the last concentration step between filtration, centrifugation or direct drying.
For other side the damp biomass could be processed by direct liquefaction (Vishwanath et al, 2008) to obtain fuel. This involves working directly with water in critical conditions and using the high water activity to decompose the organic material into molecules of high-density energy and valuable chemical compounds.
Although it seems that this option should be viable, the energy balances and costs of processing the water to reach these conditions, which are super critical, are not provided.

1.2.5 Microalgae lipid extraction

Lipids are substances that usually are soluble in organic solvents (such as ether, hexane, chloroform, etc) but insoluble in water. This group of substances includes neutral lipids such as triglycerides (TAG), diglycerides (DAG), monoglycerides (MAG), free fatty acids (FFA) and polar lipids such as phospholipids, sterols, carotenoids, vitamins A and D, etc.

The lipid fraction of microalgae cells, therefore, contains a complex mixture of different types of molecule. Triglycerides can be the largest component of most microalgae cells, although this depends on several parameters applied to the production and harvesting processes such as nutrients, starvation, growing phase, harvesting phase, method of harvesting, etc.

The lipids that are useful for biodiesel production are a fraction of all the saponifiable ones, the neutral, and they are usually in the cell cytoplasm in the form of 30 nm or so droplets (Ranjan, Patil et al. 2010). In contrast, saponifiable polar lipids such as phospholipids, glycolipids, etc. are constituents of the cell wall and organelle wall and together with the unsaponifiable fraction which consisted of small amounts of carotenoids, sterols, tocopherols and retinoids are not suitable for biodiesel due to their emulsifying properties and negative effect on Cold Soak Filtration times and consequently cold weather fuel performance (Knothe 2008)

The extraction of lipids basically consists of a mass transfer operation whose precise nature is determined by both process component, the nature of the solutes and the solvent polarities (Ranjan, Patil et al. 2010). The extraction mechanism might be diffusion across the cell wall when the biomass is suspended in a high lipids affinity solvent and/or the physically disruption of the cell walls to reach the lipids. To improve the lipid extraction all these parameters should be optimized.

Several extraction methods based in the classic Folch and Bligh and Dyer (Iverson, Lang et al. 2001) methods are still used for lipid quantification. Other techniques include fluorimetric estimation (i.e. nile red, BODIPY) to directly quantify the neutral fraction of the lipids (Govender, Ramanna et al. 2012). Several solvent mixes using soxhlet apparatus or, more recently, supercritical extraction by CO_2 (Halim, Gladman et al. 2011) or solvent combined with CO_2 supercritical extraction (Halim, Danquah et al.).

None of above methods can qualify or separate the proportion of each lipid fraction present in the mix; for this liquid chromatography and thin layer chromatography need to be used (Molina Grima, Belarbi et al. 2003).

Some authors (Pruvost, Van Vooren et al. 2009; Ranjan, Patil et al. 2010) claim that microalgae have the potential as a raw material for use in biodiesel using gravimetric analysis of lipids leading to an overestimation of the feedstock's suitability and that it should therefore only be used to provide complementary data rather than being a determinant factor when evaluating the potentiality .

Triglycerides are esters derived from free fatty acids and a glycerol molecule. The fatty acids normally found in microalgae vary in chain length, degree of unsaturation and position on the glycerol molecule. Consequently, the triglycerides fraction itself consists of a complex mixture of different types of molecules. Each type of fat has a different profile of lipids which determines the precise nature of its physiochemical properties.

To quantify and identify the fatty acid methyl esters (FAMEs), a reaction of the lipids known as transesterification is usually carried out. This process includes an alcohol (methanol) in the presence of a catalyst (acidic or basic) and should be performed before the gas chromatography (GC) analysis to determine the FAME/TAG or FAME/TL or FAME/DW average.

The FAME composition can depend on several biomass variables (microalgae species, nutrients, salinity of the medium, time of harvesting, etc) the biomass harvest methods (sedimentation with or without chemical flocculation, physical method of separation, etc), the storage method (freeze, dry, freeze-dry, wet, etc) and the lipid extraction method (supercritical extraction, solvent extraction, type of solvent used, temperature conditions, etc).

Whichever lipid extraction method is chosen, the sample should be prepared and characterized and the process variables (reaction time, temperature, amount of sample or solvent, etc.) should be optimized.

The sample preparation must take into account the different elements of biomass in the sample because the harvesting method may have added or reduced the levels of ash or organic matter, especially if chemical flocculation is used. Alternatively, the levels of organic matter in the sample can be standardized or the ash can be removed.

Many studies (Guckert, Cooksey et al. 1988; Volkman, Jeffrey et al. 1989; Servel, Claire et al. 1994; Fajardo, Cerdán et al. 2007) on different microalgae from different mediums and water quality have used the dry weight of the biomass as the base of the study underestimating the lipid yield, as example fresh water specie have less ash than sea waters species and so the biomass must be standardized by ash-free dry weights. Also some other species have high silica content that makes a lot of final ash.

Direct transesterification (DT) method have been reported as a single step extraction and FAME production reaction (Vicente, Bautista et al. 2009; Hempel, Lau et al. ; McNichol, MacDougall et al. 2012) to analyze FAME content, but DT could be not only an analysis method but also a potential way to overcome the biodiesel production directly from the dry solid biomass, especially using membrane reactors.

1.3.- References

- Acien Fernandez, F., G., Fernandez Sevilla, J., M., Sanchez Perez, J., A., Molina Grima, E., Chisti, Y. 2001. Airlift-driven external-loop tubular photobioreactors for outdoor production of microalgae: assessment of design and performance. Chemical Engineering Science 56, 2721-2732.
- Agarwal, A., K., 2007. Biofuels (alcohols and biodiesel) applications as fuels for internal combustion engines. Progress in Energy and Combustion Science 33:233-271.
- An, J-Y, Kim, B-W, 2000. Biological desulfurization in an optical-fiber photobioreactor using an automatic sunlight collection system. J Biotechnol, 80:35-44.
- Aragon, A., B., Padilla, R., B., Ursinos J., A., 1992. Experimental study of the recovery of algae cultured in effluents from the anaerobic biological treatment of urban wastewaters. Resources, Conservation and Recycling, 6, p: 293-302.
- Asenjo, J., A., Merchuk, J., C., 1994. Bioreactor system design. Marcel Dekker, New York, pp 139–552.
- Barclay, W., K. Terry, N. Nagle, J. Weissman, and R.P. Goebel, 1987. "The Potential of New Strains of Marine and Inland Saline-Adapted Microalgae for Aquaculture Applications", J. World Aquaculture SOC.. 18: 216 - 228.
- Bayless, D., J., Morgan, L., Kremer, G., G., 2003. Enhanced practical photosynthetic CO2 mitigation. United States Patent 6667171 with the assignee of Ohio University, OH (US).
- Becker, W. 2004. Microalgae for aquaculture. The nutritional value of microalgae for aquaculture. In: A. Richmond (ed.). Handbook of microalgal culture. Blackwell Science, Oxford, pp. 380-391
- Beijerinck, M.W., 1890. Kulturversuche mit Zoochloren, Lichenengonidien und anderen niederen Algen. Bot. Ztg. 48, 725–785.
- Belfort, G., R. H. Davis, et al. (1994). "The behavior of suspensions and macromolecular solutions in crossflow microfiltration." Journal of Membrane Science **96**(1-2): 1-58.
- Benemann JR, Oswald WJ (1996), Systems and economic analysis of microalgae ponds for conversion of CO2 to biomass. Final report. US DOE http://www.osti.gov/bridge/servlets/purl
- Benemann, J., R., Kopman, B., L., Weismsman, D., E., Eisenverg, D., E., Goebel, R., P., 1980. Development of microalgae harvesting and high rate ponds technologies in California. from "Algal Biomass" by Shelef, G. & Soeder, C.J. p. 457.
- Biodiesel 2020, Global Market Survey, Feedstock Trends and Market Forecasts" Introduction and Executive Summary, Emerging Markets Online. www.emerging-markets.com.
- Bolto, B., Gregory, J., 2007. Organic polyelectrolytes in water treatment. Water Reserach, 41, p: 2301 – 2324.
- Borowitzka, M., A., 2005. Carotenoid production using microorganisms. Cohen, Z. & Ratledge, C. (eds.) Single Cell Oils. AOCS Press, Champaign, Illinois. pp. 124-137.
- Bough, W., A., Salter, W., L., Wu, A., C., M. & Perkins, B., E., 1978. Influence of manufacturing variables on the characteristics and effectiveness of chitosan products. I. Chemical composition, viscosity and molecular-weight distribution of chitosan products. Biotech. Bioeng., 20, 1931-43.
- Boussiba, S., E. Sandbank, G. Shelef, Z. Cohen, A. Vonshak, A. Ben-Amotz, S. Arad and A. Richmond, 1988. Outdoor cultivation of the marine microalga *Isochrysis galbana* in open raceways. Aquaculture, 72: 247-253.

- BP, Statistical Review of World Energy, June 2011 (www.bp.com/statisticalreview)
- Buelna, G., Bhattarai, K.K., Noue, J., Taiganides, E.P., 1990. Evaluation of various flocculants for the recovery of algal biomass grown on pig-waste, Biological Wastes 31, p :211-222.
- Carlsson, A., Van Bilein, J., B., Möller, R., Clayton, D., Bowles, D., 2007. Outputs from EPOBIO Project: Micro and macroalgae utility for industrial application. York, UK:CPL Press.
- Carvalho, A., P., Meireles L., A., and Malcata F. X., 2006. Microalgal Reactors: A Review of Enclosed System Designs and Performances. Biotechnol. Prog., 22, p: 1490-1506.
- Chen, C-Y, Lee, C-M, Chang, J-S, 2006. Feasibility study on bioreactor strategies for enhanced photohydrogen production from Rhodopseudomonas palustris WP3-5 using optical-fiberassisted illumination systems. Int J Hydrogen Energy 31:2345-2355.
- Chen, C-Y, Saratale, GD, Lee, C-M, Chen, P-C, Chang, J-S, 2008. Phototrophic hydrogen production in photobioreactors coupled with solar-energy-excited optical fibers. Int J Hydrogen Energy 33:6886-6895.
- Chisti, Y., 2007. Biodiesel from microalgae. Biotechnology Advances 25 (3): 294-306.
- Chisti, Y, Moo Young, M, 1991. Fermentation technology, bioprosessing, scale-up and manufacture. In Moses M, Cape RE, editors. Biotechnology: The science and the business. New York: Hardwood Academic Publishers p.167-209.
- Danquah M., K., Gladman, B., Moheimani, N., Forde, G., F., 2009. Microalgal growth characteristics and subsequent influence on dewatering efficiency. Chemical Engineering Journal, vol. 151, nº1-3, pp. 73-78.
- Demirbas, A., 2009. Progress and recent trends in Biodiesel fuels. Energy Conversion and Management 50, p:14-34.
- Demirbas, A., 2008. Biofuels sources, biofuel policy, biofuel economy and global biofuel projections. Energy Conversion and Management, Volume 49, Issue 8 Pages:2106-2116.
- Demirbas, A. 2007. Importance of biodiesel as transportation fuel. Energy policy, 35, 4661-4670.
- Divakaran, R., Sivasankara Pillai, V.N., 2002. Flocculation of algae using chitosan. Journal of Applied Phycology 14, p: 419-422.
- Donghui, S., Jingjuan, F., Dingji, S., 2008. Exploitation of Oil-bearing Microalgae for Bio-diesel. Chinese Journal of Biotechnology, 24(3), 341-348.
- Doucha, J. and Livansky, K., 1995. Novel outdoor thin-layer high density microalgal culture system: Productivity and operational parameters. Algol. Stud. **76**, pp. 129–147.
- Doucha, J. and Livansky, K., 2000. Productivity of the microalga *Chlorella kessleri* in outdoor open thin-layer batch cultures. Algological studies 97, Arch. Hydrobiol Suppl. 103-122.
- Dodd, J., C., 1979. Algae production and harvesting from animal wastewater. Agric Wates 1:23-37.
- U.S Energy Information Administration (EIA) in International Energy Outlook Projection 2011, (www.eia.doe.gov/oiaf/ieo/index.html).
- Feuermann, D., Gordon, J., M., 1999. Solar fiber-optic mini-dishes: a new approach to the efficient collection of sunlight. Solar Energy, 65:159-170.
- F.O.Lichts World Ethanol and Biofuel Report "World ethanol production growth to slow down in 2009" October 23, 2008. Vol.7 No.4.

- Frappart, M., A. Massé, et al. (2011). "Influence of hydrodynamics in tangential and dynamic ultrafiltration systems for microalgae separation." Desalination **265**(1-3): 279-283.
- Frondel, M. and Peters, J., 2007. Biodiesel: A new Oildorado?. Energy Policy 35, p: 1675-1684.
- The Gallagher review of the indirect effects of bioofuel production (2008) Renewable Biofuel Agency (www.renewablefuelsagency.org).
- Gordon, J., M., 2002. Tailoring optical systems to optimized photobioreactors. Int J Hydrogen Energy, 27:1175-1184.
- Granada, C., Zhu L., and Hotzapple M., 2007. Sustainable Liquid Biofuels and Their Environmental Impact. Environmental Progress Vol.26, no:3 , p:.233-238.
- Granado Lorencio, F., Herrero Barbudo, Acien Fernandez, G., Molina Grima, E., Fernandez Sevilla, J., M., Pérez Sacristán, B., Blanco Navarro, I., 2009. In vitro bioaccesibility of lutein and zexanthin from microalgae Scenedesmus almerienses. Food Chemistry 114:747-752.
- Gudin, C. and Thepenier, C. 1986. Bioconversion of solar energy into organic chemicals by microalgae. Adv. Biotechnology Processes, 6, 73-110.
- Gutierrez, L.,F., Sánchez Ó.,J., Cardona, C., A, 2009. Process intergration possibilities for biodiesel production from palm oil using ethanol obtained from lignocellulosic residues of oil palm industry. Bioresource Technology 100:1227-1237.
- Hase, R., Oikawa, H., Sasao, C., Morita, M., **Watanabe** Y, 2000. Photosynthetic production of microalgal biomass in a raceway system under greenhouse conditions in Sendai city. Journal of bioscience and bioengineering, Vol.89, No:2, 157-163.
- Heasman, M., Diemar, J., O'Connor W., Sushames, T., Foulkes, L., Nell, J., A., 2000. Development ofextended shelf-life microalgae concentrate diets harvested by centrifugation for bivalves mollusks - a summary". Aquaculture Research 31(8-9):637-59.
- Heasman, M., P., Sushames, T., M., Dionar, J., A., O'Connor, W., A. and Foulkes L., A., 2001. Production of micro-algal concentrater for aquaculture (part 2). Development and evaluation of harvesting, preservation, storage and feeding technology. NSW fisherie final report series Nº34 ISSN 1440-3544.
- Henderson, R., Parson, S.,A., Jefferson, B., 2008. The impact of algal properties and pre-oxidation on solid-liquid separation of algae. Water Research 42:1827-184.
- Hill, J., Nelson, E., Tilman, D., Polansky, S., Tiffany, D., 2006. Environmental, economic, and energetic costs and benefits of biodiesel and ethanol biofuels. PNAS,vol 103, no. 30, Pages 11206-11207.
- Hu, Q., Guterman, H, and Richmond A., 1996. A Flat Inclined Modular Photobioreactor for Outdoor Mass Cultivation of Photoautotrophs. Biotechnology and Bioengineering, Vol. 51, p. 51-60.
- International Energy Agency2011 http://www.worldenergyoutlook.org/
- Jacob-Lopes, E., Ferreira Lacerda, L., M., C., Teixeira Franco, T., (2008) Biomass production and carbon dioxide fixation by *Aphanothece microscopica Nageli* in a bubble column photobioreactor. Biochemical Engineering Journal 40, p: 27–34.
- Jaffrin, M. Y. (2008). "Dynamic shear-enhanced membrane filtration: A review of rotating disks, rotating membranes and vibrating systems." Journal of Membrane Science **324**(1-2): 7-25.
- Jaouen P, Vandanjon L, Quémémeneur F (1998) "The shear stress of microalgal cell suspensions (*Tetraselmis suecica*) in tangencial flow filtration sistems: the role of pumps" Bioresource Technology 68:149-154.

- Jiménez, C., Cossío, B., R., Labella, D. and Niell, F., X., 2003. The Feasibility of industrial production of *Spirulina* (*Arthrospira*) in Southern Spain. Aquaculture 217, 179-190.
- Jiménez, C., Cossío, B., R., Niell F., X., 2003. Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: a predictive model of algal yield. Aquaculture 221, 331-345.
- Kaewpingtong, K., Shotipruk, A., Powtongsook, S., Pavasant, P., 2007. Photoautotrophic high-density cultivation of vegetative cells of Haematococcus pluvialis in airlift bioreactor. Bioresourse Technol.98, 288-295.
- Kawaguchi, 1980 Microalgae production systems in Asia, In G.Shelaf & C.S. Soeder (Eds.) Algae Biomass. Elsevier/North-Holland Biomedical Press, Amsterdam: 25-33
- Kim S. -G., Choi A., Ahn C. -Y., Park C. -S., Park Y. -H. and H. -M., Oh., 2005. Harvesting of Spirulina platensis by cellular flotation and growth stage determination. Letters in Applied Microbiology, 40, 190–194.
- Kleimann, J., Gehin-Delval, C., Auweter, H., Borkovec, M., 2005. Super- stoichiometric charge neutralization in particle-polyelectrolyte systems. Langmuir 21 (8), 3688–3698.
- Knuckey, R.M., Brown, M.R., Robert, R. and Frampton D.M.F, 2006. Production of microalgal concentrates by flocculation and their assessment as aquaculture feeds. Aquacultural Engineering vol: 35, p: 300-313.
- Kwon, G., S., Moon, S., H., Hong, S., D, 1996. A novel flocculant biopolymer produced by *Pestalotiopsis sp*. KCTC 8637 P. Biotechnology Letters 18, p: 1459-1464.
- Lamers, P., P., Janssen, M., De Vos, R., C., H., Bino, R., J., Wijffels, R., H., 2008. Exploiring and exploiting carotenoid accumulation in Dunaliella salina for cell-factory application. Cell Press doi:10.1016/j.tibtech.2008.07.002.
- Leary, D., Vierros, M., Hamon, G., Arico, S., Monagle, C., 2009. Marine genetic resources: A review os scientific and commercial interest. Marine Policy 33, 183-194.
- Lee, S.J., Kim, S.-B., Kwon, G.-S., Yoon, B.-D. and Oh, H.-M., 1998. Effects of harvesting method and growth stage on the flocculation of the green alga Botryococcus braunii. Letters in Applied Microbiology 27, 14–18.
- Lehr, F. and Posten, C., 2009. Closed photo-bioreactors as tools for biofuel production. Current Opinion in Biotechnology 20: 280-285.
- Mazzuca Sobczuk, T., Garcia Camacho, F., Molina Grima, E., Chisti, Y., 2006. Effects of agitation on the microalgae *Phaeodactylum tricornutum* and *Porphyridium cruentum*. Bioproc. Biosyt. Eng. 28, 243-250.
- Mendola, D., 2003. Aquaculture of three phyla of marine invertebrates to yield bioactive metabolites: process developments and economics. Biomolecula Engineering 20:441-458.
- Mengual, J. A. I. (1989). Fundamentos de los procesos de transporte y separación en membranas. Murcia, EDITUM-Universidad de Murcia. **1:** 171 páginas.
- Mohn, F., H., 1980. Experiences and strategies in the recovery of biomass from mass culture of microalgae. in Shelaf G, Soeder CJ, editors. Algae Biomass. Amsterdam: Elsevier p.547-71.
- Molina Grima, E., Belarbi, E., H,, Acién Fernández, F., G., Robles Medina, A., Chisti, Y. 2003. Recovery of algal biomasa and metabolites: process options and economic. Biotechnology Advanced 20, 492-515.

- Molina Grima, E., Acien Fernandez, F., Garcia Camacho, G., F., Chisti, Y., 1999. Photobioreactors: light regime, mass transfer, and scaleup Journal of Biotechnology 70 231–247.
- Molina Grima, E., Acien Fernandez, F., Chisti, Y., 2001. Tubular photobioreactor design for algal cultures Journal of Biotechnology 92, 113–131.
- Morist, A., Montesinos, J., L., Cusidó, J., A., Gódia, F., 2001. Recovery and treatment of spirulina platensis cells cultured in a continuous photobiorreactor to be used as food. Process Bioceistry 37:535-547.
- Muzzarelli, R. A. A., 1977. Chitin. Pergamon Press Ltd., Oxford, 247- 253, 207-211.
- Narayanan, D., Zhang, Y. and Mannan, M. S., 2007. Engineering for sustainable development (ESD) in Bio-Diesel production. Institution of Chemical Engineers, DOI:10.1205/psep07016.
- Ochirkhuyag, B., T. Mori, et al. (2008). "Development of a high-performance cake-less continuous filtration system." Chemical Engineering Science **63**(21): 5274-5282.
- Olaizola, M., 2003. Commercial development of microalgal biotechnology: from the test tube to the marketplace. Biomolecular Engineering 20:459-466.
- Oh, H-M., Lee, S.J., Park, M-H, Kim, H-S., Kim, H-C., Yoon, J-H., Kwon G-S., Yoon, B-D., 2001. Harvesting of Chlorella vulgaris using a bioflocculant from Paenibacillus sp. AM49. Biotechnology Letters, Volume 23, Number 15, pp. 1229-1234(6).
- Ono, E, Cuello, JL, 2004. Design parameters of solar concentrating systems for CO2-mitigating algal photobioreactors. Energy, 29:1651-1657.
- Packer, M., 2009. Algal capture of carbon dioxide;biomass generation as a tool for greenhouse gas mitigation with reference to New Zeland energy strategy and policy. Energy Policy doi:10.1016.
- Palligarnai, T.,V. and Briggs, M., 2008. Biodiesel production- current state of the art and challenges. J Ind Microbiol Biotechnol 35, p: 421-430.
- Petruesevski, B., Bolier, G., Van Breemen, A., N., Alaerts, G., J., 1994. Tangential flow filtration: a method to concentrate freshwater algae. Water Research 29:1419-1424.
- Phoochinda, W., White, D. A., Briscoe, B.J., 2004. An algal removal using a combination of flocculation and flotation processes. Environmental Technology, Vol: 25, p: 1385- 1395.
- Powell, E., E., Hill, G., A., 2009. Economic assessment of an integrated bioethanol- biodiesel-microbial fuel cell facility utilizing yeast and photosynthetic algae. Chemical Engineering Research and Design 8 7: 1340–1348.
- Pushparaj, B., Pelosi, E., Torzillo, G., Materassi, R., 1993. Microbial biomass recovery using a synthetic cationic polymer. Bioresource technology, vol. 43, nº1, pp. 59-62.
- Reijnders, L., 2006. Conditions for the sustainability of biomass based fuel use. Energy Policy, Volume 34, Issue 7, Pages 863-876.
- Richmond, A., 2004a. Handbook of Microalgal Culture Biotechnology and Applied Phycology, Blackwell Publishing Company, USA, p:179.
- Richmond, A., 2004b. Handbook of Microalgal Culture Biotechnology and Applied Phycology, Blackwell Publishing Company, USA, p:183
- Richmond, A., 1992. Open systems for the mass production of photoautotrophic microalgae outdoors: physiological principles. Journal of Applied Phycology 4: 281-286.
- Ríos, S.D., Salvadó, J., Farriol, X., Torras, C. 2012. Antifouling microfiltration strategies to harvest microalgae for biofuel. *Bioresource Technology*.

- Rosenberg, J., N., Oyler, G.,A., Wilkinson, L., Betenbaugh, M.,J., 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Current Opinion in Biotechnology 19:430-436.

- Rossignol, N., Vandanjon, L., Jaouen, P., Quéméneur, F., 1999. Membrane technology for the continuous separation microalgae/culture medium: compared performances of cross-flow microfiltration and ultrafiltration. Aquaculture Engineering 20:191-208.

- Rossi, N., Jaouen, P., Legentilhomme, P., Petit, I., 2004. Harvesting of *Cianobacterium Arthrospira platensis* using organic filtration membrane. Institution of Chemical Engineers 82:244-250.

- Rossi, N., Derouiniot-chaplain, M., Jaouen, P., Legentilhomme, P., Petit, I., 2008. *Arthrospira platensis* Harvesting with membranes: Fouling phenomenon with limiting and critical flux. Bioresource Technology 99:6162-6167.

- Ruane, M., 1977. Extraction of caroteniferous material from algae. Australian patent No 7, 239, 574.

- Rupprecht , J., 2009. From systems biology to fuel- *Chlamydomonas reinhardtii* as a model for a systems biology approach to improve biohydrogen production. Journal of Biotechnology 142: 10–20

- Samasundaran, P., 2006. Encyclopedia of Surface & Colloidal Science, 2nd Edition, Taylor and Francis Press, 4th volume, p: 2527

- Sanchez Miron, A., Contreras Gomez, A., Garcia Camacho, F., Molina Grima, E., Chisti, Y., 1999. Comparative evaluation of compact photobioreactors for large-scale monoculture of microalga. iotechnology 70 , 249–270.

- Schneider, D., 2008. http://www.americanscientist.org/issues/num2/grow-your-own/1 © Sigma Xi, The Scientific Research Society

- Schugerl, K., Lucke, J., 1977. Bubble column bioreactors: tower bioreactor without mechanical agitation. Adv Biochem Eng 7:1–84

- Scragg, A., H., Illman, A., M., Carden, A., Shales, S., W., 2002. Growth of microalgae with increased calorific values in a tubular bioreactor. Biomass and Bioenergy 23, p: 67 – 73.

- Scragg, A.,H., Morrison, J., Shales, S.,W., 2003. The use of a fuel containing *Chlorella vulgaris* in a diesel engine . Enzyme and Microbial Technology 33, 884–889.

- Sheenan, J., Dunahay, T., Benemann, J., Roessler, P., 1998. A look back at the US Department of Energy`s Aquatic Species Program – biodiesel from algae. National Renewable Energy Laboratory, Golden, Co. Report NREL/TP-580-24190.

- Shelef, G., Susenik, A., Green, M., 1984. Microalgae harvesting and processing: A literature review. US. Department of Energy NoDE-ACO2-83CH10093

- Spolaore, O., Joannis-Cassan, C., Duran, E., Isambert, A., 2006. A Commercial application of micro-algae. Journal of Bioscience and Bioengineering, 101:87-96.

- Suh, IS, Lee, SB, 2003. A light distribution model for an internally radiating photobioreactor. Biotechnol Bioeng, 82:180-189.

- Torzillo, G., Pushparaj, B., Bocci, F., Balloni, Materassi, W., R., Florenzano G., 1986. Production of *Spirulina* Biomass in Closed Photobioreactors. Biomass l 1, 61-74

- Tran, N., H., Bartlett, J., R., Kannangara , G., S., K., Milev , A., S., Volk , H., Wilson M., A. ,(2010) "Catalytic upgrading of biorefinery oil from micro-algae" Fuel 89: 265–274

- Tredici M., R., Zittelli G., C., 1998. Efficiency of Sunlight Utilization: Tubular Versus Flat Photobioreactors. Biotechnology and Bioengineering, vol. 57, p: 187-197
- Ugwu, C., U., Ogbonna, J., C., Tanaka ,·H. , 2002. Improvement of mass transfer characteristics and productivities of inclined tubular photobioreactors by installation of internal static mixers. Appl Microbiol Biotechnol 58, p:600–607
- Ugwu, C., U., Ogbonna J., C. and Tanaka ,· H. , 2005. Light/dark cyclic movement of algal culture (*Synechocystis aquatilis*) in outdoor inclined tubular photobioreactor equipped with static mixers for efficient production of biomasa Biotechnology Letters 27, p: 75–78
- Ugwu, C., U., Aoyagi, H., Uchiyama, H., 2008. Photobioreactors for mass cultivation of algae. Bioresource Technology 99, 4021–4028.
- Um, B-H, and Kim Y-S, 2009. Review: A chance for korea Advance algal-biodiesel technology. Journal of Industrial and engineering Chemistry doi10.1016/j.jiec.2008.08.002
- US Department of Energy "Energy efficiency and renewable Energy (biomass program) http://www1.eere.energy.gov/biomass/integrated_biorefineries.html
- Vilchez, C., Garbayo, I., Lobato, M., V., Vega, J., M., 1997. Microalgae-mediated chemicals production and wastes removal. Enzyme and Microbial Technology 20, 562-572.
- - Vishwanath Patil, Khanh-Quang Tran and Hans Ragnar Giselrød (2008) Towards Sustainable Production of Biofuels from Microalgae *Int. J. Mol. Sci* , *9*, 1188-1195; DOI: 10.3390/
- Warburg, O., 1919. U8 ber die Geschwindigkeit der Kohlensa urezusammensetzung in lebenden Zellen. Biochemische Zeitschrift 100, 230–270.
- Watanabe, Y. and Saiki, H., 1997. Development of a Photobioreactor Incorporating *Chlorella* sp. For Removal of CO_2 In Stack Gas, Energy Convers. Mgmt Vol. 38, Suppl., pp. 499-503,
- Wiffels, R., H., 2007. Potential of sponges and microalgae for marine biotechnology. Cell Press doi:101016/j.biotech.2007.10.002
- Xiaoling, M. and Quingyu, W., 2006. Biodiesel production from heterotrophic microalgal oil. Bioresour Technol; 97, 841-6
- Xin, M., Jianming, J., Xin, X., Lei, Z., Qingjuan, N., Mo, X., 2008. Biodiesel production from oleaginous micro-organisms. Renewable Energy, doi: 10.1016/ j.renene.2008.04.014
- Zhang, K., Miyachi, S. & Kurano, N., 2001. Photosynthetic performance of a cyanobacterium in a vertical flat-plate photobioreactor for outdoor microalgal production and fixation of CO_2 . Biotechnology Letters 23: 21–26, 2001.
- Zhang, K., Kurano, N., Miyachi, S., 2002. Optimized aeration by carbon dioxide gas for microalgal production and mass transfer characterization in a vertical flat-plate photobioreactor. Bioprocess Biosyst Eng 25, 97–101
- Zhang, X. Z., Q. Hu, et al. (2010). "Harvesting algal biomass for biofuels using ultrafiltration membranes." Bioresource Technology **101**(14): 5297-5304.

- Zijffers, J-WF, Janssen, M., Tramper, J., Wijffels, R.H,2008. Design process f an area-efficient photobioreactor. Mar Biotechnol 10:404-415.
- Zijffers, J-WF, Salim, S., Janssen, M., Tramper, J., Wijffels, R., H., 2008. Capturing sunlight into a photobioreactor: ray tracing simulations of the propagation of light from capture to distribution into the reactor. Chem Eng J, 145:316-327.

- Fajardo, A. R., L. E. Cerdán, et al. (2007). "Lipid extraction from the microalga Phaeodactylum tricornutum." European Journal of Lipid Science and Technology **109**(2): 120-126.
- Govender, T., L. Ramanna, et al. (2012). "BODIPY staining, an alternative to the Nile Red fluorescence method for the evaluation of intracellular lipids in microalgae." Bioresource Technology **114**: 507-511.
- Guckert, J. B., K. E. Cooksey, et al. (1988). "Lipid sovent systems are not equivalent for analysis of lipid classes in the microeukaryotic green alga, Chlorella." Journal of Microbiological Methods **8**(3): 139-149.
- Halim, R., M. K. Danquah, et al.2011 "Extraction of oil from microalgae for biodiesel production: A review." Biotechnology Advances.
- Halim, R., B. Gladman, et al. (2011). "Oil extraction from microalgae for biodiesel production." Bioresource Technology **102**(1): 178-185.
- Iverson, S., S. Lang, et al. (2001). "Comparison of the bligh and dyer and folch methods for total lipid determination in a broad range of marine tissue." Lipids **36**(11): 1283-1287.
- Knothe, G. (2008). ""Designer" biodiesel: Optimizing fatty ester composition to improve fuel properties." Energy and Fuels **22**(2): 1358-1364.
- McNichol, J., K. M. MacDougall, et al. (2012). "Suitability of soxhlet extraction to quantify microalgal fatty acids as determined by comparison with in situ transesterification." Lipids **47**(2): 195-207.
- Molina Grima, E., E. H. Belarbi, et al. (2003). "Recovery of microalgal biomass and metabolites: process options and economics." Biotechnology Advances **20**(7-8): 491-515.
- Pruvost, J., G. Van Vooren, et al. (2009). "Investigation of biomass and lipids production with Neochloris oleoabundans in photobioreactor." Bioresource Technology **100**(23): 5988-5995.
- Ranjan, A., C. Patil, et al. (2010). "Mechanistic assessment of microalgal lipid extraction." Industrial and Engineering Chemistry Research **49**(6): 2979-2985.
- Servel, M.-O., C. Claire, et al. (1994). "Fatty acid composition of some marine microalgae." Phytochemistry **36**(3): 691-693.
- Velasquez-Orta, S. B., J. G. M. Lee, et al. (2012). "Alkaline in situ transesterification of Chlorella vulgaris." Fuel **94**(0): 544-550.
- Vicente, G., Bautista, L.F., Rodríguez, R., Gutiérrez, F.J., Sádaba, I., Ruiz-Vázquez, R.M., Torres-Martínez, S., Garre, V. 2009. Biodiesel production from biomass of an oleaginous fungus. *Biochemical Engineering Journal*, **48**(1), 22-27.
- Volkman, J. K., S. W. Jeffrey, et al. (1989). "Fatty acid and lipid composition of 10 species of microalgae used in mariculture." Journal of Experimental Marine Biology and Ecology **128**(3): 219-240.

2.- Microalgae species characterization and production

Microalgae cells can be described as particles with distinct physical characteristics - morphology, surface charge, and density. To know such characteristics may provide advantage on the treatment requirements. This section provides information on cells production of the used microalgae species in this thesis; ***Phaeodactylum tricornutum*** **(Pht),** ***Nannochloropsis gaditana*** **(Nng) and** ***Chaetoceros calcitrans*** **(Chc)*;*** characteristics, and how those properties may influence downstream process requirements.

Figure 1 Vertical tubular 300L fotobiorreactors from different microalgae species tested in this work. *Phaeodactylum tricornutum, Nannochloropsis gaditana* and *Chaetoceros calcitrans*.

Figure 1 shows the microalgae slurry produced in vertical tubular photobiorreactors of 300L each for all species tested, the brownish one are the diatom species Pht and Chc; and the greenish one is the green algae specie Nng. The color variance is giving by the cell wall and different intracellular pigments which characterize the species where diatomaceous are more brownish than others microalgae types. The production sequences methodologies are giving at the end of the chapter.

This chapter was developed with the invaluable collaboration of Dr. Esther Clavero and Maria Pilar Rey Varela from IREC and the researchers and technicians of the Aquatic Ecosystems Research Group of IRTA (Sant Carles de la Ràpita, Tarragona, Spain) who help with the process of microalgae production for all the experiments performed.

2.1.- *Phaeodactylum tricornutum*

2.1.1 Taxonomy

Table 1: Taxonomy of *Phaeodactylum* species

TAXONOMY	
Phylum	Bacillariophyta
Class	Bacillariophyceae
Order	Naviculales
Family	Phaeodacthlaceae
Genus	*Phaeodactylum*
Species	*Phaeodactylum tricornutum* is the only species in genus *Phaeodactylum*
Studied species in our work	*Phaeodactylum tricornutum.*

(Lewin et al., 1958)

2.1.2 Biology

Phaeodactylum belongs to the pennate diatoms (Bacillariophyceae). It grows fast and has emerged as a model system for physiological, biochemical, and molecular studies mainly because of its ease of culture and the ability to be routinely genetically transformed (Scala et al., 2002, Montsant et al., 2005). *P. tricornutum* is one of the few microalgae species whose entire genome has been fully sequenced (Bowler, Allen et al. 2008) thus facilitating possible genetic modification for the purpose of increasing lipid productivity.

2.1.3 Structural and morphological features

Pht is unicellular or forms small cell clusters. Cells are circa 3 μm wide and 8 to 20 μm long (Lewin et al., 1958), and contain a single plastid (Round et al., 1990) (Figure 1). Unlike other diatoms it can exist in different morphotypes (*i.e.* fusiform, triradiate or oval) (Borkowitzka and Volcani, 1978).

In our studies fusiform morphotype of *P. tricornutum* was studied. Figure 2 shows microscope photos of the species.

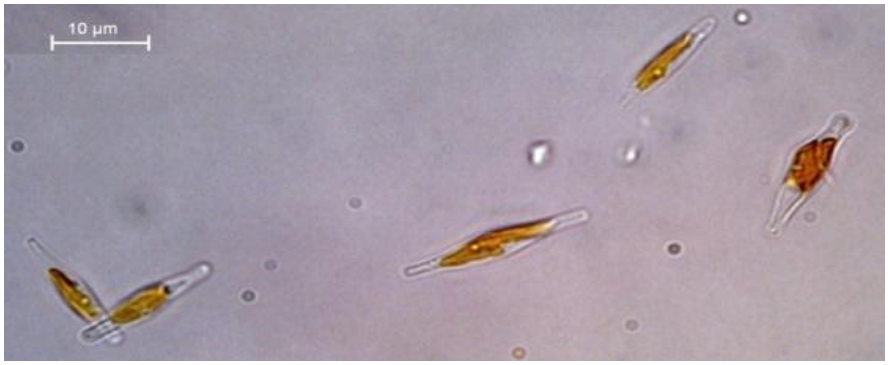


Figure 2 Microscopic view of the specie *Phaeodactylum tricornutum.*

2.1.4 Physiological characteristics

P. tricornutum has been found in several locations around the world, typically in coastal areas with wide fluctuations in salinity as well as in inland waters (Rushforth et al., 1988). They have ability of adaptation according to changing environmental conditions which could be related to the pleiomorphic character of the cells. The different morphotypes are also thought to be adapted for survival in different habitats. It is shown that the three morphotypes are physiologically different. Fusiform forms are common when grown in liquid media. Fusiform cells tend to transform into the ovoid morphotype when they are transferred on a solid medium (agar) while the reverse transformation occurs upon transfer in liquid medium. It has also been noted that fusiform and triradiate cells are more buoyant than oval cells (Lewin et al., 1958)

2.1.5 Biochemical composition

For *P. tricornutum*, different culture conditions result in significant variations in the biochemical composition of the cells of and, therefore, in their nutritious value.
The biomass of *Pht* contains on average; 36.4% crude protein; 26.1% available carbohydrates; 18.0% lipids, 15.9% ash. At low external irradiance, it is richer in protein and eicosapentaenoic acid (EPA) (Rebolloso-Fuentes et al., 2001).
The total lipid content and the fatty acid and lipid class (polar membrane lipids *vs.* neutral storage lipids) composition are dependent on different chemical (nutrient starvation, salinity, growth-medium pH) and physical stimuli (temperature and light

intensity). In addition growth phase and/or aging of the culture have an effect on oil content and composition.

It can see, in figure 3, a fluorescence microscopic view of the specie *Phaeodactylum tricornutum* after Nile Red dye, where the useful lipids for biodiesel production are a fraction of all neutral ones (saponifiable) and they usually are in the cell cytoplasm as a droplet of size around 30 nm or so (Ranjan, Patil et al. 2010) in figure 2 the shiny droplets are those neutral lipids.

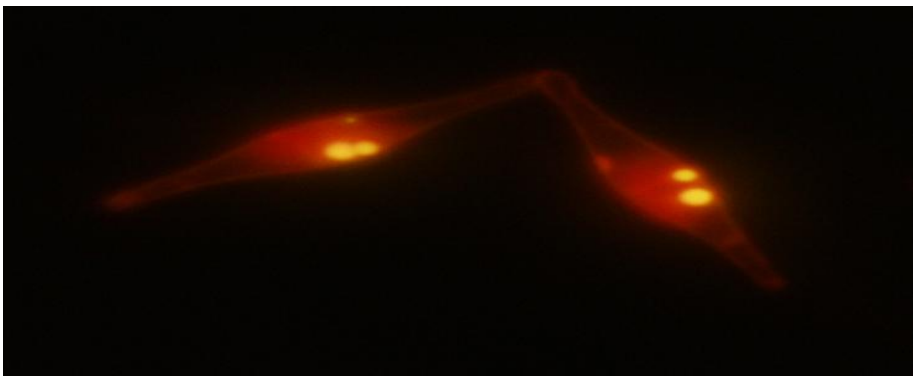


Figure 3 Florescence microscopic view of the specie *Phaeodactylum tricornutum* after Nile Red dye.

In *Pht* species, the major fatty acids could be the very-long-chain (VLC) fatty acids (VLC-Polyunsaturated fatty acids (PUFA)), arachidonic acid (AA) (C20:4ω6), eicosapentaenoic acid (EPA) (C20:5 ω3) and docosahexaenoic acid (DHA) (C22:6ω3). They compose approximately 30% of the total fatty acid content (Hu et al., 2008).

Pht possess also important advantages as a potential commercial producer of EPA (3.9–5% of cell dry weight (CDW)) (Meiser, Schmid-Staiger et al. 2004). The TAG yield from *Pht* is about 14% of total dry weight (Yu et al., 2009). Unlike in some other diatoms, culture age has almost no influence on the total fatty acid content in *P. tricornutum* that remained around 11% of CDW. Conversely, culture age has a greater impact on lipid classes, producing changes in amounts of triacylglycerols (TAG) which ranged between 43% and 69%, and galactolipids (GLs) that oscillated between 20% and 40%. In general, the content of polar lipids of the biomass decreased with culture age (Alonso *et al.*, 2000).

Carotenoids in *Phaeodactylum tricornutum* are low, ranging from 0.115 to 0.45 g/100g dry biomass. In *P. tricornutum*, chlorophylls are the main pigments (1.17 – 2.87g) (Rebolloso-Fuentes et al., 2001).

2.1.6 Growth kinetics and productivity

Growth rate and biomass productivity are influenced by environmental conditions, available resources and choice of culture system.

In general, *P. tricornutum* shows good growth at temperatures between 15 and 25 °C. For most isolates, growth ceases at temperatures above 30 °C. Furthermore, aeration affects to the growth of *P. tricornutum*, with higher growth rates under aerated conditions (Perez et al., 2008).

Temperatures above 35 °C are lethal for the algal culture; temperatures above 30 °C severely affect the growth rate but, by keeping the temperature below 28 °C, significant growth occurs. In order to achieve maximum productivity of 1.3 g/L in batch mode the following measures needs to be taken: pH must be kept at 7.7 by automatic CO2 injection, nutrient limitation must be prevented and oxygen saturation kept at less than 350 %. For a continuous mode, productivity of 1.4 g/L d was achieved (Fernández Sevilla, J.M., et al 2004).

2.1.7 Summary:

P. tricornutum is a popular species mostly used as a food source for the aquaculture industry because of its ease of cultivation and its rich oil content. It is widely used i as feed for penaid shrimp larve, freshwater, prawn larvae, bivalve larvae and postlarvae and marine zooplankton (Tredici et al., 2009).

P. tricornutum has been proposed as a source of eicosapentaenoic acid (EPA, 20:5ω3) (Veloso et al., 1991; Molina Grima et al., 1994). *Phaeodactylum* extracts are also used in cosmetics (Nizard et al., 2007) Fatty acids from *Phaeodactylum* have shown antibacterial activity (Desbois et al., 2009) and they were found in higher amounts in the fusiform than in the oval cell form (Desbois et al., 2010).

It is one of the popular species of interest for biodiesel production because of its ease of cultivation and its rich oil content. It is one of the few microalgae species whose entire genome has been fully sequenced that let also possible genetic modification for the purpose of increasing lipid productivity.

It is an example of a single celled, non-clumping, medium-sized diatom. It is known that fusiform morhphotypes are more buoyant than others.

2.2.- *Nannochloropsis gaditana*

2.2.1 Taxonomy

Table 2: Taxonomy of *Nannochloropsis* species

TAXONOMY	
Phylum	Heterokontophyta
Class	Eutigmatophyceae
Order	Eustigmatales
Family	Monodopsidaceae
Genus	*Nannochlopsis*
Species	*N.gaditana, N.granulata, N. limnetica, N. oculata, N. salina*
Studied species in our work	*Nannochloropsis gaditana*

Karlson *et al.*, 1996

2.2.2 Biology

Nannochloropsis cells (Karlson et al., 1996; Fawley and Fawley, 2007) are non-motile, spherical to ovoid, 2–4 μm in diameter algae cells. This genus includes a variety of algal species which has taken the attention of aquaculture, food and biofuels industries as well as the nutraceutical market. *Nannochloropsis* has especially been proposed as feedstocks for biodiesel production because of its ability to accumulate up to 60% lipid under nitrogen starvation (Rodolfi et al., 2009). *Nannochloropsis* is considered a promising alga for industrial applications because of its ability to accumulate high levels of polyunsaturated fatty acids (eicosapentaenoic acid). *Nannochloropsis* is also used as an ingredient in cosmetic products (Tredici et al., 2009).

2.2.3 Structural and morphological features & Physiological characteristics

Nannochloropsis cells are non-motile, spherical to ovoid, 2–4 μm in diameter (Figure 4). They have a single chloroplast lacking a pyrenoid and containing chlorophyll*a*. Major carotenoids are violaxanthin, vaucheraxanthin and neoxanthin, besides β-carotene. Among others, algae of this genus can synthesize low amounts of canthaxanthin and astaxanthin. The composition of the cell wall is still unclear. Small

retractile bodies can also be observed within the cytoplasm and can be both mobile (Brownian motion) or immobile.

Among the microalgae the most important group from the biotechnological point of view is represented by the eustigamotphytes.

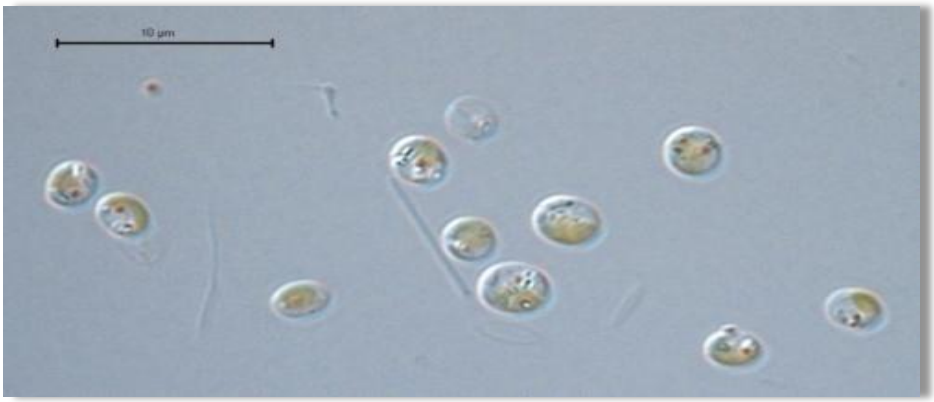


Figure 4 Microscopic view of the specie *Nannochloropsis gaditana*

2.2.4 Biochemical composition

Under artificial light in a photobioreactor, an average dry biomass composition of *Nannochloropsis* species was found as 29% protein, 36% carbohydrate, 18% lipid, 9% ash, and plamitic, palmitoleic, oleic and eicosapentaenoic acid (on average 2.2%) as major fatty acids (Rebelloso Fuentes et al. 2001) where (Brown et al. 1998) report a different composition for *Nannochloropsis* CS-246 tested for oyster rearing: 17% protein, 23% carbohydrate, 26% lipid, 16% ash.

Nannochloropsis is widely cultivated for its high content of eicosapentaenoic acid (EPA; 20:5 ω3), present in amounts up to 4-5 % of the biomass, and because of its small cells (2-3 µm diameter) as feed for rotifers in the "green water" technique. Modulation of fatty acid composition by culture parameters such as light intensity, light-dark cycles, temperature, salinity and nutrients are also possible.

Patterns of variation in the lipid class and fatty acid composition during batch cultivation of *N. oculata* have also been investigated (Hodgson, et al. 1991). Generally, the higher the biomass productivity, the higher is EPA productivity (Chini Zittelli et al. 1999). *Nannochloropsis* has been also proposed as source of lipid for biodiesel

production because it is able to reach over 60% lipids after nitrogen starvation (Rodolfi et al., 2009) mainly as TAGs containing saturated and monounsaturated fatty acids (Bondioli, et al. 2012).

Shiny yellow drops at figure 5, shows the neutral lipid content of the cell after Nile Red dye and with florescence microscopy.

Figure 5 Florescence microscopic view of the specie *Nannochloropsis gaditana* after Nile Red dye.

2.2.5 Growth kinetics and productivity

Nannochloropsis grows in seawater, with maximal growth rate in the salinity range from 25 to 30 g/L, but it can tolerate salinities between 10 and 35 g/L (Renaud and Parry, 1994).

Experiments with artificial or mixed (artificial and natural) light sources aiming to evaluate biomass productivity in different culture conditions have been carried out *Nannochloropsis* has also been proposed as source of oil for biodiesel production. (Rodolfi et al. 2009) have suggested a “two phase strategy” (a first-stage cultivation of the microalga in the presence of nitrogen carried out in closed photobioreactors, followed by a second stage of nitrogen starvation, preferably carried out in open ponds), and have estimated a productivity of 20 ton of lipids per ha per year in the Mediterranean region. Lipid content increment after nitrogen starvation was not observed in *N. salina* (Boussiba et al., 1987), thus the selection of the right strain is of crucial importance for this application.

2.2.6 Summary:

The genus Nannochloropsis are particularly interesting because of their ability to accumulate large amounts of lipids, which can reach concentrations up to 65–70% of total dry weight (Boussiba et al., 1987; Hodgson et al., 1991; Rodolfi et al., 2009; (Simionato, Sforza et al. 2011). *Nannochloropsis gaditana* is a microalga specie that belongs to the class Eustigmatophycea which stands out as an important source of pigments of great commercial value besides its lipid content. Therefore, this species is also considered as promising candidate for industrial applications of biofuel production where studies on its morphology, ultra-structure and growth physiology of its system have already been described (Lubian, 1982).

Nannochloropsis small cell size allows less energy consumption for mixing, but more energy is required for separating the cells from the culture medium. Thick cell wall, that allows mixing of the culture even with centrifugal pumps, but increases difficulty of cell breakage, which is necessary for oil extraction.

The most adequate system for biomass recovery is announced to be centrifugation that gives a product (paste) with 25-50% dry material. To reduce the volume to be centrifuged, autoflocculation can be used. The pH increase, consequent to the interruption of CO_2 inlet in the culture exposed to sunlight, can cause cell aggregation and thus sedimentation, which allows concentrating the culture 10 times, the same occurs by addition of NaOH (Sema Sirin et al.; 2011)

2.3.- *Chaetoceros calcitrans*

2.3.1 Taxonomy

Table 3: Taxonomy of *Chaetoceros* species

TAXONOMY	
Phylum	Heterokontophyta
Class	Bacillariophyceae
Order	Centrales/Biddulphiineae
Family	Chaetocerotaceae
Genus	*Chaetoceros*
Species	*C. affinis, C. curvisetus, C. castracanei, C. brevis, C. atlanticus, etc*
Studied species in our work	*Chaetoceros calcitrans*

(Paulsen) Takano 1968

2.3.2 Biology

Chaetoceros is probably the largest genus of marine planktonic diatoms with approximately 400 species described. Although a large number of these descriptions are no longer valid. It is often very difficult to distinguish between different *Chaetoceros* species (Quillfeldt, 2001).

The genus belongs to the bi- or multipolar centric class (Mediophyceae), with *Chaetoceros* having a bipolar valve outline. The cells occur in chains held together by long spines called setae that extend from the corners of the cells. Many species form resting spores, which are often abundant in the fossil record. Therefore, this genus is important within the disciplines of marine biology, marine geology, oceanography, and aquaculture. *Chaetoceros* is usually divided in 2 subgenera which are further divided in numerous sections:

Subgenus *Chaetoceros* with species characterized by numerous chloroplasts throughout the cell and robust spiny setae containing chloroplasts.

Subgenus *Hyalochaete* consisting of species having many parietal plastids and thin setae lacking chloroplasts.

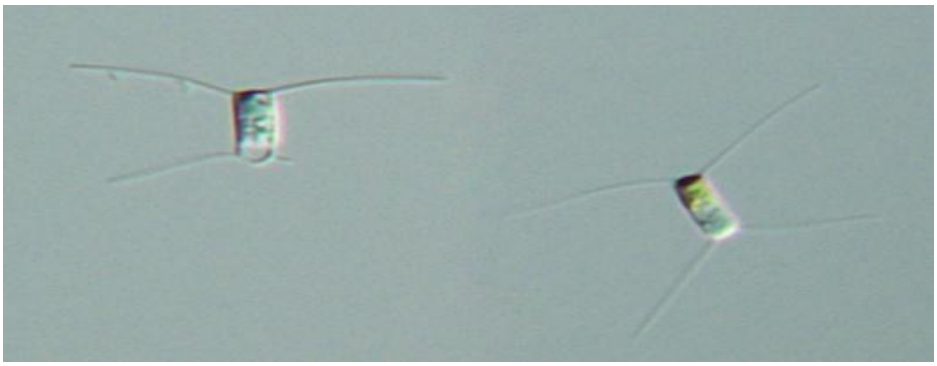


Figure 6 Microscopic views of the specie *Chaetoceros calcitrans*

The evolutionary history of the genus *Chaetoceros* is poorly known, and the classification largely based on observations of the morphology and ultrastructure of some species. Molecular data will be necessary to unravel phylogenetic relationships.

Cells of *Chaetoceros* are joined in chains that are coiled, curved or straight. Occasionally the cells are solitary. Cells are narrowly to broadly elliptical in valve view and rectangular in girdle view and are united by fusion or interlocking of setae produced

from the valve. They contain one or more small plate-like plastids. Cell width (apical axis) varies with species, roughly ranges from <10 μm to 50 μm. See figure 6.

C.calcitrans has oval cylinder shape with setae, the size vary with the specie from 3x3 to 9x9 μm, some *Chaetoceros* cells volume will be underestimated by about 10 %, when the volume of setae is not included, the volume of *C.calcitrans* is calculated around 20 to 360 μm^3 (Paulsen) Takano 1968

Chaetoceros is an important marine planktonic genus and some species are major bloom formers in both oceanic and coastal habitats. Only a few species have been recorded in fresh water. *Chaetoceros muelleri* has been reported mainly from brackish waters of varying salinities and temperatures in Europe and North America. The species is only rarely reported from freshwater habitats and has never been recorded from true marine systems (Becker, 2004).

The diatom *C.calcitrans* is considered one of the most popular strains used as a feed for shrimp larvae.

2.3.3 Biochemical composition

Vegetative cells/colonies are weakly silicified. It can be difficult to identify *Chaetoceros* taxa because they are notoriously variable in morphology. *C. muelleri* is also a variable taxon, both in vegetative cells and resting spores and it has been reported under more than 10 different specific names. Most populations of nonmarine, non-colonial *Chaetoceros* species have been reported as *C. muelleri*. Two different forms of *C.muelleri* have been described: the nominate form *C. muelleri* var. *muelleri* is characterized by the frequent presence of a small process on the valve face associated with chain formation (chains of only 2-4 cells) and smoothly curved setae, while *C.muelleri* var. *subsalsum* lacks valvar processes and consequently does not form colonies. Both forms have no ornamentation on their resting spores. Proximate composition (dry weight percentage) in *C. muelleri* is 43.1% crude protein, 17.1 % carbohydrates, 21.5% lipids and 18.3% ash.

C.calcitrans is a diatom with chlorophyll content and it is usually cultivated in a similar fashion to single cell algae where, apart from other common nutrients, light plays a significant role in controlling its growth rate.

For *C. calcitrans*, different culture conditions result in significant variations in the biochemical composition of the cells of and, therefore, in their nutritious value, in this

order, and total lipid content is the same as carbohydrate and around 12±7.5%, and the protein around 45±20% which is specially dependent on temperature, salinity and CO_2 administration.

Shiny yellow drops at figure 7, shows the neutral lipid content of two *C. calcitrans* cells after Nile Red dye and with florescence microscopy view.

Chaetoceros calcitrans offers high content of fatty acids (EPA), antioxidants e immune system stimulants substances (vitamin C and B2), and its small size (2.5 to 6 μm of diameter) makes it strongly appropriated to feed mollusks, echinoderms, crustaceans and some zooplankton like Artemia (Brown et al., 1997, 1999; Becker, 2004; Krichnavarruk et al., 2005; Khoi et al., 2006).

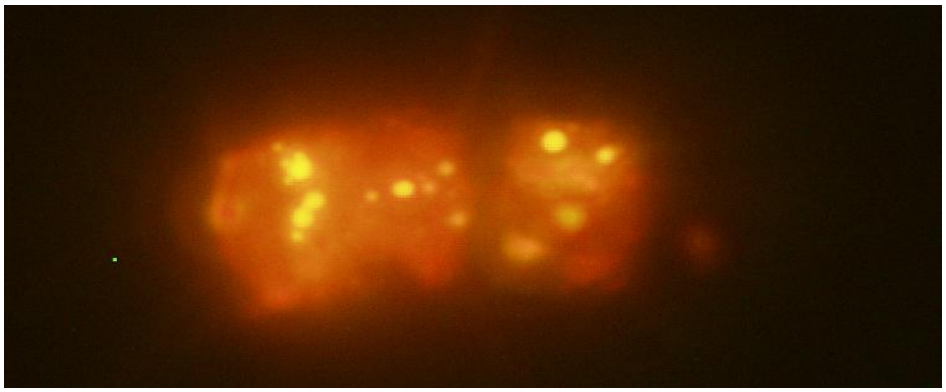


Figure 7 Florescence microscopic views of the specie *Chaetoceros calcitrans* after Nile Red dye.

2.3.4 Growth kinetics and productivity

C calcitrans grows in seawater, with maximal growth rate in the salinity range from 25 to 35 g/L and ultra centrifugation seems to be the proper concentration method to obtain sludge of 20-30% dry solid. Cream separator or bucket centrifuge are not enough efficient concentration equipments, although cell viability is around 100% whichever centrifuge tested by (Heasman et al 2001).

According to Heasman to, natural sedimentation of the *C calcitrans* specie is not proper to obtain high concentration factors.

Higher growth rates for marine diatoms, such as 4.2 d)1 for *Chaetoceros gracilis*, or close to 3.5 d)1 for *C. calcitrans* have been reported, although these rates were

obtained under continuous light conditions (Ichimi, Kawamura et al. 2012) and the higher growth of *C. calcitrans* 4.5d at 25ºC and with CO_2 contribution.

2.3.5 Summary

Chaetoceros calcitrans is a marine diatom widely used in aquaculture industries, is probably the largest genus of marine planktonic diatoms with approximately 400 species described. Although a large number of these descriptions are no longer valid. It is often very difficult to distinguish between different *Chaetoceros* species (Tomas, C. R et al 1997). Several attempts have been made to restructure this large genus into subgenera and this work is still in progress (Tomas, C. R et al 1997, Rines and Theriot 2003). However, most of the efforts to describe species have been focused in boreal areas, and the genus is cosmopolitan, so there are probably a large number of tropical species still not described (Rines et al 2000)

Proteins are the major component of the *C. calcitrans* cells, the final choice of algal species is governed by the culture system used, resources available, location and prevailing environmental conditions, as well as the scope and aims of the individual project in question. In addition to the key indicator of lipid productivity, characteristics such as ease of cultivation and harvesting are vital to the success of any large-scale algae culture facility and a sufficient data inventory of these factors remains to be generated (Griffiths and Harrison 2009) and *C. calcitrans* can be a good option to produce economically viable biodiesel.

2.4.- Biological materials production

The three microalgae species tested: *Phaeodactilum tricornutum*, *Nannochloropsis gaditana and Chaetoceros calcitrans* have all long been used in aquaculture as it was describes above, mainly as a food source for fish, crustacean and bivalve production (Fajardo, Cerdán et al. 2007),(Servel, Claire et al. 1994),(Volkman, Jeffrey et al. 1989)

Particularly the species used in this work were isolated and produced in the IRTA laboratories in Sant Carles de la Ràpita (Tarragona, Spain).

All of the three species are well known; *Phaeodactilum tricornutum* (Pht) is fusiform in shape with a measured mean size of 3.5 x 40 µm; *Nannochloropsis gaditana* (Nng) is a sphere-shaped alga with a diameter of 3 micrometers; and *Chaetoceros Calcitrans* (Chc) which is cylindrical in shape with setae (a stiff hair, bristle-like appendix).

The species Pht and Nng were choosing because their high potentiality of improvement, due to the genome is already published and it might be easy to manipulate according to the end product needed. On the other side, the specie Chc was choose because it is a fragile cell and, in order to have a representative case of the capability of those kind of cells to be concentrated by filtration, centrifugation, etc.

The growth was started in agar plates, with 15mL cultures. The medium used was f/2, obtained by reducing the concentration of "f Medium" (Guillard 1962) by half and substituting iron sequestrate with $Na_2EDTA.2H_2O$ and $FeCl_3.6\ H_2O$.

Cultures between 200mL (conical flask) and 4L (6L volumetric flasks) were grown with Walne's medium (Walne 1970) under the continuous illumination of day light fluorescents (TDL18W-840) with a 100-140$\mu Em^{-2}s^{-1}$and mixed with air.

For larger volume cultures (300L), seawater was filtered through four filter cartridges with 25, 10, 5 and 1μm pore sizes (3M/Cuno, polyKLEAN and MICRO-KLEAN) and also treated with UV light (PURION 1000) in order to eliminate biological contamination.

Aeration was connected and the following nutrients were added: 0.3mL/L of phosphorus pentoxide (P_2O_5) from a stock solution of 85.5%(P/V) (CODAFOL from Sustainable Agro Solutions S.A.) and 0.02mL/L of B group vitamins (Vitamin B1, 5 mg. Vitamin B2, 10 mg. Vitamin B6, 7.5 mg. Vitamin B12, 30 mcg. Nicotinic acid, 20 mg. Calcium pantothenate, 10 mg. Folic acid, 1 mg. Biotin, 20 mcg. Vitamin C, 50 mg and suitable diluents, s.q.f., 1 ml. from Aquavita (Syva lab).

For the diatom species (*Phaeodactilum tricornutum* and *Chaetoceros calcitrans)*, 1mL/L silicate (Sigma- Aldrich) was also added from a stock solution of 40g/L to produce the biogenic silica or to synthesize silicic acid (silicate) intracellularly which then forms the cell wall (frustule). They cannot divide without silicon in their environment (Martin-Jézéquel, Hildebrand et al. 2000) although it has been reported that some Pht can (Tesson, Gaillard et al. 2008).

Photosynthesis was induced by continuous illumination from cool white fluorescents (TLD36W-54) with a 355-450 $\mu Em^{-2}s^{-1}$ (24h light cycles)

For all experiments, cultures were harvested at the end of the exponential growth, which was after 7 days of inoculation for diatoms (Pht and Chc), and 10 days for Eustygmatoficea algae species (Nng). The culture properties can be seen in table 4, which shows the concentrations measured by ash free dry weight AFDW (g/L), by

Coulter counter (cells/mL) and indirectly by spectrophotemeter (cells/mL) at least in triplicate and with a confidence interval of 95%.

2.4.1 Microalgae characterization

The initial concentration of the three microalgae tested in next chapters were measured directly by AFDW and a Coulter counter, and indirectly by absorbance at 750nm, concentration measurement methodology are indicated in section 4.2.2. Table 4 briefly shows the characteristics of each one. As can be seen, their geometries are different. While the eustigmatophyceae *N.gaditana* is spherical, Bacillariophyta *P. tricornutum* and *C.calcitrans* species are fusiform and cylindrical, respectively. The initial concentration range by mass is between 0.06-0.095g/L (AFDW) for all three species.
Although the concentrations of cells/mL are different, which is quite normal because the cells are different sizes, both methods (Coulter counter and absorbance) show good accuracy. Error is less than 20%.

Table 4: Culture properties

Class-phylum	species	length (µm)	width (µm)	f width (µm)	µ max (div/day)	Initial cell density @750nm x10^{-6} (cells/mL)	Coulter counter x10^{-6} (cells/mL)	AFDW Concentration (g/L)
Bacillariophyta	*Chaetoceros calcitrans*	4.50 ± 1.95	4.75 ± 2.45	7.75 ± 0.50	2.0	1.40 ± 0.20	1.35 ± 0.30	0.0625 ± 0.005
Eustigmatophyceae	*Nannochloropsis gaditana*	3.75 ± 0.50	3.50 ± 1.00	3.50 ± 1.00	1.5	11.5 ± 0.10	14.5 ± 0.10	0.0925 ± 0.005
Bacillariophyta	*Phaeodactylum tricornutum*	19.00 ± 1.00	3.75 ± 0.50	3.75 ± 0.50	2.5	3.95 ± 0.50	1.60 ± 0.60	0.0750 ± 0.010

The concentration of cells/mL in the various species is different. This is normal because the cells are different shapes and sizes, and also because the stationary phase for the various species takes place at different times. Despite this, the number of cells counted by both measuring methods is similar for the smaller and spherical species (≈10%) and a little higher for the fusiform species Pht, for which the error was around 50%. One possible explanation for the size of this error has been given by (Bakker, Prins et al. 1985): "Coulter counter tends to underestimate the biomass of cells with a shape differing strongly from sphere"

2.5.- References:

- Alonso D.L., Belarbi E.H., Fernandez-Sevilla J.M., Rodriguez-Ruiz J., Molina Grima E. (2000) Acyl lipid composition variation related to culture age and nitrogen concentration in continuous culture of the microalga *Phaeodactylum tricornutum*.*Phytochemistry* 54: 461-471.
- Bondioli, P., L. Della Bella, et al. (2012). "Oil production by the marine microalgae Nannochloropsis sp. F&M-M24 and Tetraselmis suecica F&M-M33." Bioresource Technology.
- Becker, W. 2004. Microalgae for aquaculture. The nutritional value of microalgae for aquaculture. In: A. Richmond (ed.). Handbook of microalgal culture. Blackwell Science, Oxford, pp. 380-391.
- Borowitzka M.A., Volcani B.E. (1978) The polymorphic diatom *Phaeodactylum tricornutum* - ultrastructure of its morphotypes.*Journal of Phycology* 14: 10-21.
- Boussiba S., Vonshak A., Cohen Z., Avissar Y., Richmond A. (1987) Lipid and biomass production by the halotolerant microalga *Nannochloropsis salina*. *Biomass* 12: 37-47.
- Bowler, C., A. E. Allen et al. (2008). "The Phaeodactylum genome reveals the evolutionary history of diatom genomes." Nature **456**(7219): 239-244.
- Brown, M., S. Jeffrey, J. Volkman & G. Dunstan. 1997. Nutritional properties of microalgae for mariculture. Aquaculture, 151: 315-331.
- Brown M.R., McCausland M. A., Kowalsi K. (1998) The nutritional value of four Australian microalgal strains fed to Pacific oyster *Crassostrea gigas* spat. *Aquaculture* 165: 281-293.
- Brown, M., M. Mular, I. Miller, C. Farmer & C. Trenerry. 1999. The vitamin content of microalgae used in aquaculture. J. Appl. Phycol., 11: 247-255
- Chini Zittelli G., Lavista F., Bastianini A., Rodolfi L., Vincenzini M., Tredici M.R. (1999) Production of eicosapentaenoic by *Nannochloropsis* . cultures in outdoor tubular photobioreactors. *Journal of Biotechnology* 70: 299-317.
- Desbois A.P., Mearns-Spragg A., Smith V.J. (2009) A fatty acid from the diatom *Phaeodactylum tricornutum* is antibacterial against diverse bacteria including Multi-resistant *Staphylococcus aureus* (MRSA). *Marine Biotechnology* 11: 45-52.
- Desbois A.P., Walton M., Smith V.J. (2010) Differential antibacterial activities of fusiform and oval morphotypes of *Phaeodactylum tricornutum* (Bacillariophyceae). *Journal of the Marine Biological Association of the United Kingdom* 90: 769-774.
- 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.
- Fawley K.P., Fawley M.W. (2007) Observations on the diversity and ecology of freshwater *Nannochloropsis* (Eustigmatophyceae), with descriptions of new taxa. *Protist* 158: 325-336.
- Fernández Sevilla, J.M., et al. Pilot Plant Scale Outdoor Mixotrophic Cultures of Phaeodactylum tricornutum Using Glycerol in Vertical Bubble Column and Airlift Photobioreactors: Studies in Fed-Batch mode. *Biothechnology Progress.* 2004, Vols. 20, 728-736.
- Griffiths, M. and S. Harrison (2009). "Lipid productivity as a key characteristic for choosing algal species for biodiesel production." Journal of Applied Phycology **21**(5): 493-507.
- Guillard RRL, Ryther JH (1962) Studies of marine planktonic diatoms I. *Cyclotella nana* Hustedt and *Detonula confervacea*

- Cleve. Can J Microbiol 8:229–239Michael Phillip Heasman, Tanya Maree Sushames, John Alfred Diemar, Wayne Andrew O'Connor and Lynne Adele Foulkes. 2001 "Production of Micro-algal Concentrates for Aquaculture Part 2: Development and Evaluation of Harvesting, Preservation, Storage and Feeding Technology" NSW Fisheries Final Report Series No. 34 ISSN 1440-3544
- Hodgson P.A., Henderson R.J., Sargent J.R., Leftley J.W. (1991) Patterns of variation in the lipid class and fatty acid composition of *Nannochloropsis oculata* (Eustigmatophyceae) durting batch culture. I The growth cycle. *Journal Applied Phycology* 3: 169- 181.
- Hu Q., Sommerfeld M., Jarvis E., Ghirardi M., Posewitz M., Siebert M., Darzins A. (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. *The Plant Journal* 54: 621-639
- Ichimi, K., T. Kawamura, et al. (2012). "Extremely high growth rate of the small diatom chaetoceros salsugineum isolated from an estuary in the eastern seto inland sea, japan." Journal of Phycology.
- Karlson B., Potter D., Kuylenstierna M., Dersen M.A. (1996) Ultrastructure, pigment composition, and 18S rRNA gene sequence for *Nannochloropsis granulata* sp. nov. (Monodopsidaceae, Eustigmatophyceae), a marine ultraplankter isolated from the Skagerrak, northeast Atlantic Ocean. *Phycologia* 35: 253-260.
- Khoi, C., V. Guong & R. Merckx. 2006. Growth of the diatom Chaetoceros calcitrans in sediment extracts from Artemia franciscana ponds at different concentrations of nitrogen and phosphorus. Aquaculture, 259: 354-364.
- Krichnavarruk, S., W. Loataweesup, S. Powtongsook & P. Pavasant. 2005. Optimal growth conditions and the cultivation of Chaetoceros calcitrans in airlift photobioreactors. Chem. Engin. J., 105: 91-98.
- Scala S., Carels N., Falciatore A., Chiusano M.L., Boweler C. (2002) Genome properties of the diatom *Phaeodactylum tricornutum*. *Plant Physiology* 129: 993-1002.
- Monsant A., Jabbari K., Maheswari U., Bowler C. (2005) Comparative genomics of the pennate diatom *Phaeodactylum tricornutum*.*Plant Physiology* 137: 500–513.
- Lewin J.C., Lewin R.A., Philpott D.E. (1958) Observations on *Phaeodactylum tricornutum*. *Journal of General Microbiology* 18: 418-426.
- Lubián, L.M. (1982). *Nannochloropsis gaditana* sp. nov., una nueva Eustigmatophyceae marina. *Lazaroa* : 287-293.
- Martin-Jézéquel, V., M. Hildebrand, et al. (2000). "Silicon metabolism in diatoms: Implications for growth." Journal of Phycology **36**(5): 821-840.
- Meiser, A., U. Schmid-Staiger, et al. (2004). "Optimization of eicosapentaenoic acid production by<i>Phaeodactylum tricornutum</i>in the flat panel airlift (FPA) reactor." Journal of Applied Phycology **16**(3): 215-225.
- Molina Grima E., García Camacho F., Sánchez Pérez J.A., Urda Cardona J., Acién Fernández F.G., Fernández Sevilla J.M. (1994) Outdoor chemostat culture of *Phaeodactylum tricornutum* UTEX 640 in a tubular photobioreactor for theproduction of eicosapentaenoic acid. *Biotechnology and Applied Biochemistry* 20: 279-290.
- Nizard C., Friguet B., Moreau M., Bulteau A.L., Saunois A. (2007) Use of *Phaeodactylum* algae extract as cosmetic agent promoting the proteasome activity of skin cells and cosmetic composition comprising same. WO02/080876-US Patent No. 7,220,417.

- Paulsen (Takano) M.D. Guiry in Guiry, M.D. & Guiry, G.M. 2012. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. http://www.algaebase.org; searched on 08 August 2012.

- Perez E.B., Pina I.C., Rodriguez L.P. (2008) Kinetic model for growth of *Phaeodactylum tricornutum* in intensive culture photobioreactor. *Biochemical Engineering Journal* 40: 520-525.

- Ranjan, A., C. Patil, et al. (2010). "Mechanistic assessment of microalgal lipid extraction." Industrial and Engineering Chemistry Research **49**(6): 2979-2985.

- Rebolloso-Fuentes M.M., Navarro-Pérez A., Ramos-Miras J.J., Guil-Guerrero J.L (2001) Biomass nutrient profiles of the microalga*Phaeodactylum tricornutum*. *Journal of Food Biochemistry* 25: 57-76.

- Renaud S.M., Parry D.L. (1994) Microalgae for use in tropical aquaculture. II Effect of salinity on growth, gross chemical composition and fatty acid composition of three species of marine microalgae. *Journal of Applied Phycology* 6: 347-356.

- Rines J. E. B., Theriot E. C., (2003). *Systematics of Chaetocerotaceae (Bacillariophyceae). I. A phylogenetic analysis of the family*, Phycological research 51: 83-98.

- Rines J.E.B., P. Boonruang and E.C. Theriot., (2000). *Chaetoceros phuketensis sp. nov. (Bacillariophyceae): a new species from the Andaman Sea*. Phycological Research **48** (3): 161-168

- Rodolfi L., Chini Zittelli G., Bassi N., Padovani N., Biondi N., Bonini G, Tredici MR. (2009) Microalgae for oil: strain selection,induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor. *Biotechnology and Bioengineering* 102: 100-112.

- Round F.E., Crawford R.M., Mann D.G. (1990) The diatoms - biology and morphology of the genera. Cambridge University Press,Cambridge, UK.

- Rushforth S.R., Johansen J.R., Sorensen D.L. (1988) Occurrence of *Phaedactylum tricornutum* in the Great Salt Lake, Utah, USA.*Great Basin Naturalist* 48: 324-326.

- C.H. von Quillfeldt., (2001). *Identification of Some Easily Confused Common Diatom Species in Arctic Spring Blooms.* Botanica Marina Vol. 44: 375 - 389

- M.-O. Servel, C. Claire, A. Derrien, L. Coiffard, Y. De Roeck-Holtzhauer, Fatty acid composition of some marine microalgae, Phytochemistry, 36 (1994) 691-693.

- Simionato, D., E. Sforza, et al. (2011). "Acclimation of Nannochloropsis gaditana to different illumination regimes: Effects on lipids accumulation." Bioresource Technology **102**(10): 6026-6032.

- Sema Sirin, R. T., Carles Ibanez, Joan Salvadó (2011). "Harvesting the microalgae Phaeodactylum tricornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity induced flocculation." Journal of Applied Phycology 1-14.

- B. Tesson, C. Gaillard, V. Martin-Jézéquel, Brucite formation mediated by the diatom Phaeodactylum tricornutum, Marine Chemistry, 109 (2008) 60-76.

- Tomas, C. R., Hasle G. R., Syvertsen, E. E., Steidinger, K. A., Tangen, K., Throndsen, J., Heimdal, B. R., (1997). *Identifying Marine Phytoplankton*, Academic Press.

- Tredici M.R., Biondi N., Chini Zittelli G., Ponis E., Rodolfi L. (2009) Advances in microalgal culture for aquaculture feed and other uses. In: Burnell G., Allan G., (eds.) New Technologies in Aquaculture: Improving production efficiency, quality andenvironmental management. Woodhead Publishing Ltd, Cambridge, UK, and CRC Press LLC, Boca Raton, FL, USA. pp. 610–676.

- Veloso V., Reis A., Gouveia L., Fernandes H.L., Empis J.A., Novais J.M. (1991) Lipid production by *Phaeodactylum tricornutum.Bioresource Technology* 38: 115-119.
- Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, **128**(3), 219-240.
- Walne (1970). "Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria, and Mytilis. ." Fishery Investigations. **Series 2, London.** : pp 26–62.
- Yu E.T., Zendejas F.J., Lane P.D., Gaucher S., Simmons B.A., Lane T.W. (2009) Triacylglycerol accumulation and profiling in the model diatoms *Thalassiosira pseudonana* and *Phaeodactylum tricornutum* (Baccilariophyceae) during starvation. *Journal of Applied Phycology* 21: 669-681.

CHAPTER **III**

3.- Dynamic microfiltration in microalgae harvesting for biodiesel production

3.1.- Introduction

The process for producing biodiesel from microalgae consists roughly of three stages. Firstly, a growth followed by a harvesting process has to be performed; this stage includes an operation to concentrate the microalgae after it has grown. Secondly, the lipids have to be extracted from the microalgae and thirdly, a reaction and separation process must be performed in order to obtain the final product (Chisti, 2007; Meng, et al 2009). Traditional methods to separate solids from liquids are membrane processes such as micro- or ultra-filtration processes. These methods have been used to recover the microalgae biomass from the growth medium together with other common methods such as centrifugation, flocculation, etc., which often are combined (Rossignol, et al 1999; Rossi, et al 2004; Molina Grima, et al 2003). Membrane processes can play different roles in the production of biodiesel, not only on microalgae concentration but also in the transesterification process (Cao, et al 2009; Cao, et al 2007), for example.

Due to the nature of the microalgae, a large amount fouling and concentration polarization occurs during the microfiltration process that causes a substantial decrease in the permeation flux, similar to other close applications (Sur, et al 2005). As will be demonstrated, this reduction can be up to 95% of the initial flux after less than 2 hours. Therefore, a large amount of energy and time are wasted during microfiltration, compromising the feasibility of the process. To reduce fouling, dynamic filtration can be used instead, as it maximizes the turbulence over the membrane and thus, the shear stress on it (Torras, et al 2009; Brou, et al 2002). There are several types of dynamic filtration schemes such as rotational systems, vibratory systems, etc. In this work, a study with a rotational disk set-up, which contains the membrane, was performed by studying the effect of the rotational speed of the disk in order to concentrate microalgae, thus, removing water from the feed suspension.

The goal of this chapter is therefore to provide new experimental results that show the enhancement that it is achieved in the microfiltration of microalgae through dynamic processing.

3.2.- Experimental

3.2.1 Biological material

The microalgae strain *Phaeodactylum tricornutum* (Pht) has been used in this work, cells properties and concentration can be seen at section 2.4. Because of the nature of the suspended matter that is alive and in contrast with inorganic solutions, it should be noted that is practically impossible to achieve exact conditions. Therefore, a similar initial concentration was used in all of the experiments.

3.2.2 Methods

The filtration efficiency was evaluated by measuring the ash free dry weight (AFDW) and the absorbance at 750 nm of the culture (initial suspension), retentate and final permeate, in order to evaluate the cell concentration. For AFDW, a known volume of sample was filtered through a pre-combusted glass fiber filter, (Whatman GF/F, 0.2 µm). The filtered material was washed with ammonium bicarbonate in order to eliminate the remaining salts whilst avoiding osmotic shock. It was then dried at 100ºC for 24 hours, weighed and, after being combusted in a muffle furnace for 4 h at 450°C, reweighed in order to determine the organic content in weight. The AFDW was calculated by withdrawing the final filter weight from the dried filter weight. For absorbance measurements, 1 mL of the sample was fixed with 10 µL of a 37 % formaldehyde solution. Absorbance at 750 nm was read with a Synergy HT Multi-Mode Microplate Reader (Biotek). Results are given as cells/mL for better understanding. The cell concentration was estimated by interpolating absorbance in a least squares regression ($Y = 65.135 \cdot 10^6 \cdot X + 640519$, $r^2 = 0.988$, where Y is the concentration in cells/mL and X is the absorbance measured) obtained by correlating cell counts from a Neubauer haemocytometer with absorbance. Additionally, a least squares regression was used to obtain an expression to calculate the concentration in terms of mass by interpolating the results obtained for AFDW and absorbance ($Z = 1.5838 \cdot 10^{-11} \cdot Y$, $r^2 = 0.987$, where Z is the concentration in g/mL and Y is the concentration in cells/mL).

Rejection (also named rejection coefficient) and volumetric flux reduction (VFR) have been evaluated in this work. They were calculated by applying equations 1 and 2 respectively. In these equations, "initial" means at time zero and "plateau" is referred to that period in which no large variation of the flux (J) occurs.

$$[Eq.1] \qquad Rejection = 100\% \times \left(1 - \frac{[\mu-algae]_{permeate}}{[\mu-algae]_{initial}}\right)$$

$$[Eq.2] \qquad VFR = 100\% \times \left(1 - \frac{J_{plateau}}{J_{initial}}\right)$$

The confidence interval has been calculated by applying equation 3, where $\bar{x}$ is the mean value, *z* is the normal distribution, *s* is standard deviation and *n* the number of samples. In all cases a confidence level of 95 % was used.

$$[Eq.3] \qquad Confidence\ interval = \bar{x} \pm z_{\frac{\alpha}{2}} \times \frac{s}{\sqrt{n}}$$

Membrane surface micrographs were obtained by Scanning Electron Microscopy (SEM). The samples were subjected to a gold sputtering process in order to make them conductive. The SEM equipment used was a Jeol JSM-6400 Scanning Microscopy, operating with a voltage of 15kV.

Shear stress over the membrane can be estimated from the rotation and operating and geometric parameters using a same procedure described in a previous paper (Torras, et al 2009), by applying equation 4.

$$[Eq.4] \qquad \tau_{membrane} = 0.057 \cdot \rho \cdot \nu^{1/5} \cdot (k \cdot \omega)^{9/5} \cdot r^{8/5}$$

3.2.3 Equipments

Scheme and description of the equipment used can be seen in next chapter, section 4.2.3. In this chapter four different commercial membranes made by KMPT were tested: two ceramics (Al_2O_3) with mean pore size of 0.5 and 2 µm and two polymeric ones made in Teflon (PolyTetraFluoroEthylene, PTFE) with a mean pore size of 0.5 and 1 µm. Each membrane has a filtration area of 0.023 m^2, and with a diameter of 152 mm. Membrane water permeabilities were tested before using and after every wash, and the

cleaning ratio was evaluated by measuring flux recovery. The manufacturer only provided water permeability for the ceramic membranes, which were 1600 L/h/m^2/bar for the 2 µm one and 1200 L/h/m^2/bar for the 0.5 µm one.

3.2.4 Design of experiments

All the experiments were carried out at a recirculation flow rate of 200 L/h and the effective membrane area was 0.023 m^2.

The variables studied were the rotational speed, the TMP and the type of membrane. Four rotational speeds were tested: 0, 560, 830 and 1110 rpm (which correspond to 0, 50, 75 and 100% of the module capacity). Whenever rotation is applied, a turbulent regime occurs because Reynolds higher than $1.3 \cdot 10^5$ apply. The mean shear stress applied to the membrane can be estimated by using equation 4 referred to above (Torras, et al 2009). Considering the initial condition of the fluid, assuming that it did not change significantly during the experiments and using a k factor of 0.4 (Bouzerar, et al 2000), the mean shear stress on the membrane were 0, 8.86, 18.00 and 30.37 Pa respectively.

Two TMPs were tested: 1 and 2 bar. Four different membranes were tested: two ceramic ones with a mean pore size of 0.5 and 2 µm and two polymeric ones with mean pore size of 0.5 and 1 µm.

In general, all of the experiments were carried out from fresh initial suspension and extracting the permeate in order to evaluate the concentration process. An exception was made for those in which the rotational speed was studied in the same test. In these cases, a fresh initial suspension was also used but the permeate was driven to the feed vessel in order to keep the concentration constant.

At least, three experiments per test were conducted to study reproducibility. After each experiment, membranes were cleaned by soaking them into a 400 mg/L NaClO solution in demineralized water, during 12 hours. Afterwards, they were assembled again and demineralized water was pumped for 1 hour at 1 bar. Finally, water permeability was measured again. In those cases that permeability was less than 90% of theoretical one (the one measured before the first use of the membrane with test fluid), the above mentioned NaClO solution was pumped for 1 hour at 1 bar followed by demineralized water pumping for ½ hour at 1 bar.

The temperature of the bath containing the recirculating fluid was being measured but not controlled. Fluctuations up to 8 º C occurred at a rate of 3 ºC / h which is expected to have a limited effect on membrane permeability due to viscosity changes. As the experiments were performed with similar period of time, systematic effect of temperature change in results was expected and also, comparative results are analyzed. Moreover, once the plateau was reached, in all experiments and despite of the temperature increment, no significant changes in permeability were observed.

3.3.- Results and discussions

The variables studied were the rotational velocity of the disk, TMP and the type of membrane. Their effects on permeability and rejection were evaluated. In these experiments, the type of membrane had effects on both parameters but rotational velocity and TMP only affected permeability. Table 1 shows the flux results for all of the membranes tested at the different rotational speeds and TMP. Values of water permeability measured at 1 bar and for all the membranes used were 1769 ± 20 for the 2 micrometers ceramic membrane, 1445 ± 45 for the 0.5 micrometers ceramic membrane, 1046 ± 186 for the 1 micrometer polymeric membrane and 1302 ± 269 for the 0.5 micrometers polymeric membrane. Values indicate that reproducibility in polymeric membranes is lower than in commercial ones. Also, it is noticeable that for polymeric membranes, permeability is similar for both.

With regard to the rotational velocity of the disks, several experiments were performed at 0, 560 and 1110 rpm for each TMP. When using the microalgae suspension, at 1110 rpm permeability was 212 ± 68 L/m2/h/bar, at 560 rpm it was 109 ± 48 L/m2/h/bar and with no rotational speed it was 68 ± 25 L/m2/h/bar. Values show the important VFR measured and caused by the particulates that promotes fouling and concentration polarization as well as the enhancement obtained by applying rotation. Looking at the mean values of all the experiments performed, VFR at 1110 rpm was 79 % ± 7 %, at 560 rpm it was 91 % ± 5 % and at 0 rpm it was 94 % ± 2 %. Figure 1 shows a representative case of flux decline with time caused by fouling and concentration polarization at different rotational speeds (ceramic membrane, mean pore size of 2 µm, TMP of 2 bar). The experiment was started with 100% of the rotational speed measuring an initial permeability of about 1240 L/h/m^2/bar. After 40 minutes, the plateau was reached with a permeability of about 240 L/h/m^2/bar, and therefore a VFR

of 80% was measured. Afterwards, the rotational speed was reduced stage by stage obtaining a higher decline of fluxes, up to a VFR of 95 % (60 L/h/m^2/bar) with a rotational speed of 0. In each reduction of rotational speed and in all the experiments performed, a sharp and substantial decrease of the flux was observed. It should be stated that in this experiment, permeate was driven into the feed tank in order to maintain the concentration constant along all the experiment.

Table 1: Membrane fluxes

		Flux L / h / m^2			
TMP	**Membrane**	**Initial**	**Plateau at 1110 rpm**	**Plateau at 550 rpm**	**Plateau at 0 rpm**
2 bar	Ceramic, 2 µm	2520 ±73	460 ± 73	222 ± 59	130 ± 18
	Ceramic, 0.5 µm	1830 ± 47	431 ± 44	268 ± 28	105 ± 33
	Polymeric, 1 µm	1227 ± 16	200 ± 62	67 ± 16	69 ± 30
	Polymeric, 0.5 µm	3629 ± 298	207 ± 45	99 ± 17	75 ± 40
1 bar	Ceramic, 2 µm	1286 ± 96	403 ± 64	211 ± 35	130 ± 15
	Ceramic, 0.5 µm	943 ± 21	370 ± 25	265 ± 38	127 ± 23
	Polymeric, 1 µm	867 ± 180	127 ± 19	44 ± 4	33 ± 15
	Polymeric, 0.5 µm	1154 ± 162	195 ± 66	99 ± 14	54 ± 27

Regarding to the TMP, performance was better at 1 bar than at 2 bar, in terms of permeability. Initially and in all experiments, the permeability was almost double at 2 bar (as theoretically expected) but at the plateau, the permeabilities at 1 and 2 bar were similar. Considering the mean values of all the experiments performed, VFR at 2 bar was 91 % ± 4 %, whilst at 1 bar it was 84 % ± 6 %. Table 2 shows the results for each rotational speed. In this case, it can be stated that fouling effects, which reduces the permeability of the membrane, increase when the TMP also increases.

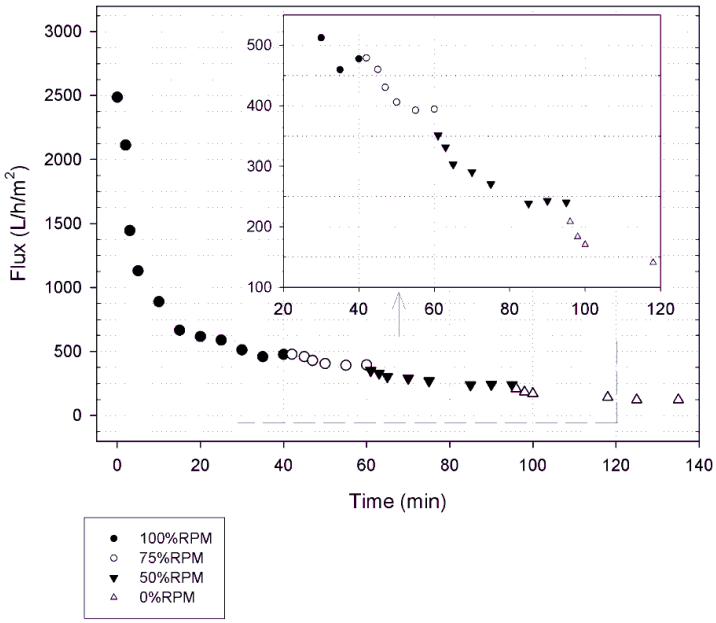


Figure 1. Decline of the permeation flux with time at different rotational speeds. Case corresponding to a ceramic membrane with a mean pore size of 2 µm, a TMP of 2 bar and a recirculation volumetric flow rate of 200 L/h.

In the case of a ceramic membrane with a mean pore size of 0.5 micrometers and 1110 rpm, the final flux at 1 bar was 370 ± 25 L/h/m^2, whilst at 2 bar it was 431 ± 44 L/h/m^2. Initial fluxes were 943 ± 21 and 1830 ± 47 L/h/m^2 respectively. It is a promising result as it indicates that good performance is obtained at a lower energy input.

Table 2: Volumetric flux reduction (VFR) of membranes at different TMP and rotational speeds.

		Mean value ± confidence interval		
		1110 rpm	*560 rpm*	*0 rpm*
VFR (%)	*1 bar*	75.3 ± 9.7	87.8 ± 8.2	91.7 ± 3.7
	2 bar	83.6 ± 8.4	93.4 ± 4.1	95.2 ± 1.9

Concerning the type of membrane, polymeric and ceramic membranes were evaluated (Figure 2). In terms of permeability, ceramic membranes had better performance than polymeric membranes because of three factors: VFR due to fouling and concentration polarization was less for ceramic ones (for example, considering the case of the 0.5 µm mean pore size membranes, initial permeability was higher for the polymeric

membrane, but the ceramic one had a larger value at the plateau), reproducibility was better and cleaning was easier for ceramic membranes. In all cases, the initial permeability measured with water recovered after cleaning. A main cause for this behavior could be because of the different type of porous structure that ceramic and polymeric membranes have. To show this difference, SEM micrographs were obtained and are shown in Figure 3.

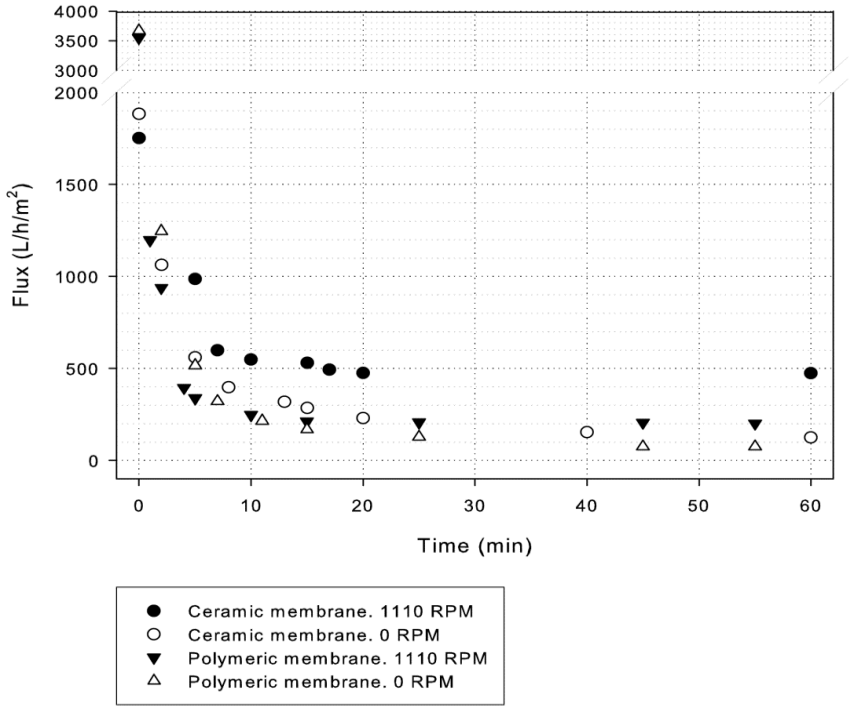


Figure 2. Variation of the permeation flux with time of polymeric and ceramic membranes with a mean pore size of 0.5 µm at different rotational speeds, a TMP of 2 bar and a recirculation volumetric flow rate of 200 L/h.

It can be seen in Figure 4 that whilst pores in ceramic membranes correspond to the spaces that are between the solid particles, in Teflon polymeric membranes the fibers create a net that constitute the barrier. Although the mean rejection of the membrane is the one stated, due to the geometry of the net, which has holes that are much larger than the mean theoretical value corresponding to the membrane cut-off, more biomass can reach the inside of the membrane and is trapped inside, increasing the level of fouling. Another cause may be due to electrical interactions between the membrane material and

part of the biomass being filtered, particularly exopolysaccharides (Morineau-Thomas, 2002).

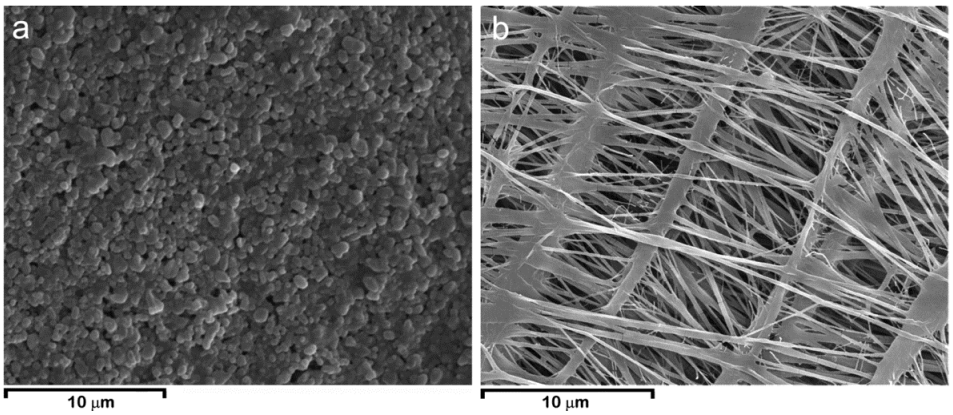


Figure 3. Scanning electron surface micrographs of the (a) 0.5 μm ceramic membrane and the (b) 0.5 μm polymeric one.

Another study focused on this phenomena would be needed in order to discuss this further, but Teflon membranes are reported to have higher biofouling due to their roughness and lower surface potential (Kang, et al 2006), so it can be expected that this cause could be of more importance for the polymeric membrane rather than the ceramic one. In terms of rejection, Table 3 shows the results as a function of the material and mean pore size. Firstly, the performance of the ceramic membranes was better than the polymeric ones. Ceramic membranes offered an almost complete rejection (although the mean rejection is higher for the membrane with a mean pore size of 0.5 μm than the 2 μm one, it is because of the experimental error as the statistics show). Secondly, the reproducibility of ceramic membranes was higher (lower standard deviation) than the polymeric membranes.

Table 3: Rejection of membranes calculated from AFDW.

	Mean value ± confidence interval			
	Ceramic 2 μm	*Ceramic 0.5 μm*	*Polymeric1 μm*	*Polymeric0.5 μm*
Mean rejection (%)	98.4 ± 1.0	99.2 ± 0.6	90.1 ± 5.7	99.4 ± 0.5

On the other hand, membranes with the same mean pore size offered different rejections.

Therefore, rejection depends largely on several parameters and can differ greatly from what is to be expected based on the calculation of the membrane's nominal cut-off. In this case, the shape of the particulate combined with the structure of the pores of the membrane can play an important role in the selectivity of the membrane.

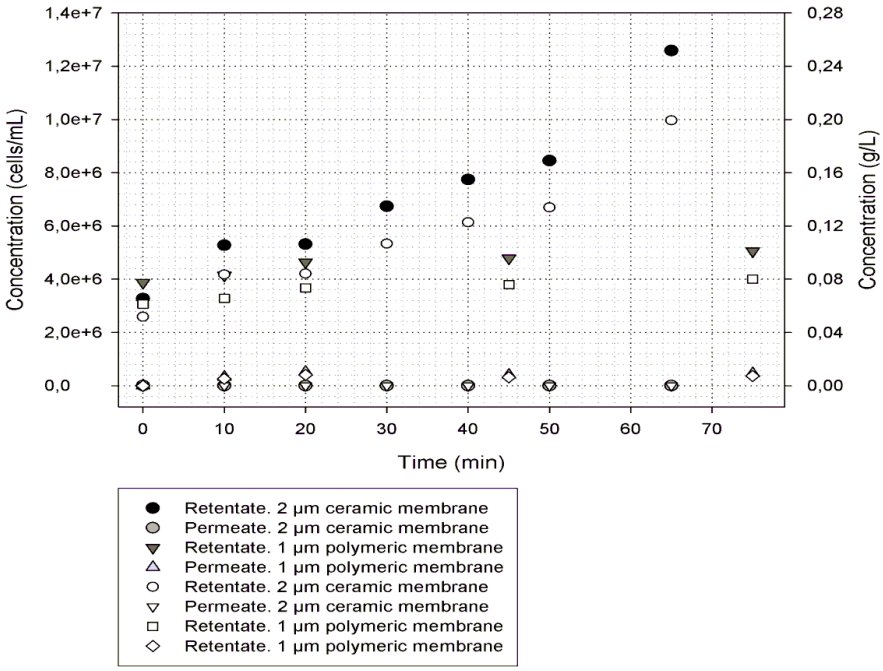


Figure 4. Evolution of microalgae concentration in the retentate and permeate streams for polymeric and ceramic membranes, a TMP of 2 bar and a recirculation volumetric flow rate of 200 L/h.

Figure 4 shows the concentration profiles of the retentate and permeate streams for polymeric and ceramic membranes. The increase in the cell concentration of the retentate was higher for the ceramic membrane than for the polymeric membrane. Consequently, whilst the concentration of microalgae in the permeate stream was null in the case of the ceramic membrane, significant values were determined for the same stream for the one concerning the polymeric membrane.

3.4.- Conclusions

Microfiltration of microalgae by using dynamic systems provides a clear enhancement of the process performance. As fouling and concentration polarization effects obtained

with the use of this suspended matter are very important, increasing the shear stress over the membrane significantly improves the permeability of the membrane. Optimum conditions can be found by a proper combination of variables such as the TMP or the rotational speed.

Results demonstrate that a higher TMP does not improve microfiltration performance due to fouling effects. Therefore, the process can be carried out with lower energy costs and can be made more cost-effective.

Membranes with similar nominal cut-offs but that are made of different materials offer different performance in terms of selectivity. For the *Phaeodactilum Tricornutum* microalgae strain, ceramic membranes with a mean pore size of two microns offer complete rejection at low TMP and reasonable permeability by using rotational enhancement.

Further experiments are being performed in order to continue this study, including more microalgae species, experiments at different TMP's and other tests, as well as an economic balance of the process.

3.5.- References

Bouzerar, R.; Ding, L. H.; Jaffrin, M. Y., Local Permeate Flux-Sheer-Pressure Relationships in a Rotating Disk Microfiltration Module: Implications for Global Performance. *J. Membr. Sci.* **2000,** *170*, 127.

Brou, A.; Ding, L. H.; Boulnois, P.; Jaffrin, M. Y., Dynamic Microfiltration of Yeast Suspensions Using Rotating Disks Equipped with Vanes. *J. Membr. Sci.* **2002,** *197*, 269.

Cao, P. G.; Tremblay, A. Y.; Dube, M. A., Kinetics of Canola Oil Transesterification in a Membrane Reactor. *Ind. Eng. Chem. Res.* **2009,** *48*, 2533.

Cao, P. G.; Tremblay, A. Y.; Dube, M. A.; Morse, K., Effect of Membrane Pore Size on the Performance of a Membrane Reactor for Biodiesel Production. *Ind. Eng. Chem. Res.* **2007,** *46*, 52.

Chisti, Y., Biodiesel from Microalgae. *Biotechnol. Adv.* **2007,** *25*, 294.

Kang, S.; Hoek, E. M. V.; Choi, H.; Shin, H., Effect of Membrane Surface Properties During the Fast Evaluation of Cell Attachment. *Sep. Sci. Technol.* **2006,** *41*, 1475.

Meng, X.; Yang, J. M.; Xu, X.; Zhang, L.; Nie, Q. J.; Xian, M., Biodiesel Production from Oleaginous Microorganisms. *Renew. Energ.* **2009,** *34*, 1.

Molina Grima, E.; Belarbi, E. H.; Acien Fernandez, F. G.; Robles Medina, A.; Chisti, Y., Recovery of Microalgal Biomass and Metabolites: Process Options and Economics. *Biotechnol. Adv.* **2003,** *20*, 491.

Morineau-Thomas, O.; Jaouen, P.; Legentilhomme, P., The Role of Exopolysaccharides in Fouling Phenomenon during Ultrafiltration of Microalgae (Chlorella sp and Porphyridium Purpureum): Advantage of a Swirling Decaying Flow. *Bioprocess Biosyst. Eng.* **2002,** *25*, 35.

Rossi, N.; Jaouen, O.; Legentilhomme, P.; Petit, I., Harvesting of Cyanobacterium Arthrospira Platensis using Organic Filtration Membranes. *Food Bioprod.* **2004,** *82*, 244.

Rossignol, N.; Vandanjon, L.; Jaouen, P.; Quemeneur, F., Membrane Technology for the Continuous Separation Microalgae/Culture Medium: Compared Performances of Cross-Flow Microfiltration and Ultra-Filtration. *Aquacult. Eng.* **1999,** *20*, 191.

Sur, H. W.; Cui, Z. F., Enhancement of Microfiltration of Yeast Suspensions using Gas Sparging - Effect of Feed Conditions. *Sep. Purif. Technol.* **2005,** *41*, 313.

Torras, C.; Pallares, J.; Garcia-Valls, R.; Jaffrin, M. Y., Numerical Simulation of the Flow in a Rotating Disk Filtration Module. *Desalination* **2009,** *235*, 122.

CHAPTER **IV**

4.- Antifouling microfiltration strategies to harvest microalgae for biofuel.

4.1.- Introduction

Biological feeds are normally difficult to filter because the cake is really compressible. They can be highly fouling precursors especially because they are commonly a mix of organic matter of different sizes, shapes and compressibility. Also, concentration polarization phenomena may arise because the surface charge of the cells affects the interaction between the cell, the exogenous matter and the membrane surface.

The purpose of tangential flow filtration, therefore, is to prevent fouling by creating a turbulent flow tangential to the membrane, which causes good mixing.

An increase in microalgae concentration factor has a direct effect on membrane fouling. It leads to a considerable amount of energy and wasted time, which compromises the viability of the process.

Antifouling strategies are critically important for sustainable biomass concentration by filtration, and permeate flux must be high at continuous processes and industrial scale.

Large velocities and pressure gradients not only require too much energy from the pump, but also decrease the trans-membrane pressure (TMP), which means that the membrane process is not optimal.

The economic viability of microfiltration depends on the flux and the capacity of the module. The higher the flux, the faster the feed can be processed, which leads to higher productivities.

To enhance flux capacity and optimal module operation, a variety of approaches can be used, focusing on the membrane (type parameters, including material, pore size, etc.), the module (hydrodynamic parameters) or the feed slurry (physical parameters) (Belfort, et al. 1994)

Hydrodynamic parameters, such as velocity distribution, shear stress over the membrane, etc., can be modified by designing the membrane module or changing the operation conditions

Chemical changes can be made to membranes to improve particle repulsion and physical changes (such as particle size distribution using flocculation, aggregation, etc.) can be made to the feed solution to reduce membrane fowling.

In order to flush the foulants from the 50kDa PVC surface membrane (Zhang, Hu et al. 2010) proposed back wash with compressed air antifouling strategies. They also developed a mathematical model from the process and reported a volumetric reduction factor (VRF) of 150 in 559 min. with an average flow rate of (45.5 L/m^2h), a surface membrane area of 0.12 m^2 and a transmembrane pressure of (34 kPa)

They evaluated the cost of reaching the VRF in these conditions but their approach does not seem to me to be very reliable. If the initial flux remained constant ($90L/hm^2$) the VRF could not be 150. They may not have taken into account the membrane module price only the membrane price. Moreover the TMP they use (34.5 kPa) is very low for an ultra-filtration process according to the bibliography it should be between 100-500 kPa (Mengual 1989).

To reduce the fouling effect, dynamic filtration can be used to maximize the velocity gradient over the membrane (shear stress) and turbulence.

Dynamic cross flow filtration (DCF) reduces fouling phenomena, which can substantially increase the permeate flux (Jp). Also, unlike in cross flow filtration, the inlet flow does not need to be much higher than this Jp.

There are several types of dynamic filtration: for example, rotational system membranes or disks, vibratory systems, etc (Jaffrin 2008)

Dynamic membrane filtration is gaining importance in a wide variety of applications such as chemicals, biotechnology, pharmaceuticals, etc

(Ochirkhuyag, Mori et al. 2008) briefly described how dynamic cross flow filtration can be used with microalgae. They used a $0.1m^2$ surface mono-disk, with 1μm filter paper over sintered polyethylene plates and a trans-membrane pressure (TMP) of 0.2 MPa. They obtained a permeate flux of $25L/h/m^2$ at 400RPM.

(Frappart, Massé et al. 2011) compared the performances and influence of hydrodynamic factors in tangential and dynamic ultrafiltration for microalgae separation. These authors reported about two times improvement on fluxes of dynamic filtration compared with tangential filtration.

According to (Kim, Akeprathumchai et al. 2001), flocculation might enhance the filtration process because the increasing size of the treated particles has a direct effect on the permeate flux.

The present paper studies such hydrodynamic factor effects as TMP, membrane pore size or speed of a rotational bi-shaft overlapping disk, which contains the membrane over the fluxes. The permeate fluxes were more than three times higher than in static cross flow filtration.

Flocculation/sedimentation should also be used as a pre-concentration step in an overall harvesting process so that treated volumes are reduced. A dynamic cross flow filtration of a concentrate obtained from pH induced flocculation sedimentation was tested.

The volume concentration factor was over 80 when enhanced dynamic filtration was used with the tested species and over 150 (v/v) if the feed was pretreated.

The chosen microalgae—*Nannochloropsis gaditana* (Nng), *Chaetoceros Calcitrans* (Chc) and *Phaeodactylum tricornutum* (Pht)—have been reported to be lipid precursors, specially of high-value lipids such as omega 3 and omega 6

4.2.- Experimental

4.2.1 Biological material

The microalgae strains —*Nannochloropsis gaditana* (Nng), *Chaetoceros Calcitrans* (Chc) and *Phaeodactylum tricornutum* (Pht)— have been used in this work, cells properties and concentration can be seen at section 2.4.

Because of the nature of the suspended matter that is alive and in contrast with inorganic solutions, it should be noted that is practically impossible to achieve exact conditions. Therefore, a similar initial concentration was used in all of the experiments.

4.2.2 Methods

The filtration efficiency was evaluated by measuring the ash free dry weight (AFDW) of the culture (initial solution), retentate and final permeate and the absorbance at 750 nm and samples for coulter counter (Multisizer 3 from Beckman Coulter). Samples were taken at different times during the experiments.

AFDW aliquots of algal suspension were filtered through pre-weighed, pre-combusted (400^{o}C, 2 h) glass-fiber filters (Whatman GF/F Glass Microfiber filter 4.7cm, nominal pore size 0.7µm). During the filtration, the vacuum pressure differentials were maintained at 35 to 55 mm Hg in order to prevent the biomass from passing through the

membrane. Algal samples were washed with 20 mL 0.5 M ammonium bicarbonate to rinse the sea water salts away (Zhu and Lee 1997).

The filters were dried at 100°C to a constant weight, cooled in a vacuum desiccator, and then weighed. Dry weight (DW) was calculated by subtracting the oven dried filter weight from the pre-weighed, pre-combusted filter weight. These oven dried filters were then placed in a furnace at 400° C for 4 h, cooled in vacuum desiccators, and weighed to obtain the ash free dry weight. The ash free dry weight (AFDW) was obtained by subtracting the final filter weight from the pre-weighed, pre-combusted filter weight,.

For absorbance measurements, 1 mL of sample was fixed with 10 µL of a 37% formaldehyde solution. Absorbance at 750 nm was measured with a Synergy HT Multi-Mode Microplate Reader (Biotek). For better understanding results were given as cells/mL. Cell concentration was estimated by interpolating absorbance in a least squares regression for:

[Eq. 1] **Pht** $Y = 65.135 \cdot 10^6 \cdot X + 640{,}519$, ($r^2 = 0.988$)

[Eq. 2] **Chc** $Y = 33.07 \cdot 10^6\ X + 128{,}000$, ($r^2 = 0.992$)

[Eq. 3] **Nng** $Y = 1 \cdot 10^8\ X + 928{,}994$, ($r^2 = 0.988$)

"Y" is the concentration in cells/mL and "X" the absorbance measured at 750 nm. Cells/mL was obtained by correlating cell counts from a Neubauer haemocytometer with absorbance at 750 nm.

A least squares regression was also used to calculate the concentration in terms of mass by interpolating the results obtained for AFDW and absorbance

[Eq. 4] **Pht** $Z = 1.60 \cdot 10^{-11} \cdot Y$, ($r^2 = 0.987$)

[Eq. 5] **Chc** $Z = 3.00 \cdot 10^{-11} \cdot Y$, ($r^2 = 0.9949$)

[Eq. 6] **Nng** $Z = 8.00 \cdot 10^{-12} \cdot Y$, ($r^2 = 0.9937$)

Where Z is the concentration in g/mL and Y is the concentration in cells/mL.

For the Coulter counter (Multisizer 3 from Beckman Coulter), 40 mL of sample was fixed with a stock solution of formaldehyde 37% to a final concentration of 4% and stored at 4ºC until it was required for measurement.

Samples were diluted with the electrolyte ISOTON® II (Beckman Coulter) to a solute concentration of 10% and volumetric control mode was used to obtain the concentration and the particle diameters.

The tube apertures were 50 µm for small species (*N. gaditana* and *C. Chalcitrans*) and 100 µm for *P. tricornutum* and current gain was 800 µA, suitable for cell measurements.

Rejection (also named rejection coefficient) and volumetric flux reduction (VFR) were calculated from equations 4 and 5 respectively.

$$\textbf{[Eq. 7]} \qquad Rejection = 100\% \times \left(1 - \frac{[\mu - algae]_{permeate}}{[\mu - algae]_{initial}}\right)$$

$$\textbf{[Eq. 8]} \qquad VFR = 100\% \times \left(1 - \frac{J_{plateau}}{J_{initial}}\right)$$

Confidence intervals were calculated from equation 3, where $\overline{x}$ is the mean value, *z* is the normal distribution, *s* is the standard deviation and *n* the number of samples. In all cases a confidence level of 95 % was used.

$$\textbf{[Eq. 9]} \qquad Confidence\ interval = \overline{x} \pm z_{\frac{\alpha}{2}} \times \frac{s}{\sqrt{n}}$$

Membrane surface micrographs were obtained by scanning electron microscopy (SEM). The samples were subjected to a gold sputtering process in order to make them conductive.

The SEM equipment used was a Jeol JSM-6400 scanning microscope, operating at a voltage of 15 kV. Membrane surface micrographs were treated and analyzed by IFME software (Torras and Garcia-Valls 2004)

The mean shear stress applied to the membrane can be estimated by using eq. 10 (Torras, Pallares et al. 2009). Considering the initial condition of the fluid, assuming that it did not change significantly during the experiments, and using a k factor of 0.4 (Bouzerar, Ding et al. 2000), the mean shear stress on the membrane was 0, 8.93, 18.14, and 30.61 Pa, for 0, 560, 830 and 1110 rpm respectively (which correspond to 0, 50, 75 and 100% of the module rotation speed capacity).

$$[Eq.10] \qquad \tau \text{membrane} = 0.057\rho\upsilon 1/5(\kappa\omega)9/5r\ 8/5$$

The energy consumption of the membrane equipment and the concentration process was measured with a quality energy controller (Fluke 1735)

4.2.3 Equipment

Figure 1 show the experimental set-up used. It consists of a monoblock screw pump (2.6ID10, PCM Moineau) used specifically with microalgae to minimize shear (Jaouen, Vandanjon et al. 1999).

The pump motor drive is regulated by a frequency regulator (VDF-E, Delta Electronics, Inc) to control the flow rate.

A back-pressure controller (Stübbe DHV 712R), which maintained the trans-membrane pressure (TMP) constant throughout the experiment was connected to the outflow of the membrane filtration system (rotational membrane module).

The model used was the Dynamic Cross-flow Filter (DCF) 152/0.14, a commercial module made by KMPT (Krauss Maffei Process Technology). The external diameter of the membrane is 152 millimeters and the total surface area is 0.14 (square meters). The equipment consists of a closed cavity with two parallel shafts that each has three overlapping membrane disks attached. Permeate is collected inside the shafts and driven out. The disks rotate at a particular angular speed, which can be adjusted by means of a switch/potentiometer unit (E82ZBU for 8200 motec).

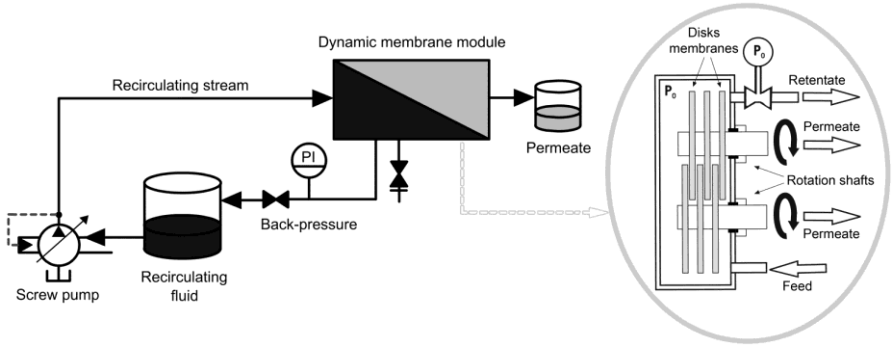


Figure 1: Experimental set-up

The membrane module is also equipped with a security valve set at 5.5 bar (Stübbe, DHV-716). Two commercial membranes provided by KMPT were tested: both were

ceramic (Al2O3) with a mean pore size of 0.5 and 2 μm. Each membrane has a filtration area of 0.023 m^2, and a diameter of 152 mm.

Membrane water permeability was tested before use and after every wash, and the cleaning ratio was evaluated by measuring flux recovery. The theoretical water permeability was 1600 L/h/m^2/bar for the 2 μm membrane and 1200 L/h/m^2/bar for the 0.5 μm membrane (table 2).

4.2.4 Experimental design

Several pre-experiments were carried out at a recirculation flow rate of 250 L/h and an effective membrane area of 0.023 m^2 to find the optimal operation condition for dynamic microfiltration.

The variables studied were the rotational speed, the TMP and the pore size of the membrane. Four rotational speeds were tested: 0, 560, 830 and 1110 rpm (which correspond to 0, 50, 75, and 100% of the module capacity). Whenever rotation is applied, a turbulent regime occurs because the Reynolds numbers used are higher than 1.3×10^5.

Various TMPs between 0.4 and 3 bar were tested, and in particular the interaction of variables at 1 and 2 bar.

In general, all pre-experiments were carried out using a fresh initial suspension (25L) and extracting the permeate flow so that variation with the concentration process could be evaluated. An exception was made for those experiments in which the rotational speed and TMP screening were studied in the same test. In these cases, a fresh initial suspension (25L) was also used but the permeate flow was driven to the feed vessel in order to keep the concentration constant. At least three experiments per test were conducted to study reproducibility.

Once a compromise had been reached between variables several large-scale experiments were carried out (250-500 or 900L).

The optimal operation conditions found for the dynamic cross flow filtration equipment and these kinds of slurry were applied to such other antifouling strategies as sediment biomass.

After each experiment, membranes were cleaned by soaking in a 400 mg/L NaClO solution in demineralised water, for 12 h. Then, they were assembled again, and demineralised water was pumped for 1 h at 1 bar.

Finally, water permeability was measured again. In those cases that permeability was less than 90% of the theoretical value (measured before the first use of the membrane with test fluid) the above-mentioned NaClO solution or common acid/base membrane cleaning was pumped for 1 h at 1 bar followed by demineralised water pumping for 1/2 h at 1 bar until the theoretical value was reached

The temperature of the bath containing the re-circulating fluid was measured but not controlled. Fluctuations up to 8 ºC occurred at a rate of 3 ºC/h which is expected to have a limited effect on membrane permeability due to viscosity changes. As the experiments were performed within a similar period of time, temperature change was expected to have a systematic effect on the results, and the comparative results were analyzed. Moreover, once the plateau was reached, in all experiments and despite the temperature increment, no significant changes in permeability were observed.

pH induced flocculation/sedimentation

The experiments were carried out by using NaOH (1N) solution to adjust the pH of the stock microalgae solution to an optimum alkaline pH. This was done using the sedimentation experimental procedure (Sema Sirin 2011). Only the base (NaOH 1N) was added to the culture at a high speed of agitation by air to get a homogeneous pH (9-10). The culture was then allowed to settle for one hour.

After sedimentation, the supernatant was removed and the concentrate fraction was allowed to filtrate. For the analyses, an aliquot from every representative fraction (initial, sedimented concentrate and supernatant) was fixed with 37% formaldehyde solution samples so that the final concentration was 4% formaldehyde.

4.3.- Results and discussion

4.3.1 Water permeability

Values of water permeability (when no fouling occurs) measured at 1 bar and for the two membrane pore sizes used are shown in table 2.

Water permeability was measured at 0 rpm when the flux is maximum. Extra tests of permeability at different rpm (550 and 1110) show that permeability decreases when the rotational speed increases but that results were not significantly different (table 1). Also

water permeability measured at higher pressures does not interfere with the permeability of ceramic membranes.

Table 2: Membranes water permeability

Water permeability (L/h/m2/bar)		
Value provided by supplier	1600	1200
revolution (%)	**ceramic 2µm**	**ceramic 0,5µm**
0	1770 ± 20	1445 ± 45
50	1700 ± 45	1335 ± 25
100	1650 ± 40	1295 ± 25

4.3.2 Variable optimization

In order for the dynamic cross flow microfiltration of three species of microalgae (Pht, Nng and Chc) to work in optimal conditions, several variables were studied: the rotational velocity of the disk, TMP and the membrane pore size. The effects of every variable on permeability and rejection have been studied. It was found that TMP and rotation velocity influenced permeability but not rejection for which only the membrane pore size underwent a slight, but not significant, variance (between 0.5 and 2 µm).

Table 3: Membrane fluxes (L/h/m^2)

		2bar (mean value ± confidence interval)		**1bar (mean value ± confidence interval)**	
	membrane speed (rpm)	***Ceramic 2 µm***	***Ceramic 0.5 µm***	***Ceramic 2 µm***	***Ceramic 0.5 µm***
	initial	2500 ± 75	1830 ± 45	1300 ± 95	945 ± 20
Pht	***1110***	460 ± 75	430 ± 45	405 ± 65	370 ± 25
	560	220 ± 60	270 ± 30	210 ± 35	265 ± 40
	0	130 ± 20	105 ± 35	130 ± 15	130 ± 25
	initial	2350 ± 50	2000± 120	1240 ± 35	1080 ± 60
Nng	***1110***	725 ± 165	520 ± 100	705 ± 70	715 ± 100
	560	360 ± 105	365 ± 105	340 ± 90	385 ± 25
	0	170 ± 25	150 ± 45	135 ± 35	170 ± 60
	initial	1990 ± 50	1900 ± 50	1030 ± 60	970 ± 30
Chc	***1110***	690 ± 35	395 ± 45	635 ± 40	405 ± 100
	560	355 ± 75	215 ± 50	320 ± 120	220 ± 50
	0	135 ± 75	95 ± 10	155 ± 115	115 ± 25

Table 3 shows the fluxes for every species tested for both membranes at different rotation velocities and 1 and 2 bar of TMP.

4.3.2.1 TMP

There are no significant differences between the fluxes at the plateau between 1 and 2 bar transmembrane pressure although, as theoretically expected, the initial fluxes were twice as high at 2 bar than at 1 bar. This prompted us to study the effect of this variable in an attempt to find the optimum work pressure.

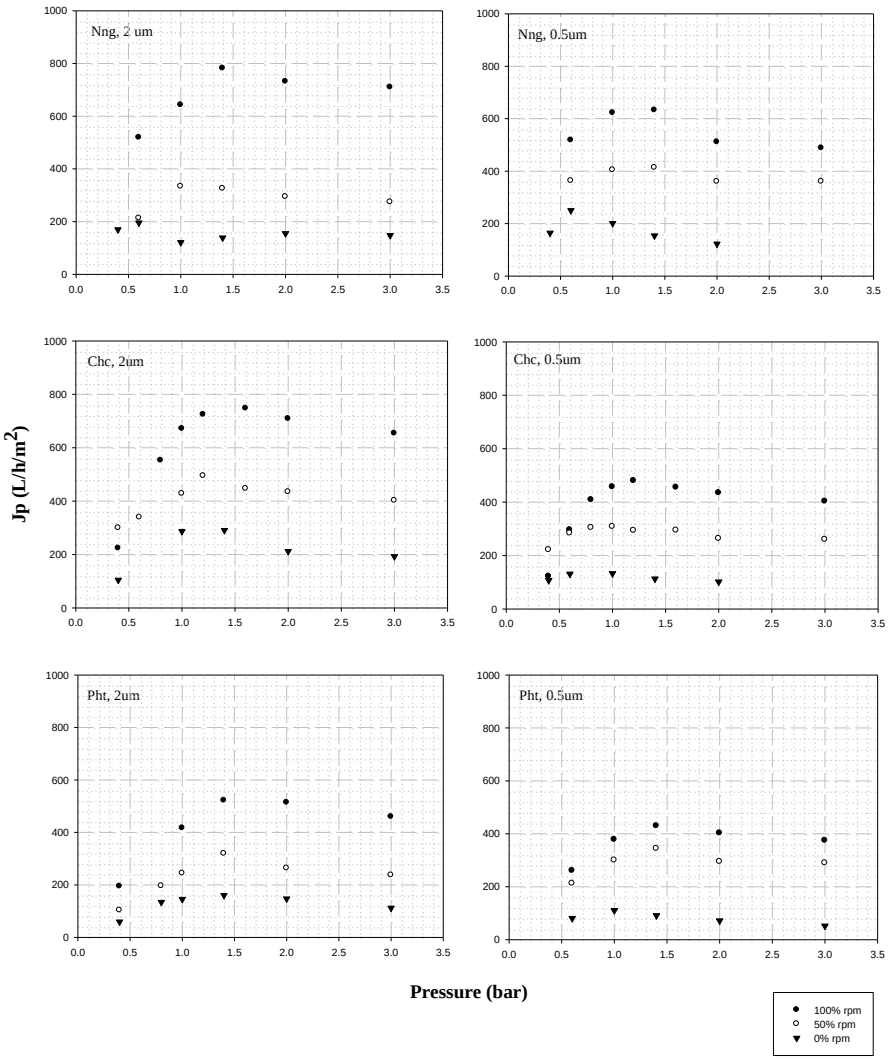


Figure 2: Permeate flux variation vs pressure and rotational membrane speed for three species *Phaeodactilum tricornutum* (Pht), *Nannochloropsis gaditana* (Nng) and *Chaetoceros Calcitrans* (Chc) and ceramic membrane pore sizes of 0.5 and 2 µm.

Figure 2 shows the effects of TMP on the permeate flux (Jp) for each species, ceramic membrane pore size (2 or 0.5µm) and membrane rotation speed (0, 50 and 100% of total revolutions) for an inlet volumetric flow rate of 250L/h.

Each point represents the stabilized permeate flux after the plateau had been reached at constant pressure. In these cases, for all three species the maximum flux is between 1 and 2 bar when the permeate flux starts to decrease or remains constant. It should be pointed out that in these experiments, the permeate was recycled into the feed tank so that the concentration would remain constant throughout the experiment.

The optimum TMP increases as a function of the rotational membrane speed. This is also the case for water permeability (when no fouling occurs): the fluxes are higher when the membranes are static and decrease when the membrane speed increases (table 2). The explanation may be that higher rotational speeds produce higher global TMP in agreement with (Serra and Wiesner 2000). (Ding, Jaffrin et al. 2006) also explain this phenomenon, which increases at lower module cavity pressure.

The mean VFR of all the experiments performed was 85% ± 3% at 2 bar and 70% ± 7% at 1 bar. Table 3 shows the results for each species at different rotational speeds. In this case, fouling effects or cake compressibility reduce the permeability of the membrane, and these effects increase when TMP also increases.

4.3.2.2 Rotating speed

Permeate flux increases with the rotational speed of the membrane: the higher the rotational speed, the higher the Jp. It can be seen that the performance is similar for the small species (Nng and Chc) at a high permeate flux of about 800L/h/m^2 (figure 2) and at optimum pressure (between 1 and 2 bar). For Pht, however, the maximum permeate flux was 550L/h/m^2.

Performance is probably affected not by the size of the cell or the class phylum (table 1) but by the shape. As (Mota, Teixeira et al. 2002) described, rod-like particles in cross-flow filtration may be affected by the shear-induced arrangement and become more densely packed, which gives rise to brick-like wall structures, and higher tortuosity. In turn this leads to higher cake resistance and the tortuosity may become more significant when the filtration pressure increases.

For the ceramic membrane pore size of 2µm, spherical shape algae (Nng and Chc) and 1110 rpm, the final flux at 1 bar and 2 bar, respectively, was 671 ± 52 and 707 ± 88

L/h/m^2. For fusiform algae (Pht) the final flux was 403 ± 64 at 1 bar and 460 ± 73 L/h/m^2 at 2 bar.

Table 4: Volumetric Flux Reduction (%) of Membranes at Different TMP and Rotational Speeds for all three microalgae studied at lab scale

			mean value ± confidence interval		
			1110 rpm	*560 rpm*	*0 rpm*
Volumetric flux reduction (%)	**Pht**	1 bar	64 ± 4	78 ± 6	89 ± 2
		2 bar	79 ± 3	88 ± 3	95 ± 1
	Nng	1 bar	38 ± 4	69 ± 5	85 ± 4
		2 bar	72 ± 4	84 ± 2	93 ± 1
	Chc	1 bar	48 ± 10	86 ± 3	88 ±5
		2 bar	72 ± 6	86 ± 3	94 ± 2

Initial fluxes were not significantly different between the species but were twice as high at 2 bar as at 1 bar (2284 ± 157 and 1184 ± 86 L/h/m^2, respectively). This is promising because it indicates that performance and microalgae viability is better at lower pump energy input.

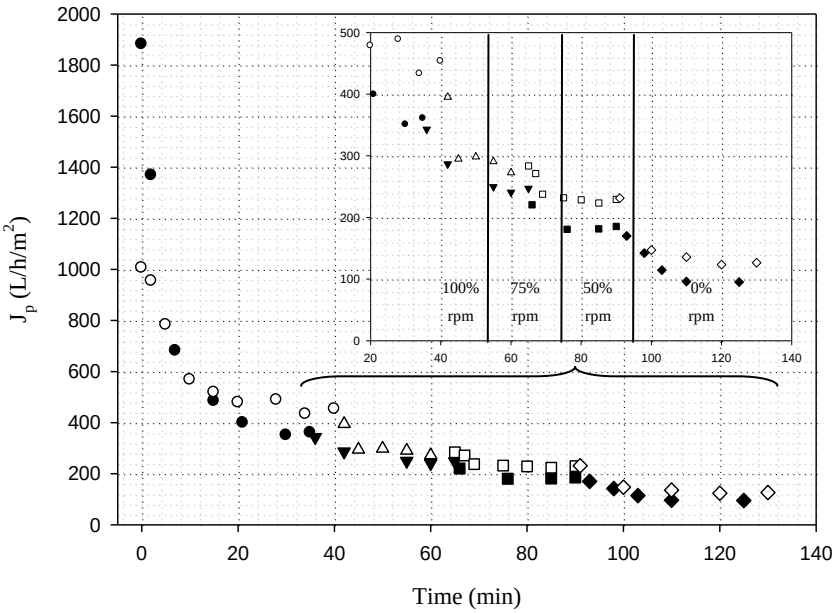


Figure 3: Decrease in the permeate flux with time at different rotational speeds (100%-1100rpm, 75%-835rpm, 50% - 560rpm and 0%-0 rpm). The case of Chc specie, a mean ceramic membrane pore size of 0.5µm, a TMP of 2 bar (full symbols) and 1 bar (empty symbols), and a recirculation volumetric flow rate of 250 L/h.

The rotational velocity of the disks has the greatest effect, particularly on permeate flux. The values in table 4 show the VFR measured for all three species and the enhancement obtained by applying high rotational membrane speeds, which decreases fouling and concentration polarization.

Several experiments were performed at 0, 550 and 1110 rpm for each TMP using fresh microalgae suspensions.

Figure 3 shows a representative case of how flow decreases over time as a result of membrane fouling and concentration polarization at different rotational speeds (ceramic membrane, mean pore size of 0.5µm and 1 and 2 bar for the species *Chaetceros calcitrans*).

The experiments were started at maximum membrane revolution (1110 rpm) and the initial permeability was measured at 1 and 2 bar (in both cases approximately 975 ± 46 L/h/m^2/bar). After 40 min the plateau was reached. The permeability at 1 bar was double that at 2 bar: 458 ± 28 L/h/m^2/bar (53%VFR) and 185 ± 13 (81% VFR), respectively. Then, the rotational speed was gradually reduced to increase flux reduction to 128 ± 6 L/h/m^2/bar, 87% of VFR at 1 bar and 50 ± 5 L/h/m^2/bar, 95% of VFR at 2 bar with a rotation membrane speed of 0 rpm.

For all tested pressures, membrane pore sizes, species and rotational speeds, a sharp and substantial decrease in flux was observed in all experiments. It should be stated that permeate was driven into the feed tank so that the concentration remained constant throughout the experiment.

The mean fluxes for all the experiments carried out at maximum rpm are 56 ± 3%. These values are higher than at the medium revolution values, which were 47 ± 4 % higher than at 0 rpm. This shows the importance of creating turbulence that is not proportional to the rpm and the antifouling strategy improves when rpm is high.

Considering mean values of the experiments at 1110 rpm, the VFR for small species (Nng and Chc) was 54%± 2% at 1bar, at least 20% better than for Pht.

In all cases the initial permeability measured with water was recovered after cleaning.

4.3.2.3 Membrane pore size

The ceramic membranes almost completely rejected all three species of microalgae (>98%) and there were no significant differences between the membrane pore sizes.

Rejection depends on such parameters as electrical interaction between particles and membrane, the shape of the biomass, and the hexogen material from the biomass. The

permeate fluxes are higher for ceramic membranes with large pore sizes, and are independent of the species, rotational speed and pressure.

Table 5: Rejection of membranes (%). calculated from AFDW

	mean value ± confidence interval	
	Ceramic 2um	*Ceramic 0.5um*
Pht mean rejection (%)	98.8 ± 1.0	99.2 ± 0.6
Nng mean rejection (%)	99.5 ± 0.3	98.8 ± 1.2
Chc mean rejection (%)	98.3 ± 0.9	98.8 ± 0.5

The volumetric flux reductions are the same for both the membranes tested (table 6) which means that, as the initial flux for the 2µm membrane is 25% higher than for 0.5 µm, the plateau flux is also proportional higher.

Table 6: Volumetric flux reduction of membranes (%) at different TMP and rotational speeds for both pore size membranes studied at lab scale

			VFR (%)	
		rpm	**Cer 2 µm**	**Cer 0.5 µm**
Volumetric flux reduction (%)	2bar	100%	72 ± 5	76 ± 1
		50%	86 ± 3	86 ± 2
		0%	94 ± 1	94 ± 1
	1bar	100%	49 ± 9	51 ± 8
		50%	75 ± 4	71 ± 4
		0%	89 ± 1	86 ± 2

This behavior can be explained by the fact that smaller pore size membranes can be blocked more quickly than bigger ones. SEM micrographs (figure 4) show selective surface of both pore size membranes.

The results of the preliminary analysis show that 2 µm ceramic membranes perform better for all three species tested in terms of fluxes and rejection.

As there were no significant differences between the pressures tested (1 and 2 bar), we recommend that the lowest pressure be used in order to save energy during pumping and avoid high cake compression.

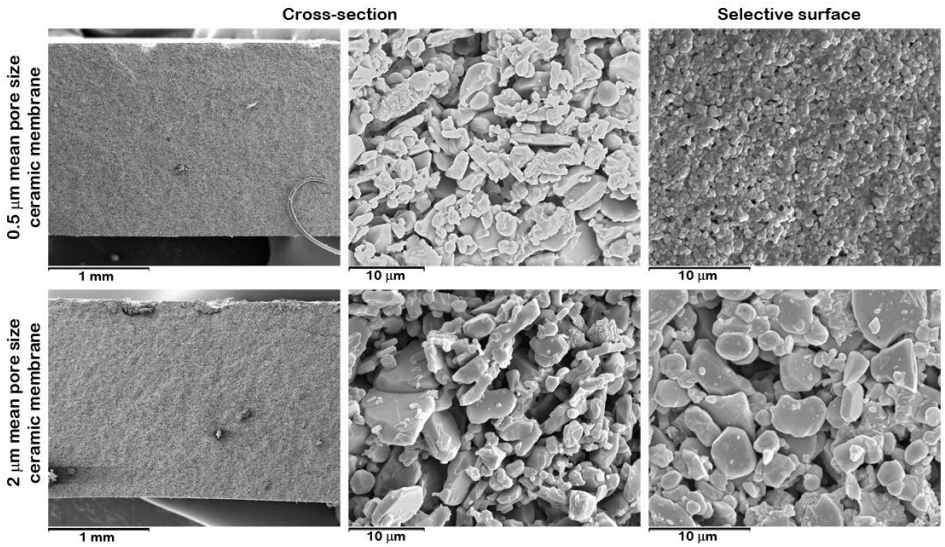


Figure 4: Cross-section and surface SEM micrographs of the ceramic membranes used

Figure 4 shows cross-section and surface SEM micrographs of the ceramic membranes used in this study. They can be used to study the morphology of the membranes.

Both membranes are of very nearly the same thickness. For the 0.5 μm membrane, the thickness is 1.91 ± 0.01 mm, while for the 2 μm membrane, it is 1.98 ± 0.01 mm. No differences in permeability, then, can be expected due to this factor.

Table 7: IFME results obtained from surface micrographs of the membranes used

	0.5 μm membrane	**2 μm membrane**
Number of pores	5507	198
Porosity	12%	9%
Mean pore size	0.095 μm	0.701 μm
Largest pore size	0.705 μm	2.538 μm
Smallest pore size	0.028 μm	0.262 μm
Pore size standard deviation	0.00351	0.1191
Horizontal irregularity	0.00053	0.00087
Vertical irregularity	0.00014	0.00047
Overall irregularity	0.00033	0.00067

The porous structure of both membranes is also similar. The numerical results of the main morphology parameters, obtained with the IFME software from the selective surface micrographs, are presented in table 7. The mean pore size difference is clearly seen in the micrographs and from the value of the largest pore sizes. Also, the

membrane with the highest mean pore size has the greatest distribution of pore sizes. However, both the porosity and the regularity are similar for the two membranes. Regularity is similar because they have the same order of magnitude; the absolute difference is due to the differences in pore size and the number of pores. The large difference observed in the surface is not distinguished in the cross-section micrographs, which present similar pore sizes.

Considering the above, a significant difference in the selectivity of the membranes is expected because the biggest pore sizes are quite different. No great differences are expected, however, in water permeability because they are of similar thicknesses and the hydraulic coefficient given by Darcy's Law, which models the permeability across a membrane (assuming all other factors to be constant), is expected to be similar (Bear 1988).

4.3.3 Pilot-scale concentration experiments

4.3.3.1 Direct dynamic filtration of the slurry

To test membrane performance in optimal conditions (1110 rpm, 1 bar, 2 µm ceramic membranes and a flow rate of 250 L/h) several experiments were performed with high initial volumes for all three species so that the concentration factor was maximum.

Figure 5 shows the experiment done for all three species in the optimal conditions found. Although the concentration factors are 20 times higher than in preliminary studies for Pht and 80 times higher for Nng and Chc, the VFR were only 20% higher than in preliminary studies where the concentration factor was only 4. This means that the plateau variation is not proportional to the retentate concentration.

Table 8: Experimental data for optimum process conditions (1bar, ceramic membranes with a mean pore size of 2 µm at 1110 rpm and a recirculation volumetric flow rate of 250 L/h) for three species tested where C_f and V_f are concentration factors by cell density and volume, respectively, and Jp_o and Jp_f are the fluxes in L/hm^2 at the beginning of the process and after the plateau was reached, respectively.

Specie	Initial volume (L)	Initial cell density (cells/L) **x10^{-6}**	Rejection (%)	C_f	V_f	Jp_o (L/h/m2)	Jp_f (L/h/m2)
Pht	230	3.7 ± 0.3	85%	20	19	1377 ± 35	129 ± 15
Chc	560	1.5 ± 0.3	89%	104	102	1349 ± 5	269 ± 65
Nng	550	9.9 ± 0.9	97%	83	85	1203 ± 28	365 ± 54

In this way for the species Pht, when the concentration factor is 20 the VFR is 90%, 3 times higher than reported in previous work (Rios, Clavero et al. 2011) As far as the other species are concerned, when the concentration factor by volume is 80 and 100 for Nng and Chc, respectively, the VFR is reduced to 80% for Chc and 70% for Nng.

One possible explanation for this phenomenon is that Pht produces a considerable amount of exogenous organic matter such as exopolysaccharides and other insoluble material (Tesson, Gaillard et al. 2008) and in a polydisperse feed, the presence of smaller particles in the microfiltration process often leads to a high flux reduction. The shape and size of the microalga may also be responsible.

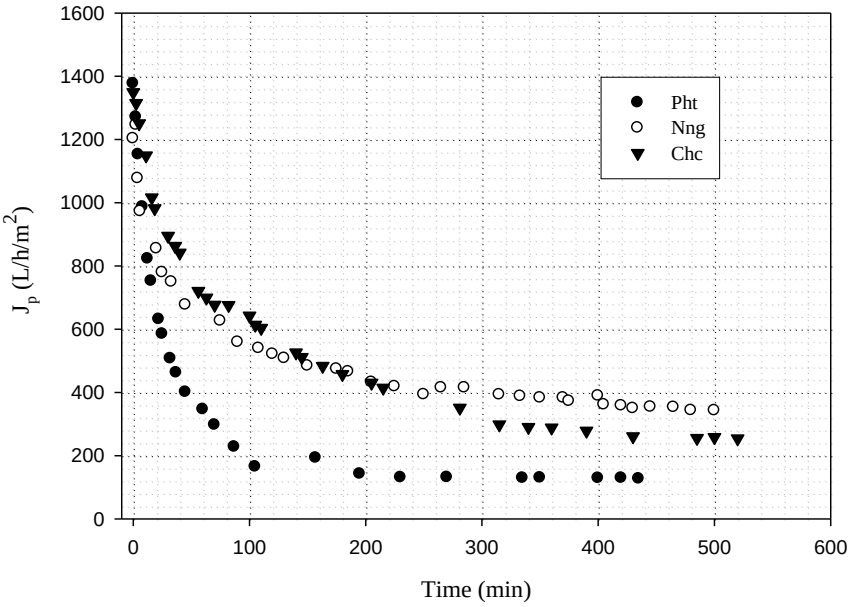


Figure 5: Variation of the permeate flux (at pilot scale) with time for the different species tested (Nng, Pht and Chc) at 1bar, ceramic membranes with a mean pore size of 2 μm at 1110 rpm and a recirculation volumetric flow rate of 250 L/h

Rejection also decreases in these experiments as can be seen in table 8. For Pht, rejection was 85%; for Chc it was more or less the same (89%); and for the smallest species that was also from a different class-phylum, Nng, rejection was the same as in preliminary studies (97%). However, it should be pointed out that no serious cell damage was observed by microscope in the retentate fraction at the end of the experiments (500 min) except in some of the Chc cells, which lost some of their setae.

4.3.3.2 Dynamic filtration from pre-concentrated slurry

The economic viability of the microfiltration process depends on the flux and the capacity of the module. The higher the flux, the faster the feed can be processed (Kim, Akeprathumchai et al. 2001) or the smaller the area of permeate needs to be.

Another antifouling strategy was tested in an attempt to get higher fluxes in a high-scale process. The strategy consists of a pre-concentration step before dynamic filtration in which the volume is reduced about 10 times at a sedimentation speed of about 130cm/h and a relatively low economic cost (data shown in annex table).

A pH-induced flocculation-sedimentation was carried out to reduce the treated volume and increase particle size for dynamic microfiltration. For the two tested species (Nng and Pht) the initial concentration was 10 times higher than the initial culture concentration conditions.

As can be seen in figure 6 a and b, the fluxes of pre-treated slurry are two times higher than those of non-treated slurry for the species Nng and more than three times higher for the species Pht.

Although after the first flocculation-sedimentation step the initial concentration was 10 times higher (3.61 E+07 for Pht and 1.18E+08 for Nng) than for non-sedimented slurry and this was expected to affect the decrease in the flux, this was not the case here. The fluxes in dynamic filtration from pre-treated slurry were more or less the same for both species (Nng and Pht). When the microalgae were not pre-treated the flux for Nng was more than twice as high as for Pht, which may be because the flocculation-sedimentation step is not so important when the cells are larger or because the conglomerate of the external organic matter was soluble before the chemical change.

Under alkaline conditions, some chemical ions in the medium precipitated together with the algal biomass. Two major reactions are effective when the pH is elevated to alkaline values: i.e. calcium carbonate $CaCO_3$ precipitation and magnesium hydroxide $Mg(OH)_2$ precipitation (Alexeyev 1979). However, the role played by each reaction depends on the primary particles and on the ions contained in solution.

According to (Sur and Cui 2005) another possible explanation for the improvement in filtration is that the pH of the solution is one of the main parameters that affects permeate flux. They studied how the solution pH affects the cake characteristics because of the effect it has on the electrostatic interaction between particles and, hence, the permeate flux.

The surface charge of the cells affects the interaction between the cells, the EPS, and the membrane surface and, as occurs in the microalgae sedimentation step, the isoelectric point of the slurry should be reached for cells to be neutralized and precipitated. These conditions improve the filtration flux. Further experimental characterization is required to clarify this phenomenon.

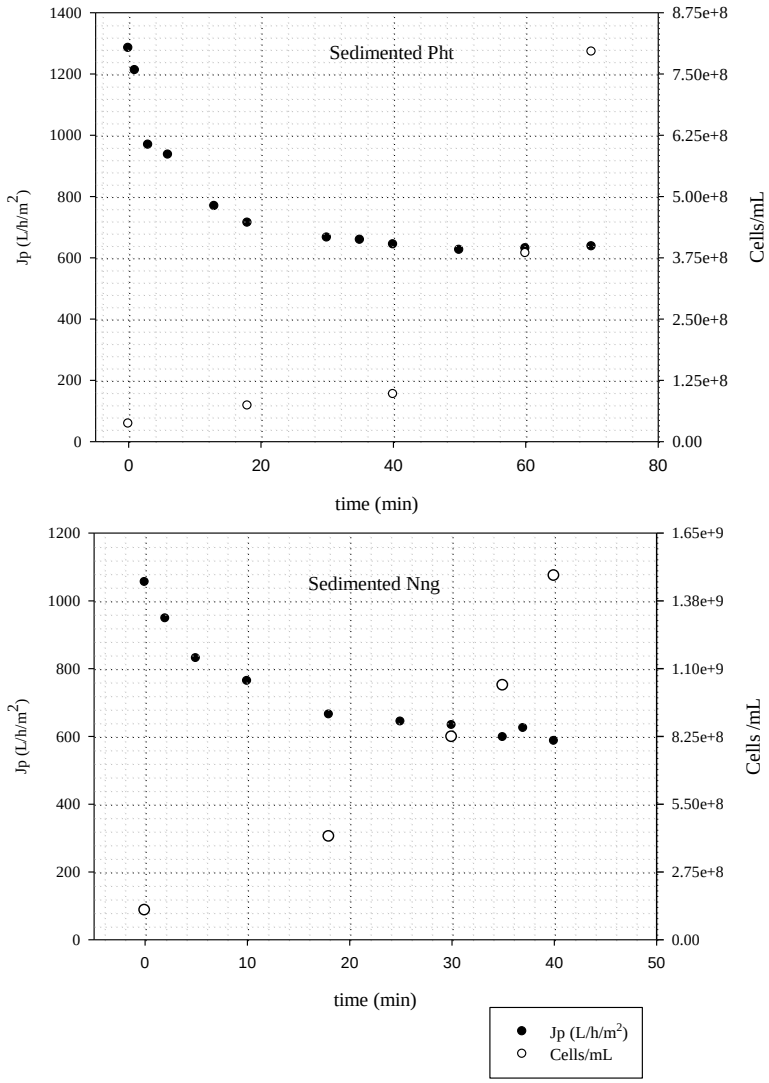


Figure 6: Variation of the permeation flux (L/h/m^2) and cell density (cells/mL) with time for the pre-treated species tested (a) Pht and (b) Nng at 1 bar, ceramic membranes with a mean pore size of 2µm at 1110 rpm and a recirculation volumetric flow rate of 250 L/h

Table 9 shows a summary of the whole pilot-scale concentration process: an initial pre-concentration step by alkali induction followed by dynamic microfiltration in optimal conditions.

As can be seen, the initial slurry volume was 900L for both species treated (Nng & Pht) after one hour of sedimentation. The final sedimented volume for Nng was smaller than for Pht and concentrations were higher perhaps because the size of the cells led to smaller flocs and a more compact. More studies should be carried out to confirm this hypothesis.

The concentration factors by volume or cells/mL V_f and C_f after the pre-concentration step were 9 and 13.5 for Pht and for Nng, respectively, and after dynamic filtration 21.5 and 11.5. The result of the overall process is a 170-fold increase in concentration in two hours.

It should also be said that pre-concentration by pH-induced flocculation is environmentally friendly because the variation in pH is not very high and the pH of sea water is now becoming more acidic because of green house gases (specially CO_2). This is one of the challenges of the new millennium (Hofmann and Schellnhuber 2010). The harvesting technique we propose, then, can be used to solve energy and environmental problems.

Table 9: Summary of the major properties of the pilot-scale process with the pre-treated species tested (Nng and Pht).

Species	Initial volume (L)	Initial cell density (cells/L) **x10^{-6}**	Rejection (%)	C_f	V_f	J_{po} (L/h/m^2)	Process time (h)	J_{pf} (L/h/m^2)
Pht sedimented	900	3.65 ± 0.25	-	10	8	-	1	-
Nng sedimented	900	9.65 ± 0.55	-	12	15	-	1	-
Pht S+F	115	36 ± 10	99%	22	21	1283 ± 60	1.2	636 ± 20
Nng S+F	62	131 ± 14	99%	12	11	1054 ± 110	0.7	565 ± 45

The concentration factor also depends on the method used to measure the variable. In figure 6 pilot experiments are measured by different methods and it can be seen that the accuracy is good enough if it is borne in mind that we are dealing with live organisms.

Table 10: Plateau permeability for several studies done with different membranes and algae species

Membrane	Microalgae species	Plateau permeability (L/h/m2/bar)	References.
Milipore 0.45 um	*Scenedesmus, Monoraphidium sp-. Navicula sp.*, etc	**20**	Petruevski et al (1995)
Polyacrylonitrile 40 kDa	*Haslea ostrearia*	**30**	Rossignol et al (1999)
Polyacrylonitrile 40 kDa	*Skeletonema costatum* (freeze dryed)	**60**	Rossignol et al (1999)
Polyacrylonitrile 40 kDa	*Arthrospita platensis*	**16.5**	Rossi et al (2004)
PVC UF 50kDa	*Scenedesmus cuadricauda*	**150**	Zhang et al (2010)
Ceramic 2um (DCF)	*N gaditana, P tricornutum* (Pre-treated)	**600**	Rios et al (2012)

Further experiments should be performed to search for the optimum method to measure the concentration variable, which also depends on the species studied and the concentration range of the slurry.

As can be seen in figure 7 the VFR can vary between 10 and 20% for the different methods tested and it is difficult to conclude which of the methods is the best. The only conclusion that can be drawn is that variability depends on such parameters as cell concentration, size and shape of the cells and probably the extracellular organic matter in suspension, especially with AFDW, where the differences are higher in pre-concentrated experiments because with the chemical change produced, not only do we sediment the cells, but also ions from the medium.

Table 10 presents the results of several recent studies. A comparison of VFR and the permeabilities at the plateau shows the great performance reached with the pre-concentrated slurry and the 600L/h/m^2/bar of permeability. These high concentrations and permeabilities are necessary for scale-up.

The results show that the use of microfiltration with different antifouling strategies is a promising method for concentrating microalgae.

The study shows that the microalgae concentration process needs a combination of solid-liquid separation techniques and that in our case there is a synergy between methods.

The *Chaetoceros calcitrans* species is not suitable for pH-induced pre-concentration because after sedimentation the cells undergo a cell-wall lysis which causes the death of the cell and the exudation of internal cell components that buffer the slurry. More studies should be carried out to optimize the pre-concentration step of this species.

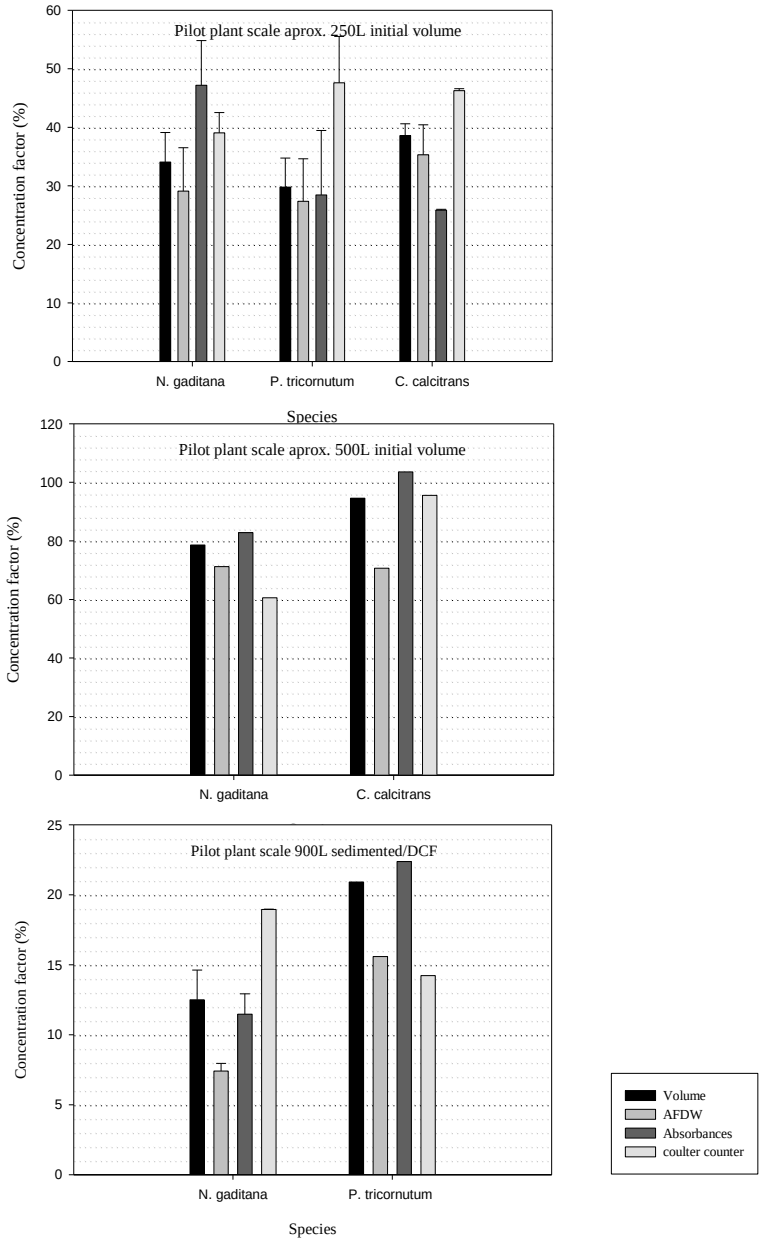


Figure 7: Variation of the concentration factor (Cf) for the different methods analyzed, where volume is the volumetric reduction factor, AFDW is ash free dry weight, the absorbance is cells/mL measured by spectrophotometer absorbance at 750nm, and the multisizer Coulter counter is used to measure particles/mL) for different pilot-scale concentration processes

4.3.4 Economic study

In the sections above, dynamic filtration has been shown to increase performance more than tangential cross-flow filtration. This technique considerably increases permeability, which means processing time is reduced; fewer units are required to treat the same volume, etc. However, more electrical energy is required for the rotor to work. Therefore, an economic analysis should also be carried out to study whether the change proposed decreases the cost of the process or not.

4.3.4.1 Economic study of DCF not considering pre-concentration

We based our economic study on a previous economic balance by (Zhang, Hu et al. 2010) since it deals with the same process and uses tangential cross-flow filtration with a back-flush antifouling technique. On this basis, then we set up a reference scenario using the same values and only adapting the cost of the membrane modules (which also include the membranes themselves).

The base investment costs of membrane set-up have been taken from the information provided by GEA-Westfalia, KMPT and New Logic Research. Thus, for tangential cross-flow filtration, the base cost of \$1500 m^2 has been used, while for dynamic filtration, the base cost is \$2140 m^2.

As far as the power consumption of dynamic filtration is concerned and on the basis of the information published on the New Logic Research web page (VSEP 2011), we adopted a conservative approach similar to that described in case studies of the Portland waste oil installation, In this case the power required to process 20 gal/min is about \$3113/year and the highest cost is that of system maintenance and cleaning.

Table 11 shows a summary of the economic balance for both scenarios and the complete balance is presented in appendix 1. The total investment cost of the harvesting process was evaluated. It includes all the equipment needed (membranes, membrane modules, pumps and air compressor), land cost and construction per square meter including the capital cost of the tanks. Also, all the services (electricity for pumping, membrane rotation, air compression and chemicals, labor, etc) are included in the operational cost.

Table 11: Economic study of harvesting processes using the DCF antifouling strategy in comparison with Zhang et. al 2010

	Category		Cost (cross-flow filtration) (ref)		Cost (dynamic filtration)
Capital cost	Land cost ($)	$	184,893	$	182,930
	Construction ($)	$	74,826,317	$	74,031,956
	Total capital cost ($)	**$**	**6,855,828,834**	**$**	**4,076,977,834**
Operational Cost	Electricity & chemicals ($/year)	$	4,585,113	$	42,970,143
	Membrane replacement ($/year)	$	1,625,471	$	672,238
	Labor ($/year)	$	1,050,000	$	1,050,000
	Total operational cost ($/year)	**$**	**7,260,583**	**$**	**44,692,381**
Total cost	Capital cost ($/year)	$	597,722,400	$	355,449,506
	Operational cost ($/year)	$	7,260,583	$	44,692,381
	Total cost ($/year)	**$**	**604,982,983**	**$**	**400,141,887**
	Overall cost ($/kg dry biomass)		**0.75 $/kg**		**0.50 $/kg**

For the comparison, the new scenario was to process the same volume within the same time as the reference scenario, but reducing the number of membrane modules required because the permeabilities improvements by DCF. In this way, the investment cost was reduced quite considerably, because the number of modules required was reduced from 41,000 of 33m^2 each (1.55E+6 m^2 of membranes) to 4000 DCF modules of 140m^2 each (5.6E+5 m^2 of membranes). The DCF modules require a permeate area that is 40% smaller than the other modules, which reduces the number of modules, the footprint area, etc.

The new scenario is more energy intensive and the electricity consumed increased by almost 90% (4E+7 $/year). However, the impact is much less than the increase would suggest because the most sensitive value of the overall cost is the capital cost and, in particular, the membrane area although the depreciation rate which have been taken into account (20 years) is more favorable for a high capital cost process.

A sensitivity study was carried-out for three key variables throughout the process of biomass production by DCF and TFF. Two variables are directly related to the capital

cost, particularly to the membrane module construction (flux and membrane price), and one variable is directly related to the operational cost (energy consumption).

As can be seen in figure 8, the variables that have a direct effect on capital cost (flux, membrane prices) have the highest impact on the overall cost of microalgae biomass production by DCF. Particularly important is the permeate flux, which determines the number of membrane modules, the footprint, etc.

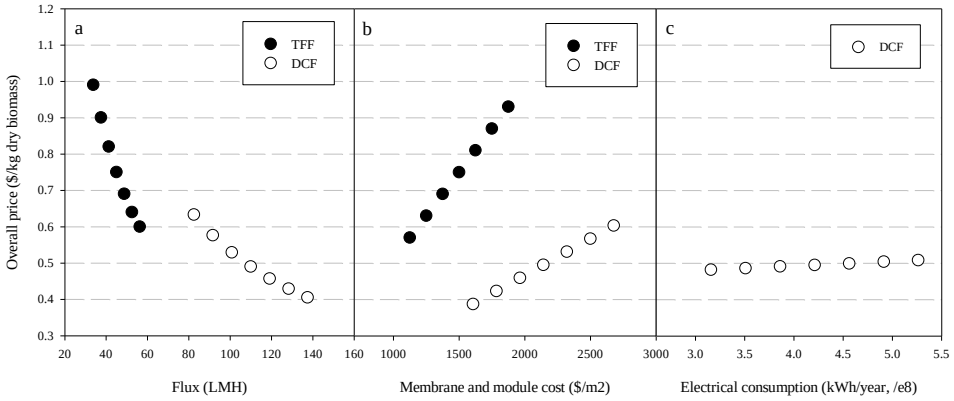


Figure 8: DCF sensitivity analysis for three variables (power consumption for cross flow, module and membrane price and fluxes)

This explains why the overall cost of producing microalgae biomass is cheaper for DCF than TFF, although the former is more energy intensive than the latter. As can be seen in figure 8, in the cross flow model energy has little effect on the final cost of biomass production: a 100% variation has less than a 5% impact on final cost.

According to figure 8, the overall price ($/kg dry biomass) is more sensitive for TFF than DCF in the scenarios tested (flux and membrane module cost) where the steepness of the slope indicates the degree of sensitivity.

4.3.4.2 Economic study of DCF considering pre-concentration

A third antifouling strategy—pH induced flocculation-sedimentation—was evaluated. Not only does it reduce the initial volume to be filtered at a low investment cost, the chemical change that takes place also considerably enhances the permeate flux. These two advantages considerably reduce the number of membrane modules and pumps required, so the capital cost is reduced by at least one order of magnitude.

For the comparison, the same volume was processed in the same time as the reference strategy, (Zhang, Hu et al. 2010) and by applying the permeability increment advantage and the reduction in the volume to be filtered by the pre-concentration process, considerably fewer membrane modules are required.

For the sedimentation process, we have included the price of the decanter tanks in the construction cost at the same price as for the filtration construction (this is a very conservative scenario, because tank construction should be more economic than vertical construction). Also, the sedimentation process can be considered to be continuous if the tank volume is proportional to the filtration feed needed and the number of tanks is multiplied by the same ratio.

Table 12: Economic study of harvesting processes by pH induced flocculation sedimentation followed by dynamic cross flow filtration and a hypothetic scenario for Zhang et all 2010 (Zhang, Hu et al. 2010)

	Category	**Cost (sedimentation+cross-flow filtration)**	**Cost (sedimentation+dynamic filtration)**
Capital cost	Equipment cost ($)	$ 378,656,971	$ 199,459,216
	Land cost ($)	$ 190,850	$ 190,733
	Construction ($)	$ 77,237,141	$ 77,189,451
	Total capital cost ($)	**$ 456,084,963**	**$ 276,839,400**
Operational cost	Electricity & chemicals ($/year)	$ 43,731,857	$ 51,662,365
	Membrane replacement ($/year)	$ 90,621	$ 33,392
	Labor ($/year)	$ 1,050,000	$ 1,050,000
	Total operational cost ($/year)	**$ 44,872,477**	**$ 52,745,757**
Total cost	Capital cost ($/year)	$ 39,763,565	$ 24,136,120
	Operational cost ($/year)	$ 44,872,477	$ 52,745,757
	Total cost ($/year)	**$ 84,636,043**	**$ 76,881,878**
	Overall cost ($/kg dry biomass)	**0.0085 $/kg**	**0.0077 $/kg**

It was taken into account not only the chemicals needed for the pH induction sedimentation, but also those required for neutralizing the waste water and the cleaning process. As can be seen in table 12, the capital cost was still greater, but the difference was not as high as with only filtration, where it is two orders of magnitude higher than the operational cost.

As can be seen in table 12, the combination of two solid-liquid separations improves the final overall cost of dry biomass by two orders of magnitude.

A hypothetic scenario combining the antifouling strategy carried out by Zhang et al 2010 (Zhang, Hu et al. 2010) and the improvement achieved by flocculation sedimentation technique was evaluated. The scenario description includes the estimation of flux obtained from our work and Kim et al (Kim, Akeprathumchai et al. 2001). Table 12 shows the substantially improvement of the overall cost of biomass concentration from pre-concentrated microalgae slurry and confirm that DCF is still 10% less cost effective than cross flow filtration moreover when pre-concentration is performed

Schenk et al 2008 mentioned that harvesting is one of the major limiting factors because to be viable for biodiesel production the cost of the biomass had to be less than $300 US/ton dry weight and also have high lipid content. This study has proved that the proposed techniques to reduce fouling (pH induces flocculation-sedimentation + dynamic cross flow filtration) can lead, at a conservative estimate, to a biomass cost of less than $8 US/ton dry weight (table 10).

Although most reviewers (Amer, Adhikari et al. 2011) (Davis, Aden et al. 2011) point out that production improvements—especially in the microalgae slurry concentration—are essential if the downstream cost (harvesting) is to be reduced, we believe that concentration could be the key point in biodiesel production. As this study has shown, non concentrated initial microalgae slurry (open pond) can be pre-concentrated using an economic, low-energy and environmentally-friendly technique to obtain high concentrated biomass after sedimentation followed by dynamic cross flow filtration.

In the appendix, we suggest a scenario with an initial microalgae slurry concentration of 0.5g/L (typical of open ponds) and a production of $8.5 US/ton dry weight by combining two separation techniques (sedimentation by induced pH followed by DCF).

The initial microalgae concentration is not the most sensitive parameter in obtaining microalgae biomass. The lipid content or lipid quality may play a fundamental role in the production of biodiesel from microalgae on an industrial scale.

4.4.- Conclusions:

This chapter aimed to propose a possible solution to the microalgae harvesting bottleneck in the production of biomass from microalgae.

The results of the lab-scale experiments showed that microfiltration does not perform better because increasing the TMP causes fouling effects. Therefore, the process can be carried out with a lower energy cost. Additionally, with lower TMPs, it might possible to use low-cost materials to design membrane modules, which reduces the capital cost.

The increase in rotational membrane speed clearly enhances the process performance because the share stress over the membrane increases. As a result, there is an increase in membrane permeability owing to less fouling and concentration polarization.

The VFRs calculated for the membrane pore sizes tested (2 and 0.5 μm) are almost the same and although there was not a great deal of difference in the plateau flux of both membrane sizes, the trend was for the flux to be greater for the bigger pores. The results show that membranes with bigger pore sizes undergo less fowling and concentration polarization than membranes with small pore sizes.

Pilot-scale experiments showed that dynamic cross flow filtration is a promising way to concentrate biomass from diluted suspensions. The process can enhance fluxes and optimum filtration conditions can be found by using the right combination of variables.

The fact that concentration factors were higher than 100 when plateau fluxes were also high (>300L/h/m^2) is promising and suggests that the design process can be scaled up.

When the algae cultures were pre-concentrated by alkalinity induced flocculation and then filtered, the permeabilities obtained were almost two times higher (≈600L/h/m^2/bar) than without pre-concentration. The increase in initial concentration was almost 200 times higher than the initial slurry.

Hence, it could be concluded that alkalinity induced flocculation followed by dynamic filtration can improve the overall process by making a synergy between processes.

Although more energy is required for dynamic filtration than tangential filtration, the improvement in permeability can make up for this cost difference. The high capital cost of filtration is mostly due to the modules and the replacement of membranes. Therefore, by improving the permeability the number of modules used and/or membrane area can be reduced substantially.

The sensitive variable analysis showed that the energy consumption is trivial in comparison with membrane replacement and membrane module investment.

From the results, it could be concluded that biomass can be obtained from microalgae at a low price, less than $10 US/ton, with initial concentrations of algae that can even be found in open ponds as reported.

Further research should be performed to maximize harvesting efficiency and minimize the cost. Particular areas of investigation could be the optimization of filtration variables with pre-concentrated slurry and the characterization of the final product, among other things, so that an overall economic balance can be obtained. Life cycle analysis should also be done to determine whether the process is economically feasible and environmentally friendly.

4.5.- References:

Amer, L., B. Adhikari, et al. (2011). "Techoeconomic analysis of five microalgae-to-biofuels processes of varying complexity." Bioresource Technology **102**(20): 9350-9359.

Bear, J. (1988). "Dynamics of fluids in porous media." Courier Dover Publications and American Elsevier Publishing Company, Inc.

Belfort, G., R. H. Davis, et al. (1994). "The behavior of suspensions and macromolecular solutions in crossflow microfiltration." Journal of Membrane Science **96**(1-2): 1-58.

Bouzerar, R., L. Ding, et al. (2000). "Local permeate flux-shear-pressure relationships in a rotating disk microfiltration module: implications for global performance." Journal of Membrane Science **170**(1): 127-141.

Bowler, C., A. E. Allen, et al. (2008). "The Phaeodactylum genome reveals the evolutionary history of diatom genomes." Nature **456**(7219): 239-244.

Davis, R., A. Aden, et al. (2011). "Techno-economic analysis of autotrophic microalgae for fuel production." Applied Energy **88**(10): 3524-3531.

Ding, L. H., M. Y. Jaffrin, et al. (2006). "Investigation of performances of a multishaft disk (MSD) system with overlapping ceramic membranes in microfiltration of mineral suspensions." Journal of Membrane Science **276**(1-2): 232-240.

Frappart, M., A. Massé, et al. (2011). "Influence of hydrodynamics in tangential and dynamic ultrafiltration systems for microalgae separation." Desalination **265**(1-3): 279-283.

Hofmann, M. and H. J. Schellnhuber (2010). "Ocean acidification: A millennial challenge." Energy and Environmental Science **3**(12): 1883-1896.

Jaffrin, M. Y. (2008). "Dynamic shear-enhanced membrane filtration: A review of rotating disks, rotating membranes and vibrating systems." Journal of Membrane Science **324**(1-2): 7-25.

Jaouen, P., L. Vandanjon, et al. (1999). "The shear stress of microalgal cell suspensions (Tetraselmis suecica) in tangential flow filtration systems: the role of pumps." Bioresource Technology **68**(2): 149-154.

Kim, J.-S., S. Akeprathumchai, et al. (2001). "Flocculation to enhance microfiltration." Journal of Membrane Science **182**(1-2): 161-172.

Martin-Jézéquel, V., M. Hildebrand, et al. (2000). "Silicon metabolism in diatoms: Implications for growth." Journal of Phycology **36**(5): 821-840.

Meiser, A., U. Schmid-Staiger, et al. (2004). "Optimization of eicosapentaenoic acid production by<i>Phaeodactylum tricornutum</i>in the flat panel airlift (FPA) reactor." Journal of Applied Phycology **16**(3): 215-225.

Mengual, J. A. I. (1989). Fundamentos de los procesos de transporte y separación en membranas. Murcia, EDITUM-Universidad de Murcia. **1:** 171 páginas.

Molina Grima, E., E. H. Belarbi, et al. (2003). "Recovery of microalgal biomass and metabolites: process options and economics." Biotechnology Advances **20**(7-8): 491-515.

Mota, M., J. A. Teixeira, et al. (2002). "Influence of cell-shape on the cake resistance in dead-end and cross-flow filtrations." Separation and Purification Technology **27**(2): 137-144.

Ochirkhuyag, B., T. Mori, et al. (2008). "Development of a high-performance cake-less continuous filtration system." Chemical Engineering Science **63**(21): 5274-5282.

Pruvost, J., G. Van Vooren, et al. (2009). "Investigation of biomass and lipids production with Neochloris oleoabundans in photobioreactor." Bioresource Technology **100**(23): 5988-5995.

Rios, S. D., E. Clavero, et al. (2011). "Dynamic Microfiltration in microalgae harvesting for biodiesel Production." Industrial and Engineering Chemistry Research **50**(4): 2455-2460.

Şirin, S., Trobajo, R., Ibanez, C., Salvadó, J. 2012. Harvesting the microalgae Phaeodactylum tricornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. *Journal of Applied Phycology*, **24**(5), 1067-1080.

Serra, C. A. and M. R. Wiesner (2000). "A comparison of rotating and stationary membrane disk filters using computational fluid dynamics." Journal of Membrane Science **165**(1): 19-29.

Sur, H. W. and Z. F. Cui (2005). "Enhancement of microfiltration of yeast suspensions using gas sparging – effect of feed conditions." Separation and Purification Technology **41**(3): 313-319.

Tesson, B., C. Gaillard, et al. (2008). "Brucite formation mediated by the diatom Phaeodactylum tricornutum." Marine Chemistry **109**(1-2): 60-76.

Torras, C. and R. Garcia-Valls (2004). "Quantification of membrane morphology by interpretation of scanning electron microscopy images." Journal of Membrane Science **233**(1-2): 119-127.

Torras, C., J. Pallares, et al. (2009). "Numerical simulation of the flow in a rotating disk filtration module." Desalination **235**(1-3): 122-138.

VSEP (2011). "membrane filtration of waste oil: A cost effective and environmentally sound processing solution"." Vibratory Shear Enhanced Processing (VSEP)" New Logic Research Inc**. http://www.vsep.com/pdf/WasteOil.pdf**

Walne (1970). "Studies on the food value of nineteen genera of algae to juvenile bivalves of the genera Ostrea, Crassostrea, Mercenaria, and Mytilis. ." Fishery Investigations. **Series 2, London.** : pp 26–62.

Zhang, X., Q. Hu, et al. (2010). "Harvesting algal biomass for biofuels using ultrafiltration membranes." Bioresource Technology **101**(14): 5297-5304.

Zhu, C. J. and Y. K. Lee (1997). "Determination of biomass dry weight of marine microalgae." Journal of Applied Phycology **9**(2): 189-194.

CHAPTER V

5.- Lipid extraction methods from microalgal biomass harvested by two different paths: screening studies toward biodiesel production

5.1.- Introduction

Triglycerides are esters derived from three fatty acids and a glycerol molecule. The fatty acids normally found in microalgae vary in chain length, degree of unsaturation and position on the glycerol molecule. Consequently, the triglycerides fraction itself consists of a complex mixture of different types of molecules. Each type of fat has a different profile of lipids which determines the precise nature of its physiochemical properties.

To quantify and identify the fatty acid methyl esters (FAMEs), a reaction of the lipids known as transesterification is usually carried out. This process includes an alcohol (methanol) in the presence of a catalyst (acidic or basic) and should be performed before the gas chromatography (GC) analysis to determine the FAME/TAG or FAME/TL or FAME/DW average.

Whichever lipid extraction method is chosen, the sample should be prepared and characterized and the process variables (reaction time, temperature, amount of sample or solvent, etc.) should be optimized.

The sample preparation must take into account the different elements of biomass in the sample because the harvesting method may have added or reduced the levels of ash or organic matter, especially if chemical flocculation is used. Alternatively, the levels of organic matter in the sample can be standardized or the ash can be removed.

Direct transesterification DT method have been reported as a single step extraction and FAME production reaction (McNichol, MacDougall et al. 2012; Velasquez-Orta, Lee et al. 2012) to analyze FAME content, but DT could be not only an analysis method but also a potential way to overcome the BD production directly from the dry solid biomass, especially using membrane reactors.

This chapter focuses on the lipid extraction and quantification of the FAME fraction from three microalgae species obtained by two different harvesting paths, providing a critical point of view according to the suitability of the microalgae as a source of BD production in a scale up.

5.2.- Materials and Methods

5.2.1 Materials

5.2.1.1 Reagents

The chloroform and n-hexane were purchased from J.T Baker Chemical Co., Europe. The hydrochloric acid and methanol were obtained from Sigma-Aldrich Inc. and Scharlab S.L. respectively. All reagents were of analytical grade and used as received without further purification.

The fatty acid standard was Supelco 37 component FAME mix and was purchased from Sigma-Aldrich Inc., Spain.

Deionised water was further purified by passing it through a Milli-Q Plus purification system (Millipore SA, Spain).

5.2.1.2 Microalgae Cultures

Three microalgae species were tested, these being *Phaeodactilum tricornutum* (PhT), *Nannochloropsis gaditana* (Nng) and *Chaetoceros calcitrans* (ChC). They have all long been used in aquaculture mainly as a food source in the production of fish, crustacean and bivalve (Fajardo, Cerdán et al. 2007),(Volkman, Jeffrey et al. 1989). They were isolated and produced in the IRTA laboratories in Sant Carles de la Ràpita (Tarragona, Spain). PhT is fusiform in shape with a mean size of 3.5 x 40 µm; Nng is a sphere-shaped alga with a diameter of 3 micrometers; and ChC which is cylindrical in shape with setae (a stiff hair, bristle-like appendix).

Algae culture were growing from agar plate to cultivated in 300L photobiorreactors as it was explained in chapter 2.

5.2.2 Methods

5.2.2.1 Microalgae harvesting

For all experiments, cultures were harvested at the end of the exponential growth. This was carried out 7 days after inoculation for the diatom algae (PhT and ChC), and after 10 days after inoculation for the Eustygmatoficea alga species (Nng). Two paths were followed to concentrate the microalgae (Figure 1).

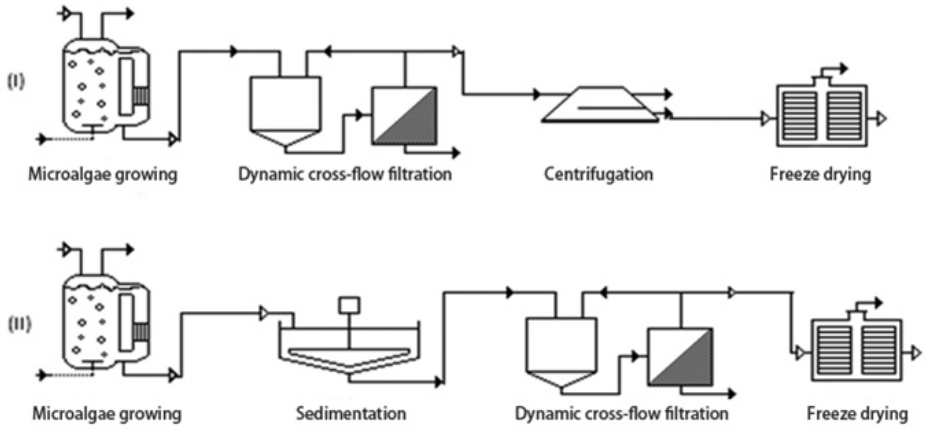


Figure1. Diagrams of concentration paths (I) from photo bioreactor to final freeze drying passing through dynamic microfiltration and centrifugation (II) from photo bioreactor to final freeze drying passing through pH induce flocculation sedimentation and dynamic microfiltration.

(I) *PhT, Nng and ChC* species grown in photo bioreactors were harvested by dynamic cross flow microfiltration at the optimum conditions described by Rios et. al (Rios, Clavero et al. 2011). Afterwards, the concentrate obtained was further dewatered using a bench top centrifuge (Jouan MR 23-i). The final paste was quickly frozen at -80ºC and then freeze-dried (Lyoquest ecoplus -80)

(II), *PhT* and Nng species grown in photo bioreactors were pre-concentrated by pH induced flocculation-sedimentation (Sema Sirin 2011) in photo bioreactors. The pre-concentrated algae culture obtained was filtrated using dynamic cross flow microfiltration at optimum conditions as described elsewhere (Rios, Clavero et al. 2011; Ríos, Salvadó et al. 2012). Then, a fraction of that paste was quickly frozen at -80ºC and then freeze-dried (Lyoquest ecoplus -80).

5.2.2.2 Dry cells analysis by Thermogravimetric Analyzer (TGA)

For the lipid analysis, first of all the biomass needs to be standardized (McNichol, MacDougall et al. 2012) because of differences in humidity, ash and organic matter, differences between species and the chosen harvesting method. In this study, the biomass was taken as a dry matter (DW) or the same matter free of humidity and ash (AFDW) base.

Dry matter composition was evaluated using a Leco TGA701 instrument in order to identify the humidity and the organic and ash content. The microalgae samples were homogenized, weighed (0.15±0.01g), and then put into pre-weighed cresols for analysis. The temperature was increased by 2ºCmin^{-1} up to 100ºC. Samples were maintained at this temperature until no more mass variation was observed. If all humidity had evaporated, the temperature was raised from 100^{0}C to 450^{0}C by 10^{0}Cmin^{-1}. Samples were kept at 450^{0}C until a constant mass was reached. The differences between the data were used to calculate the humidity and the organic and ash fractions. The samples were replicated three times.

5.2.2.3 Lipid extraction

Three independent aliquots of each species and each harvesting method were used to provide replicates for each lipid extraction tested.

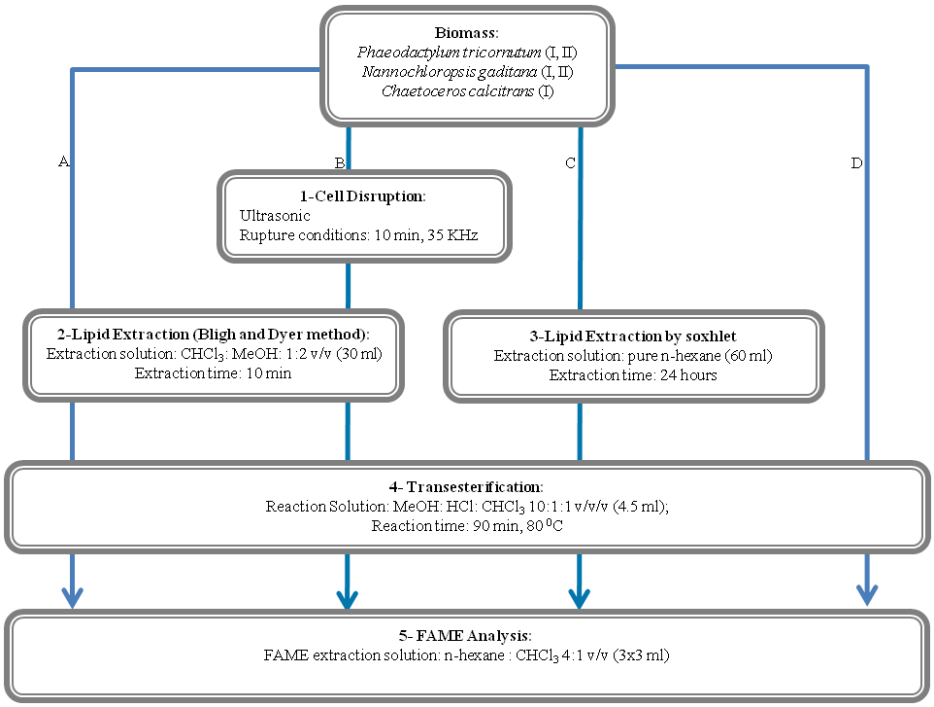


Figure2. Diagram of the different methodologies tested for dry microalgae lipid extraction

1g dry weight and 0.5 g dry weight of concentrated algae cells from a homogeneous mixture were randomly assigned to each replicate of Bligh and Dyer's method and to each replicate of soxhlet extraction respectively. Blanks containing no cells were

running in triplicate for each extraction procedure. Samples and blanks were coded and processed in random order.

Figure 2 shows the experimental design for the lipid extraction methods. All the experiments were analyzed in triplicate. (Figure 2) represents the lipid extraction method carried out by modifying the Bligh and Dyer (Bligh and Dyer 1959) extraction method (m B&D) followed by trans-esterification and FAMEs analysis (Lewis, Nichols et al. 2000). Extraction path (B) is the same as path (A) but also contains the additional step of cell wall breaking by ultrasound (um B&D). Extraction path (C) and path (D) represent a direct lipid extraction method with n-hexane in soxhlet apparatus and direct trans-esterification (DTE-m) of the initial dry biomass respectively. FAME analysis was performed after each extraction path.

Extraction methods were tested for all 3 species of microalgae and for the two concentration paths. This was followed by biomass concentration I and II (Figure 1). The methodology of each lipid extraction method will be detailed next.

5.2.2.3.1 Ultrasonic disruption followed by Modified Bligh and Dyer method

1g of freeze-dried biomass was blended with 7.5 ml of distilled water in a 100 ml flask and then sonicated using a thermostatic ultrasonic bath (frequency 35 kHz) for 10 min at 25 ºC. Then 30 ml solvent mixture (chloroform: methanol ratio of 1:2 v/v) was added to form a single phase solution with a final ratio of chloroform-methanol-water (1:2:0.75, v/v/v).

The mixture was homogenized with a magnetic stirrer (Multipoint) at 300 rpm for 10 minutes. Then 10 ml chloroform and 5 ml of distilled water were added to form the two phase system.

The phases were separated by 5 min centrifugation at 3500 rpm. The chloroform phase was collected with a Pasteur pipette by inserting the pipette through the upper phase with gentle positive-pressure (i.e., gentle bubbling) in order to avoid contaminating the pipette tip. When the pipette tip was at the bottom of the tube, the bottom phase was withdrawn carefully and then filtered with a Whatman glass fiber filter of 0.45 µm. Then, the filtrate solvent was evaporated in a rotaevaporator (Heidolph instrument at -25KPa and 60ºC). When all the solvent had evaporated, lipids were determined gravimetrically (Zubir and Chin 2010).

5.2.2.3.2 Modified Bligh and Dyer method

1g of freeze-dried biomass was blended with 7.5 ml of distilled water in a 100 ml flask and treated as explained above at 2.2.3.1 but without the ultrasonic step.

5.2.2.3.3 Soxhlet lipid extraction method with (n-hexane)

0.5g of freeze-dried biomass was weighed. It was transferred to a 28 mm id X 80 mm length cellulose extraction thimble (Whatman) which was plugged with silanized glass wool. 60 ml of pure n-hexane was used as solvent. It was refluxed over the thimbles about 20 times per hour for 20 hours in a standard soxhlet apparatus.
The lipid-containing solvent was then recovered in the attached round bottom flask which was then used to dry the lipids in a rotaevaporator (Heidolph at -25 KPa, 60 ºC). After the solvent had completely evaporated, the lipids were determined gravimetrically (Zubir and Chin)

5.2.2.3.4 Direct Transesterification.

The methylation method referred by Lewis et al. 2000 (Lewis, Nichols et al. 2000) was performed in triplicate with different amounts of dry biomass. 15, 30, 100 and 300 mg were weighed in 10 ml test tube and 4.5 ml of reaction mix (methanol: hydrochloric acid: chloroform (10:1:1 v/v/v)) was added. The samples were re-suspended by vortex for 10 seconds and placed in a preheated ultrasonic bath at a constant ultrasonic pulse (35 kHz) at 80 ^{0}C for 90 minutes in order to carry out transesterification.
Once the reaction was completed, the samples were allowed to cool down to room temperature. Then 1.5 ml of distilled water was added and mixed by tube inversion. Fatty acids (FA) were extracted by adding 3 x 4 ml aliquots of a mix of hexane-chloroform (4:1 v/v) and vortexing for 10s, thus separating the two layers and allowing the recovery of the organic phase that contains the FAME fraction.
1 ml of each sample was transferred to clean 1.5ml test tubes and then 0.5ml internal pattern of Methyl heptadecanoate (C17:0) (from 0.75 g/l of stock solution) was added in order to calculate the FAME concentration. The samples were then ready for fatty acid composition analysis.

5.2.2.3.5 Fatty Acid composition analysis.

The fatty acids were analyzed for mB&D, umB&D and soxhlet extraction paths were carried out using the same method as direct transesterification. However, extracted crude lipids (20 ± 2 mg) were used instead of dry biomass.

Analyses were performed using an Agilent 6890 GC (Agilent Technologies instrument) equipped with an Innowax polyethylene glycol capillary column (30 m, 0.32 mm id and 0.25 µm film thicknesses) from Sigma Aldrich, Spain. Both the injector and detector temperatures were kept constant at 150 ^{0}C. The oven temperature was raised from 150 ^{0}C by 10 ^{0}C·min^{-1} up to 260 ^{0}C and maintained for 5 min. Each sample was analyzed in triplicate using a flame ionization detector. The peaks were identified and quantified using Agilent Chem Station software (version B.01.03 (204)). Components were identified by comparing retention time with a standard pattern provided by the 37 Component FAME Mix, SupelcoTM.

5.3.- Results and discussions

5.3.1 Culture and Sample Characteristics

Table 1 shows the characteristics of the cultures and samples used in our study. The initial slurry concentrations before harvesting were measured and determine their ash free dry weight (AFDW) (g/l) and were also measured in triplicate and indirectly by spectrophotometer (cells/ml) (Ríos et al., 2012), it must highlight the representativeness of the pilot scale volume of slurry take it as a base for the study (table 1).

The freeze dried algae biomass compositions were also studied and the data for each culture and sample is shown in table 1.

It can be concluded from the composition differences between the samples (Table 1) that the chosen concentration method has a big effect on composition. It can be seen that ash content is inversely proportional to the organic matter content. For instance, the sedimented (dried) sample had 71(5)% ash content whereas all non-sedimented ones had 41(13) %; and if only Nng and PhT species are compared, non-sedimented biomass have half of the calculated ash 35(5) % on dry biomass respects to sedimented samples.

It can be explained due to when the pH is raised to alkaline, two chemical ions precipitated together with the algal biomass: calcium carbonate $CaCO_3$ and magnesium hydroxide $Mg(OH)_2$ (Vandamme et al., 2012). Each reaction depends on the primary

particles and on the ions contained in the solution. Chemical ions in the culture medium became insoluble after adjusting the pH to alkaline and appeared as ash fraction in the pre-concentrated samples.

Table1. Characteristics of microalgae cultures and samples used for analysis

		Initial biomass properties (Before harvesting)			*Dry biomass properties measured by TGA (After harvesting and freeze dried)*		
	Species	***Initial concentrated volume (L)***	***Initial cell density (cells/ml)***	***Initial AFDW Concentration (g/l)***	***%ash***	***%humidity***	***%organic***
Harvesting Path (I)	PhT	230	3.70×10^6 (0.30×10^6)	0.0625 (0.005)	39 (0.3)	7 (0.2)	54 (0.5)
	ChC	560	1.50×10^6 (0.30×10^6)	0.0925 (0.005)	54 (2.2)	10 (1.2)	36 (1.2)
	Nng	550	9.90×10^6 (0.90×10^6)	0.0750 (0.010)	31 (0.9)	3 (0.6)	66 (0.4)
Harvesting Path (II)	PhT	900	3.65×10^6 (0.25×10^6)	0.0620 (0.005)	69 (1.2)	10 (0.8)	21 (0.4)
	Nng	900	9.65×10^6 (0.55×10^6)	0.0755 (0.010)	73 (0.2)	7 (0.2)	19 (0.1)

However, despite this, ChC species had the higher ash content from the non-sedimented species. This may be due to its structure; ChC is a diatom and has silica frustules and setae which can turn into ash during the drying process. PhT is also a diatom, but the silica content is lower than that of other diatoms. Nng has the lowest ash content when it is obtained without prior sedimentation.

The lipid content directly depends on the ash content and organic matter, etc. of the sample analyzed because the lipids are extracted from the organic fraction of the cells. Therefore, it may be necessary to standardize the data as $mg_{(extracted\ lipids)}/g_{(AFDW)}$ (lipids extracted (mg) per gram of ash free dry weight) instead of the currently favored $mg_{(extracted\ lipids)}/g_{(DW)}$ (lipids extracted (mg) per gram of dry biomass), despite washing the biomass with deionized water to remove residual salts (Borges et al., 2011; Halim et al.; Servel et al., 1994; Volkman et al., 1989)

5.3.2 Total gravimetrically lipid extraction

Figure 3a and 3b represent the amount of total lipids (mg of oil / $g_{(DW)}$) and (mg of oil / $g_{(AFDW)}$) respectively, for each species and each of the different harvesting methods mentioned in figure 1 (method I = harvesting- dynamic microfiltration followed by centrifugation; method = II harvesting-sedimentation followed by dynamic microfiltration).

For the lipid analysis, the biomass must be standardized by the fraction of the processed organic matter; to compare lipid content against species, however, it should be remembered that at industrial scale all the microalgae composition must be valuable in order to have feasible final products.

At industrial scale, biomass washing, ash separation and probably drying steps cannot be performed to make the process feasible, although the drying step can be an exception if residual heat (Xu et al., 2011) from CO_2 cogeneration plant chimney can be used to dry and cool the injection font for the microalgae photo bio-reactors .

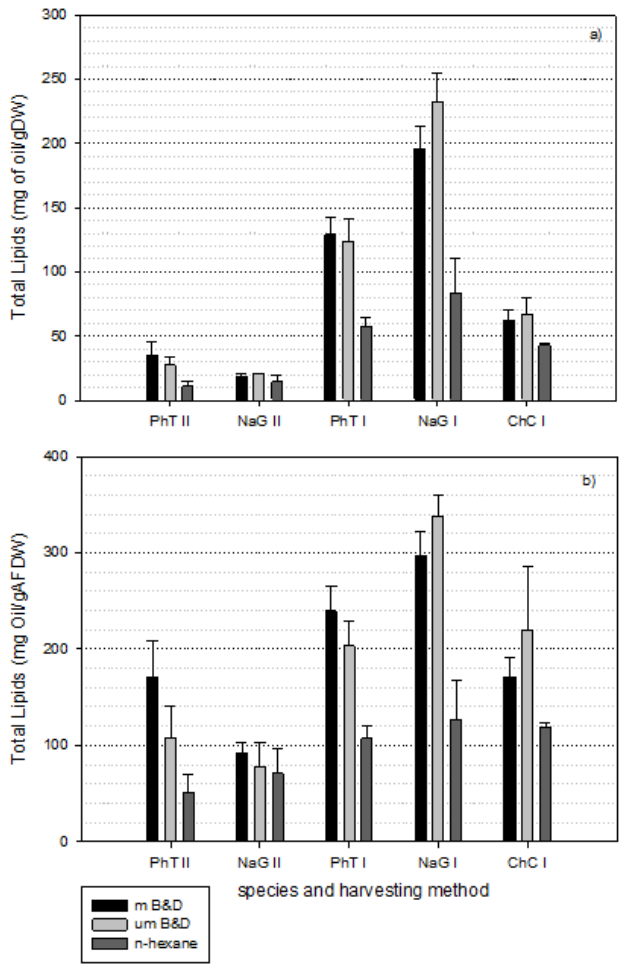


Figure3: Total lipid extracted gravimetrically from different the freeze-dried biomasses, a) values not standardized by ash free dry weight, b) values standardized by ash free dry weight. (PhT I), (NaG I) and (ChC I) are the samples obtained by concentration path I, and PhT II and NaG II are the samples obtained by concentration path II

Figure 3a shows that the total lipid content is higher for harvesting path I than for harvesting path II, and that the non-sedimented *NaG* species (21(1%)) is about 2 and 4 times greater than the lipid content from the non-sedimented *PhT* (12(0.8%)) and *ChC* (6.4(0.2%)) species respectively. The total lipids in the AFDW base (Figure 3b) were higher than in the DW base. However, the lipid content differences between the species were not significant in the AFDW base. Significant differences were obtained between the harvesting and extraction methods.

Figure 3a shows that there are no significant differences in the crude lipid extraction results of any of the species when cell disruption (sonication) is performed.

It may be the case that the high polarity of the mix chloroform-methanol can already easily extract the polar lipids from the cell membrane and due to that no improvement is obtained when the cell wall is disrupted mechanically (McNichol et al., 2012). The initial contact with the solvents may weaken the association between the lipids and cell structure prior to lipid dissolution in the mono-phase system.

Soxhlet extraction with n-hexane showed a significantly lower extraction capacity than the solvent mix (B&D), and no significant extraction differences were found between species. This result was expected because it is known that n-hexane is most suitable to extract neutral lipids.

The neutral lipid content was similar for all three species. The polarity index for n-hexane is zero, which is sufficient for neutral lipid extraction but not for polar lipids such as chloroform (polarity index 4.1) or methanol (polarity index 5.1)

The solvent polarities had a significant impact on the yield and composition of the total lipid extracted from microalgae (Guckert et al., 1988)

The total lipid content of the sedimented species was 10% and 20% lower than the total lipid content of the non-sedimented *NaG* and *PhT*, respectively. This difference in the lipid content did not overlap with the ash/organic matter average. More characterization studies need to be carried out to understand this behavior.

Some interaction between the ions and lipid content may be the cause. Borges et al 2011 (Borges et al., 2011) reported a 4% lipid content in the dry biomass extracted from *Nannochloropsis oculata* using Bligh and Dyer method after flocculation/sedimentation, whereas other studies have reported a 30-50% lipid content in the dry biomass for this specie when it is concentrated by centrifugation (Andrich et al., 2005; Suen et al., 1987)

5.3.3. Extracted fatty acid methyl ester content

Table 2 shows the total FAME of each species for each of the harvesting and extraction methods studied.

It can be concluded from Table 2 that solvent polarities had a significant impact on the yield and composition of the microalgae extracts.

Table2 Total Fatty Acid Methyl Ester (FAME) of each species for each harvesting and extraction method, (TL is the gravimetrical total lipid measurement without the direct esterification results because the FAME were measured using GC)

Extraction method	Species & treatment	(1) (%) TL/DW	(2) (%) FAME/TL
Soxhlet (n-hexane)	PhT I	5.8 (0.9)	95.0 (4.0)
	NaG I	6.5 (0.8)	88.2 (9.4)
	ChC I	4.3 (0.1)	94.0 (4.8)
	NaG II	1.1 (0.5)	87.3 (10.4)
	PhT II	1.4 (0.7)	93.9 (4.8)
B&D ultrasound	PhT I	12.9 (1.9)	50.4 (2.6)
	NaG I	19.6 (2.4)	43.5 (1.7)
	ChC I	6.2 (1.2)	43.6 (1.7)
	NaG II	1.8 (0.4)	46.2 (1.2)
	PhT II	3.5 (1.6)	24.7 (1.1)
B&D	PhT I	13.1 (2.4)	41.8 (0.5)
	NaG I	24.5 (2.5)	37.5 (1.7)
	ChC I	8.7 (2.3)	29.5 (0.8)
	NaG II	2.2 (0.7)	34.1 (0.5)
	PhT II	3.1 (1.0)	25.8 (1.0)
Direct trans-esterification (15, 30, 100 and 300 mg)	PhT I	5.4 (0.4)	100.0 (0.0)
	NaG I	6.2 (1.3)	100.0 (0.0)
	ChC I	2.3 (0.3)	100.0 (0.0)
	NaG II	0.5 (0.2)	100.0 (0.0)
	PhT II	0.4 (0.2)	100.0 (0.0)

1- (%) TL/DW Average Total Fatty Acid in relation to total biomass dry weight

2- (%) FAME/TL Average FAME in relation to total lipid dry weight

The amount of FAMEs obtained by harvesting path I was between 3-9% of the dry biomass (ChC < PhT < Nng), whereas for harvesting path II this was below 1% of the dry biomass (Figure 3)

Although the Bligh & Dyer method extracted significantly more crude lipids for all three microalgae species, the FAME average was half that of the crude lipids (table 2). The crude lipids extracted by n-hexane with soxhlet apparatus and direct transesterification (DTE-m) are suitable for BD production (FAME).

Figure 4 shows the FAME content per one gram of dry biomass. No differences in FAME content were observed between the different extraction methods. The FAME content increased a little only when high ash content biomass was treated, especially by soxhlet extraction method.

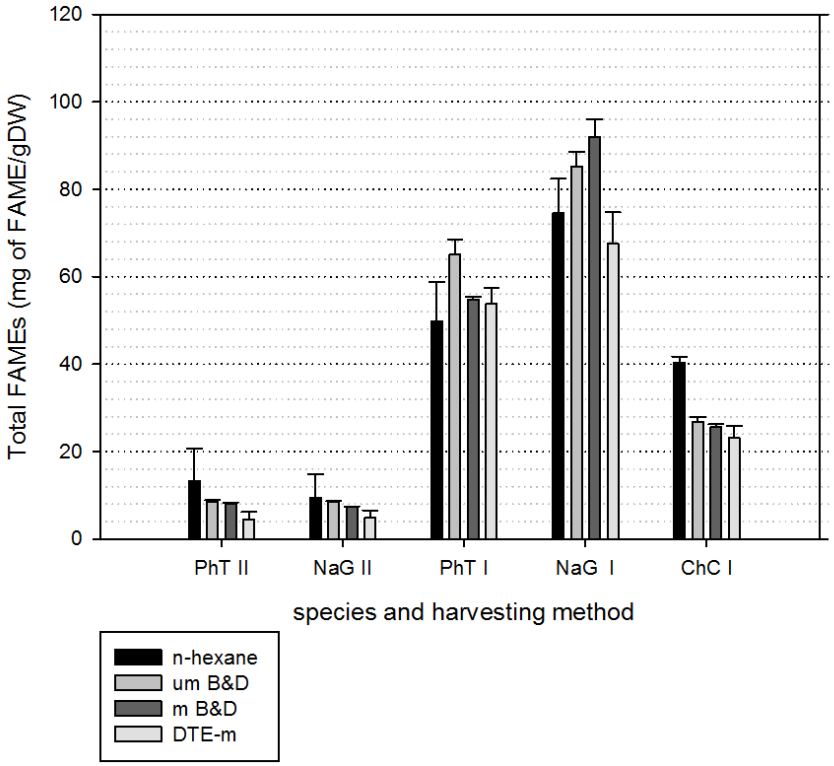


Figure4: Total FAME obtained by different extraction methods for each species and each harvesting method

Soxhlet extraction with n-hexane seems to be the best option for FAME extraction of high ash content BM, probably because it involves a better hot solvent diffusion through the biomass composition. It should be also kept in mind that the soxhlet extraction took longer than the B&D or direct transesterification methods. The Nng specie had the highest FAME content (Figure 4).

The direct transesterification method not only can be an analytic method to quantify the FAME content of the dry biomass, moreover it could be a method for producing BD on an industrial scale. Direct transesterification has been received a lot of attention firstly because the simultaneous lipid extraction and esterification reaction, which reduces the process time, and secondly because it uses a lower volume of solvent which in turn reduces the impact on the environment. McNichol et al 2012 (McNichol et al., 2012) also found that direct transesterification increased fatty acid recovery.

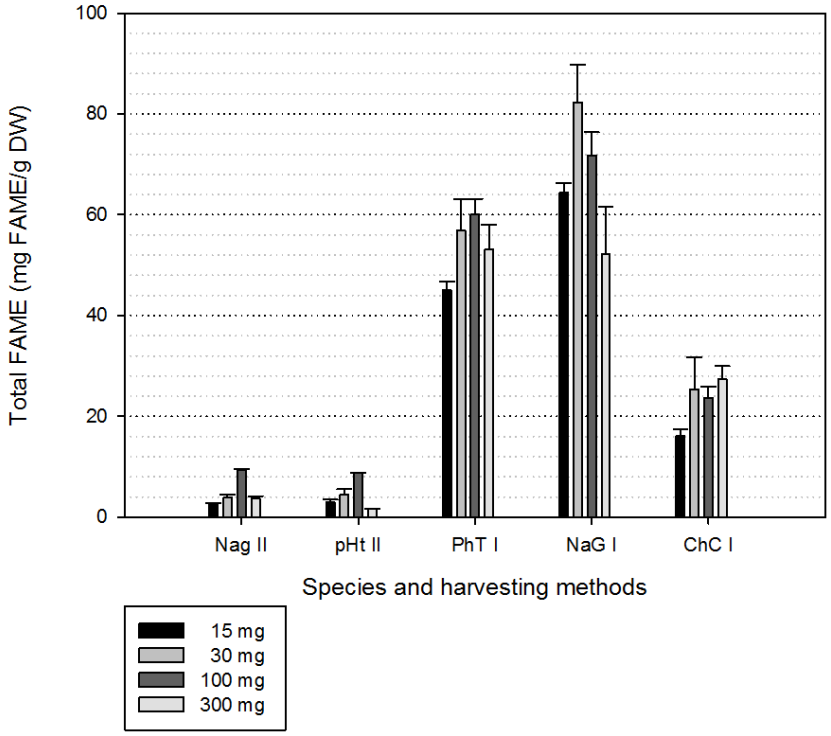


Figure5: Total FAME obtained by DTE-m by different biomass weights with the same volume of reaction solvent mix for each three species under studied harvesting methods harvesting

In this study different biomass weights in the same volume of reaction solution was evaluated in order to find the optimum ratio of biomass/extraction solution for direct transesterification method (Figure 5). These biomass weights were 15, 30, 100 and 300mg which mean a biomass concentration of 3.3, 6.6, 22.2 and 66.6 respectively.

Figure 5 shows the total FAME obtained by DTE-m for the different biomass weights in the same volume of reaction solvent mix for each three species and for each harvesting path. It shows that the best biomass/solvent ratio was between 30 and 100mg for all

species and harvesting path. Any quantity of biomass greater than 100 mg seemed to lead to a diffusion problem or defect in the reaction solution mix or time of reaction which resulted in less FAME than 30-100mg biomass ratio.

Direct transesterification could be a suitable method for producing BD from dry biomass, especially with the development of new technologies such as membrane reactors and different solid liquid separation techniques (Dubé et al., 2007)

More studies need to be performed in order to optimize reaction time, solvent mix and the scale of the process.

5.3.4. Fatty acids composition

The neutral/saponifiable lipids of each microalga species were characterized to determine the FAME compositions and suitability for BD production.

The main fatty acids in all three species of microalgae are myristic acid (C14:0), palmitic acid (16:0), palmitioleic acid (16:1), omega 3 (ω3) (eicosapentaenoic acid (EPA C20:5ω3) and docosahexanoic acid (DHA C22:6ω3) (except PhT species)

The three microalgae species showed almost the same FAME composition with different ratios (Tables 3, 4 and 5). The results for all species were also compared with those of other studies in the tables. The FAME compositions from other studies are quite heterogeneous because FAME quality and quantity depends on growth, harvesting, storage, extraction method, etc.

No significant differences between lipid extraction methods can be observed (Figure 4a), although the data were collected from different studies. As can be seen the highest differences with the other studies correspond to saturated fatty acid content, where the high proportion of C14:0 is compensated for by the lower content of C16:0 that we found in the present study for the ChC species.

It must be highlighted that the non-identified FAME average from n-hexane extraction is the higher (more than 10%) than the other extraction methods.

The lowest average FAME was not shown in the final results but it normally occurred in samples with a large amount of ash in the biomass (sedimented Pht and Nng) (tables 4-5respectively).

Table 3. Fatty Acid Methyl Ester (FAME) profile recovered from ChC species harvested by path (I) for each extraction method (u-B&D, B&D, sohxlet extraction with n-hexane and DTE-m)

Fatty Acid	ChC I (%)								References			
	u-B&D		B&D		n-hexane		DTE-m		*C. calcitrans.* (Servel et al., 1994)	*C. mulleri.* (Zhukova & Aizdaicher, 1995)	*C. constrictus.* (Zhukova & Aizdaicher, 1995)	*C. calcitrans.* (Volkman et al., 1989)
Saturated												
C14:0	29.32	(0.07)	30.34	(0.80)	21.48	(0.46)	30.18	(3.60)	13	15	14	17.50
C16:0	6.28	(0.03)	6.52	(0.27)	9.32	(0.33)	7.55	(0.05)	18	17.3	16.4	10.70
C18:0	1.48	(0.09)	1.48	(0.01)	2.13	(0.15)	1.83	(0.14)		0.8	4.8	0.80
Monosaturated												
C16:1	14.97	(0.12)	15.29	(0.40)	16.69	(0.32)	15.91	(1.34)	28.8	30	14.3	30.00
C18:1 n7	2.54	(0.05)	2.54	(0.03)			2.57	(0.24)		0.5	1.5	0.2
C18:1n9	1.25	(0.01)	1.18	(0.04)			2.11	(0.00)	0.3	1.4	4.7	0.8
Polyunsaturated												
C18:2	6.63	(0.01)	5.80	(0.69)			5.26	(0.84)	0.7	0.7	1.8	0.80
C20:4	2.03	(0.02)	2.17	(0.02)			2.17	(0.16)	2.3	1.7	3.3	5.7
C20:5 (EPA)	11.66	(0.01)	12.33	(0.53)	11.05	(0.72)	12.10	(1.14)	34	12.8	18.8	11.1
C22:6(DHA)												
Others	23.01	(0.40)	22.36	(1.22)	32.39	(2.78)	21.04	(4.60)				

All the extraction methods found that the saturated fatty acids (SFA) were the high percentage of the total FAME (~40% of total FAME) for the Chc specie. This result is promising for BD production because polyunsaturated fatty acids have poor oxidative stabilities and cold flow properties. Also, the cetane numbers usually fell outside the specification for oils rich in unsaturated fatty acids, which leads to SFAs that are more suitable for BD production.

Knothe et al (Knothe, 2008) report that palmitic, stearic, oleic, and linolenic acid as the most common fatty acids contained in BD. In particular, oils with high oleic acid content have been reported to have a reasonable balance of fuel properties.

The *PhT* species (Table 4) is composed mostly of mono-unsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA), and a lower proportion of saturated fatty acid (SFA). The MUFA and PUFA averages did not show significant differences for any of the extraction methods.

The PhT species included over 3% of the docosahexanoic acid (DHA) in the total FAME composition. This is a uncommon fatty acid which is essential to growth and functional development of the brain in infants (Horrocks & Yeo, 1999). However, no

C20:4 was detected in the final FAME mix, in contrast to the findings of some other studies (Fajardo et al., 2007; Zhukova & Aizdaicher, 1995).

Moreover, *Nanochloropsis gaditana* (Table 5) had a higher content of PUFA, MUFA and a lower content of SFA.

Although the PUFA, MUFA and SFA averages did not differ significantly between the extraction methods, soxhlet extraction with n-hexane obtained a lower EPA content in the FAME profile for sedimented Nng. As explained above, this solvent might have some problems with low lipid content, ash in the biomass or the extraction of unsaturated fatty acids. The whole neutral lipid stream could be transformed into BD by transesterification. However, the recovered lipid stream can be further fractionated in order to separate polyunsaturated fats, eicosapentaenoic acid or docosahexaenoic acid for further commercialization and saturated fatty acids for BD production. Liquid chromatography and thin layer chromatography should be used to separate the each fraction of lipid present in the mix (Molina Grima et al., 2003).

Furthermore, EPA plays an important role in preventing human diseases and has a high economic value (Molina Grima et al., 2003). Figure 6 shows the amount of EPA/total FAME in *PhT*, *Nng* and *Chc*.

The lowest EPA content from all tested species is for *ChC*, which contained (~ 12%) of total FAME (Figure 6). There was no significant difference between the extraction methods (≈3.1 mg/ g DW); however, as explained above, this species has the most suitable FAME average for BD production.

For *PhT*, the higher EPA mass per gram of biomass was obtained using the umB&D method (≈20.5 mg/g DW), whereas the highest EPA content was found in *NaG* using the mB&D method (≈24.7 mg/g DW).

The data suggest that it is feasible to produce BD from microalgae and/or to investigate lower energy harvesting paths to overcome the economic drawbacks.

Although *ChC* had the most suitable lipid profile for producing BD, in absolute numbers, *NnG* has the highest lipid content, moreover both *NnG* and *PhT* can be pre-concentrated by sedimentation, as previous studies have shown (Ríos et al., 2012)

Table4. FAME profile recovered from Pht species harvested by path (I) and (II) for each extraction method (u-B&D, B&D, soxhlet extraction with n-hexane and DTE-m)

Fatty Acid	PhT I (%)								PhT II (%)								References			
	u-B&D		B&D		n-hexane		DTE-m		u-B&D		B&D		n-hexane		DTE-m		*P. tricornutum* **(Liang et al., 2006)**	*P. tricornutum* **(Fajardo et al., 2007)**	*P. tricornutum* **(Zhukova & Aizdaicher, 1995)**	*P. tricornutum* **(Viso & Marty, 1993)**
Saturated																				
C14:0	4.62	(0.05)	4.35	(0.12)	2.38	(0.03)	4.20	(0.21)	4.38	(0.03)	4.71	(0.28)			4.12	(0.43)	7	7.2	7.40	8.8
C16:0	10.35	(0.05)	9.54	(0.17)	10.71	(0.05)	14.51	(0.80)	13.78	(0.26)	14.85	(0.64)	17.76	(3.80)	18.01	(1.31)	15	16.1	11.30	22.3
C18:0	0.47	(0.02)	0.43	(0.00)	4.09	(0.06)			0.61	(0.05)					0.60	(0.00)		0.6	0.40	1.7
Monosaturated																				
C16:1	30.21	(0.28)	30.42	(1.00)	30.27	(0.05)	31.54	(2.25)	32.71	(0.13)	34.52	(2.27)	16.31	(3.74)	35.58	(3.40)	26	19.2	21.50	32.7
C18:1 n7	3.75	(0.02)	3.93	(0.08)	3.17	(0.03)	3.06	(0.92)	3.65	(0.06)	3.82	(0.18)	1.21	(0.56)	3.36	(0.01)				
C18:1n9	3.23	(0.00)	3.34	(0.07)	1.05	(0.08)	3.17	(0.11)	2.81	(0.04)	2.94	(0.15)	1.67	(0.63)	3.44	(0.20)		1.5	2.30	1.5
Polyunsaturated																				
C18:2	7.01	(0.03)	7.39	(0.22)	1.27	(0.12)	6.50	(0.38)	5.86	(0.06)	6.35	(0.48)			5.93	(0.03)		2.8	1.50	4.1
C20:4																		2.3	0.6	
C20:5 (EPA)	24.60	(0.24)	25.51	(0.38)	24.51	(0.20)	24.57	(1.73)	20.00	(0.06)	20.71	(1.21)	37.83	(14.00)	23.64	(3.20)	20	23.7	28.4	12.5
C22:6 (DHA)	2.76	(0.08)	2.66	(0.08)	3.10	(0.08)	3.40	(0.22)	2.72	(0.08)	2.89	(0.20)					1.8	2.5	0.7	1.8
Others	13.00	(0.01)	12.65	(1.87)	19.47	(0.14)	13.00	(3.47)	13.47	(0.34)	12.11	(2.12)	25.23	(5.72)	8.81	(0.71)				

Table5. FAME profile recovered from Nng species harvested by path (I) and (II) for each extraction method (u-B&D, B&D, soxhlet extraction with n-hexane and DTE-m)

	Nng I (%)								Nng II (%)								References					
Fatty Acid	u-B&D		B&D		n-hexane		DTE-m		u-B&D		B&D		n-hexane		DTE-m		*N. salina* CS-190 (Volkman et al., 1989)	*N. oculata* CS-216 (Volkman et al., 1989)	*N. oculata* CS-179 (Renaud et al., 1991)	*N. oculata* CS-170 (Renaud et al., 1991)	*N. Gaditana* (Mourente et al., 1993)	*N. oculata* (Borges et al., 2011)
Saturated																						
C14:0	6.96	(0.48)	8.16	(2.68)	8.03	(0.44)	7.69	(1.18)	8	(0.04)	1.06	(0.05)	5.68	(0.37)	5.2	(1.45)	5	3.3	4.6	5.4	1.9	5.57
C16:0	16.7	(1.58)	20.2	(0.72)	13.86	(0.02)	21.15	(1.79)	20.49	(0.13)	22.03	(0.60)	29.17	(1.48)	23.09	(0.71)	27.8	17.8	14.2	26	13	31.91
C18:0	0.25	(0.02)	0.3	(0.00)			0.34	(0.00)									1	0.9	0.6	1.2	0.1	
Monosaturated																						
C16:1	21.15	(1.24)	25.58	(0.59)	24.58	(0.70)	18.33	(6.58)	22.75	(0.03)	24.15	(0.64)	22.89	(1.95)	22.28	(1.91)	31.8	26.6	29.4	22	14.3	26.32
C18:1 n7	0.68	(0.06)	0.79	(0.06)			0.8	(0.11)	0.63	(0.02)												
C18:1n9	0.51	(0.04)	0.66	(0.00)			0.58	(0.03)	0.47	(0.02)							8.3	7.7	6.3	5.8	1.5	9.63
Polyunsaturated																						
C18:2	0.43	(0.03)	0.52	(0.00)	1.66	(0.08)	2.65	(1.70)	0.42	(0.02)							1.5	2.9	2	3.3	1.1	
C20:4	5.35	(0.42)	6.64	(0.18)			5.72	(0.83)	5.62	(0.16)	6.17	(0.20)	6.26	(0.91)	6.45	(0.19)	4	7.1	8.8	5.5	3.2	
C20:5 (EPA)	24.43	(1.38)	30.21	(0.76)	32.26	(0.99)	30.78	(2.57)	26.53	(0.26)	28.41	(0.51)	11.86	(1.54)	29.02	(5.42)	24.2	28.4	28.8	24.9	23.5	24.11
C22:6(DHA)																						
Others	15.42	(4.61)	7.35	(0.30)	17.85	(0.30)	16.05	(0.60)	15.08	(0.10)	18.18	(1.90)	24.15	(2.36)	20.93	(4.96)						

It is necessary to find a compromise between economics and the environment. On one hand, there is the economic improvement provided by pre-concentration, but on the other there is the high quantity of solvent needed to extract the same amount of lipids if sedimentation is not carried out previously. This leads to high capital and operational costs (land, equipment, construction, chemicals, electricity, etc) which in turn increase the impact on the de environment.

In order to reduce the solvent requirement, direct transesterification could represent an alternative method for producing BD directly from dry biomass because produces equal or higher levels of FAME than the two-step extraction/reaction of biomass. More studies need to be done in order to determine the best way of achieving maximum efficiency.

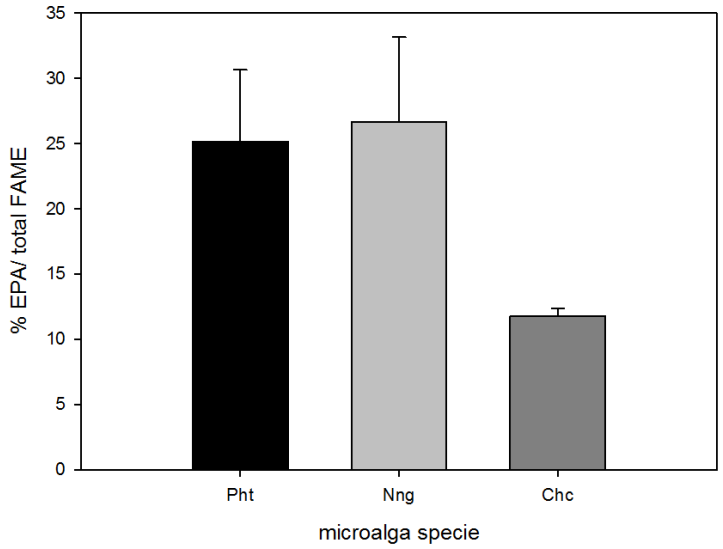


Figure6: EPA content of each species studied, by all extraction methods tested (mean of total FAME (SD)).

Biorefinery should also be considered. This involves taking BD as a byproduct from other high value products such as proteins, hydrocarbons or high value lipids such as omega 3 or 6, otherwise high investment and optimization would be necessary.

5.4.- Conclusion

The efficacy of the solvent extraction methods for characterizing and quantifying lipids in microalgae depends on the species tested as well as the harvesting method.

Characterization or standardization of the biomass for determining lipid content should be carried out in order to compare the results with other studies.

Direct transesterification of dry biomass is an effective method for determining the FAME profile for all the species and harvesting methods tested in the present study. It could also be a suitable method for producing BD directly from biomass it uses less solvent and thus has less impact on the environment.

5.5.- References

Andrich, G., Nesti, U., Venturi, F., Zinnai, A., Fiorentini, R. 2005. Supercritical fluid extraction of bioactive lipids from the microalga Nannochloropsis sp. *European Journal of Lipid Science and Technology*, **107**(6), 381-386.

Araujo, G.S., Matos, L.J.B.L., Gonçalves, L.R.B., Fernandes, F.A.N., Farias, W.R.L. 2011. Bioprospecting for oil producing microalgal strains: Evaluation of oil and biomass production for ten microalgal strains. *Bioresource Technology*, **102**(8), 5248-5250.

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.

Borges, L., Morón-Villarreyes, J.A., D'Oca, M.G.M., Abreu, P.C. 2011. Effects of flocculants on lipid extraction and fatty acid composition of the microalgae Nannochloropsis oculata and Thalassiosira weissflogii. *Biomass and Bioenergy*, **35**(10), 4449-4454.

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

Dubé, M.A., Tremblay, A.Y., Liu, J. 2007. Biodiesel production using a membrane reactor. *Bioresource Technology*, **98**(3), 639-647.

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.

Govender, T., Ramanna, L., Rawat, I., Bux, F. 2012. BODIPY staining, an alternative to the Nile Red fluorescence method for the evaluation of intracellular lipids in microalgae. *Bioresource Technology*, **114**, 507-511.

Guckert, J.B., Cooksey, K.E., Jackson, L.L. 1988. Lipid sovent systems are not equivalent for analysis of lipid classes in the microeukaryotic green alga, Chlorella. *Journal of Microbiological Methods*, **8**(3), 139-149.

Halim, R., Danquah, M.K., Webley, P.A. Extraction of oil from microalgae for biodiesel production: A review. *Biotechnology Advances*.

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

Horrocks, L.A., Yeo, Y.K. 1999. HEALTH BENEFITS OF DOCOSAHEXAENOIC ACID (DHA). *Pharmacological Research*, **40**(3), 211-225.

Iverson, S., Lang, S., Cooper, M. 2001. Comparison of the bligh and dyer and folch methods for total lipid determination in a broad range of marine tissue. *Lipids*, **36**(11), 1283-1287.

Knothe, G. 2008. "Designer" biodiesel: Optimizing fatty ester composition to improve fuel properties. *Energy and Fuels*, **22**(2), 1358-1364.

Lewis, T., Nichols, P.D., McMeekin, T.A. 2000. Evaluation of extraction methods for recovery of fatty acids from lipid-producing microheterotrophs. *Journal of Microbiological Methods*, **43**(2), 107-116.

Liang, Y., Beardall, J., Heraud, P. 2006. Effects of nitrogen source and UV radiation on the growth, chlorophyll fluorescence and fatty acid composition of Phaeodactylum tricornutum and Chaetoceros muelleri (Bacillariophyceae). *Journal of Photochemistry and Photobiology B: Biology*, **82**(3), 161-172.

McNichol, J., MacDougall, K.M., Melanson, J.E., McGinn, P.J. 2012. Suitability of soxhlet extraction to quantify microalgal fatty acids as determined by comparison with in situ transesterification. *Lipids*, **47**(2), 195-207.

Molina Grima, E., Belarbi, E.H., Acién Fernández, F.G., Robles Medina, A., Chisti, Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances*, **20**(7-8), 491-515.

Mourente, G., Rodriguez, A., Tocher, D.R., Sargent, J.R. 1993. Effects of dietary docosahexaenoic acid (DHA; 22:6n−3) on lipid and fatty acid compositions and growth in gilthead sea bream (Sparus aurata L.) larvae during first feeding. *Aquaculture*, **112**(1), 79-98.

Naylor, R.L., Liska, A.J., Burke, M.B., Falcon, W.P., Gaskell, J.C., Rozelle, S.D., Cassman, K.G. 2007. The Ripple Effect: Biofuels, Food Security, and the Environment. *Environment: Science and Policy for Sustainable Development*, **49**(9), 30-43.

Pruvost, J., Van Vooren, G., Cogne, G., Legrand, J. 2009. Investigation of biomass and lipids production with Neochloris oleoabundans in photobioreactor. *Bioresource Technology*, **100**(23), 5988-5995.

Ranjan, A., Patil, C., Moholkar, V.S. 2010. Mechanistic assessment of microalgal lipid extraction. *Industrial and Engineering Chemistry Research*, **49**(6), 2979-2985.

Renaud, S., Parry, D., Thinh, L.-V., Kuo, C., Padovan, A., Sammy, N. 1991. Effect of light intensity on the proximate biochemical and fatty acid composition of <i>Isochrysis</i> sp. and <i>Nannochloropsis oculata</i> for use in tropical aquaculture. *Journal of Applied Phycology*, **3**(1), 43-53.

Rios, S.D., Clavero, E., Salvadó, J., Farriol, X., Torras, C. 2011. Dynamic Microfiltration in microalgae harvesting for biodiesel Production. *Industrial and Engineering Chemistry Research*, **50**(4), 2455-2460.

Ríos, S.D., Salvadó, J., Farriol, X., Torras, C. 2012. Antifouling microfiltration strategies to harvest microalgae for biofuel. *Bioresource Technology*.

Şirin, S., Trobajo, R., Ibanez, C., Salvadó, J. 2012. Harvesting the microalgae Phaeodactylum tricornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. *Journal of Applied Phycology*, **24**(5), 1067-1080.

Servel, M.-O., Claire, C., Derrien, A., Coiffard, L., De Roeck-Holtzhauer, Y. 1994. Fatty acid composition of some marine microalgae. *Phytochemistry*, **36**(3), 691-693.

Suen, Y., Hubbard, J.S., Holzer, G., Tornabene, T.G. 1987. TOTAL LIPID PRODUCTION OF THE GREEN ALGA NANNOCHLOROPSIS SP. QII UNDER DIFFERENT NITROGEN REGIMES1. *Journal of Phycology*, **23**, 289-296.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., Muylaert, K. 2012. Flocculation of Chlorella vulgaris induced by high pH: Role of magnesium and calcium and practical implications. *Bioresource Technology*, **105**, 114-119.

Velasquez-Orta, S.B., Lee, J.G.M., Harvey, A. 2012. Alkaline in situ transesterification of Chlorella vulgaris. *Fuel*, **94**(0), 544-550.

Viso, A.-C., Marty, J.-C. 1993. Fatty acids from 28 marine microalgae. *Phytochemistry*, **34**(6), 1521-1533.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, **128**(3), 219-240.

Xu, L., Wim Brilman, D.W.F., Withag, J.A.M., Brem, G., Kersten, S. 2011. Assessment of a dry and a wet route for the production of biofuels from microalgae: Energy balance analysis. *Bioresource Technology*, **102**(8), 5113-5122.

Zhukova, N.V., Aizdaicher, N.A. 1995. Fatty acid composition of 15 species of marine microalgae. *Phytochemistry*, **39**(2), 351-356.

Zubir, M.I., Chin, S.Y. 2010. Kinetics of modified Zirconia-catalyzed heterogeneous esterification reaction for biodiesel production. *Journal of Applied Sciences*, **10**(21), 2584-2589.

CHAPTER **VI**

6.- Microalgae-based biodiesel: economic analysis of downstream process realistic scenarios

6.1.- Introduction

Microalgae oil has been identified as a reliable resource for biodiesel production due to its high lipid productivity and potential cultivation in non-fertile locations. However, high scale production of microalgae based biodiesel depends on the optimization of the entire process to be economically feasible.

To recognize the microalgae as a true feasible feedstock for biodiesel production, a critical requirement implies not only ensuring high levels of desirable fatty acids, but also being able to provide high biomass concentrations within short growth cycles on a sustainable and cost-competitive basis.

Biodiesel production from microalgae can be divided in four steps: (1) microalgae production (strain, growth method selection, etc.), (2) harvesting/concentration (solid-liquid separation method or combination of, mechanical/thermal methods), (3) intracellular high value compound extraction (cell wall disruption or not, lipid/proteins/carbohydrates extraction method, etc.), and (4) transesterification of the lipids to produce final biodiesel.

It is necessary to find a compromise between all stages in the microalgae transformation to optimize the global process. From the harvesting step when more separation steps are used, the overall energy demand decreases.

Transesterification is commonly used as the technology to transform the vegetable and animal oils into biodiesels. In this study two different transesterification options are analyzed and compared with the production of petroleum-based diesel: direct transesterification from the dry biomass and transesterification of the oil extracted from the wet and dry biomass.

What was a difficulty during the biomass concentration might be an advantage during the lipid extraction and transesterification, because the microalgae size provides a high

surface contact with solvents, forming a homogeneous phase, with the subsequent advantage for mixing and pumping in a direct transesterification process.

According to (Wahlen et al., 2011) direct transesterification might yield more biodiesel than the expected regarding the triacylglycerides (TAG) content, probably due to the capture of fatty acids from membrane phospholipids. It is worth mentioning that free fatty acids (FFA) are feasible to biodiesel conversion, especially with acid catalyzed transesterification of the dry biomass, although this explanation is not considered by (Wahlen et al., 2011). Moreover, FFA can be the major saponifiable lipid in microalgae (Volkman et al., 1989) depending on the strain, but most of the species have a significant fraction of the saponifiable lipids as FFA (Vicente et al., 2009).

Within the literature consulted, the feasibility studies of the microalgae as a resource of biodiesel conducted by (Davis et al., 2011; Merit Lassing, 2008; Xu et al., 2011) must be highlighted. However, the present work aims to shed a constructive critical point of view about certain assumptions taken in the mentioned studies. This is the case of a possible overestimation of the algae as biodiesel precursor due to the assumed lipid ratio in the dry biomass that can range between 30 to 50 % dry weight. The suitable lipids for biodiesel are only the neutral and saponifiable ones; and therefore, the studies must specify the ratio of this lipids as well as if it is referred to the dry weight base biomass or not, and if this dry weight base biomass accounts for the ash content or only the organic matter (OM). The characterization of the microalgae biomass is of great importance because, according to the microalgae strain selected, the production medium or the concentration method, the lipid fraction can vary as a function of the accumulated ash in the biomass, which implies higher amounts of biomass to obtain the same fraction of neutral/saponifiable lipids for their conversion to biodiesel. Therefore, in scale up or feasibility studies, the mass fraction of OM, ash and water in the biomass should be clearly stated.

The scope of this study is to analyze from a critical point of view the biodiesel production using the experience acquired at pilot scale, especially from the microalgae concentration step by sedimentation, filtration or centrifugation, and the lipid extraction and biodiesel production. In this analysis computational tools are used in the modeling and economic evaluation of the process alternatives to assess a realistic scenario regarding the current state of the technologies used.

This chapter was developed with the invaluable collaboration of Carmen Torres from SUSCAPE group in the Universitat Rovira i Virgili, who performed the rigourous

simulations in AspenHysys V7.1® and others such as the automated environmental evaluation tool (AEET) programmed in Matlab® R2010b (Torres et al., 2011)

6.2.- Materials and methods

In the economic evaluation of the global process to obtain biodiesel from microalgae, certain assumptions have been made regarding our base case, a production plant with a capacity of 40000 metric tons (MT)/year ($4 \cdot 10^7$ kg/year) of biodiesel.

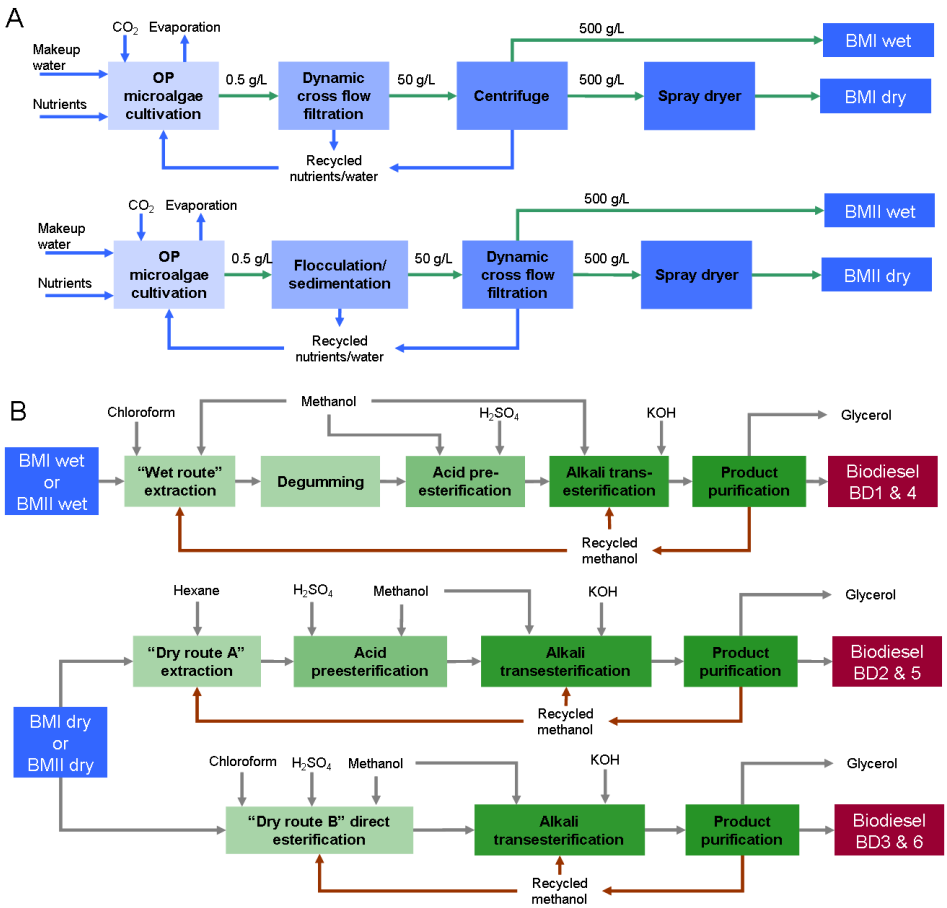


Figure 1. Simplified flow diagrams of the process alternatives considered during (A) the microalgae biomass cultivation and harvesting, and (B) the oil extraction and biodiesel production.

The proposed routes, or process alternatives, aim at minimizing the capital investment and energy consumption in the biomass concentration process, oil extraction and

transesterification. The studied pathways combine effective and complementary solid-liquid separation techniques experimentally tested, state of the art lipids extraction methods used in laboratories and at industrial scale, and finally an effective and less solvent demanding technique to direct extract and transesterified the neutral/saponifiable lipids from the dry biomass.

All routes are intended to convert the chemical energy contained in the microalgae into high-value biodiesel with minimal fixed and variable cost and a lower environmental impact. In total, six process alternatives are studied by the combination of two different paths during the biomass harvesting (Figure 1A), and three different options for the extraction and transesterification (Figure 1B). It should be noted that Figure 1B is simplified and it only shows the main inputs and outputs, for detailed information see the AspenHysys® simulation cases included in the supplementary information.

This study tries to provide an easy, critical and fair cost comparison of the six proposed alternatives of biodiesel production from microalgae with other biodiesels sources and fossil diesel.

6.2.1 Microalgae cultivation approach and assumptions

Autotrophic algae growth in open pond was considered in this work, where sea water medium is enriched with nutrients (urea and diammonium phosphate) and CO_2 is supplied from a nearby power plant. An adequate radiation to achieve a productivity of 30 g/m^2/day is assumed during 330 days per year of operation (Williams & Laurens, 2010). A microalgae cell density of 0.5 g/L based on dry weight (DW) biomass is commonly reached in OP technology; this is achieved due to the higher surface area to volume ratio and the shorter path length versus ponds.

Nutrients and CO_2 requirements for algae growth were calculated by stoichiometry assuming an algae composition of [$C_{106}H_{181}O_{45}N_{15}P$] and a CO_2 diffusivity efficiency of 20% (Clarens et al., 2010). Besides, a water evaporation loss of 0.3 cm/day was assumed in the OP with a liquid depth of 0.2 m to maximize solar radiation (Davis et al., 2011).

6.2.2 Concentration approaches and assumptions

To determine the optimal processing steps (*i.e.* number of concentration stages that best minimize cost) new process configurations were proposed with the aim of establishing a

baseline analysis. It should be highlighted that everything concerning biomass is referring to the entire mixture of different fractions (organic matter, ash, water, etc.). For instance, the salinity in the Mediterranean sea water is almost 40 g/L, moreover under alkaline conditions, some chemical ions in the medium precipitate together with the algal biomass, *i.e.* calcium carbonate $CaCO_3$ and magnesium hydroxide $Mg(OH)_2$ precipitation (Vandamme et al., 2012). These conditions leads to more ash content when the method to pre-concentrate is pH induced flocculation-sedimentation (Ríos et al., 2013). Although most of the reviewed papers include a sedimentation step in their studies, it seems that the authors are not paying attention to this fact that directly affects the final lipid content (Davis et al., 2011; Xu et al., 2011). It is important to note that the selected approaches are not claimed to be the best or global optimized process, but they represent the more likely options to be feasible on a large scale considering the available technology.

As shown in Figure 1A, two paths for microalgae concentration tested in our laboratories at pilot plant scale (Ríos et al., 2012) are studied: (BMI) two physical concentration steps (dynamic cross flow microfiltration followed by continuing centrifugation), and (BMII) one chemical step (pH induced sedimentation) followed by a physical concentration step (dynamic cross flow microfiltration). These two paths are used to obtain both dry and wet biomass and therefore, four different configurations are analyzed.

Depending on the harvesting method applied, the ash content is different in the final dry biomass. In this sense, the ash content is 35% based on DW for BMI, and 75% based on DW for BMII. Furthermore, the lipid content depends on parameters such as the nitrogen starvation, the microalgae strain, the growth phase when harvesting, etc. and can reach almost 75% of the total lipids at laboratory scale (Chisti, 2007). In the presented study, a lipid content of 50% DW of the organic matter is assumed, which means 30% for BMI and 12.5% for BMII. Besides, the lipids suitable for biodiesel production, the saponifiable/neutral ones, are around 50% of the total lipids which means that after the concentration step 15% DW for BMI and 6.25% DW for BMII are suitable lipids.

With respect to the harvesting configurations, some assumptions have also been taken based on the experimental data obtained in the pilot plant. In the concentration path BMII, the pH induced flocculation thickens the material to 5% (50 g/L) and virtually any algae is carried over into the clarified effluent, which is treated and recycled to the

cultivation stage. It was assumed that pH induction performed with KOH has the same effect than with the alkali used in our previous work (Ríos et al., 2012). After the pre-concentration step, the clarified effluent is neutralized with HNO_3, since both potassium and nitrogen are nutrients. This means that accumulation of byproduct from this step is avoided and at the same time the nutrients costs are lowered due to the reutilization of 75% of nutrients required in the growth stage (Merit Lassing et al., 2008).

As a first step in the concentration path BMI, the dynamic filtration concentrates the biomass three times more than the sedimentation method (15%) and as a second step (in the path BMII), it is able to concentrate the sedimented biomass to 50% (w/w) (500g/L), the same as the continuing centrifugation method. We considered that the filtration fluxes are constant over time, being 360 L/h/m^2 for BMII and 600L/h/m^2 for BMI, at 1 bar.

By coupling waste heat from a nearby power plant to the process, the energy balance can be improved. In these study and based on current available technologies, the dry biomass is obtained by spray dryer using flue gas since it is a widely used method to dry similar compounds (Merit Lassing et al., 2008). However, the sensitivity analysis performed in section 3.3 includes the study of the process if this flue gas resource is not available.

6.2.3 Lipid extraction approaches and assumptions

Two microalgae-to-biofuel concepts are assessed: the so called “dry route” with dry route A (oil extraction from dry biomass followed by esterification) and “dry route B” (direct esterification from dry biomass); and the so called “wet route” that includes oil extraction in the water phase and esterification of obtained lipids.

About the six paths studied to obtain biodiesel it must be said that basically the difference between the pathways to obtain BD1-3 and BD4-6 is the harvesting process, where BMI and BMII differ in the fraction content of the biomass (Figure 1A and 1B).

Therefore, two dry routes are considered; on the one hand, the “dry route A” includes a lipid extraction with n-hexane at high temperature followed by the transesterification of the oil to produce BD2 and BD4 from BMI and BMII respectively. The n-hexane to oil molar ratio considered in the simulation is 360:1, which is equivalent to 16 L of n-hexane per kg of dry biomass. On the other hand, “dry route B” consists in a direct transesterification, where the dry biomasses BMI and BMII are extracted and esterified

in one step obtaining BD3 and BD6 with the subsequent solvent and time saving. A 0.5:1 molar ratio of chloroform to oil is used considering that it might assist the cell wall breakage combined with the presence of the acid catalyst (Vicente et al., 2009). The amounts of methanol and acid catalyst with respect to oil is 50:1.3:1 molar (CH_3OH: H_2SO_4:TAG). However, a 600:1 methanol to oil ratio is also analyzed taking into account the results reported by (Velasquez-Orta et al., 2012)

The wet route extracts the lipids from wet biomass (50% w/w water) using a mixture of chloroform/MeOH following the Bligh and Dyer lipids extraction method (Bligh & Dyer, 1959). Although 90% extraction efficiency is assumed, the extraction of all the lipid content, including the polar ones, (Ríos et al., 2013) forces us to consider a degumming operation before the transesterification of the oil to produce BD1 and BD3 (Knothe, 2008).

6.2.4 Biodiesel production approaches and assumptions

Biodiesel is usually produced from vegetable oils through alkali-catalyzed transesterification. The main drawback of alkaline catalysts is their sensitivity to the FFA content in the oil (Mittelbach & Remschmidt, 2004) FFA reacts with alkali catalysts forming soaps that decrease the catalytic activity, reducing the reaction yield and emulsifying the final product impeding the glycerol separation. Therefore, a pretreatment step is required in order to decrease the initial acid value of the oil, an acid catalyzed esterification followed by an alkali catalyzed transesterification. It is assumed that microalgae oil has the same behavior as other vegetable oil (*e.g.* C*ynara cardunculus*) with an approximate FFA content of ≈10% (Torres et al., 2013).. The acid catalyzed pre-esterification is carried out at 60ºC with a methanol to oil molar ratio of 6:1 and 0.5 % v/v sulfuric acid, the reaction extend of the FFA esterification is almost 100% while some transesterification also occurs to produce methyl ester and glycerol. The pretreated oil is sent to an alkali catalyzed transesterification reaction with sodium hydroxide. The reaction is simulated at 60 ºC with a methanol to oil ratio of 6:1 and 1 % w/w NaOH.

Direct transesterification is an example of process intensification as it reduces the number of steps to produce biodiesel combining the extraction of the lipids and their transesterification in only one step with the subsequent time, energy, capital cost and solvent saving. The assumptions applied in the direct transesterification have been taken

from (Ehimen et al., 2010; Velasquez-Orta et al., 2012; Wahlen et al., 2011). They reported higher FAME yields with the acid catalyzed direct transesterification due to the acid action in the breakage of the microalgae cell wall, besides some species or solvents can produce more FAME than the corresponding regarding the TAG content (Vicente et al., 2009). From our experience, after the cell wall breakage and the subsequent reaction of the total FFA, an alkali catalyzed transesterification might reach the final biodiesel and glycerol with best cost and time efficiency(Ríos et al., 2013; Zhang et al., 2003a).

It should be noted that the intermediate steps (solvent separation and reutilization, biomass separation, oil clarification, etc) are not described, but they are taking into account in the economic evaluation study together with the necessary storage facilities. Detailed information about the simulation of the biodiesel production is included in a previous work (Torres et al., 2013).

6.2.5 Economical approaches and assumptions

The profitability analysis of the process includes the calculation of the Net Present Value (NPV) and the discounted payback period. The calculations of the capital and production costs are based in Spain/European Union conditions, a 6% rate of interest for the capital investment and a plant life cycle of 20 years were assumed. Detailed information about the equipment module costing technique used to estimate the total bare module of the plant can be found in the literature (Turton et al., 2003)

All process units are viable for their operation, for example, the DCF equipment can process high volume of slurry in a continuous way if the time of each batch is tuned as a function of the end wet biomass. The base investment costs of membrane set-up have been taken from the information provided by GEA-Westfalia, KMPT and New Logic Research. Thus, for dynamic filtration, the base cost is 2140 $\$/m^2$ (Ríos et al., 2012).

It should be noted that if any of the assumptions proves to be false, this will have extensive effects on the overall process. Therefore, a sensitivity analysis is performed and presented in subsection 3.3.

6.2.6 Computational approaches

Each process alternative is modeled and evaluated using computational tools (Figure 2). The four different harvesting configurations are simulated using mathematical models

created in spreadsheet that include every unit operation of the cultivation and biomass concentration steps. The three different configurations of oil extraction and biodiesel production units are simulated in AspenHysys V7.1®. The rigorous simulation of the process alternatives with this software tools allows the user an easy, fast and automatic calculation of process variables when modifications are made in the initial assumptions and the operating conditions.

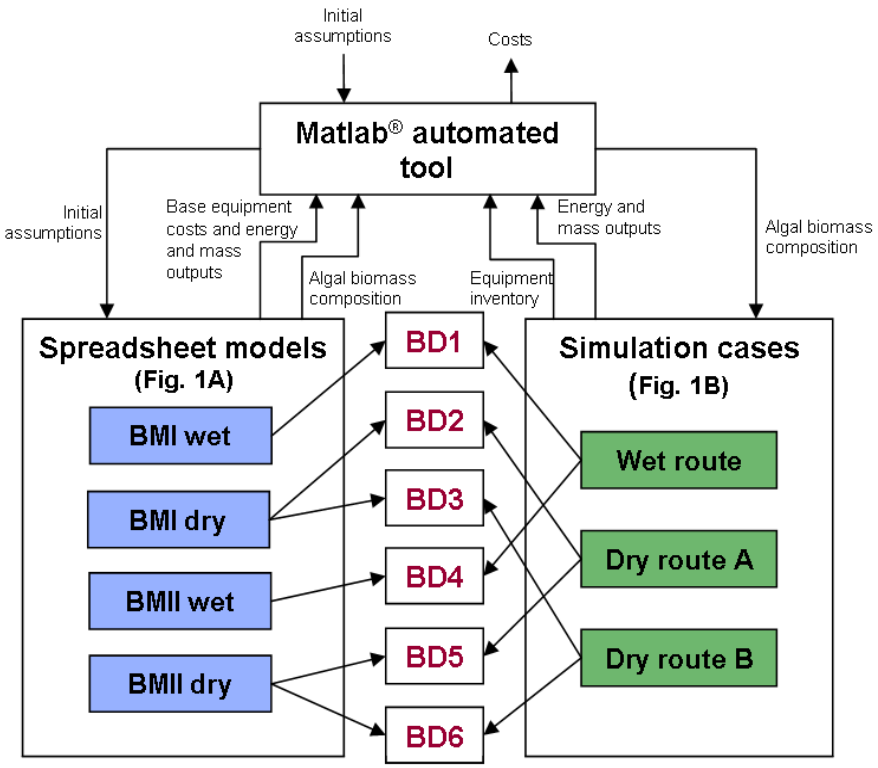


Figure 2. Flow diagram of the process alternatives considered during (A) the microalgae biomass harvesting, and (B) the oil extraction and biodiesel production.

The modular automated evaluation tool programmed in Matlab® (R2010b) (Torres et al., 2012) was modified accordingly with the case study to retrieve the inventory of mass and energy inputs from the results of the spreadsheet and the simulation. Figure 2 shows the interaction between both parts through Matlab®. The initial assumptions related with biomass composition are set in the mathematical model of microalgae biomass production and harvesting, in this way they can be automatically analyzed for a sensitivity analysis, together with other process variables. After the harvesting process, the data of algal biomass composition are sent to the corresponding simulation case

depending on the selected extraction-transesterification alternative and thus, the combination of model spreadsheet and simulation case. Finally, the outputs data are processed by the automated tool to perform the profitability analysis. In this way, different scenarios are assessed for the six process alternatives, changing critical variables in the economic performance, such as the microalgae productivity in the OP, the ash content in the dry biomass, the saponifiable/neutral lipids fraction, the concentration degree in each step of the biomass harvesting, the plant capacity, etc.

6.3.- Results and discussions

The results of the profitability analysis are presented in this section: first, the results obtained for the base case are evaluated in detail for the different harvesting configurations (subsection 6.3.1) and for the extraction-transesterification alternatives (subsection 6.3.2); finally, different scenarios are analyzed in a sensitivity analysis (subsection 6.3.3).

6.3.1 Harvesting pathways comparison

The costs of producing the microalgae biomass needed to achieve a plant capacity of 40000 (MT)/year ($4 \cdot 10^7$ kg/year) of biodiesel are presented in Table 1 for the different harvesting pathways studied. As shown, the operating cost of the BMI path is almost twice the BMII path, besides there is not a significant difference between the production of wet or dry biomass taking into account that for our base case the dry process is performed by residual flue gas. However a sensitivity analysis was performed and presented in section 3.3 in order to understand the influence of the drying process on the economic results if the flue gas resource is not available. Nevertheless, this increase in the manufacturing cost might be damped due to the high influence that capital investment has on the total operating costs through the depreciation.

The economic results of the four cases are driven by the capital costs. Specifically, the harvesting pathway BMI is responsible of more than half of the total investment; while in the BMII pathway is less than 10% of the total capital cost of biomass production. The first step of the harvesting pathways is the most cost demanding, because of the high volume of water that has to be handled, which request a huge investment in membrane area and settling tanks for BMI and BMII respectively, where the operating cost is 30 and 7 times higher for the first step of harvesting compared with the second

step (110 and 70 ($/t) for the BMI and BMII respectively). Therefore, it is necessary to reduce the initial harvested volume by settling because the capital investment and the manufacturing costs of DCF is not viable when high volume of slurry must be processed. However, if is compared with centrifugation, it is less cost intensive.

Table 1. Capital costs and operating costs of the cultivation and harvesting process alternatives for the base case scenario ($ x 10^{-6}).

	BMI wet	BMI dry	BMII wet	BMII dry
Capital costs				
Equipment	2 539.27	2 545.00	1 374.51	1 380.80
Cultivation (%)	49.55	49.44	91.54	91.12
1st concentration (%)	50.40	50.29	7.28	7.25
2nd concentration (%)	0.05	0.05	1.18	1.18
Drying (%)	-	0.25	-	0.46
Land	6.31	6.31	6.72	6.72
Storage and facilities	38.02	38.02	0.99	0.99
Bare module capital costs	*2 583.62*	*2 589.35*	*1 382.25*	*1 388.51*
Manufacturing cost (yearly)				
Raw materials	35.76	35.76	21.76	21.76
Utilities	26.94	26.94	1.44	1.44
Operating labor	11.14	12.19	11.14	12.19
Total manufacturing costs	*73.84*	*74.89*	*34.34*	*35.39*
Depreciation	225.25	225.80	120.51	121.06
Total operating costs	*299.09*	*300.69*	*154.85*	*156.44*

Although the harvesting in the BMII pathway, by flocculation/sedimentation, reduces considerably the cost of the first concentration of the biomass which according to other studies are responsible of 20 to 30% of the biomass production (Davis et al., 2011; Molina Grima et al., 2003) , it seems mandatory to reduce the cost of the cultivation step. In this sense, in section 3.3 the influence of the cultivation conditions on the costs are analyzed taking into account that conditions set in the base scenario are pessimistic compared with the considered by other authors, especially regarding the actual neutral/saponifiable lipid content.

6.3.2 Biodiesel production alternatives

The resulting extraction and/or transesterification costs for the production of 40000 (MT)/year ($4 \cdot 10^7$ kg/year) of biodiesel are presented in Table 2. The economics for this

step of the process are driven by the manufacturing costs; especially when treating biomass of BMII pathway, because of the high amount of mass that have to be handled (solvents and reactants) to avoid the diffusion problems derived from the higher ash content.

The process intensification applied on the concentration step (wet biomass) might not lead to an economical improvement since the dry biomass is less solvent demanding but, as it was explained in previous section, a sensitive analysis must be performed in order to understand the influence that the cost of the drying process has if the flue gas resource is not available. Apart from being more solvent demanding and more expensive, wet extraction also raises the risk and environmental concern related with the type of solvent needed.

Table 2. Equipment and manufacturing costs of extraction and transesterification alternatives ($ x 10^{-6}).

Item	BD1	BD2	BD3	BD4	BD5	BD6
Capital costs						
Reactors	0.84	0.80	1.41	0.84	0.80	2.01
Distillation columns	1.24	1.07	0.36	1.55	1.34	0.36
Washing columns	0.14	0.14	0.14	0.15	0.14	0.14
Other separators and vacuum system	2.15	2.64	0.41	3.82	4.73	0.70
Heat exchangers	0.52	0.15	0.05	0.94	0.15	0.05
Pumps	0.13	0.11	0.07	0.15	0.11	0.09
Storage	0.87	0.87	3.15	0.87	0.87	5.56
Total bare module cost	*5.88*	*5.77*	*5.59*	*8.29*	*8.14*	*8.90*
Manufacturing costs (yearly)						
Raw materials	53.68	14.81	27.25	136.43	35.31	52.95
Waste treatment	8.21	9.24	0.18	23.58	26.25	0.18
Utilities	4.69	16.06	0.96	11.10	40.61	2.01
Operating labor	0.66	0.66	0.66	0.66	0.66	0.66
Total manufacturing costs	*67.24*	*40.77*	*29.05*	*171.77*	*102.83*	*55.80*

On the other side, the intensification process of direct transesterification leads to cost savings when compared with other pathways. As it can be seen in Table 2, the BD3 and BD6 are the less cost intensive alternatives, although the BD3 is almost half of the BD6 cost.

A global economic balance must be performed to reach the total cost of the production of biodiesel from microalgae. Figure 3 shows the economic indicators of the global process of biodiesel from microalgae. Regarding the total investment cost (TIC),

pathway BMI is almost twice pathway BMII, while for each pathway the differences between the extraction and transesterification are not significant. This shows that the steps to be improved are the biomass production, especially the cultivation step which is responsible of more than 90% of the investment in the biomass production step by the pathway BMII.

The total operating cost (TOC) has the BD6 as the most competitive option from the economic point of view, and this supports the point discussed above. Regarding the intensification process, *i.e.* whether wet or dry, the second option seems to be the appropriate one, because is less cost intensive if the drying is performed by residual streams of flue gas.

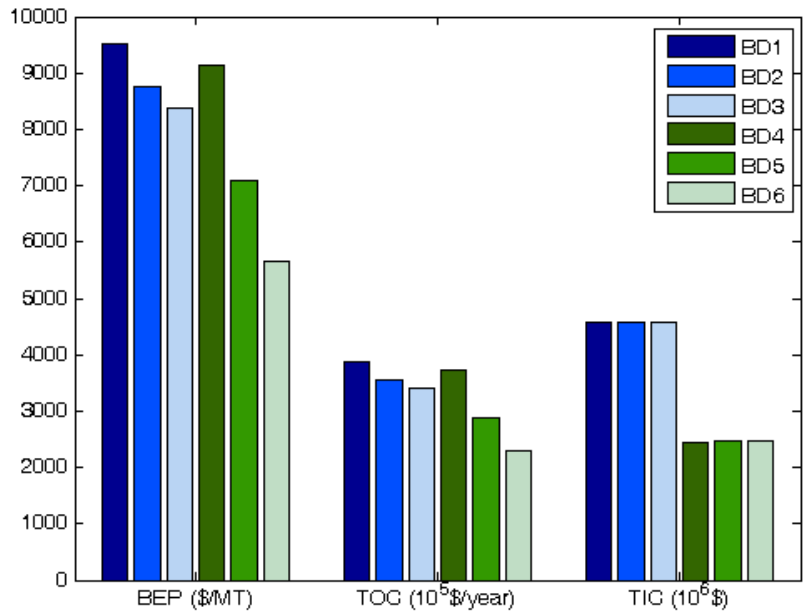


Figure 3. Break-even price (BEP), total operating costs (TOC) and total investment cost (TIC) for the six different biodiesel production alternatives considering the base case scenario.

The break-even price (BEP) is in the best pathway of the base case scenario, higher than 5000$/MT, which means that there is still a lot of improvement to be achieved in the biodiesel production from microalgae oil to be competitive with respect to the fossil diesels (1500$/MT).

It should be highlighted that in this work we only considered the possibility to produce biodiesel from microalgae, but without taken advantage from the taxes that

governments are taking off from the process and also without considered other possible routes to use the biomass such as in the biorefinery concept.

6.3.3 Sensitivity analysis

In Figure 4 the relative importance of key operational variables is analyzed using a tornado plot. The figure shows the increases and reductions of the BEP compared with the base case that is depicted in the y axis. The absolute values in $/MT are also indicated for each process alternative and new scenario, allowing the identification of the best alternative for each variable/uncertainty considered. For instance, the BMII alternatives (BD4-6) using flocculation/sedimentation are in general the less cost intensive pathways.

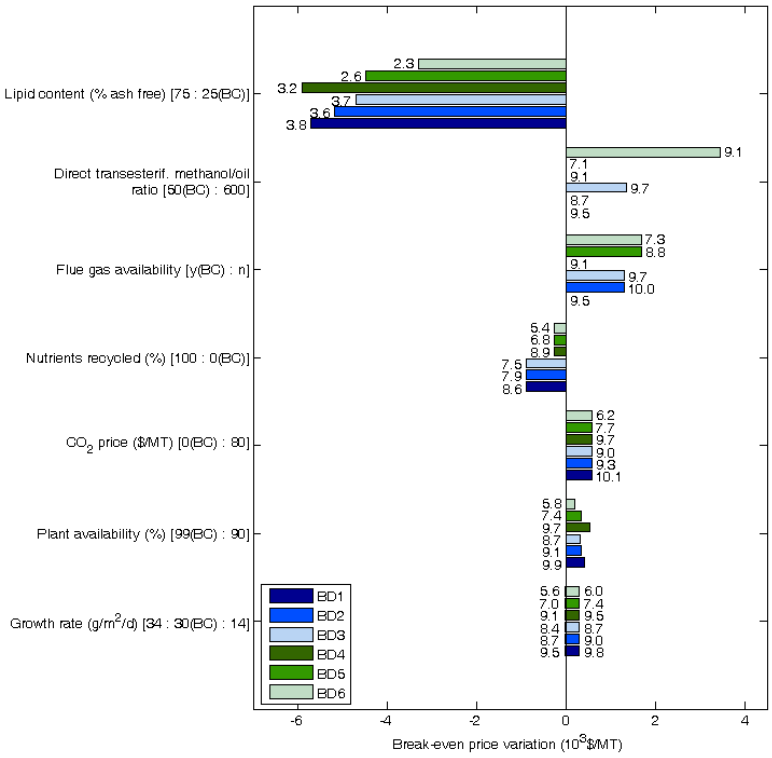


Figure 4. Tornado plot for the sensitivity analysis of the process, where BC indicates the base case condition corresponding to the vertical axis.

Figure 4 shows the impact that some variables have in the entire process and, as expected, the lipid content variance is the most sensitive parameter because with a higher lipid yield the entire process needs less capital investment, especially during the cultivation and the harvesting, and also less operating costs to produce the same amount of biodiesel.

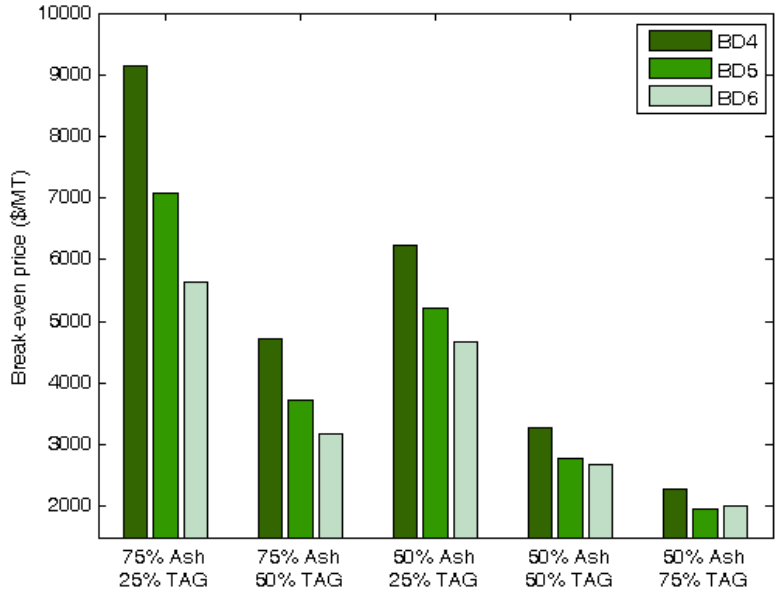


Figure 5. Analysis of four different scenarios for the process alternatives of the BMII harvesting path, regarding the oil content (TAG content in the organic matter) and ash content.

In the best case, BD6 with 75% lipid content (ash free), the BEP is around 2300 $/MT, but it can be improved if the settling process is optimized reducing the ash content till 50%. For instance, using a mixture of seawater and waste water might reduce the ash content as well as reducing the costs in nutrients (Schenk et al., 2008). With this improvement the BEP can be around 2000 $/MT (see the fourth scenario of Figure 5). Another option to reduce the ash content can be the use of tap water, but it poses some disadvantages like higher manufacturing cost, lower flocculation/sedimentation efficiency (Schenk et al., 2008) and also the increase of the environmental impact.

As it was highlighted in the above sections, the use of residual flue gas saves around 1300 and 1700 $/MT for the dry routes of BMI and BMII, respectively. The different responses registered between the pathways are supported by the fact that a higher

amount of biomass of BMII pathway needs to be dried to produce the same amount of biodiesel due to the different ash content.

As explained in section 3.1, the analysis clearly points out that is extremely mandatory to reduce the investment cost of the biomass production, especially in the cultivation step in order to increase the overall efficiency. This must be achieved by improving the lipid content of the microalgae and the cell productivity. Regarding the former, in Figure 4 the algae productivity seems to have less influence on the BEP than the expected, but only because very low lipid content is considered (our base case), the more lipid content in the biomass the more influence of the productivity. However, in the OP technology the growth rate cannot be improved due to several operational constraints (mixing, light efficiency, strain contamination, water evaporation, etc.). Therefore, the photobiorreactor (PBR) technology, which now needs three times more capital investment than OP (Davis et al., 2011), must be improved. In this sense, great effort is being done by the scientific community to find better technologies for the algae growth. This is the case of the research work on the field of alternative materials for the PBR, such as flexible plastic tubes, reducing significantly the cost investment. Moreover, coupled systems of the OP and PBR have been found to be very reliable. These systems take advantage from a "nursery" stage in PBR, which allows maintaining pure cultures, and a "grow on" phase in large area raceways (Williams and Laurens, 2010).

Figure 5 shows different scenarios for the processes that include the flocculation/sedimentation as the first concentration step of the harvesting. Although the lipid content is directly related to the biomass ash content, the higher response comes from the variation of the lipid content.

As shown in the Figure 5, a 25% increase in the lipids content in the organic matter has more influence than the reduction of 25% in the ash. This is a consequence of the impact that the higher lipid content has in the cultivation step cost, unlike the amount of ash because it is generated after the cultivation during the flocculation/sedimentation.

After all the improvement that can be reached in the harvesting step, the BEP might not overcome the barrier of around $1500 per MT needed for the cultivation step with the current less cost intensive cultivation technology (OP) and the biological improvements, such as high lipid content (75% ash free) and growth productivities (35 $g/m^2/day$).

As explained above, it seems that the OP cultivation technology has reached the biological and engineering production ceiling, therefore new technology must be

developed, such as for the photobiorreactor, which still have the opportunity to improve the production rates and lipid content from the selected strain due to the possibility to work with single species (no contamination) and also from the engineering point of view, with new materials specifically developed for this application.

6.4.- Conclusions

Although flocculation/sedimentation technology increases the biomass ash content, it is of capital importance to pre-concentrate the microalgae in order to reduce the operating cost of the harvesting process. Dynamic cross flow filtration of the pre-concentrated biomass seems to be more suitable and less cost demanding that centrifugation technology. Also, intensification process such as dry biomass direct extraction/transesterification has been identify as promising option that reduces cost and environmental impact due to the lower amount of process units and solvent required and.

The results obtained point out that a cost reduction of the cultivation step is mandatory to consider the microalgae-based biodiesel competitive with respect to the fossil diesels, regardless the Common Agricultural Policy (CAP) subsidies. To cover the production costs, the required biodiesel selling price for the best alternative assessed is $5700 per MT where only the biomass production (cultivation/harvesting) is responsible of the 65% of the cost.

6.5.- References

Amin, S. 2009. Review on biofuel oil and gas production processes from microalgae. *Energy Conversion and Management*, **50**(7), 1834-1840.

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.

Clarens, A.F., Resurreccion, E.P., White, M.A., Colosi, L.M. 2010. Environmental Life Cycle Comparison of Algae to Other Bioenergy Feedstocks. *Environmental Science & Technology*, **44**(5), 1813-1819.

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

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

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

Knothe, G. 2008. "Designer" biodiesel: Optimizing fatty ester composition to improve fuel properties. *Energy and Fuels*, **22**(2), 1358-1364.

Merit Lassing, Hulteberg, C., Mårtensson, P., Karlsson, H.T., Børre, E.O., Børresen, T., Svensson, M., Eklund, H. 2008. Final Report on Biodiesel Production from Microalgae : A Feasibility Study *Presented to StatoilHydro ASA Oslo, Norway*.

Mittelbach, M., Remschmidt, C. 2004. *Biodiesel : the comprehensive handbook*. Martin Mittelbach, Austria.

Molina Grima, E., Belarbi, E.H., Acién Fernández, F.G., Robles Medina, A., Chisti, Y. 2003. Recovery of microalgal biomass and metabolites: process options and economics. *Biotechnology Advances*, **20**(7-8), 491-515.

Reijnders, L. 2008. Do biofuels from microalgae beat biofuels from terrestrial plants? *Trends in Biotechnology*, **26**(7), 349-350.

Ríos, S.D., Castañeda, J., Torras, C., Farriol, X., Salvadó, J. 2013. Lipid extraction methods from microalgal biomass harvested by two different paths: screening studies toward biodiesel production *Bioresource Technology*, **submitted**.

Rios, S.D., Clavero, E., Salvadó, J., Farriol, X., Torras, C. 2011. Dynamic Microfiltration in microalgae harvesting for biodiesel Production. *Industrial and Engineering Chemistry Research*, **50**(4), 2455-2460.

Ríos, S.D., Salvadó, J., Farriol, X., Torras, C. 2012. Antifouling microfiltration strategies to harvest microalgae for biofuel. *Bioresource Technology*, **119**(0), 406-418.

Schenk, P., Thomas-Hall, S., Stephens, E., Marx, U., Mussgnug, J., Posten, C., Kruse, O., Hankamer, B. 2008. Second Generation Biofuels: High-Efficiency Microalgae for Biodiesel Production. *BioEnergy Research*, **1**(1), 20-43.

Şirin, S., Trobajo, R., Ibanez, C., Salvadó, J. 2012. Harvesting the microalgae Phaeodactylum tricornutum with polyaluminum chloride, aluminium sulphate, chitosan and alkalinity-induced flocculation. *Journal of Applied Phycology*, **24**(5), 1067-1080.

Torres, C.M., Gadalla, M., Mateo-Sanz, J.M., Jiménez, L. 2012. An automated environmental and economic evaluation methodology for the optimization of a sour water stripping plant. *Journal of Cleaner Production*(0).

Torres, C.M., Ríos, S.D., Torras, C., Salvadó, J., Mateo-Sanz, J.M., Jiménez, L. 2013. Sustainability analysis of biodiesel production from Cynara Cardunculus crop. *Fuel*, **submitted**.

Turton, R., Bailie, R.C., Whiting, W.B., Shaeiwitz, J.A. 2003. Analysis, Synthesis, and Design of Chemical Processes, 2/E. *Prentice Hall • Cloth Bound w/CD-ROM, 987 pp*.

Vandamme, D., Foubert, I., Fraeye, I., Meesschaert, B., Muylaert, K. 2012. Flocculation of Chlorella vulgaris induced by high pH: Role of magnesium and calcium and practical implications. *Bioresource Technology*, **105**, 114-119.

Velasquez-Orta, S.B., Lee, J.G.M., Harvey, A. 2012. Alkaline in situ transesterification of Chlorella vulgaris. *Fuel*, **94**(0), 544-550.

Vicente, G., Bautista, L.F., Rodríguez, R., Gutiérrez, F.J., Sádaba, I., Ruiz-Vázquez, R.M., Torres-Martínez, S., Garre, V. 2009. Biodiesel production from biomass of an oleaginous fungus. *Biochemical Engineering Journal*, **48**(1), 22-27.

Volkman, J.K., Jeffrey, S.W., Nichols, P.D., Rogers, G.I., Garland, C.D. 1989. Fatty acid and lipid composition of 10 species of microalgae used in mariculture. *Journal of Experimental Marine Biology and Ecology*, **128**(3), 219-240.

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.

Williams, P.J.l.B., Laurens, L.M.L. 2010. Microalgae as biodiesel & biomass feedstocks: Review & analysis of the biochemistry, energetics & economics. *Energy & Environmental Science*, **3**(5), 554-590.

Xu, L., Wim Brilman, D.W.F., Withag, J.A.M., Brem, G., Kersten, S. 2011. Assessment of a dry and a wet route for the production of biofuels from microalgae: Energy balance analysis. *Bioresource Technology*, **102**(8), 5113-5122.

Zhang, Y., Dubé, M.A., McLean, D.D., Kates, M. 2003a. Biodiesel production from waste cooking oil: 1. Process design and technological assessment. *Bioresource Technology*, **89**(1), 1-16.

Zhang, Y., Dubé, M.A., McLean, D.D., Kates, M. 2003b. Biodiesel production from waste cooking oil: 2. Economic assessment and sensitivity analysis. *Bioresource Technology*, **90**(3), 229-240.

CHAPTER **VII**

7.- Sustainability analysis of biodiesel production from *Cynara Cardunculus* crop

7.1.- Introduction

Second-generation biofuels (those that do not threaten food supplies) were developed to overcome the direct competition for land that is required for producing food. Several groups (Green Peace, Intermon Oxfam, etc) claim that the growing production of biofuels is responsible for the increasing food prices, and the destruction of forests.

Biofuels are hardly 3% of the world's energy consumption for transport purposes (Um and Kim, 2009). In this sense, *Cynara Cardunculus* production can reach 30-35 t/ha·year, however the average production is about 20 t/ha·year where around 6% are seeds and some authors obtained 25% of oil per dry matter of seed after soxhlet extraction with hexane and with the same thermodynamic and chemical characteristics of sunflower oil, that is 60.9% linoleic, 23.6% oleic, 12.1% palmitic and 3.4% stearic acid (Benjelloun-Mlayah at al., 1997; Curt et al., 2002). A review of the above publication shows that is possible to obtain at large scale cultivation from 400 to 650 kg/ha·year of *Cynara Cardunculus* oil and therefore yields are comparable to those obtained with sunflower or soybean, 800 and 375 kg/ha·year respectively (Mittelbach and Remschmidt, 2003). In addition, *Cynara Cardunculus* does not need arable land quality or fertilizer, none plowing, herbicide and insecticide pre-treatment or at least significantly less than other raw material as sunflower or soybean (Curt et al., 2002). After cardoon harvesting the seed should be separated from the crop and processed to extract the oil. Biodiesel (fatty acid alkyl ester) is produced through the transesterificaction of the oil or fat with a short chain alcohol (usually methanol or ethanol) in the presence of catalyst (typically sodium hydroxide or potassium hydroxide) and glycerol is produced as a byproduct.

Modeling of biodiesel production has been focused the attention of research works during last years. Most of them include the economic evaluation and technological assessment of the process for different oils and oils blend (Santana et al., 2010; West et al., 2008; Zhang et al., 2003a, 2003b), or predicting the final characteristics of the product specially that including in standards (García et al., 2010). To perform rigorous

process simulation of biodiesel production it is necessary to include parameters regarding the quality of the oil fraction, and the kinetics and thermodynamic packages for the transesterification reaction and esterification, if necessary. Most of the biodiesel simulation processes are based on data from the literature, for instance, from the work of Noureddini and Zhu (1997) and Lee et al. (2007) about the transesterification reaction, and from the work of Berrios et al. (2007) about the esterification of FFA (free fatty acids). However, in most of the simulations published, the kinetic data are applied without considering the type of oil feedstock.

Biodiesel is usually manufactured from vegetable oils through alkali-catalyzed transesterification. The main drawback of alkaline catalysts is their sensitivity to the FFA contained in the oil (Mittelbach and Remschmidt, 2003). FFA reacts with basic catalysts forming soaps that decrease the catalytic activity, reduce the reaction yield and emulsify the final product impeding the glycerol separation. Therefore, a pretreatment step is required in order to decrease the initial acid value of the oil.

This chapter was developed with the invaluable collaboration of Carmen Torres from SUSCAPE group in the Universitat Rovira i Virgili, who performed the rigourous simulation in AspenHysys V7.1® and others such as the automated environmental evaluation tool (AEET) programmed in Matlab® R2010b (Torres et al., 2011; Torres et al., in press).

The scope of this chapter is to develop a realistic simulation of biodiesel production using empirical thermodynamic and kinetic data (Pasqualino, 2004) for the specific oil source, vegetable oil extracted from *Cynara Cardunculus* (cardoon), a wild robust perennial plant, native from the Mediterranean basin. The aim of this chapter is to analyze the economic and environmental feasibility of a plant with a capacity of 5000 t/year biodiesel production. Starting from a base case of combined acid-catalyzed pretreatment and alkali-catalyzed transesterification, four potential alternatives are rigorously simulated using AspenHysys® from which an automated evaluation tool programmed in Matlab® retrieves the inventory of relevant energy and material inputs, as well as the environmental releases. Then, the Eco-indicator 99 methodology is used for the impact assessment, achieving results in the line of other vegetable sources of biodiesel, particularly to those corresponding to the transesterification of rapeseed oil. With respect to the economic criterion, a profitability analysis is performed calculating the net present value (NPV), which includes the initial investment, manufacturing costs and products revenues.

7.2.- Materials and methods

A production plant with a capacity of 5000 t/year of biodiesel produced from *Cynara Cardunculus L.* oil is studied. The existing biodiesel facilities in Spain range between 1500 t/y and 250000 t/year (APPA, 2008), and therefore, this is a plant with a small capacity. The sustainability analysis is performed using indicators to measure the economic and environmental behavior of the process. A quantitative assessment is possible using the net present value (NPV) to include the profitability analysis and the Eco-indicator 99 methodology for the impact assessment of a process.

The characterization of the process presented in this work includes the automated environmental evaluation tool (AEET) programmed in Matlab® R2010b (Torres et al., 2011; Torres et al., in press). In this tool the calculations are grouped into different modules: the inventory module retrieves data from the process simulation related with the inputs and outputs of materials and energy, the environmental module implements the life cycle impact assessment methodology with the standard Eco-indicator 99, while the economic module also computes the capital investment, operating costs and profitability indicators.

7.2.1 Process simulation

The procedure is based on the simulation of the plant of biodiesel in AspenHysys V7.1®. The calculation procedure connects the AEET modules with the process simulator to retrieve the inventory of energy and material inputs and environmental releases. This software tool allows the user an easy, fast and automatic calculation of process variables (*e.g.*, changes in the operating conditions).

Most of the chemical components involved in the simulation are defined in the AspenHysys® component library, such as glycerol, methanol, water, sodium hydroxide and sulfuric acid. M-oleate was selected to represent the biodiesel product and the oleic acid to represent the FFA content (Zhang et al., 2003a; West et al., 2008). The oil derived from cardoon (*Cynara Cardunculus L.)* crop was simulated according to the characterization made by Pasqualino (2006) (0.08% diglycerides, 2.78% monoglycerides, and 5.84% FFA) using triolein ($C_{57}H_{104}O_6$) as the main component. Those chemicals not available in the simulator library, such as diolein, monoolein, phosphoric acid, sodium phosphate, calcium oxide, potassium hydroxide and

dipotassium phosphate, were defined as hypothetical components in the AspenHysys® database (see Table S1 in the supplementary data).

The Peng-Robinson Soave (PRSV) equation of state was the fluid package selected to predict all physic-chemical properties. PRSV can predict more accurately the phase behavior of hydrocarbon systems, and handle non-ideal systems with equivalent, or better, accuracy than traditional activity coefficient models. Furthermore, PRSV performs rigorous three-phase flash calculation for aqueous systems containing water, methanol or glycols, as well as systems containing hydrocarbons in the second liquid phase.

The process simulation is structured in two sections: acid-catalyzed esterification pretreatment and the alkali-catalyzed transesterification (Figure 1). The first section is required because if the free fatty acids (FFA) content in the cardoon oil is higher than 5% soaps will be produced during the reaction, inhibiting the separation of glycerol and ester phases and forming emulsions with water washes. Both process sections are similar to the works presented by Zhang et al. (2003a), West et al. (2008), Morais et al. (2010) and Othman et al. (2010), where the main process units are the reactor (esterification pretreatment or transesterification), the methanol recovery system using distillation columns, the oil phase separation using liquid-liquid extraction columns, and glycerine and FAME purification in distillation columns.

7.2.2 Acid-catalyzed pretreatment

The process simulation of the esterification pretreatment and the transesterification sections are shown in Figure 1. The esterification reaction is carried out in a CSTR reactor (CSTR-100) using the kinetic data reported by Pasqualino (2006). The best reaction conditions are 60ºC, 400 kPa, with a methanol to oil molar ratio of 6:1 and 0.5 % v/v sulfuric acid as catalyst. The pressure value selected is extracted from Zhang et al. (2003a) and West et al. (2008) although simulations with atmospheric pressure were also conducted obtaining identical reaction results. It is expected that the reaction pressure essentially affects the fixed capital costs and, to a lesser extent, the fugitive emissions which, as it is explained in section 3.2, are not key factors in the profitability and environmental indicators. All five operational variables are adjusted simultaneously using the *Set* tool of the process simulator. The reaction mechanism is described in eq. 1-8. Some transesterification also occurs to produce methyl ester and glycerol (eq. 1-6),

while the reaction extend of the FFA esterification (eq. 7-8) is almost 100%. The Arrhenius parameters are shown in Table S2 of the supplementary data.

$$TG + MeOH \xrightarrow{k_1} DG + FAME \quad (1)$$

$$DG + FAME \xrightarrow{k_2} TG + MeOH \quad (2)$$

$$DG + MeOH \xrightarrow{k_3} MG + FAME \quad (3)$$

$$MG + FAME \xrightarrow{k_4} DG + MeOH \quad (4)$$

$$MG + MeOH \xrightarrow{k_5} GL + FAME \quad (5)$$

$$GL + FAME \xrightarrow{k_6} MG + MeOH \quad (6)$$

$$FAA + MeOH \xrightarrow{k_7} H_2O + FAME \quad (7)$$

$$H_2O + FAME \xrightarrow{k_8} FAA + MeOH \quad (8)$$

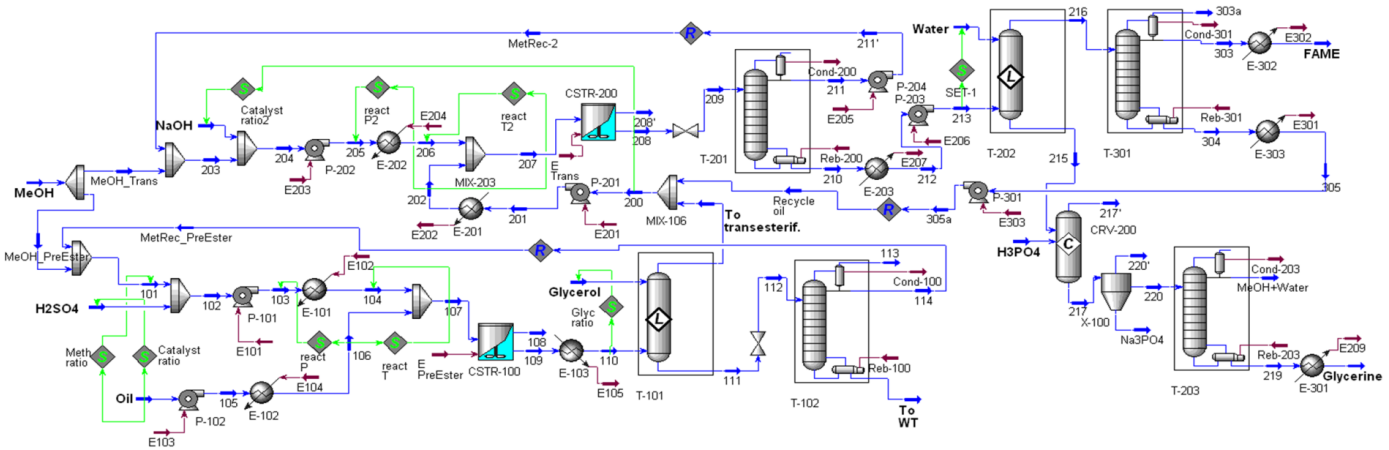
where *TG* is triglyceride, *DG* is diglyceride, *MG* is monoglyceride, *MeOH* is methanol and *GL* is glycerol.

The methanol and the sulfuric acid are mixed and heated to 60 ºC (*E-101*) before being pumped into the reactor. The cardoon oil stream is also preheated before entering the esterification, where the FFA are converted to methyl esters (*CSTR-100*) at 400 kPa. The products are cooled to 45 ºC (*E-103*) and forwarded to the glycerin washing column (*T-101*) to remove the sulfuric acid and water. The glycerol to sulfuric acid mass ratio is adjusted to 2:1. The light phase containing the oil is sent to the transesterification section. The methanol-rich stream (57 % w/w) is fed to a distillation column (*T-102*) with 9 theoretical stages that operates 20 – 30 kPa. The methanol entering the column is virtually recovered and recycled, while the bottom stream cannot be reused due to the presence of sulfuric acid (near 30%) and it is considered as hazardous waste to be treated. However, the neutralization and recovering of glycerol from this stream is studied as a process alternative (see subsection 2.1.3).

7.2.3 Alkali-ctalyzed transesterification

In the transesterification section (see Figure 1) sodium hydroxide is used as catalyst (eq. 1-6). In this case, five operational variables are adjusted simultaneously and just the optimal values are reported. The reaction is simulated in a CSTR (CSTR-200) at 60 ºC and 400 kPa, with a methanol to oil ratio of 6:1 and 1 % w/w NaOH. Zhang et al. (2003a) and West et al. (2008) fixed the reaction pressure to 400 kPa in their simulations, however during the presented study simulations with atmospheric pressure were also conducted obtaining identical reaction results. The kinetic data is shown in Table S2 of the supplementary data.

Prior to entering the reactor, the alkali-catalyst and methanol are mixed and as the oil stream, they are heated to 60 ºC (*E-202* and *E-201*). In CSTR-200 the oil is converted to FAME achieving a yield of 91 %, and producing glycerol as by-product. In order to recover the excess methanol, a distillation column (*T-201*) of 5 theoretical stages was simulated recovering the 98 % of the methanol feed. As glycerol is susceptible of thermal decomposition, vacuum conditions are needed to keep the temperature of the bottom stream below 150 ºC (Zhang, 2003a). After cooled (*E-203*) to 60º C the bottom stream enters the water washing extraction column (*T-202*) to separate the FAME and unconverted oils (top) from glycerol and catalyst (bottom) using a water to glycerol mass ratio of 1:5. Then, the FAME is separated from the unconverted oil in a 6 theoretical stages column (*T-301*) to obtain the biodiesel final product to the ASTM specifications (99.6 % w/w). The unconverted oils are recycled achieving 99.8 % of conversion of the cardoon oil to FAME in the overall process. On the other hand, the catalyst (sodium hydroxide) is neutralized adding phosphoric acid in the conversion reactor (*CRV-200*) before the glycerol is purified. The resulting sodium phosphate is removed by gravity (*X-202*). Nevertheless, potassium hydroxide is studied as an alternative catalyst (see subsection 2.1.3), obtaining in the neutralization dipotassium phosphate that may be considered as a valuable by-product. Finally, the catalyst-free stream has a glycerol content of 78 % which is purified in a distillation column to 96.7 % purity in order to achieve the commercial quality.



Stream name	101	104	107	109	To transesterif.	111	To WT	114	200	203	204	207	208	210	211	215	216	219	304	303
Temperature (°C)	65.6	60.0	57.9	60.0	40.0	46.3	144.2	36.2	50.3	30.8	28.46	57.24	60.0	122.2	35.5	59.8	50.0	191.9	552.7	178.2
Pressure (kPa)	100.0	400.0	400.0	400.0	190.0	200.0	30.0	20.0	101.3	101.3	101.3	400.0	400.0	30.0	20.0	120.0	110.0	110.0	30.0	20.0
Mass flow (kg/h)	141.4	148.9	732.4	732.4	585.7	210.5	73.42	132.8	697.4	139.2	147.9	845.3	845.3	768.8	75.5	66.2	700.5	59.9	111.7	587.2
Component mass fraction																				
Triolein	0.000	0.000	0.727	0.727	0.909	0.000	0.000	0.000	0.815	0.000	0.000	0.761	0.131	0.144	0.000	0.000	0.158	0.000	0.990	0.000
Diolein	0.000	0.000	0.022	0.022	0.028	0.000	0.000	0.000	0.031	0.000	0.000	0.021	0.001	0.001	0.000	0.000	0.002	0.000	0.010	0.000
Monoolein	0.000	0.000	0.001	0.001	0.001	0.000	0.000	0.000	0.001	0.000	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
OleicAcid	0.000	0.000	0.047	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Methanol	0.974	0.925	0.188	0.188	0.001	0.793	0.000	0.973	0.017	1.000	0.941	0.165	0.095	0.005	1.000	0.024	0.003	0.000	0.000	0.001
NaOH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.059	0.010	0.010	0.011	0.000	0.000	0.000	0.000	0.000	0.000
Glycerol	0.000	0.000	0.000	0.000	0.000	0.132	0.868	0.000	0.000	0.000	0.000	0.000	0.069	0.075	0.000	0.874	0.000	0.967	0.000	0.000
M-Oleate	0.000	0.000	0.000	0.049	0.061	0.000	0.000	0.000	0.136	0.000	0.000	0.042	0.694	0.763	0.000	0.000	0.838	0.000	0.000	0.999
H_2O	0.026	0.024	0.005	0.008	0.000	0.061	0.030	0.027	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.101	0.000	0.033	0.000	0.000
H_2SO_4	0.000	0.051	0.010	0.010	0.000	0.015	0.102	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_3PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Na_3PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure 1. Acid-catalyzed pretreatment and alkali-catalyzed transesterification of process I.

7.2.4 Process alternatives

Three process alternatives are studied and compared with the base case (process I) described in the previous subsections. The first modification, process II, includes the neutralization of the sulfuric acid in the waste stream of the pretreatment section (stream *To WT* in Figure 1) by the addition of calcium oxide. The resulting solid is removed and the neutralized stream is sent to a distillation column in which the glycerol is purified to 96 % w/w. This stream is cooled and recycled to the washing glycerol column (*T-101*) (see Figure S1 of the supplementary data). The additional equipment in process II allows reducing the waste stream and the glycerol consumption.

In the second modification potassium hydroxide replaces sodium hydroxide as catalyst (process III). The flowsheet is identical to Figure S1 (see Figure S2 of the supplementary data) but the reaction is simulated in a CSTR at 60 ºC and 400 kPa, with a methanol to oil ratio of 6:1 and 1.5 % KOH. The kinetic data is shown in Table S2 of the supplementary data, obtaining a conversion yield of 93 % of cardoon oil to FAME.

The last modification (process IV) substitutes the washing column with glycerol in the acid-catalyzed pretreatment section for a gravity settler (see Figure S3 of the supplementary data). A stream with 93 % of pretreated oil, 6 % of FAME and 1 % of methanol is obtained in the heavy phase of the decanter, and it is sent to the transesterification section. The light phase, with a 91 % of methanol, 4 % of water and 5 % of sulfuric acid is sent to a distillation column where 97 % of the unreacted methanol is recovered and recycled to the esterification feed. In this way, the supply of fresh glycerol is not required, in addition, the methanol recovery and further treatments are simplified.

7.2.5 Environmental module

The process and its design alternatives are evaluated using the LCA methodology (Guinée 2002). The calculation of potential environmental impacts is carried out using the Eco-indicator 99 (Eco-Indicator 99, 2000) described by Goedkoop and Spriensma (2000). The following considerations and assumptions have been taken:

- The aim of the study is to evaluate the materials consumption, emissions, energy use and the main environmental impacts related to the process, to determine the most sensitive stages and to compare in detail different process alternatives.

- Figure 2 shows a schematic overview of the process life cycle considered, and the principal mass flows and stages involved. The system boundaries include: cultivation, transportation, oil extraction, transesterification plant and products distribution. The Ecoinvent report for life cycle inventories of bioenergy (Jungbluth et al., 2007) is used as a reference.
- The functional unit selected is 1000 kg of biodiesel produced in a plant of 5000 t/year capacity.

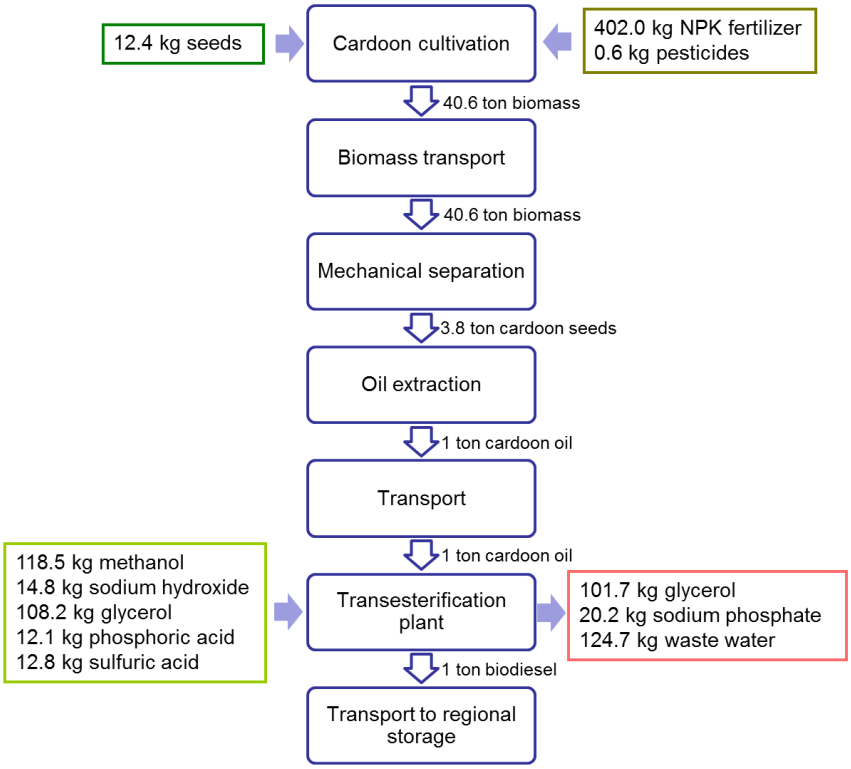


Figure 2. Supply chain overview of the biodiesel production from cardoon.

- The materials and energy inputs and outputs related to the transesterification plant are retrieved from the process simulation. The AEET performs the emission inventory using emission factors and methods based on correlations. Releases of all chemicals to air, water and land are accounted, including fugitive emissions from the equipment and storage tanks (for a detailed description, please check Torres et al., 2011). Then, the damage assessment is performed following the panel procedure

(Goedkoop and Spriensma, 2000) using the operational damage factors corresponding to the hierarchist perspective and the average weighting set.

- The materials flows for the stages previous to the transesterification plant have been calculated based on the experimental works available in the literature. Gominho et al. (2011) reported a sowing requirement corresponding to 4.6 kg/ha of seed. Fernández et al. (2006) described in detail the fertilization and weed control required. For low-fertility soils about 1000 kg/ha 9:18:27 complex fertilizer are recommended in the first year and a restoration fertilization from the second year onwards with rates of 12.6 kg N, 3.5 kg P_2O_5 and 20.8 K_2O/t dry matter biomass. The weed control needs 1.5 kg/ha alachlor and 0.4 kg/ha linuron and generally no pesticides application is required from the second year onwards. Gominho et al. (2011) also reported a seed yield of 1.4 t/ha for a standard production of 15 t for the whole biomass. And a seed oil content of 26.4% was found by Curt et al. (2002). The LCI of the construction phase is approximated by the mass of steel contained in the process units.
- Data of the energy and materials production, transportation and waste management are obtained from the Ecoinvent Database® V2.2 assuming that the plant is located in Europe.

7.2.6 Economic module

The profitability of the process is measured using the NPV, which is defined as the difference between the present values of all cash inflows and the present value of all cash outflows. The calculation of NPV includes the evaluation of the total capital cost (C_{TCI}) and the total manufacturing cost (C_{TM}). Detailed information about their calculation can be found in Turton et al. (2003). In this work the NPV calculation was implemented in the economic module with the following assumptions and considerations:

- A discount rate of 10% and a plant life cycle of 15 years were assumed.
- The C_{TCI} includes the fixed capital cost (C_{FC}) and the working capital cost (C_{WC}), where the second is usually a fraction of the first (15% is used in this work). The C_{FC} consists in the total bare module capital cost (C_{BM}), the contingencies and fees and the auxiliary facilities cost. In this work the contingencies and fees are calculated as 18% of the total bare module, while the expenses of auxiliary facilities

are estimated as 30% of the sum of the total bare module and the contingencies and fees.

Table 1. Raw materials, product, utilities and waste treatment prices.

	Price	
Raw materials & products		
Cardoon oil[a]	500	$/t
Methanol (99.85%)[b]	211	$/t
Sodium hydroxide[b]	495	$/t
Sulfuric acid (98%)[b]	67	$/t
Glycerol (92%)[b]	1500	$/t
Glycerol (85%)[c]	750	$/t
Phosphoric acid[b]	804	$/t
Calcium oxide[b]	56	$/t
Potassium hydroxide	890	$/t
Dipotassium phosphate	850	$/t
Biodiesel[b]	1360	$/t
Utilities[d]		
Cooling water	0.25	$/GJ
Electricity	25.77	$/GJ
LP steam (5 barg, 160ºC)	4.86	$/GJ
MP steam (10 barg, 184ºC)	5.61	$/GJ
HP steam (41 barg, 254ºC)	7.81	$/GJ
Makeup water	0.06	$/t
Waste treatment[f]		
Liquid	37	$/t
Solid	150	$/t

[a]Fernández et al. (2004) and Grimmelis et al. (2008).

[b]ICIS (2012).

[c]Zhang et al. (2003b).

[d]Based on the price provided by Turton et al. (1998) updated to 2011 (Chemical Engineering, 2012).

[f]Othman et al. (2010).

- The equipment module costing technique is used to estimate the C_{BM} of the plant. This technique relates all costs back to the purchase cost of equipment evaluated for some base conditions. The deviation from these base conditions are handled using multiplying factors that depend on the equipment type, the system pressure and the materials of construction.

- The C_{TM} includes three different costs: direct manufacturing cost (*i.e.*, raw materials, labor fees, utilities, maintenance and repairs, operating supplies, laboratory charges and patents and royalties), fixed manufacturing cost (*i.e.*, overheads, packaging, storage, local taxes, insurances and depreciation), and general expenses (i.e., administration, distribution and selling, and research and development). The last two categories can be calculated applying constant factors to expenses previously computed, allowing the use of the following equation for the C_{TM} calculation (eq. 9).

$$C_{TM} = 0.304 \cdot C_{TCI} + 2.73 \cdot C_{OL} + 1.23 \cdot \left(C_{RM} + C_{WT} + C_{U}\right) \quad (9)$$

where, C_{OL} are the operating labor costs, C_{RM} are the raw materials costs, C_{WT} are the waste treatment costs and C_U are the utilities costs.

- The raw materials, products and utilities prices are listed in Table 1. The cardoon oil price was estimated considering the double application of the crop: lignocellulosic biomass for energy production as solid biofuel, and vegetable oil for biodiesel production (Grammelis et al., 2008). Furthermore, Fernández et al. (2004) points out that the production cost of the cardoon oil is less than 50% of sunflower oil, regardless the Common Agricultural Policy (CAP) subsidies. The utilities include consumption of steam, cooling and makeup water, and electricity. The waste treatment prices of the liquid (MeOH+Water and To WT) and the solid (Na_3PO_4) streams are also shown.
- The technique used to estimate the operating labor cost is based on the number of operators required per equipment unit per shift. In this work it is assumed that a single operator works on the average 49 weeks/year, 5 shifts/week, 8 hours/shift. The plant operates 24 hours/day (3shifts/day). The operator salary was estimated at 30000$/year according to the Mediterranean countries salary standards.

7.3.- Results and discussion

7.3.1 Environmental assessment

The environmental analysis was applied to the system described in Figure 2, where the inventory data of the transesterification phase vary depending on the process alternative considered. This analysis yields almost identical results for the four alternatives considered. Figure 3 shows the comparison of the ten impact categories included in the

Eco-indicator 99. The impacts are shown as percentages with respect to the larger value of each category, allowing the visual comparison. Process IV shows the best environmental results with a total impact less than 1% lower than Process I, being the former process the worst alternative. However, both the total environmental impact and the categorized impacts are very similar for the four processes, with differences that never overcome the 5 %.

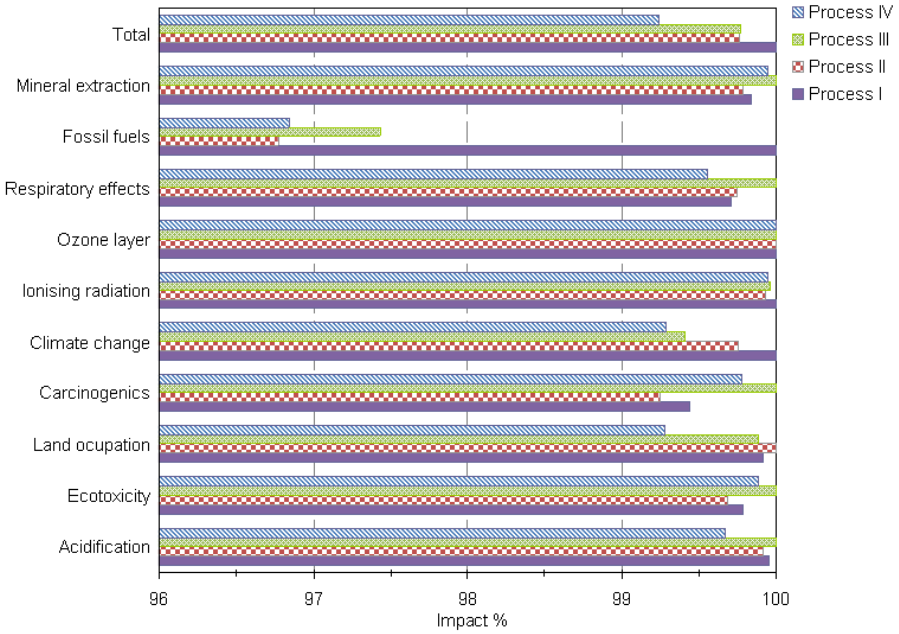


Figure 3. Comparison of the environmental impacts for the four process alternatives using Eco-indicator 99 (H, A) methodology.

On the other hand, considering the contribution that the different stages have in the LCA, the results of the four different alternatives of cardoon oil transesterification are strongly influenced by the LCI of the cultivation phase, which represents more than 80% of the total impact of the process, followed by the esterification phase (16%). These figures match with the results obtained for other types of agricultural feedstock, for instance, Gasol et al. (2012) reported a contribution between 63% and 88% in the environmental impact associated with the agricultural step of the *Brassica napus* (rapeseed oil crops). This means that a higher yield in the process, *i.e.*, less cardoon oil needed per metric ton of biodiesel produced, may lead to lower impacts. This is the case of processes III and IV (potassium hydroxide catalyst), which have slightly higher

reaction yields than process I and II (sodium oxide catalyst). However, considering the uncertainty in the data and the simulation of the reactions and that the cultivation stage is identical for the four alternatives, it is worthy a further analysis in the esterification phase, where more significant differences in the use of resources exist between the alternatives. In this sense,

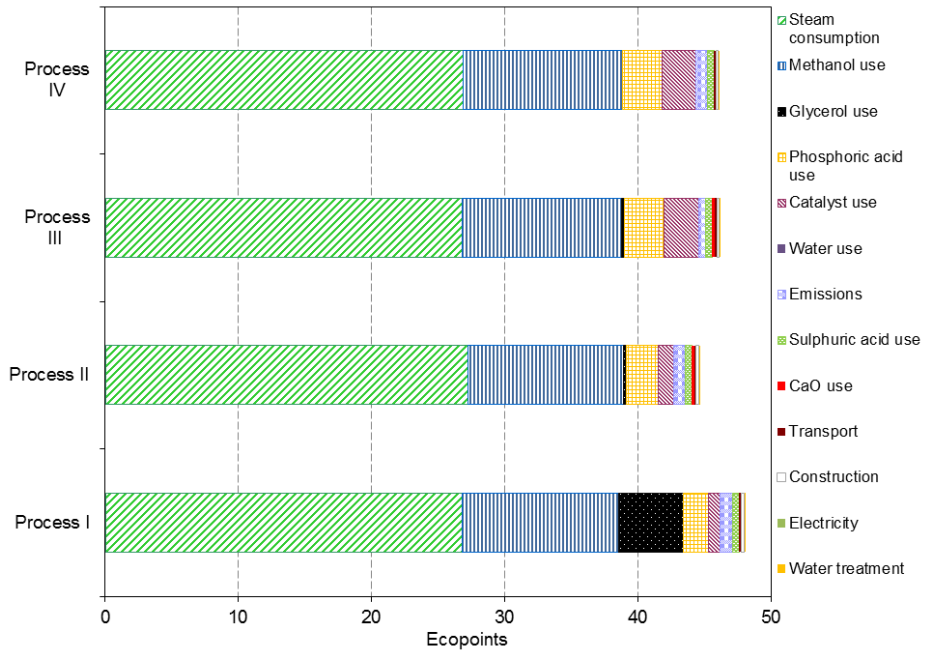


Figure 4. Contribution of the resources use to the environmental impact per metric ton of biodiesel produced of the four process alternatives.

Figure 4 shows the contribution of the resources use and utilities in the environmental impacts of the processes.

As expected, the four alternatives show similar impact distribution, being the steam and the methanol use the main contributors. Regarding the high contribution in the environmental impacts of the steam use, and considering that in this study the steam was assumed to be produced using fossil fuels, it would be interesting in future works finding alternatives that reduce this consumption. In this sense, Morais et al. (2010) point out the potential environmental advantages of using as fuel the by-product glycerol, which can be of special importance regarding the expected oversupply of glycerol due to the worldwide growth of biodiesel production.

Once again, in Figure 4, the higher environmental impact is found in process I, where the use of glycerol in the pretreatment section differentiates his performance. Processes II and III also use glycerol as solvent, but it is reused after the neutralization and separation units, reducing its consumption. Then, the catalyst choice is the key factor that makes process II the best option in terms of environment, in contrast to processes III and IV. The use of sodium hydroxide in process II yields lower impacts mainly because a lower concentration of catalyst is required. Following a similar rationale the amount required of phosphoric acid is lower.

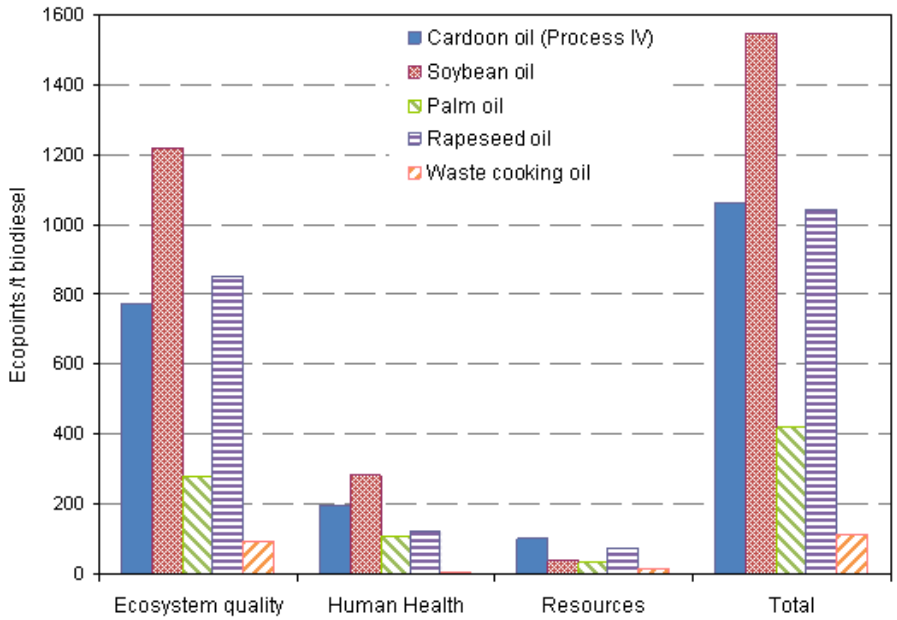


Figure 5. Comparison of the environmental impacts for different types of feedstock using Eco-indicator 99 (H, A) methodology, data extracted from Ecoinvent database v2.2 (2010).

Regarding the use of resources in the esterification phase, it seems that the advantages found in process II are not enough to compensate the disadvantage of a higher impact in the cultivation phase due to a lower conversion of cardoon oil into FAME. However, it is clear that the slight differences found between the alternatives confirm the necessity of calculating a different metric to support their comparison, in this case an economic indicator (section 3.2).

For a general view of the environmental impacts resulting from the LCA, Figure 5 shows the comparison between the values obtained in this work for the cardoon

biodiesel production through process IV and other types of feedstock extracted from Ecoinvent database v2.2 (2010).

As shown, the environmental impacts calculated are in line with those corresponding to other vegetable oils. Specifically, the presented analysis brings up similar results to those reported for the rape methyl ester production. This means that biodiesel from cardoon oil presents the same advantages when compared with conventional diesel when the use phase is considered, and especially when the use of the byproducts (glycerol and lignocellulosic biomass after oil extraction) is possible. Within these advantages, some categories stand out with high political significance in Europe, for instance, better energy balance and lower contribution to global warming (Gasol et al., 2012). Nevertheless, biodiesel seems to be worse at categories as acidification and ecotoxicity mainly due to the impacts associated with the agricultural stage, where additional efforts have to be done in the development of agricultural techniques to achieve minimum intensive production.

From Figure 5 it can be concluded that, regardless the waste oils, the palm oil transesterification process yields less environmental impact than the obtained for cardoon oil. However, cardoon cultivation is technically more adaptable and less demanding than other crops, such as sunflower or soybean, and a sustainable production when considering the use of biomass of the residual parts of the crop, resembling second generation biofuels.

7.3.2 Economic assessment

The results of the economic analysis are summarized in Table 2, where the capital costs, the manufacturing costs (without including the by-products revenue), and the profitability indicators are detailed. Regarding the total capital investment, process IV avoids the neutralization and purification units for the glycerol reutilization in the acid-catalyzed pretreatment section, reducing considerably the fixed cost by the substitution of a reactor and a distillation column by a gravity settler

Respect to the manufacturing costs, process I is clearly the most expensive due to a higher consumption of glycerol (in the other alternatives it is recycled). The waste treatment cost in the processes I and II is reduced in processes III and IV because the salt obtained in the catalyst neutralization is a by-product instead of a solid waste. Besides, the waste water produced in the pretreatment section is considerably reduced

in process IV. Furthermore, the yearly products revenue is marginally favorable to process III and IV due to the sale of dipotassium phosphate as a fertilizer.

Table 2. Capital costs, manufacturing costs and profitability analysis of the process alternatives ($ x 10^{-3}).

Item	Process I	Process II	Process III	Process IV
Capital costs				
Reactors	243.0	242.7	233.3	197.1
Distillation columns	827.0	958.3	953.4	779.7
Washing columns	291.7	291.7	291.7	135.8
Other separators and vacuum system	30.4	40.1	37.7	41.0
Heat exchangers	15.7	18.0	15.7	14.1
Pumps	64.3	73.4	69.5	65.5
Total bare module cost (C_{BM})	1462.1	1614.2	1591.4	1223.2
Contingencies and fees	263.2	290.6	286.4	220.2
Auxiliary facilities	517.6	571.4	563.4	433.0
Total fixed capital cost (C_{FC})	2242.9	2476.1	2441.2	1876.4
Working capital (C_{WC})	336.4	371.4	366.2	281.5
Total capital investment (C_{TCI})	2579.3	2847.5	2807.4	2167.9
Manufacturing costs (yearly)				
Raw materials (C_{RM})	3401.4	2639.3	2708.5	2798.4
Waste treatment (C_{WC})	40.5	31.4	12.8	6.4
Utilities (C_U)	261.1	124.4	121.9	123.1
Operating labor (C_{OL})	540.0	750.0	750.0	630
Total manufacturing costs (C_{TM})	6816.1	6354.1	6401.3	5980.3
Profitability analysis				
Products revenue (yearly)	7644.5	7640.2	7799.6	7848.0
Net present value (*NPV*)	3711.6	6923.9	7818.7	12038.0
Discounted payback period (years)	4	3	3	2
Break-even price ($/t)	1199.4	1110.5	1089.1	1000.4

The profitability indicators indicate that the process IV (gravity settler instead of glycerol washing) is the more profitable alternative, allowing a lower biodiesel price and less time to achieve benefits. However, these profitability indicators are strongly influenced by the biodiesel price, the plant capacity and the cardoon oil price. This means that, for instance, decreasing 20% the biodiesel sale price the payback period increases from two to six years.

Following this idea, Table 3 shows the results of previous economic studies of transesterification plants, where different plant capacities, oil and biodiesel prices, and raw material have been studied. The values obtained for process IV are indicated in the

last row of the table. As shown, the oil price, the capital investment and the production costs obtained in the analysis are consistent with the other studies; nevertheless, the biodiesel price considered for the calculation of the NPV and the payback period is higher with respect to the defined by the other authors, although the values from some citations should be updated to the present. For this reason, the break-even prices are indicated in the last column, allowing the comparison of the profitability with independency of the biodiesel price selected.

Table 3. Economic evaluation for biodiesel production plants from a literature review.

Raw material	Capacity (t/year)	Oil price ($/t)	Biodiesel price ($/t)	Capital investment ($x10^{-3})	Production cost ($x10^{-3}/y)	BEP ($/t)
Beef tallow[a]	100000	-	-	12000	34000	340
Canola oil[b]	50000	1100	930	3861	65900	1320[g]
Animal fats[a]	10560	-	-	3200	-	420
Vegetable oil[c]	10000	600	850	4000	7800	780[g]
Castor oil[d]	8600	1157	-	10054	14130	1560
Soybean oil[e]	8000	500	1300	1856	10693	1336[g]
Canola oil[a]	8000	500	600	2680	7760	884
W. cooking oil[f]	8000	200	600	1590	5200	650[g]
Canola oil[a]	7800	-	-	-	5950	763
Cardoon oil	5000	500	1360	2158	5197	1000

[a]Zhang et al. (2003b).
[b]Apostolakou et al. (2009).
[c]Skarlis et al. (2012).
[d]Santana et al. (2010).
[e]Othman et al. (2010).
[f]West et al. (2008).
[g]Values estimated from the available data.

From Table 3 it is possible to conclude that waste oils, such as waste cooking oils, render better economic results. However, since they are residual products, difficulties in gathering and pricing may appear and, therefore, their use cannot be considered as main raw material for biodiesel production on a large scale (Fernández and Curt, 2004). The break-even price obtained for the cardoon oil transesterification plant is in the range of previous studies, though the plant capacity is the smallest. Therefore, an analysis of the influence of the plant capacity in the economic feasibility of the process can lead to improved results.

Figure 6 depicts the influence of the plant capacity on the manufacturing costs per unit of product (unit cost) and on the break-even price of the biodiesel. Both curves show practically identical shape, as the break-even price is function of two terms, the manufacturing costs, and the by-product revenue, which allows reducing the biodiesel price beyond the unit cost. In the range of low capacities the manufacturing costs and the break-even price decrease rapidly as the production capacity increases up to 15000 t/year.

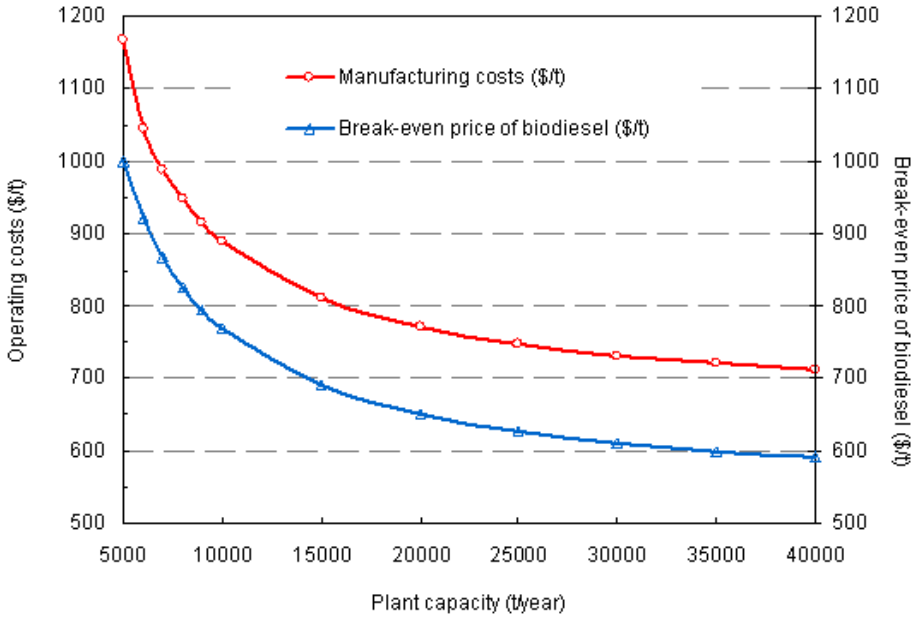


Figure 6. Manufacturing costs and break-even price of the biodiesel versus plant capacity.

From this point the decrease is less sharply, approaching to values of 711 $/t for the manufacturing costs and 591 $/t for the break-even price. For capacities higher than 40000 t/y some equations made in the economic analysis cannot be applied and thus they were not calculated. Finally, Figure 6 demonstrates that, considering the data of Table 3, the cardoon transesterification plant shows an economic behavior similar or even better than other vegetable oils; for instance, similar to the results reported by Skarlis et al. (2012) for a capacity of 10000 t/year, and slightly lower break-even price than the value obtained by Zhang et al. (2003b) for a capacity of 8000 t/year.

7.4.- Conclusions

Mediterranean basin *Cynara cardunculus* oil is a feasible alternative for the biodiesel production to replace part of the liquid fuel used in the transport sector in South.

According to a rigorous simulation, *Cynara cardunculus* biodiesel can reach, or even improve, the environmental and economic performance achieved by others raw materials that are identified as a first generation biodiesel.

The profitability of the resulting process is benefited by the additional ester amount obtained in the preesterification step. In addition, phase separation is easier for pre-treated oil transesterification, while the purification of both phases (biodiesel and glycerol) is trouble-free.

7.5.- References

Apostolakou, A. A., Kookos, I. K., Marazioti, C., Angelopoulos, K.C., 2009. Techno-economic analysis of a biodiesel production process from vegetable oils. Fuel Process. Technol. 90 (7-8), 1023-1031.

APPA, 2008. Capacidad, producción y consumo de biocarburantes en España. Situación y perspectivas. Asociación de Productores de energías renovables. Accessed the 19/08/2012. www.appa.es/descargas/Informe_Capacidad_produccion_consumo_biocarburantes.pdf.

Berrios, M., Siles, J., Martín, M.A., Martín, A., 2007. A kinetic study of the esterification of free fatty acids (FFA) in sunflower oil. Fuel, 86 (15), 2383-2388.

Benjelloun-Mlayah, B., Lopez, S., Delmas, M., 1997. Oil and paper pulp from Cynara cardunculus: preliminary results. Ind. Crop. Prod. 6 (3-4), 233-236.

Chemical Engineering, 2012. Economic indicators, April 2012. Accessed the 19/08/2012. www.che.com/business_and_economics/economic_indicators.html.

Curt, M. D., Sánchez, G., Fernández, J., 2002. The potential of Cynara cardunculus L. for seed oil production in a perennial cultivation system. Biomass Bioenerg. 23 (1), 33-46.

Eco-indicator 99, 2000. Eco-indicator 99 – Manual for Designers: A damage oriented method for life cycle impact assessment. Ministery of Housing, Spatial Planning and Environment. Den Hagg, The Netherlands.

Fernández, J., Curt, M.D., 2004. Low-cost biodiesel from Cynara cardunculus. 2nd World Conference and Exhibition on Biomass for Energy, Industry and Climate Protection, May 2004, Rome, Italy.

Fernández, J., Curt, M.D., Aguado, P.L., 2006. Industrial applications of Cynara cardunculus L. for energy and other uses. Ind. Crop. Prod. 24 (3), 222-229.

García, M., Gonzalo, A., Sánchez, J.L., Arauzo, J., Peña, J.A., 2010. Prediction of normalized biodiesel properties by simulation of multiple feedstock blends. Bioresource Technol. 101 (12), 4431-4439.

Gasol, C.M., Salvia, J., Serra, J., Antón, A., Sevigne, E., Rieradevall, J., Gabarrel, X., 2012. A life cycle assessment of biodiesel production for winter rape grown in Southern Europe. Biomass Bioenerg. 40, 71-81.

Goedkoop, M., Spriensma, R., 2000. The Eco-indicator 99: a damage oriented method for life cycle impact assessment. Methodology report. Pré Consultants B.V., Amersfoort, The Netherlands.

Guinée, J., 2002. Handbook on Life Cycle Assessment. Operational Guide to the ISO Standard. Kluwer Academic Publishing, Dordrecht, The Netherlands.

Gominho, J., Lourenço, A., Palma, P., Curt, M.D., Fernández, J., Pereira, H., 2011. Large scale cultivation of Cynara cardunculus L. for biomass production – A case study. Ind. Crop. Prod. 33 (1), 1-6.

Grammelis, P., Malliopoulou, A., Basinas, P., Danalatos, N.G., 2008. Cultivation and characterization of Cynara cardunculus for solid biofuels production in the mediterranean region. Int. J. Mol. Sci. 9 (7), 1241-1258.

ICIS, 2012. Trusted market intelligence for the global chemical, energy and fertilizer industries. Accessed the 19/08/2012. http://www.icis.com.

Jungbluth, N., Chudacoff, M., Dauriat, A., Dinkel, Doka, G., Faist Emmeneger, M., Gnansounou, E., Kljun, N., Schleiss, K., Spielman, M., Stettler, C., Sutter, J., 2007. Life cycle inventories of bioenergy. Ecoinvent report no. 17, Swiss Center for Life Cycle Inventories, Dübendorf, CH.

Lee, J.W., Yu, J.X., Mei, J.H., Yan, L., Kim, Y.W., Chung, K.W., 2007. A kinetic study on the transesterification of glyceryl monoleate and soybean used frying oil to biodiesel. J. Ind. Eng. Chem. 13 (5), 799-807.

Mittelbach, M., Remschmidt, C., 2003. Biodiesel : the comprehensive handbook, Martin Mittelbach, Graz, Austria.

Morais, S., Mata, T.M., Martins, A.A., Pinto, G., Costa, C.A.V., 2010. Simulation and life cycle assessment of process design alternatives for biodiesel production from waste vegetable oils. J. Clean. Prod.18 (13), 1251-1259.

Noureddini, H., Zhu, D., 1997. Kinetics of transesterification of soybean oil. J. Am. Oil Chem. Soc. 74 (11), 1457-1463.

Othman, M.R., Repke, J.-U., Wozny, G., Huang, Y., 2010. A modular approach to sustainability assessment and decision support in chemical process design. Ind. Eng. Chem. Res. 49 (17), 7870-7881.

Pasqualino, J.C., 2006. Cynara cardunculus as an alternative crop for biodiesel production. Ph.D. dissertation. Universitat Rovira i Vigili, Tarragona, Spain.

Santana, G.C.S., Martins, P.F., de Lima da Silva, N., Batistella, C.B., Maciel Filho, R., Wolf Maciel, M.R., 2010. Simulation and cost estimate for biodiesel production using castor oil. Chem. Eng. Res. Des., 88 (5-6), 626-632.

Skarlis, S., Kondili, E., Kaldellis, J.K., 2012. Small-scale biodiesel production economics: a case study focus on Crete Island. J. Clean. Prod. 20 (1), 20-26.

Torres, C.M., Gadalla, M.A., Mateo-Sanz, J., Esteller, L.J., 2011. Evaluation Tool for the Environmental Design of Chemical Processes. Ind. Eng. Chem. Res. (50) 23, 13466-13474.

Torres, C.M., Gadalla, M.A, Mateo-Sanz, J.M., Jiménez-Esteller, L., 2012. An automated environmental and economic evaluation methodology for the optimization of a sour water stripping plant. Submitted to J. Clean. Prod. (in press).

Turton, R., Bailie, R.C., Whiting, W.B., Shaeiwitz, J.A., 2003. Analysis, synthesis and design of chemical processes, second ed. Prentice Hall, New Jersey, USA.

Um, B.H., Kim, Y.S., 2009. Review: A chance for Korea to advance algal-biodiesel technology. J. Ind. Eng. Chem. 15 (1), 1-7.

West, A.H., Posarac, D., Ellis, N., 2008. Assessment of four biodiesel production processes using HYSYS.Plant. Bioresource Technol. 99 (14), 6587-6601.

Zhang, Y., Dubé, M.A., McLean, D.D., Kates, M., 2003a. Biodiesel production from waste cooking oil: 1. Process design and technological assessment. Bioresource Technol. 89 (1), 1-16.

Zhang, Y., Dubé, M.A., McLean, D.D., Kates, M., 2003b. Biodiesel production from waste cooking oil: 2. Economic assessment and sensitivity analysis. Bioresource Technol. 90 (3), 229-24.

CHAPTER **VIII**

8.- Conclusions

Second and/or third generation biofuel can be a proper alternative to substitute the fossil fuel used in the transport sector. Non edible oils, such as *Cynara cardunculus* oil, can contribute to a fraction of the necessity of liquid fuels, but a real alternative to the total substitution seems to be the biodiesel from microalgae. This last, can be divided in four steps: microalgae production, harvesting/concentration, intracellular high value compound extraction and transesterification of the lipids to produce final biodiesel.
In this work, several insights into the process to obtain biodiesel from second generation biomass were performed:

- Optimization of microalgae dewatering by dynamic micro/ultrafiltration, from sedimenten and non-sedimented broth (micro/ultrafiltration)
- Solvent lipid extraction methods from dry microalgae: Bligh and Dyer, soxhlet extraction with n-hexane and direct transesterification (lipid extraction).
- Economic analysis of the biodiesel production process from *Cynara cardunculus* oil and from microalgae culturing (economic analysis).

From the results obtained in the three above-mentioned parts, the following conclusions have been reached:

- **Micro/ultrafiltration**

  - The separation process on a large scale should be designed for continuous operation and a filtration process, followed by centrifugation can be the right choice. A previous flocculation can mean significant energy savings but with the disadvantage of the need of large areas for installation of settlers, increasing the land footprint.
  - Microfiltration of microalgae by using dynamic systems provides a clear enhancement of the process performance. As fouling and concentration

polarization effects using this suspended matter are very important, increasing the shear stress over the membrane significantly improves the permeability of the membrane. Optimum conditions have been found by a proper combination of variables such as the TMP, the rotational speed, membrane pores and materials, etc. Pilot experiments were carried-out, concentration factor up to 200 and permeability up to 600L/h/m^2/bar (with pre-concentrated broth) were achieved.

- TMP higher than 1.5 bar does not improve dynamic microfiltration performance due to fouling effects. In this direction, also less pumping energy is required.
- Membranes with similar nominal cut-offs but that made from different materials offer different performance in terms of selectivity. For the species *Phaeodactilum tricornutum, Nannochloropsis gaditana and Chaetoceros calcitrans* microalgae strain, ceramic membranes with a mean pore size of two microns offer complete rejection at low TMP and reasonable permeability by using rotational enhancement.
- Although dynamic filtration is more energetic intensive than tangential filtration, the permeability improvement can deal with a scenario where a significant reduction of the membrane area needed is achieved. The energy increment cost is much less than the amortization cost of the needed equipment, in case of not using dynamic filtration. Harvesting using dynamic cross flow filtration leads to a 25% improvement of the cost according to cross flow filtration.
- If a pre-concentration step is performed before the dynamic filtration, the improvement of the harvesting cost can be almost 6 times better from the capital investment and 2 times better from the yearly manufacturing cost.
- Flocculation/sedimentation technology leads to increasing the biomass ash content.

- **Lipid extraction**

  - Wet biomass was extracted taking advantage of certain solvents that can perform it (mixture of chloroform-methanol). Thus, the thermal drying step was avoided. No economic improvement was obtained. Dry biomass

obtained after thermal drying was also tested by a solvent extraction method and by a one step extraction-transesterification method (direct transesterification).

- The size of the microalgae is an advantage during the lipid extraction and transesterification, because provides a high surface contact with solvents, forming a homogeneous phase. For the same reason, it improves mixing and pumping in the direct transesterification process.
- Extraction, characterization and lipid quantification depend on the microalgae specie as well as the chosen harvesting method.
- Direct transesterification of dry biomass is an effective method to determine FAME profile for all three species tested and harvesting methods. It can also be a suitable method to produce biodiesel directly from the biomass due to less solvent requirement and its consequences on the environment.

- **Economic analysis**

  - The cost reduction of the cultivation step is mandatory to consider the microalgae oil a competitive resource for biodiesel production. To cover the production costs, the required biodiesel selling price for the best alternative assessed is 5700$/MT where only the biomass production (cultivation/harvesting) is responsible of the 65% of the cost by taking into account our realistic base case scenario.
  - Biodiesel from microalgae production process still needs to be improved; or the fossil fuel price must be higher than 6$/L to reach the breakeven price.
  - Mediterranean basin *Cynara cardunculus* oil is a feasible alternative for the biodiesel production to replace the part of the liquid fuel used in the transport sector in some region such as south Europe. *Cynara cardunculus* biodiesel can reach, or even improves; the environmental and economic performance achieved by others raw materials that are identified as a first generation biodiesel such as canola, soybean, castor oil, etc. The

profitability of the resulting process is benefited by the additional ester amount that is obtained in the pre-esterification step.

8.1 Future Work

As future work, new technology must be developed in order to improve the cultivation step. PBRs still have the chance to be improved either in the production rates and/or lipid contents. After the genetic modification of a single species, it is possible to produce high lipid contents cultures without contamination using PBR technology.
From the engineering point of view, research in new specifics materials for the process (e.g. flexible plastic photo-tubes with good mechanical properties and able to let the proper spectrum of the light to pass through) might still be developed. There is room for significant cost reduction potential through both biological and engineering improvement opportunities.

- Near-term research should focus on maximizing lipid content versus algal growth rates
- Capital cost reductions (e.g. novel low-cost equipment for a certain process stage, new materials to handled sea water, etc.) than through operating cost reductions (for example, optimizing nutrient or CO_2 requirements), especially in the PBR case.
- From a sustainability standpoint, it is important to optimize the process inputs such as carbon, nutrient, and water balances, recycle opportunities, and delivery sources, all of which are location-specific.
- Algal biofuel economics could be further improved in the near-term through means such as utilizing the spent algal biomass for more valuable co-products beyond biogas for power generation. Then, a research in high value co-products suitable for nutraceutical industry must be performed. However, the market sustainability of such co-products must be considered in the context of the envisioned commercial production volume.

9.- Supplementary data (SD)

9.1.- SD-Chapter 6

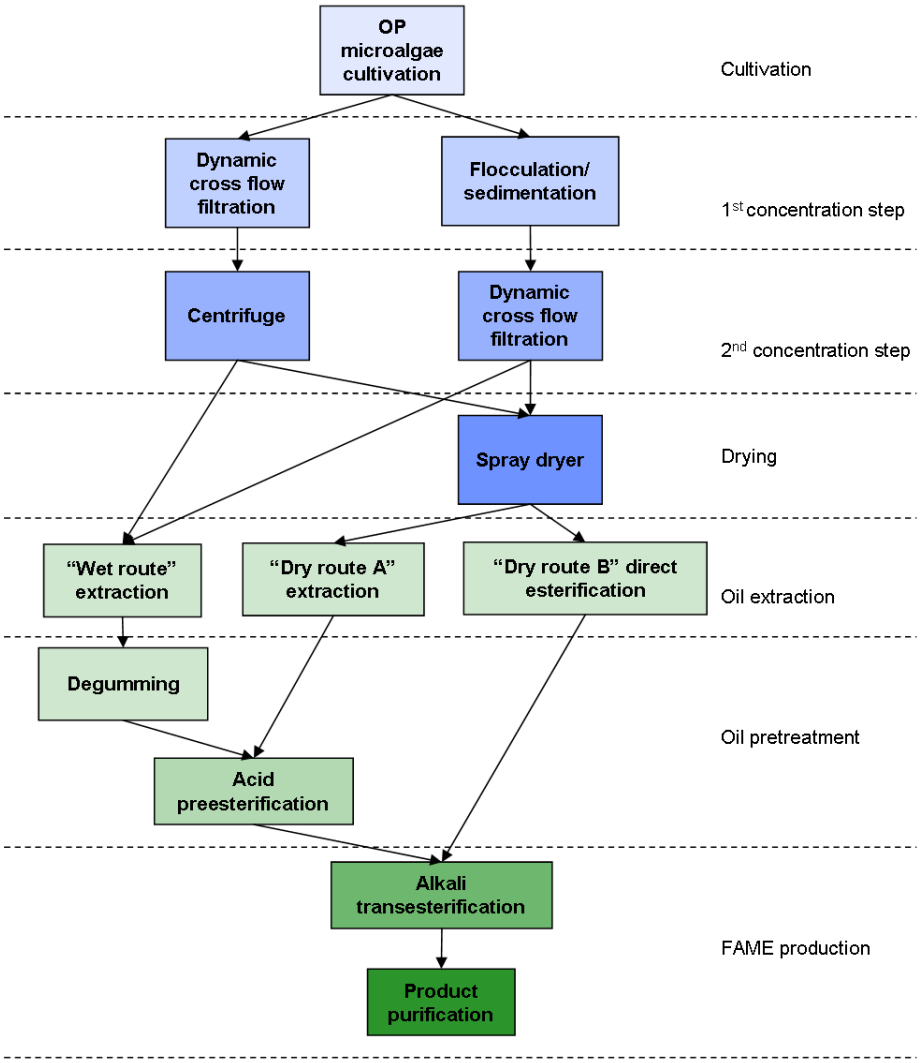
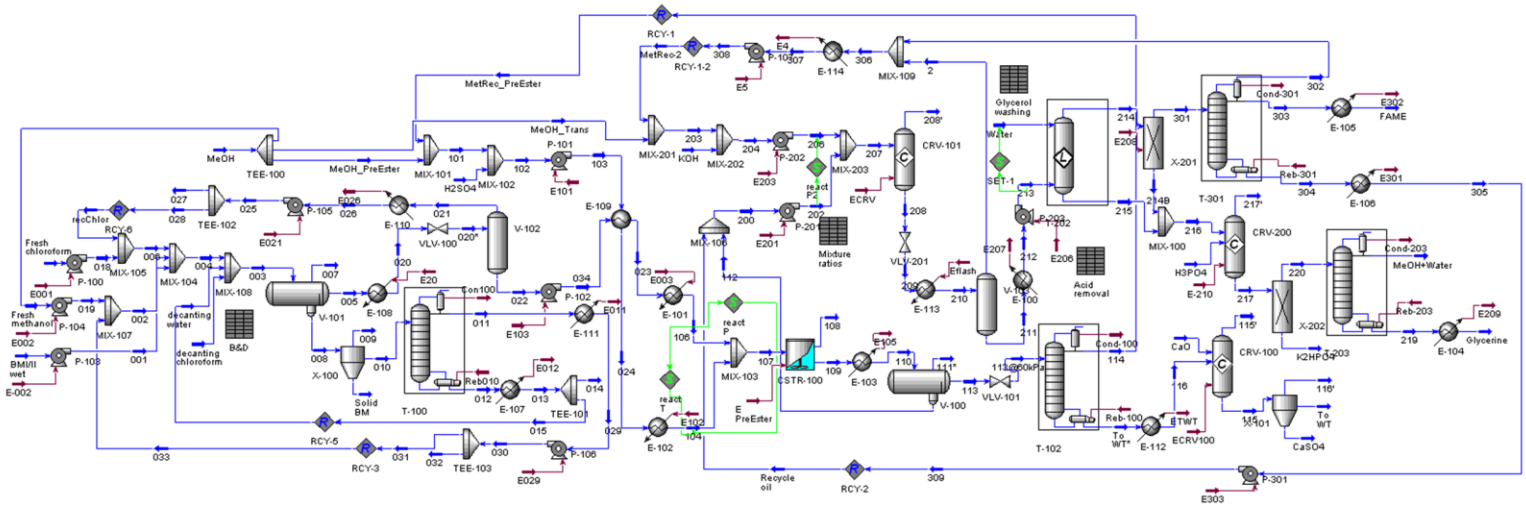
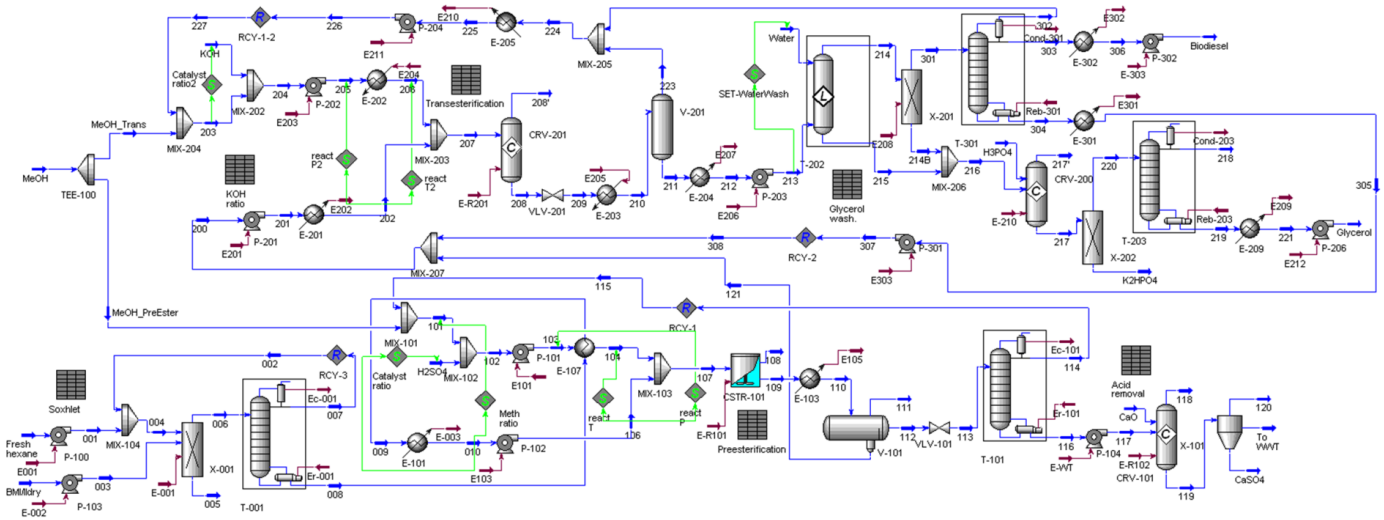


Figure S1. Superstructure for the design of the process alternatives for biodiesel production from microalgae culture.



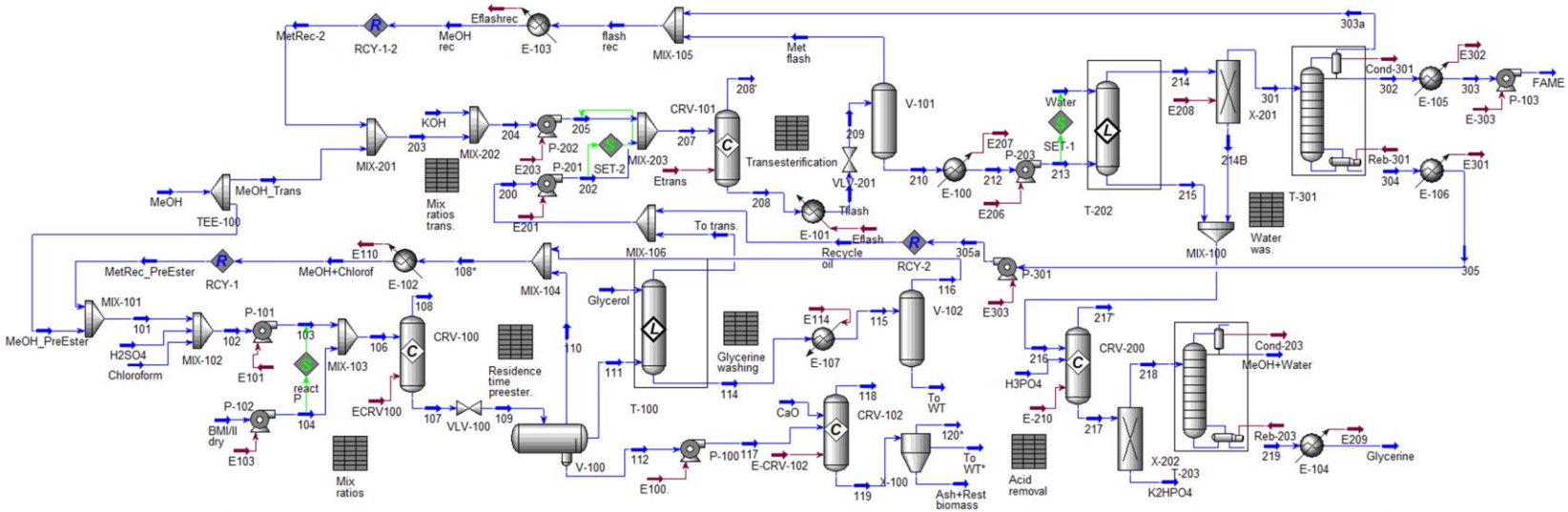
Stream name	001	003	004	005	008	013	106	107	109	112	113	116	To WT	202	207	208	211	216	219
Temperature (ºC)	20.0	38.8	33.7	38.8	38.8	20.0	60.0	57.1	60.0	45.0	45.0	50.0	50.0	48.0	35.7	60.0	91.3	55.2	143.3
Pressure (kPa)	105.0	101.3	101.3	101.3	101.3	101.3	110.0	110.0	110.0	109.0	109.0	60.0	60.0	105.0	105.0	105.0	71.0	120.0	30.0
Mass flow (kg/h)	136656.0	570554.8	434346.8	148882.5	421672.4	206780.0	5148.1	6888.9	6889.0	5262.2	1626.7	134.6	77.1	5389.4	6488.5	6488.6	6110.6	769.7	441.6
Component mass fraction																			
Triolein	0.030	0.007	0.009	0.028	0.000	0.000	0.797	0.596	0.596	0.780	0.000	0.000	0.000	0.785	0.652	0.020	0.021	0.000	0.000
OleicAcid	0.007	0.002	0.002	0.007	0.000	0.000	0.195	0.146	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ash	0.375	0.090	0.118	0.000	0.122	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rest biomass	0.087	0.021	0.028	0.000	0.028	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Water	0.500	0.366	0.169	0.007	0.493	0.985	0.000	0.001	0.010	0.000	0.044	0.475	0.997	0.000	0.000	0.000	0.000	0.079	0.033
Methanol	0.000	0.265	0.347	0.042	0.344	0.015	0.000	0.221	0.204	0.013	0.825	0.002	0.003	0.012	0.142	0.073	0.025	0.072	0.000
M-Oleate	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.153	0.200	0.000	0.000	0.000	0.195	0.162	0.798	0.847	0.000	0.000
Sulfuric acid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.000	0.043	0.523	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Potassium hydroxide	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.523	0.000	0.000	0.029	0.029	0.031	0.243	0.000
Glycerol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.066	0.070	0.555	0.967
n-Hexane	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chloroform	0.000	0.249	0.326	0.916	0.013	0.000	0.008	0.026	0.026	0.007	0.088	0.000	0.000	0.007	0.015	0.015	0.006	0.051	0.000

Figure S2. Flowsheet diagram of the AspenHysys® case for the microalgae oil extraction and transesterification through “wet route”.



Stream name	003	004	006	007	106	107	109	112	116	To WWT	202	207	208	211	216	220	221	218	301
Temperature (°C)	20.0	69.7	25.0	70.2	60.0	57.3	60.0	45.0	72.2	15.0	60.0	53.5	60.0	90.4	57.5	60.0	40.0	53.3	50.0
Pressure (kPa)	120.0	105.0	110.0	105.0	110.0	109.1	109.1	99.1	30.0	101.3	110.0	110.0	110.0	60.0	120.0	100.0	29.0	20.0	110.0
Mass flow (kg/h)	40968.0	436711.4	437200.7	432343.8	4856.9	6402.1	6402.2	1435.7	124.6	73.1	5089.9	5964.3	5964.3	5630.0	570.6	527.4	419.5	107.9	5115.6
Component mass fraction																			
Triolein	0.100	0.000	0.009	0.000	0.803	0.609	0.609	0.000	0.000	0.000	0.790	0.674	0.020	0.021	0.000	0.000	0.000	0.000	0.024
OleicAcid	0.025	0.000	0.002	0.000	0.197	0.150	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ash	0.500	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rest biomass	0.375	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Water	0.000	0.000	0.000	0.000	0.000	0.003	0.013	0.058	0.491	0.995	0.000	0.000	0.000	0.000	0.099	0.145	0.033	0.579	0.000
Methanol	0.000	0.000	0.000	0.000	0.000	0.228	0.211	0.898	0.003	0.005	0.012	0.146	0.075	0.021	0.080	0.086	0.000	0.421	0.014
M-Oleate	0.000	0.000	0.000	0.000	0.000	0.000	0.157	0.000	0.000	0.000	0.197	0.169	0.825	0.874	0.000	0.000	0.000	0.000	0.962
Sulfuric acid	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.044	0.506	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Potassium hydroxide	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.506	0.000	0.000	0.011	0.011	0.011	0.110	0.000	0.000	0.000	0.000
Glycerol	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.068	0.072	0.711	0.769	0.967	0.000	0.000
n-Hexane	0.000	1.000	0.989	1.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000	0.000	0.000

Figure S3. Flowsheet diagram of the AspenHysys® case for the microalgae oil extraction and transesterification through “dry route A”.



Stream name	102	106	107	111	112	114	To WT	119	To WT*	202	205	207	208	213	216	218	219	301	303
Temperature (ºC)	25.1	29.8	80.0	79.0	79.0	80.1	80.0	50.0	50.0	77.9	25.3	36.8	80.0	60.0	58.7	60.0	143.4	50.0	40.0
Pressure (kPa)	100.0	165.0	165.0	101.3	101.3	100.0	100.0	110.0	110.0	101.3	101.3	101.3	101.3	120.0	120.0	100.0	30.0	110.0	19.9
Mass flow (kg/h)	9443.7	72518.3	72246.9	5408.1	66100.3	157.0	157.0	66436.7	39198.8	5379.9	7688.6	13068.5	6035.1	5734.6	554.3	523.7	441.3	5237.6	5099.2
Component mass fraction																			
Triolein	0.000	0.057	0.057	0.758	0.000	0.001	0.001	0.000	0.000	0.786	0.000	0.323	0.021	0.022	0.000	0.000	0.000	0.024	0.000
OleicAcid	0.000	0.014	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ash	0.000	0.152	0.153	0.000	0.167	0.000	0.000	0.166	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Rest biomass	0.000	0.212	0.213	0.000	0.233	0.000	0.000	0.231	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Water	0.008	0.436	0.438	0.000	0.478	0.007	0.007	0.477	0.809	0.000	0.000	0.000	0.000	0.000	0.103	0.134	0.033	0.000	0.000
Methanol	0.797	0.104	0.102	0.002	0.111	0.006	0.006	0.111	0.188	0.001	0.994	0.585	0.061	0.013	0.049	0.051	0.000	0.009	0.007
M-Oleate	0.000	0.000	0.015	0.199	0.000	0.834	0.834	0.000	0.000	0.176	0.000	0.072	0.839	0.883	0.000	0.000	0.000	0.967	0.993
Sulfuric acid	0.063	0.008	0.008	0.000	0.009	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Potassium hydroxide	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.005	0.003	0.007	0.007	0.072	0.000	0.000	0.000	0.000
Glycerol	0.000	0.000	0.000	0.000	0.000	0.012	0.012	0.000	0.000	0.000	0.000	0.000	0.071	0.074	0.770	0.814	0.967	0.000	0.000
n-Hexane	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Chloroform	0.132	0.017	0.014	0.041	0.002	0.139	0.139	0.002	0.004	0.037	0.001	0.016	0.002	0.001	0.006	0.000	0.000	0.000	0.000

Figure S4. Flowsheet diagram of the AspenHysys® case for the microalgae oil extraction and transesterification through “dry route B”.

9.2.- SD-Chapter 7

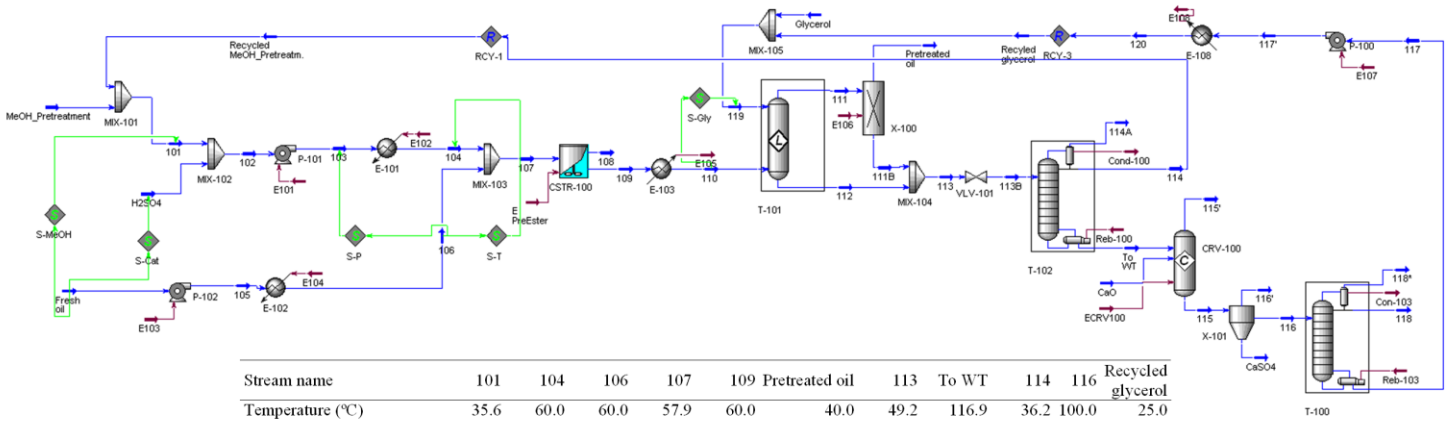
Table S1. Base properties for the hypothetical components.

Name	Diolein	Monolein	H_2PO4	Na_3PO_4 [c]	CaO [c]	$CaSO_4$ [c]	K_2HPO_4 [c]
Component class	Miscell	Miscell.	Inorganic	Inorganic	Inorganic	Inorganic	Inorganic
orm. boiling point (ºC)	491.88	401.67	213.00	-	-	-	-
Molecular weight	621.00	356.55	97.99	163.94	56.08	136.14	174.2
Liquid density (kg/m^3)	996.79	990.41	1834.00	1620.00	3350.00	2960.00	2044.00
Critical temperature (ºC) [b]	647.05	561.91	704.35	-	-	-	-
Critical pressure (kPa) [b]	505.00	1056.00	9342.95	-	-	-	-
Critical volume ($m^3/kgmole$) [b]	2.83	1.25	0.27	-	-	-	-
Acentricity [b]	1.76	1.53	0.13	-	-	-	-

[a]ChemSpider database (www.chemspider.com).
[b]Property estimated by Hysys default method.
[c]Solid hypothetical component.

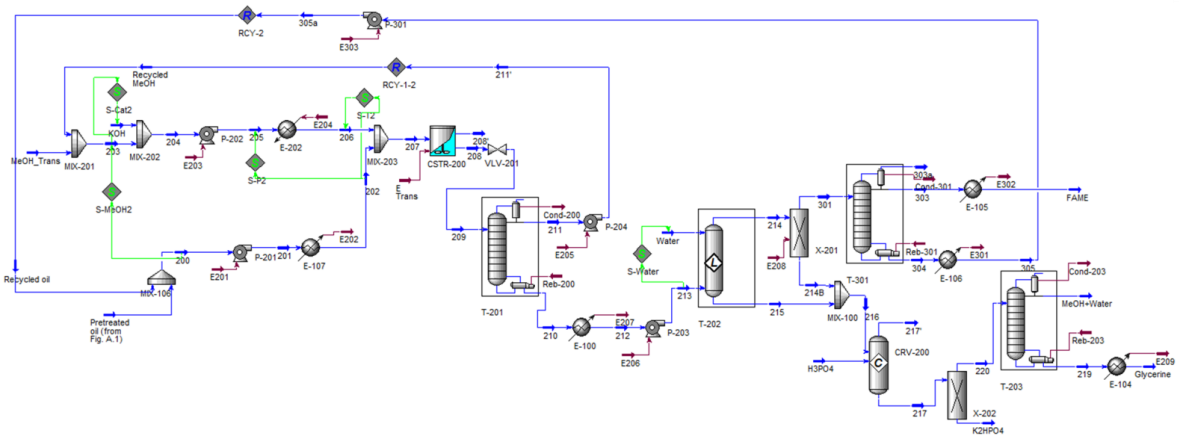
Table S2. Arrhenius parameters for pre-esterification and transesterification reactions.

	Acid-catalyzed esterification (H_2SO_4)		Alkali-catalyzed transesterification (NaOH)		Alkali-catalyzed transesterification (KOH)	
Rate constant	A (L/mol·h)	E_a (J/mol)	A (L/mol·h)	E_a (J/mol)	A (L/mol·h)	E_a (J/mol)
k1	4.11E-20	-1680	1.42E-01	-6228	1.03E+02	10499
k2	1.17E-21	-2052	1.25E-01	-13751	1.33E+00	-9681
k3	7.80E-13	-1167	2.08E+11	60314	7.47E+12	70984
k4	6.48E-11	-1253	5.11E+02	13136	6.73E+13	78154
k5	8.22E-11	-1102	2.98E+11	54185	6.39E+10	51401
k6	9.12E+11	748	6.03E+09	50909	2.83E+06	36649
k7	8.16E+05	575	--	--	--	--
k8	1.52E-05	-341	--	--	--	--



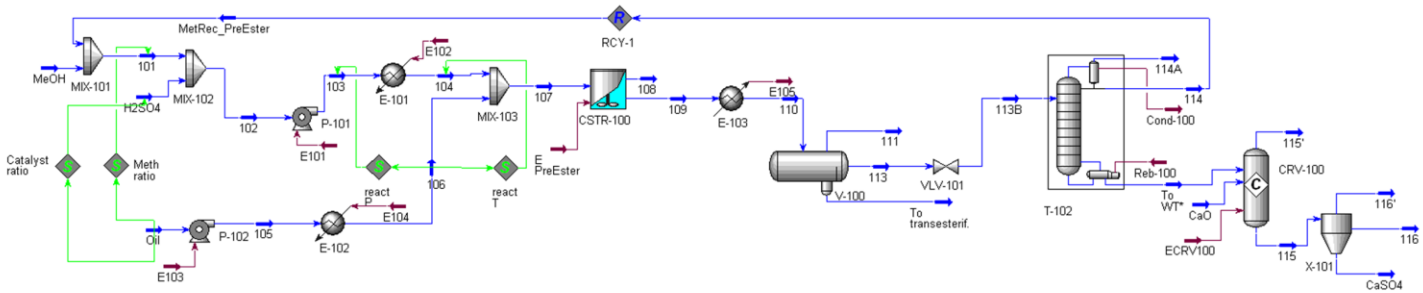
Stream name	101	104	106	107	109	Pretreated oil	113	To WT	114	116	Recycled glycerol
Temperature (°C)	35.6	60.0	60.0	57.9	60.0	40.0	49.2	116.9	36.2	100.0	25.0
Pressure (kPa)	100.0	400.0	400.0	400.0	400.0	190.0	200.0	30.0	20.0	30.0	105.0
Mass flow (kg/h)	141.4	148.9	583.5	732.4	754.4	585.2	210.9	73.41	133.3	67.3	63.9
Component mass fraction											
Triolein	0.000	0.000	0.913	0.727	0.706	0.910	0.000	0.000	0.000	0.000	0.000
Diolein	0.000	0.000	0.028	0.021	0.021	0.028	0.000	0.000	0.000	0.000	0.000
Monoolein	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.000
Oleic Acid	0.000	0.000	0.058	0.045	0.047	0.000	0.000	0.000	0.000	0.000	0.000
Methanol	0.974	0.925	0.000	0.183	0.188	0.000	0.635	0.000	0.973	0.000	0.000
NaOH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Glycerol	0.000	0.000	0.000	0.000	0.000	0.000	0.290	0.833	0.000	0.909	0.960
M-Oleate	0.000	0.000	0.000	0.000	0.047	0.061	0.000	0.000	0.000	0.000	0.000
H_2O	0.026	0.024	0.000	0.005	0.008	0.000	0.040	0.065	0.027	0.091	0.040
H_2SO_4	0.000	0.050	0.000	0.039	0.010	0.000	0.036	0.102	0.000	0.000	0.000
H_3PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Na_3PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure S1. Acid-catalyzed pretreatment section of process II.



Stream name	202	204	207	208	Recycled MeOH	210	216	217	220	MeOH + Water	Glycerine	301	FAME	Recycled oil
Temperature (°C)	60.0	29.5	53.8	60.0	35.5	126.1	58.0	25.0	60.0	58.6	40.0	50.0	40.0	159.6
Pressure (kPa)	400.0	101.3	400.0	400.0	101.3	30.0	120.0	101.3	100.0	20.0	29.0	110.0	19.9	101.3
Mass flow (kg/h)	691.9	180.6	872.5	872.5	103.8	768.8	80.5	91.9	71.7	11.8	59.9	695.9	499.0	106.7
Component mass fraction														
Triolein	0.920	0.000	0.729	0.119	0.000	0.135	0.000	0.000	0.000	0.000	0.000	0.149	0.000	0.972
Diolein	0.027	0.000	0.022	0.003	0.000	0.004	0.000	0.000	0.000	0.000	0.000	0.004	0.000	0.028
Monoolein	0.001	0.000	0.001	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
OleicAcid	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Methanol	0.000	0.928	0.192	0.124	1.000	0.006	0.024	0.021	0.027	0.164	0.000	0.003	0.001	0.000
KOH	0.000	0.072	0.015	0.015	0.000	0.017	0.162	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Glycerol	0.000	0.000	0.000	0.066	0.000	0.075	0.719	0.630	0.807	0.000	0.967	0.000	0.000	0.000
M-Oleate	0.052	0.000	0.041	0.672	0.000	0.763	0.000	0.000	0.000	0.000	0.000	0.843	0.999	0.000
H_2O	0.000	0.000	0.000	0.000	0.000	0.000	0.095	0.130	0.165	0.836	0.033	0.000	0.000	0.000
H_2SO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
H_3PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
KH_2PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.220	0.000	0.000	0.000	0.000	0.000	0.000

Figure S2. Alkali-catalyzed transesterification section of process III.



Stream name	101	104	106	107	109	To transesterif.	113	114
Temperature (°C)	35.6	60.0	60.0	57.9	60.0	45.0	45.0	36.2
Pressure (kPa)	100.0	400.0	400.0	400.0	400.0	200.0	200.0	20.0
Mass flow (kg/h)	141.4	148.9	583.5	732.4	754.4	590.9	141.6	127.9
Component mass fraction								
Triolein	0.000	0.000	0.913	0.727	0.706	0.901	0.000	0.000
Diolein	0.000	0.000	0.028	0.021	0.021	0.028	0.000	0.000
Monoolein	0.000	0.000	0.001	0.001	0.001	0.001	0.000	0.000
Oleic Acid	0.000	0.000	0.058	0.045	0.047	0.000	0.000	0.000
Methanol	0.974	0.925	0.000	0.183	0.188	0.010	0.635	0.973
NaOH	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Glycerol	0.000	0.000	0.000	0.000	0.000	0.000	0.290	0.000
M-Oleate	0.000	0.000	0.000	0.000	0.047	0.061	0.000	0.000
H_2O	0.026	0.024	0.000	0.005	0.008	0.000	0.040	0.027
H_2SO_4	0.000	0.050	0.000	0.039	0.010	0.000	0.036	0.053
H_3PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Na_3PO_4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Figure S3. Acid-catalyzed pretreatment section of process IV.

10.- List of Publications and Contributions to Conferences

Publications:

Carmen M. Torres; Sergio D. Ríos; Carles Torras; Joan Salvadó; Josep M. Mateo-Sanz; Laureano Jiménez "**Sustainability analysis of biodiesel production from *Cynara Cardunculus* crop"** Fuel (2013) Accepted.

Sergio D. Ríos; Carmen M. Torres; Carles Torras; Joan Salvadó; Josep M. Mateo-Sanz; Laureano Jiménez (2013)"**Performance of realistic scenarios for biodiesel production from microalgae: economic and environmental analysis"** Bioresource Technology; 10.1016/j.biortech. 2013.03.046.

Sergio D. Ríos, Joandiet Castañeda, Joan Salvadó, Xavier Farriol and Carles Torras.(2013) "**Lipid extraction methods from microalgal biomass harvested by two different paths: screening studies toward biodiesel production**." Bioresource Technology; 133, 378-388.

Sergio D. Ríos, Joan Salvadó, Xavier Farriol and Carles Torras. (2012). "**Antifouling microfiltration strategies to harvest microalgae for biofuel**." Bioresource Technology 119 (2012) 406–418.

Rios SD, Clavero E, Salvado J, Farriol X and Torras C (2011). **Dynamic microfiltration in microalgae harvesting for biodiesel production**. Industrial and Engineering Chemistry Research, Volume 50, Issue 4, p: 2455-2460.

Congress participations:

Poster presentation: Carmen M. Torres; Sergio D. Ríos; Carles Torras; Joan Salvadó; Josep M. Mateo-Sanz; Laureano Jiménez "Biodiesel production from *Cynara Cardunculus* crop: economic, environmental and social assessment". AIChE, Pittsburgh, USA, November 2012.

Poster presentation: Ester Clavero, Sergio Ríos, María Rey and Joan Salvadó, "Microalgae production and biorefinery". VII Barcelona Global Energy Challenges, Barcelona, Spain, 2-3 June 2012.

Poster presentation: Nurra C, Rios SD, Şirin S, Salvadó J and Torras C. A possible route for microalgae harvesting". VII Barcelona Global Energy Challenges, Barcelona, Spain, 2-3 June 2012.

Oral presentation: Rios SD, Clavero E, Salvado J, Farriol X and Torras C. "Dynamic microfiltration in microalgae concentration". International Congress on Membranes and Membrane Processes, 23-29 July 2011.

Poster presentation: Rios SD, Şirin S, Torras C, Clavero E, Salvadó J. "Harvesting studies of Phaeodactylum tricornutum microalga", 1st International Conference on Algal Biomass, Biofuels and Bioproducts, Westin St Louis, 17-20 July 2011.

Poster presentation: Rosa Trobajo, Ester Clavero, María Rey, Sergio Ríos and Joan Salvadó, "Microalgae production and biorefinery". VI Barcelona Global Energy Challenges, Barcelona, Spain, 2-3 June 2011.

Poster presentation: Rios SD, Şirin S, Nurra C, Salvadó J and Torras C. "Microalgae concentration: a step in the biorefining process". VI Barcelona Global Energy Challenges, Barcelona, Spain, 2-3 June 2011.