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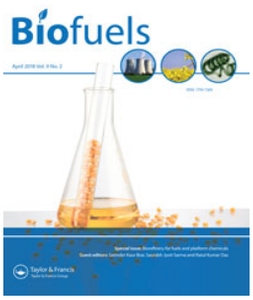

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Biodiesel from microalgae lipids: from inorganic carbon to energy production

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ABSTRACT

Following the United Nations Conference on Climate Change, COP21 (Paris, France), several countries have attempted to reduce their greenhouse gas emissions. In order to reach this objective, microalgae could be used to capture carbon dioxide and transform it into a biomass composed essentially of lipids, carbohydrates and proteins. Moreover, cultivating microalgae does not require arable land, in opposition to several oleaginous plants used to produce biofuels. Despite the fact that microalgae could be transformed into several biofuels such as bioethanol (by fermentation of hydrocarbons) and biomethane (by anaerobic digestion), transforming lipids into biodiesel could allow the reduction of oil-based diesel consumption. However, microalgae biodiesel production costs remain high for a short-term commercialization. The microalgae lipids can be transesterified into biodiesel in the presence of catalysts (homogeneous or heterogeneous). In order to commercialize biodiesel from microalgae, biodiesel physicochemical properties must respect the American Society for Testing and Materials (ASTM) standards. The aim of the study was to describe the current technologies available to produce biodiesel from microalgae.

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Microalgae; culture; lipids; extraction; transesterification; biodiesel

Introduction

Between 1980 and 2011, the world carbon dioxide (CO_2) emissions increased from 18.5 to 31.5 billion metric tons per year, which represented an increase of 420 million metric tons per year [1]. In order to reduce these emissions, several countries signed the United Nations Framework Convention on Climate Change at COP21 (Paris, France) in December 2015. Among them, by 2030, the Canadian government is committed to reduce greenhouse gases (GHG) emissions by 30% under the 2005 GHG emission level of 607 Mt CO_2 eq [2]. In Canada (2013), near 80% of all GHG, around 581 Mt CO_2 eq, was attributable to CO_2 emissions [3,4]. The Canadian CO_2 emissions related to energy consumption increased from 457 to 581 million metric tons between 1980 and 2013, which represents around 16 metric tons of CO_2 per person in 2013 [1,4]. The main sectors responsible for CO_2 emissions are the energy sector (93%) and industrial processes (6.7%) [5].

The exploitation of fossil fuel in western Canada has, among other things, contributed to increasing CO_2 emissions linked to the energy sector from 467 to 588 Mt CO_2 eq, an increase of 25% between 1990 and 2013 [4,5]. Indeed, Canada owns the second most important petroleum deposit (all forms included) in the world with 178 billion barrels, which is the 4th most important oil producer in the world with 3.3 million barrels per day (2012); most of this oil is exported to the United States of America [6].

Between 1992 and 2012, the world proven petroleum deposit evaluated by the BP petroleum company seems to have increased from 1040 to 1690 billion barrels [7]. The world petroleum deposit was evaluated at 1526 billion barrels in 2012 [8]. Some authors predict that these petroleum deposits will be sufficient to fulfill the demand for petroleum-based products until 2042 [9]. This could be attributable to the fact that the crude petroleum consumption increased from 78 to 90 million barrels per day from 2002 to 2012 [7]. In 2012, the world's most important petroleum oil consumers were the United States, China and Japan with 18.5, 10.3 and 4.7 million barrels per day, respectively [10]. According to the same source, Canada was the 10th most important oil consumer with 2.3 million barrels per day in 2012.

Alternatively to non-renewable energy sources, energy can be produced from biomass such as lignocellulosic plants, herbaceous plants, water plants and animal manure [11]. Indeed, in 2011, 82% of all the world's non-renewable energy was produced from biomass, followed by hydropower, wind, solar and other with 15, 2.0, 0.2 and 0.4%, respectively [12].

In order to find more sustainable solutions to replace oil-based fuel, other solutions are currently being developed such as liquid biofuels, gaseous

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biofuels (hydrogen (H_2), methane (CH_4), etc.), solar energy, fuel cell and hybrid technologies [13]. Consequently, several countries (mostly European countries) have voted for laws stating that the fuel used by transportation must contain at least 10% (v/v) biofuel such as bioethanol or biodiesel by 2010 [14]. However, since most biofuels are produced from plants cultivated on arable lands, the European countries reduced, in 2013, their target of biofuels in fuels to 6% (v/v) [15].

Despite the fact that the biodiesel production is around 4 times lower than the bioethanol production [16], the interest in biodiesel has constantly been rising since the fluctuation of oil prices [17]. In April 2011, the average price of a barrel of oil reached a peak value exceeding 121 US$ [18,19] while in 2015 it decreases to less than 40 US$ [20]. Furthermore, the world biodiesel production increased from 77 to 403 $\times$ 10^3 barrels per day between 2005 and 2011, but the Canadian biodiesel production remains low at 3600 barrels per day in 2012 [10].

Among the vegetable oleaginous species used as a source of biomass, microalgae are an interesting source because these microorganisms, which do not require being cultivated on arable lands, can be utilized to produce biodiesel [17].

Microalgae can also be utilized as a raw material for the production of food supplements, medical chemicals and food [21,22]. Moreover, microalgae can be transformed into energy using gasification (synthesis gas), torrefaction, anaerobic digestion, direct combustion (heat), liquefaction, pyrolysis, hydrogenation and transesterification [23].

Biodiesel from vegetable oil can produce the required energy to be used in transportation and generating low air pollutants such as sulfur and polycyclic aromatic compounds [24]. For example, increasing the biodiesel content in petrodiesel from 0 to 20% (v/v) reduces the emissions of particulate matter (−10%), carbon monoxide (−11%) and unburned hydrocarbons (−21%), but slightly increases the nitrogen oxide emissions (+2%) [25,26]. Moreover, with a 20% (v/v) biodiesel-petrodiesel blend (B20), mercaptan emissions (bad smells) can be reduced up to 18% [27]. In addition, increasing the biodiesel content from 20 to 100% (v/v) in petrodiesel can increase the distance traveled from 0.38 to 4.5 km/L [28]. According to the same source, the main problem with biodiesel is the feedstock cost, which represents 80% of the operating costs. However, the research on microalgae biodiesel must continue because the biodiesel production costs remain high between 2.4 and 6.6 $US/L (microalgae culture in ponds) [29]. Reducing the cost associated with microalgae biodiesel is an important challenge for the replacement of first-generation biodiesel, produced from oleaginous plants such as soybean.

This literature review will describe microalgae culture, composition, harvesting, lipid extraction and lipid transesterification. Moreover, the possibilities of using microalgae as a raw material for biodiesel production will be discussed through biodiesel properties, by-products, cost calculation and Canadian biodiesel production.

Microalgae: a source of sustainable development

Currently, first-generation biofuels are mainly produced from agricultural biomass such as soybean, rapeseed, palm, etc. Most of the biodiesel produced in the United States comes from soybean culture [17]. However, using arable lands to produce biodiesel can have negative effects on the economy, the environment and the society.

On an economic level, producing biofuels such as biodiesel on a large scale using arable lands in order to cultivate the biomass could lead to an increase of food prices. As an example, between 2000 and 2011, the FAO Food Price Index and FAO Oils/Fats Price Index of several food and oil prices increased by 250% [30]. This situation would, as a consequence, increase the disparity between rich and poor countries, threatening the food security of the poorest countries [31]. At an environmental level, producing biofuels on a large scale using arable land could create problems of soil impoverishment, land pollution and deforestation [32–34].

Using microalgae as a raw material for biofuel production could solve these problems as microalgae culture does not compete with human and animal food production [35]. Furthermore, this type of culture does not require heavy equipment for chemical product spreading or for watering because nutrients and water are added to the bioreactor.

One of the main advantages of microalgae is their lipid content. As an example, palm oil can contain up to 36% (dry weight) of lipids (triglycerides) with a maximal culture yield of 5.4 m^3 lipids/ha/year [36], while microalgae can contain up to 75% (dry weight) lipids with a culture yield of 137 m^3 lipids/ha/year [17].

Microalgae composition

Microalgae are composed of four main components: lipids, carbohydrates, proteins and nucleic acids [37].

Carbohydrates

Carbohydrates are structural material, with a metabolic function, of the microalga, which can be found as starch, polysaccharides, monosaccharides (like glucose) and more [38,39]. Concerning biofuels, one of the main interests of carbohydrates is to use microalgal glucose and transform it by fermentation into bioethanol with a yield up to 50 wt% (compared to the total carbohydrates) for *Chlorococcum humicola* microalgae (a 100%

conversion based on glucose). [40]. Another study stated that cyanobacteria could synthesize directly bioethanol from CO_2 with an annual production of 20 m^3/ha [41].

Proteins

Like carbohydrates, proteins contained in microalgae have metabolic and structural functions [42]. Depending on the type of microalgae, the protein content ranges from 30 to 50 wt% of the dry biomass [43,44]. Several companies (such as Cyanotech, Martek Biosciences Corporation, Mera Pharmaceutical, etc.) have cultivated microalgae for their protein content (dietary supplements) or other components like pigments [43].

Lipids

Lipids are also structural elements with an energy storage function [42]. Despite the fact that lipids are a complex group of 14 categories, such as hydrocarbons, fatty acids, phospholipids, glycolipids and sulfated lipids [45], several of these lipids cannot be transformed into fatty acids methyl esters (FAME) and are called unsaponified lipids.

Some polyunsaturated lipids (omega 3 and omega 6) such as eicosapentaenoic acid (EPA) and decohexanoic acid (DHA) showed a potential reduction of some types of cancer such as colorectal cancer [46], and have antiangiogenic, antivasoproliferative and neuroprotective properties [47].

Depending on the type of microalgae, the lipid yield obtained from microalgae can reach more than 75% (g lipid/g dry biomass) [48–50]. The microalgae with the most important lipid contents are (g lipid/g dry biomass): *Botryococcus braunii* (25–75%), *Schizochytrium* sp. (50–77%), *Dunaliella tertiolecta* (16–71%), *Dunaliella* sp. (17–67%) and *Neochloris oleoabundans* (29–65%).

Several microalgae species have a high carotenoid content, like the microalga *Dunaliella salina* that contains 10% (g/g dry biomass) of β-carotene (a kind of carotenoid) [51]. Some factors affect the carotenoid content in microalgae such as temperature, pH, and dissolved oxygen level in the culture medium [52]. As an example, using a stream of air containing 2% (v/v) of CO_2 for the growth of the microalga *Chlorococcum* sp. for 10 days at an irradiance of 200 μmol photons/m^2/s, Liu *et al.* [51] increased the initial pH from 5 to 9 and found that a pH of 8 was the best pH tested for carotenoid growth with a total carotenoid content of 7.4 mg/g.

Other studies tried to recover extracellular polymeric substances (EPSs; extracellular biomass) of microalgae [53,54]. For example, a recent study used solvents to extract a maximum concentration of EPSs in an aqueous phase of 0.944 g/L of EPSs from the microalga *Dunaliella salina* and obtained a maximal emulsifying activity of 86% EPSs [53].

Nucleic acids and chlorophyll

In microalgae, the content of nucleic acids generally varies from 4 to 6% (g/g dry biomass) [55]. On the other hand, a high level of nucleic acids (> 0.3 g microalgae/kg body weight/day) induces oral toxicity for the human body [56], which means that microalgae must be processed before human consumption. For animal food, unprocessed microalgae do not seem to be toxic [55].

Chlorophyll is a green pigment. Industrially, this pigment is mainly used as an additive by the pharmaceutical industry (cosmetic products) and also acts as a natural dying agent [57]. Moreover, according to the same source, these compounds have antioxidant and antimutagenic properties. The solvents used to extract chlorophyll are methanol, ethanol, dimethyl formamide, acetone and supercritical CO_2 [57]. As an example, Bai *et al.* [58] studied microalgae pigments (chlorophyll and carotenoids). They obtained chlorophyll contents ranging from 1.7 to 5.6% (g lipid/g dry biomass) for a few species of microalgae (including *Chlorella* sp., *Chlorella minutissima*, *Dunaliella promolecta*, *Isochrysis galbana* and *Nannochloropsis oculata*) by using methanol as an extraction solvent.

Microalgae metabolisms

There are more than 30,000 species of algae known on Earth [59], that can be classified as a function of their taxonomy, size and metabolism [34,42,60]. Based on their metabolism, there are four main classifications to identify microalgae: photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic.

Photoautotrophic

Inorganic carbon (CO_2 or bicarbonate) is transformed into biomass (microalgae) and oxygen following an input of light energy, water and nutrients [57,61]. Carbon dioxide (CO_2) can be supplied by several sources. A common source of CO_2 often cited in the literature comes from thermal power plants [62].

Despite the fact that the photoautotrophic metabolism seems to be a sustainable solution to reduce the CO_2 emissions, this process has some limits. Even if the lipid content of some microalgae (*Botryococcus braunii*, *Dunaliella tertiolecta*, *Neochloris oleoabundans*) is relatively high (around 75% (g lipid/g dry biomass), the concentrations of microalgae biomass obtained by these photoautotrophic metabolisms are low to medium (0.5–6.7 g/L) which makes microalgae biomass harvesting problematic compared to other oleaginous species because microalgae require a high volume of culture medium.

Heterotrophic

Heterotrophic microalgae transform organic carbon sources without light within fermenters [63]. Some

microalgae species with heterotrophic metabolisms were studied because they can have high lipid contents (up to 56% (g lipid/dry biomass)) [64,65]. It should be noted that heterotrophic microalgae, such as *Chlorella pyrenoidosa*, can reach high biomass concentrations (up to 216 g/L), but rheological problems in fermenters (mass transfer and mixing problems) were observed at biomass concentrations higher than 50 g/L [66,67].

Despite the fact that glucose is the most common carbon source [65], other carbon sources were tested for microalgae growth, such as fructose, galactose, acetic acid, sodium acetate, glycerol and various hydrolysis products [64,68]. Among the hydrolysis products are sugarcane [69], artichoke [70,71], tapioca [72] and rice straw [65]. A study demonstrated that hydrolysis products have a higher potential than glucose does. For example, growing the microalga *Chlorella pyrenoidosa* with glucose and rice straw hydrolysate, as carbon sources, at a concentration of 10 g/L, Li *et al.* [65] obtained a higher lipid content of 56% (g lipid/g dry biomass) using rice straw hydrolysate than glucose 50% (g lipid/g dry biomass). Furthermore, these authors obtained respective biomass concentrations of 2.83 g/L and 0.92 g/L for rice straw hydrolysate and glucose as carbon sources.

Despite the potential of heterotrophic microalgae for biodiesel production, no commercial scale plant of microalgae for biodiesel purpose has been built [64] up to now. This could be explained by the fact that the first objective of microalgae culture is to decrease CO_2 emissions. Moreover, heterotrophic culture requires a cheap organic source such as organic matter hydrolysate [73], which is directly in competition with bioethanol production.

Mixotrophic and photoheterotrophic

Microalgae with a mixotrophic metabolism can have a photoautotrophic or heterotrophic metabolism. They can use inorganic or organic carbon with or without light energy [68,74]. When the light is absent, these types of microalgae can continue to grow by using inorganic or organic carbon. Liang *et al.* [75] used glucose and CO_2 (from air) in the absence of light and obtained respective biomass productivities of 151 and 10 mg/L/day. When they used glucose in similar but dark conditions, the biomass productivity increased to 254 mg/L/day. The term 'photoheterotrophic' is kept for microalgae that are able to use organic carbon and light [74].

Microalgae production

Industrial microalgae culture for lipid extraction was considered a long time ago. As an example, some studies were performed in the 1950s in order to produce the *Chlorella* algae on a wide scale as a food source [76]. On the other hand, the interest in a specific microalgae production for a biofuel purpose is linked to the increase of oil prices and to the reduction of CO_2 mentioned previously. An interesting fact is that microalgae can mitigate CO_2 emissions of several industries such as thermal power plants that burned oil fuel [77,78]. Moreover, microalgae can also be cultivated using wastewaters (as a source of nutrients) despite the abundance of heavy metals, pathogenic microorganisms, nitrogen or phosphorus [79,80].

A major problem with microalgae production is the fact that unrealistic biomass production rates reported in research articles have led to the cancellation of several microalgae production scaling-up projects [81].

On an industrial scale, four modes of culture can be considered: open ponds, photobioreactors, hybrid modes and fermenters.

Open ponds

Open ponds are divided into three categories [82]: (1) lakes, (2) lagoons and (3) ponds. Most industrial microalgae cultures are performed in raceway type ponds, shallow (less than 30 cm deep), provided with a paddle wheel, which creates a flow to avoid microalgae settling [17]. Nutrients and water are added in front of the paddle wheel and microalgae are harvested at the back of the wheel [17,83].

The main advantage of open culture systems (outside) is to use free sunlight energy [84]. However, these systems have the disadvantage of producing low biomass density (lower than 5 g/L) [85], which complicates the dewatering process. Cultivating the microalga *Chlorella* sp. in an open pond, Doucha and Lìvanský [86] obtained a high biomass concentration (40 g/L) after 18 days of production. On the other hand, to our best knowledge of the literature, no other studies have reported such a high biomass concentration in open ponds. The main advantage of open systems is linked to their facility of scaling up and their lower investment costs, even if these systems have non-negligible disadvantages compared to closed systems: risk of contamination, higher harvesting costs, loss of CO_2 (no CO_2 recycled), higher water evaporation rates and bad use of light energy [87].

Despite the fact that open ponds are used for industrial microalgae production for food and cosmetic purposes [88–90], production costs (around 3.65 US$/kg biomass) make this technology hard to apply industrially for the production of biodiesel [91].

Photobiorectors

In order to solve the problems linked to microalgae culture in ponds (biomass concentration, contamination, etc.), microalgae culture in photobioreactors is often preferred [92]. There are several types of photobioreactors [87, 93]: (1) tubular, (2) flat plate, (3) airlift, (4) bubbling column and (5) stirred tank.

Tubular photobioreactors, made of plastic or glass, can have different configurations: spiral [94,95], external looped [96] and straight [87]. These systems can be paired with a pump or an airlift system [87,96].

The flate plate photobioreactors have parallel glass plates between which microalgae grows with sunlight. Kurano *et al.* [97] cultivated the microalgae *Chlorococcum littorale* with this type of reactor and obtained biomass doubling every 2 h at a temperature of 40°C. Sometimes, this type of reactor is used with only two parallel glass plates in which the gas is fed like in a bubbling column [98].

The airlift bioreactors are a group of two vertical concentric tubes [99] in which the inside tube is perforated at the inlet and the oulet [100] or is shorter than the outside tube [87,99]. This configuration must allow microalgae to go up in the inlet tube and go down between the two tubes. Air is fed in at the bottom of the reactor and makes microalgae flow from the bottom to the top to avoid settling.

A bubbling photobioreactor works similarly to an airlift photobioreactor. The bubbles are produced with a perforated flat plate in which air and CO_2 form bubbles at the bottom of the reactor. Some of these reactors have produced interesting biomass yields. For example, Ranjbar *et al.* [101] cultivated the microalga *Hoematococcus pluvialis* in a bubbling air column of 1.1 L and obtained a biomass concentration of 6.7 g/L after 33 days of culture (illumination at 21.5 μmol/m^2/s; temperature of 20°C; pH of 7.5 by adjusting the CO_2 concentration).

Stirred tanks are photobioreactors rarely used despite a biomass productivity of 47.8 g/m^2/day (in pseudo steady-state regime) for a configuration of bioreactor in series [102]. The little interest in this type of reactor could be explained by the fact that some studies obtained low growth rates and/or low biomass concentrations. For example, using a stirred tank photobioreactor to cultivate the marine microalgae *Dunaliella salina*, Li *et al*. [103] obtained a maximum growth rate and biomass concentration of 0.119 1/h and 0.55 g/L, respectively, after around 116 h of culture (temperature of 29°C; pH of around 7.4).

Other configurations of dome-shaped photobioreactors have been tested. For example, Sato *et al*. [104] grew the microalga *Chlorococum littorale* and reached a biomass concentration of 2.5 g/L (temperature of 25°C; pH of 7.5; time of 288 h).

Hybrid culture

As technical problems occurred with both of the previous systems (costs, scaling up, contamination, biomass concentration, etc.), some researchers paired ponds and photobioreactors [105,106]. The first step is a photobioreactor in which the culture conditions are controlled to minimize the risks of contamination [105]. The temperature is controlled with a water bath (16–18°C) [106]. Then, microalgae are transferred into a pond until the harvesting with nutrient deprivation [91].

Fermenters

Fermenters are stirred reactors in which microalgae with heterotrophic metabolisms are grown. For this type of reactor, biomass concentrations and lipid content can reach 116 g/L and 56% (g lipid/g biomass), respectively [107]. On the other hand, a higher biomass concentration can be reached (up to 150 g/L) without rheological problems [66]. According to Cantin *et al.* [64], a few companies such as Solazyme Inc. (South San Francisco, USA) and Fermenlg (Libourne, France) produced biodiesel within fermenters with organic carbon and without light. Solazyme Inc. affirmed that some of their biodiesels, $Soladiesel_{RD}$ et $Soladiesel_{BD}$, could have a higher cetane number (74) or better cold properties than conventional biodiesel [108]. However, no specification is given on the production capacity or the costs of the biodiesel produced.

Microalgae culture parameters

As there are few projects of industrial scale for microalgae with heterotrophic metabolisms [64] and the production costs are high [72, 109], the present study will focus on the autotrophic culture metabolism. The main factors that influence microalgae productivity are: (1) inorganic carbon concentration, (2) light energy, (3) oxygen concentration, (4) temperature, (5) pH and (6) stirring.

- **Inorganic carbon concentration**

Inorganic carbon (such as CO_2) is the main source of carbon for autotrophic microalgae. Despite the fact that CO_2 of surrounding air can be used for microalgae growth, CO_2 concentration ranging from 2 to 15% (v/v) have been tested [84,110,111]. Some microalgae species such as the strain ZY-1 (*Chlorella*) can stand high CO_2 concentrations (up to 70% (v/v)) [112]. According to our best knowledge of the literature, the optimal CO_2 concentration is between 4 and 10% (v/v) [110,112,113].

- **Energy light intake**

One of the most important factors is the light energy intake, which is an essential factor for microalgae growth. In the laboratory, the microalga uses an absorption spectrum between 400 to 700 nm with an efficiency that depends on the photosynthetic efficiency [84,114,115].

As the sun is a free and an unlimited energy resource, microalgae culture is performed outdoors. Consequently, the biomass productivity can be influenced depending of the time of the year. For example,

Blanco *et al.* [116] cultivated the microalgae *Muriellopsis* sp. in open ponds (Isla de la Cartuja, Sevilla, Spain) and obtained a higher biomass productivity during the months of May, June and July (20 g/m^3/day), while the lowest biomass productivity was obtained during the month of November (8 g/m^3/day).

- **Oxygen concentration (O_2)**

Oxygen is a microalgae production by-product, but the O_2 concentration must not be too high because it has an inhibitory effect on microalgae growth [117]. Kitaya *et al.* [110] increased the O_2 concentration from 10 to 30% (v/v) during the growth of the microalga *Euglena gracilis* and noticed that an increase of O_2 concentration decreased the maximum specific growth rate from 0.45 to 0.14 1/h.

- **Nutrients intake**

At a laboratory scale, culture medium with water and mineral salts [101,114,115] was often used because it allows a better control of nutrient intake. The main macronutrients are nitrogen, phosphorus, hydrogen, sulfur, calcium, magnesium, sodium and potassium [84]. A modification of the nutrients has different effects depending on the microalga. The main macronutrients used for the lipid synthesis are carbon, hydrogen, oxygen, phosphorus and sulfur [42]. Carbon, oxygen and hydrogen are supplied by the transformation of CO_2 and water as discussed above.

The macronutrient that has the most important effect (except carbon, hydrogen and oxygen) is nitrogen. Some microalgae species are more sensitive to the type of nitrogen that increases their lipid content [118,119]. As an example, using different sources of nitrogen (ammonium nitrate, ammonium chloride and urea) at a concentration of 0.64 mmol-N/L with the microalga *Ellipsoidion* sp., Xu *et al.* [118] obtained similar maximum growth rates (0.29 $\pm$ 0.02 1/h), but with lipid contents of 28, 33 and 22% (g lipid/g dry biomass) for ammonium nitrate, ammonium chloride and urea, respectively. In comparison, the absence of nitrogen gave a maximum growth rate of 0.17 1/h with a lipid content of 8% (g lipid/g dry biomass). In another study, nitrogen deprivation of a particular species (NAVIC1) increased the lipid content from 22 to 58% (g lipid/g dry biomass) [120]. A more recent study showed an enhancement of lipid content after nitrogen deprivation [121].

Some studies showed that increasing the phosphorus concentration has a positive effect on biomass productivity but few effects on lipid productivity. For example, by increasing the phosphorus concentration from 0.1 to 2 mg/L in the culture medium of the microalga *Scenedesmus* sp., Xin *et al.* [119] obtained an increase of the biomass concentration from 0.14 to 0.37 g/L, while the lipid concentration remained stable at 0.08 $\pm$ 0.01 g/L. Consequently, for the range of phosphorus concentrations, the lipid content decreased from 53 to 24% (g lipid/g dry biomass).

Despite the fact that sulfur is an essential nutrient, few studies have been performed, to our best knowledge of the literature, in comparison to phosphorus or nitrogen. Giorgano *et al.* [122] tested the effect of sulfur concentrations from 6 μM to around 1.1 mM and obtained an increase of the maximum specific growth rate of the microalga *Dunaliella salina* from 0.006 to 0.02 1/h [123]. However, sulfur concentration can have an inhibitory effect on microalgae growth for concentrations higher than 1.1 mM [122,123].

Some microalgae species are also sensitive to salinity [124]. For example, by using a culture medium with salinity from 1 to 3 wt%, Renaud *et al.* [125] obtained an increase of the lipid content from 28 to 33% (g lipid/g dry biomass) for the microalga *Nannochloropsis oculata*. On the other hand, for the alga *Nitzschia* (*frustuluwn*), a similar increase of the salinity had an opposite effect, decreasing the lipid content from 33 to 29% (g lipid/g dry biomass).

Some other micronutrients are also important, such as iron, bore, manganese, copper, molybdenum, vanadium, cobalt, nickel, silica and selenium [84]. In order to decrease the production costs, some microalgae can be grown using waste water as a source of nutrients. Some species such as *Chlamydomonas globosa*, *Chlorella minutissima* or *Scenedesmus bijuga* can metabolize nitrogen, phosphorus, sulfur and metals (Al, Fe, Mn, Zn) [113,126,127].

- **Temperature**

Despite the weather conditions, the optimal conditions of temperature must remain between 20 and 30°C [17,91,128]. As the nutrient intake, the optimum temperature depends on the microalgae species [91,128]. For example, for the microalgae species *Nannochloropsis* sp., *Tetraselmis* sp. and *Isochrysis* sp., Abu-Rezq *et al.* [129] obtained maximum biomass yields with an ambient air CO_2 concentration (0.035% v/v) at respective optimal temperature ranges of 19–21, 19–21 and 24–26°C.

As the sun inevitably heats the culture medium, an external source of water must be provided to cool down the culture medium. For microalgae cultivated in ponds, cooling down is mainly done by evaporation [17]. As to the photobioreactors, cooling is performed with external input of water [130,131]. Consequently, culturing microalgae requires a non-costly source of water (river, sea, groundwater, etc.) whatever the type of bioreactor used.

- **pH**

The optimum pH varies as a function of the microalgae species. An increase of CO_2 in the gas phase has the effect of decreasing the pH of the reaction medium, as CO_2 produces carbonic acid in aqueous phase. Consequently, when microalgae consume CO_2, there is an increase of pH [84]. Using sodium hydroxide (NaOH) or hydrochloric acid for pH regulation and a temperature of 20°C, Bitaubé Pérez *et al*. [132] obtained an optimal pH of the culture medium between 8 and 9 for a specific growth rate of 7.2 1/h for the microalga *Phaeodactylum tricornutum*. However, the optimum pH varies as a function of the microalga. For example, by using microalgae strain ZY-1 (*Chlorella* sp.), Yue and Chen [112] obtained an optimal pH of 5 for a biomass growth rate of 0.08 g/L/h. They also observed few variations of the optimal biomass growth rate for pH variations between 4 and 6.

- **Stirring**

Stirring is an important parameter, as the latter prevents microalgae from settling. Moreover, stirring improves the light energy and the gas transfer, and standardizes the temperature and the concentration of nutrients [84,133]. Stirring can be performed mechanically or pneumatically. Another study tried to demonstrate (without success) that the superficial gas velocity could harm the microalgae *Dunaliella tertiolecta* and *Chlamydomonas reinhardtii* wild-type [134]. However, according to this study, the superficial velocity (0.076 m/s) alone cannot explain the death of microalgae.

Harvesting and drying methods

Figure 1 presents the different methods of biodiesel production from microalgae. The different methods will be described in the following sections. When the microalgae are harvested, the water content may vary between 0.5 g/L for open systems for photoautotrophic microalgae [135] and 116 g/L for fermenters with heterotrophic microalgae [107]. In order to reduce the production costs, water must be reduced by using microsieve, filtration, centrifugation or flocculation (aluminium chloride, iron chloride ($FeCl_3$), chitosan, organoclays etc.) or by sedimentation [82,91,136,137]. Despite the fact that some authors mention flotation as a harvesting method, this method remains technically and economically limited [91]. Otherwise, the harvesting costs can represent between 20 and 30% of the total production costs.

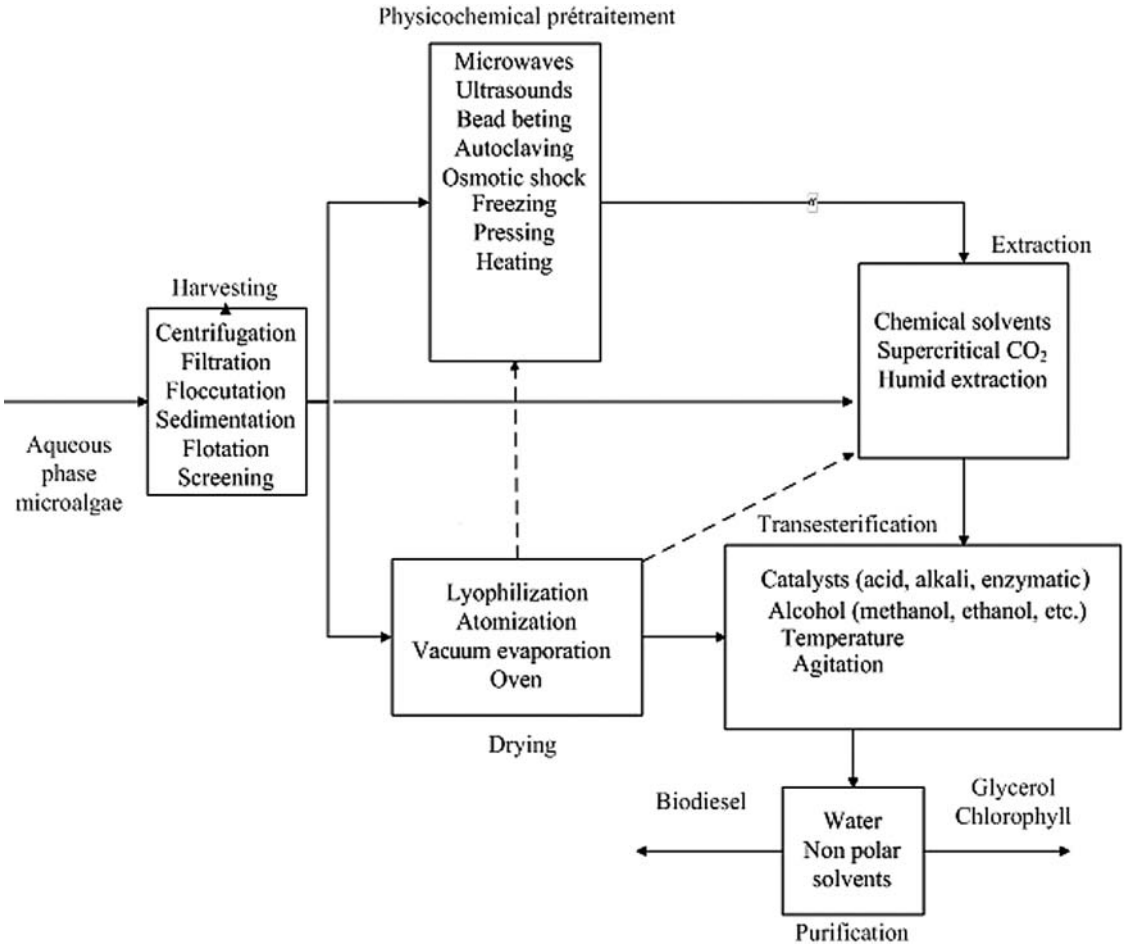

Figure 1. Process diagram of a biodiesel production process with all possible extraction methods.

Some companies stated that they have made major breakthroughs for harvesting and dehydration microalgae. As an example, Algaeventure Inc. states that they can increase microalgae solid content from 20 to 40 wt% of solid matter without forced drying by using a porous belt filtration system [138]. This company affirms that their process could reduce up to 90% of the cost linked to microalgae harvesting [139]. Evodos Inc. developed a harvesting process using a spiral plate technology (SPT), which could concentrate microalgae up to 32 wt% of solids, but few details have been released [140]. Other patents have been filed for the membrane used to concentrate the microalgae biomass [141].

Drying

In order to increase the contact between the lipids and the solvent, microalgae are sometimes dried. Several techniques have been tested including freeze [142], oven [143], spray [142] and vacuum dryings [144,145]. Furthermore, the techniques with low pressures (less than 10 kPa) such as freeze-drying are often seen as too costly at an industrial scale. These techniques allow direct transesterification because water reduces the FAME yield, as water hydrolyzes the FAME [146]. Some patents state that hot air, between 60 and 90°C, could be used to dry microalgae [147]. On the other hand, drying microalgae at these temperatures for a long period of time could induce the degradation of some microalgae components such as the peroxydation of unsaturated lipid double bounds [45]. Consequently, this type of drying is more appropriate for microalgae applications where drying is essential, such as gasification, pyrolysis or thermal oxidation. Drying from solar energy could be energetically interesting, but few studies to our best knowledge have tested this kind of drying, which could only be used during hot, dry, sunny days.

On the other hand, removing water from microalgae by evaporation implies higher costs as the lipid extraction process require 2.5 times more energy than does a process without water removal [148]. According to these authors, the biodiesel production process implying a drying step would mean a negative energy balance (−2.6 MJ/kg biodiesel) with 82 MJ/kg biodiesel linked to the drying of microalgae biomass. As the energy produced by the biodiesel is around 41 MJ/kg biodiesel [149], using drying techniques seems to be impossible for now.

Lipid extraction methods

Methods using solvents

There are two main types of solvents used to extract lipids from microalgae: organic solvents and supercritical fluids.

- Organic solvents

Extraction methods using chloroform have been known to extract and evaluate the total lipids for a long time [150]. The 'Bligh and Dyer' method [151] using a blend of chloroform-methanol-water (1:1:0.5 v/v) is one of the most used methods to determine the total lipids in biological samples [152]. This extraction method is used directly [153] or with mechanical or thermal pretreatments [142,154] in order to extract the total lipids.

The order of blending solvents (chloroform, methanol or water) with microalgae biomass also has an influence on the extracted lipids yield [143,154]. As an example, Lewis *et al.* [153] obtained a FAME yield that increased from 26 to 33% (g lipid/g dry biomass) by inverting water and chloroform in the sequence water-methanol-chloroform. They explained this difference by the fact that a non-polar solvent added first could weaken the link between the lipids and the cell walls of microalgae.

Nevertheless, using chloroform is risky because this solvent could have carcinogenic effects on the human being [155]. With fewer toxic effects, hexane is sometimes preferred to chloroform [156]. However, because of the non-polar nature of hexane, the lipid yields are lower, since microalgae often have a high content in more polar lipids such as glycolipids and phospholipids [42]. Moreover, using toxic organic solvents to extract lipids (chloroform, hexane, etc.) prevents or makes more difficult the valorization of microalgae for food purposes. For microalgae lipid extraction, some studies have attempted to replace the chloroform-methanol extraction (2:1, v/v) by different blends such as hexane-isopropanol (3:2, v/v), dichloroethane-methanol (1:1, v/v), dichloroethane-ethanol (1:1, v/v) and acetone-dichloromethane (1:1, v/v), but the lipid yield obtained was significantly lower [157].

Samorì *et al.* [158] used polar solvents such as ethanol, octanol or 1,8-diazabicyclo-[5,4,0]-undec-7-ene (DBU). In this study, despite the fact that the lipid yield obtained was almost twice as high (16 g lipid/g dry biomass), the biodiesel yield was 5 times lower (0.65% g biodiesel/dry biomass) than for hexane extraction. A patent describes a process for proteins and sugar extraction from microalgae in aqueous phase using organic solvents (acetone, methanol, ethanol, isopropanol, methyl ethyl ketone, dimethylether or propionic aldehyde) which make the lipid extraction easier [159]. This process includes: (1) centrifugation of the aqueous microalgae, (2) blending microalgae with an organic solvent, (3) centrifugation of the organic solvent/humid microalgae blend, (4) heating the organic solvent/humid microalgae blend, (5) organic solvent separation, (6) evaporation of the organic solvent and (7) separation of the lipids from the aqueous phase. On the other hand, the heating phase includes high temperatures (50 to 150°C) and pressures (0.5 to 3 MPa),

which considerably increase the extraction costs. However, in this patent, no yields were specified.

- Supercritical carbon dioxide

As some organic solvents imply risks to human health, CO_2 supercritical extraction was considered to recover lipids from microalgae such as polyunsaturated fatty acids for food and pharmaceutical purposes [160–162] and for biodiesel [156,163,164]. Otherwise, a patent describing a process using supercritical CO_2 to extract lipids from microalgae to produce biodiesel was filed by the China Green Oil Co Ltd (Hong Kong, China) [165]. Several companies still hesitate to use supercritical extraction at an industrial scale based on the high investment costs [166] and the high pressures required [156,160,167]. Still, some studies indicate that this technology has a high potential to extract lipids from microalgae, even when the latter are still wet [156]. Using supercritical CO_2 to extract lipids from the microalga *Nannochloropsis* sp., Andrich *et al.* [160] obtained a lipid yield of 26% (g lipid/g dry biomass) at a temperature of 40°C and a pressure of 70 MPa.

Physicochemical methods

- Organic solvents

As microalgae cell wall is a barrier to lipid extraction, physicochemical methods are used to increase the lipid content and the biodiesel obtained. Tables 1 and 2 present the lipid and the biodiesel yields, respectively, for different microalgae lipid extraction studies. Among the pre-treatments used, the main methods are microwave, ultrasound, bead-beating, freeze-drying, homogenization, French press, crushing, autoclave and osmotic shock. Other researchers own a patent for a cavitation system implying pressures from 0.3 to 30 MPa allowing microalgae cell walls to break up even if no extraction yield was specified in the patent filed [168,169].

Some pre-treatments such as microwave and bead-beating seem to be effective to increase the lipids extracted from microalgae when the latter is in aqueous phase [142,154,157]. As an example, after a pre-treatment by bead beating of the microalga *Botryococcus braunii* UTEX 572 in aqueous phase, Lee *et al.* [157] obtained an increase in the lipids extracted from 14 (without pre-treatment) to 29% (g lipid/g dry biomass) (bead beating). At last, the company Solazyme Inc. (South San Francisco, USA) owns a patent for a process based on lysis of microalgae cell walls and the use of an organic solvent for the lipid separation [170]. For example, the patent mentioned cell lysis with heating at temperatures ranging from 50 to 130°C, the addition of a strong alkali such as potassium hydroxide (KOH) or NaOH, a strong acid such as sulfuric acid (H_2SO_4), a mechanical lysis (1500 bar with a shrinkage), an osmotic shock, and the use of ultrasound and a pressure oscillation. Among others, they used KOH and the H_2SO_4 concentrations that varied from 0 to 160 mN with temperatures ranging from 25 to 130°C. Furthermore, Dillon *et al.* [170] also used several surfactants such as NINOL© 201 (Oleamide DEA from Stepan Company) to extract lipids of the heterotrophic microalgae *Phlorella protothecoides* at a temperature of 55°C for 5 h and obtained lipid yields of up to 92% (based on the theoretical mass of lipids).

Lyophilization is mainly used in studies focusing on solvent extraction in order to obtain a better contact between a non-polar solvent and the lipids. Other studies tried to show that lyophilization could break the cell walls of microalgae and increase the lipid yield. For example, Lewis *et al.* [153] obtained an increase of the lipid yield extracted from the microalga *Botryococcus braunii* UTEX 572 from 14 to 19% (g lipid/g dry biomass) after lyophilization.

Some techniques used with solvent extraction allowed an increase in the amount of lipids extracted. As an example, using ultrasounds on a blend of chloroform-methanol (3:1, v/v), the lipid yield obtained from the microalga *Scenedesmus* sp. was increased from 2 to 6% (g lipid/g dry biomass) compared with no treatment [171]. However, another study used ultrasounds and observed no significant effect on the lipid yield with a blend of chloroform-methanol-water (1:2:0.8, v/v) for a heterotrophic microalga (strains ACEM 6063 and ACEM A) [153].

Despite the fact there are few studies using electric energy to extract lipids from microalgae, some patents affirm that this technique could be used to reduce microalgae extraction cost. As an example, Diversified Technologies, Inc. (Bedford, MA, USA) stated that their technology of pulsed electric field (PEF) could reduce the extraction costs from 0.46 US$/L oil for a conventional drying to 0.03 US$/L oil [172].

- Solvent-free and ionic liquid extraction

In order to eliminate some steps in the lipid recovery, a separation of lipids in aqueous phase could be performed based on the difference of specific gravities and solubilities of both lipids and water. The main advantage of the lipid extraction in aqueous phase is to allow the valorization of residual biomass in agriculture. To our best knowledge of the literature, no studies have demonstrated the feasibility of this type of extraction for microalgae lipids. On the other hand, a patent from Solazyme Inc. (South San Francisco, USA) affirms that it is possible to recover lipids directly in aqueous phase following cell wall lysis due to heating (higher than ambient temperature) and an acid pre-treatment, and then a recovery of lysate with a decrease of the temperature (20°C, 24 h) and a

Table 1. Physico-chemical pre-treatments effect on lipid yield for several studies.

Microalgae species	Initial state	Pre-treatment	Parameters	Solvents	Max lipid yield (% g lipid/ g dry biomass)	FAME yield (% g FAME/ g dry biomass)	Ref.
Botryococcus sp.	Lyophilized	None	None	Water-chloroform-methanol (2:1:1 v/v/v)	7.7	1.2	[142]
		Autoclave	125 °C – 1.5 MPa		12		
		Bead beating	0.1 mm – 2800 rpm		28		
		Microwave	100°C – 2450MHz – 5 min		29		
		Ultrasound	10 kHzn– 5 min		8.5		
		Osmotic shock	Sodium chloride (NaCl) 10% (m/v) – vortex (1 min) – 48 h		11		
Chlorella vulgaris		None	None		5.1	2.9	
		Autoclave	125 °C – 1.5 MPa		9.6		
		Bead beating	0.1 mm – 2800 rpm		7.9		
		Microwave	100°C – 2450 MHz – 5min		9.8		
		Ultrasound	10 kHz – 5 min		5.9		
		Osmotic shock	NaCl 10% (m/v) – vortex (1 min) – 48 h		8.0		
Scenedesmus sp.		None	None		1.8	1.9	
		Autoclave	125°C – 1.5 MPa		5.5		
		Bead beating	0.1 mm – 2800 rpm		8.3		
		Microwave	100°C – 2450 MHz – 5min		10		
		Ultrasound	10 kHz – 5 min		7.3		
		Osmotic shock	NaCl 10% (m/v) – vortex (1 min) – 48 h		6.8		
Botryococcus braunii UTEX 572	Refrigerated (4°C)	None	None	Chloroform-methanol (2:1 v/v)	14	Unspecified	[157]
		Ultrasound	1 min		16		
		Homogenization	1 min		15		
		French press	170 atm		23		
		Lyophilization	Not specified		19		
		Bead beating	1 min – 1mm		29		
				Hexane-isopropanol (3:2 v/v)	20		
				Dichloroethane: methanol (1:1 v/v)	19		
				Dichloroethane: ethanol (1:1 v/v)	19		
				Acetone: dichloromethane (1:1 v/v)	18		
Scenedesmus sp.	Oven dried at 45°C (4–5 days)	None	None	Soxhlet with n-hexane	0.70	Unspecified	[171]
		Ultrasound	100 W – 30 min		0.80		
		None	None	Chloroform-methanol-water	2.0		
		Ultrasound (chloroform-methanol)	100 W – 30 min	(3:1:1.2 v/v/v)	6.0		
Phaeodactylum tricornutum	Frozen	Extraction time	1st extraction: 600 min	Ethanol-water-hexane (1.6: 1.9:1 v/v/v)	5.0	Unspecified	[258]
			1st extraction: 600 min – 2nd extraction: 90 min		6.2		
			1st extraction: 600 min – 2nd extraction: 90 min – 3rd extraction: 80 min	Hexane (4 × 32 mL)	6.3		
Botryococcus braunii	Refrigerated	None	None	Hexane and methanol: chloroform (1:1 v/v)	37	Unspecified	[259]
		Temperature (10 min)	75°C	Hexane	0.15		
			80°C		1.2		
			85°C		32		
			90°C		36		
			120°C		36		
Botryococcus braunii	Lyophilized	None	None	Hexane chloroform-methanol (2:1 v/v)	8.0	2.7	[158]
				3 ml 1,8-diazabicyclo-[5,4,0]-undec-7-ene (DBU)	15	0.54	
				DBU-ethanol (1:0.39 v/v)	16	0.65	
				2 mL DBU/octanol (1:1 v/v)	13	0.59	
	Fresh	Centrifugation	300 rpm – 2 h	3 mL octanol	1.2	Unspecified	
			300 rpm – 24 h		4.3		
			3000 rpm – 4 h		4.3		
			300 rpm – 2 h	3 mL n-hexane	0.64		

(*continued*)

Table 1. (*Continued*)

Microalgae species	Initial state	Pre-treatment	Parameters	Solvents	Max lipid yield (% g lipid/ g dry biomass)	FAME yield (% g FAME/ g dry biomass)	Ref.
			300 rpm – 24 h		5.6		
			3000 rpm – 4 h		5.1		
			300 rpm – 2 h	2 mL DBU/octanol (1:1 v/v)	2.4		
			300 rpm – 24 h		8.2		
			3000 rpm – 4 h		6.9		
Nannochloropsis sp.	Lyophilized	None	40°C – 70 MPa	Supercritical CO_2	26	Unspecified	[160]
			40°C – 55 MPa		25		
			40°C – 40 MPa		24		
			55°C – 70 MPa		26		
			55°C – 70 MPa		25		
			55°C – 70 MPa		25		
			Soxhlet	Hexane	24		
Chlorococcum sp.	Oven dried (85°C) for 16 h and ground	None	Agitation	Hexane-isopropanol (3:2, v/v)	4.8	Unspecified	[156]
			Agitation	Hexane	1.5		
			Soxhlet (5.5 h)		3.2		
			Soxhlet (7.5 h)		5.7		
			80°C – 30 MPa	Supercritical CO_2	4.8	1.5	
			60°C – 30 MPa		5.8	1.4	
			60°C – 40 MPa		5.9	Unspecified	
			60°C – 50 MPa		6.0		
	Fresh		60°C – 30 MPa		7.1		

FAME: fatty acid methyl esters.

centrifugation (4400 rpm) [170]. Moreover, they also noticed that the size of the emulsion increased from 0 to 30% when the temperature increased from 25 to 130°C. Even if they stated that a small amount of organic solvent (as small as 5% (v/v)) could be used, the patent does not give the lipid extraction yield. In the same sort of idea, other researchers filed to patent a process using acid hydrolysis (using H_2SO_4) of microalgae (100°C, 200 kPa) in aqueous phase including mechanical and chemical techniques in order to hydrolyze the lipids into free fatty acids (FFAs) and they esterified the FFAs in aqueous phase with methanol and H_2SO_4 [173]. The problem with this kind of technique is that the excess water could give really low lipid (triglycerides, FFAs and others) conversions because water favors the reverse reaction (hydrolysis) [174–176].

The company Alfa Laval Lund AB (Lund, Sweden) filed for a patent for a centrifugation separator with disks, which is operated using a centrifuge force between 4500 and 5000 *g* that allows separating oil and microalgae in aqueous phase [177].

Following the pre-treatments mentioned previously, ionic liquids could be used to recover lipids [178,179]. As an example, a patent described a separation process using ionic solvents such as hydronium (H_3O^+), ammonium (NH_4^+), alkyl amonmonium, imidazolium and heterocycles (cycle of carbon with another element in the cycle) [178]. The ionic solvent is then recovered with an antisolvent such as inorganic salts (like calcium chloride ($CaCl_2$)). However, the process allows recovering at least 80% of the ionic liquid, which might predict problems with the recovery of the ionic liquid. Moreover, the antisolvent has to be immiscible with the lipids.

Other patents reported the use of resins for the recovery of microalgae lipids. For example, Poenie *et al.* [180] filed a patent concerning a technique that extracts lipid from the microalga *Chlorella* sp. using resins (CHLOR 13A, NANN 13A, CHLOR 14B, CHLOR JB21, NANN JB21). The types of resins in this patent are made from polymers such as polystyrene, polyethylene, etc. After the saturation of the resin exchange column, water is evaporated using, for example, air streams. Then, an eluent, an alcohol such as methanol containing an acid (H_2SO_4) or an alkali (NaOH, KOH, sodium ethoxide (C_2H_5ONa), potassium methoxide (KCH_3O)), is fed to the column to recover microalgae lipids.

Other researchers have developed processes for breaking microalgae cell walls by mechanical techniques. For example, Thomas *et al.* [181] own a patent for a vertical ram-type extractor which involves a mass of 5.6 kg dropped with a final velocity of 8 m/s in order to break microalgae cell walls. Echevaría Parres *et al.* [182] filed a patent in which microalgae are dragged into a turbine and then crushed between a rotor and a

Table 2. Physico-chemical pre-treatments effect on the FAME yield for several studies.

Microalgae	Initial state	Pre-treatment	Parameters	Solvent	FAME yield (% g FAME/g dry)	Reference
Nannochloropsis	Fresh	Oven dried and ground	Extraction time: 20h	Chloroform-methanol-water (1:2:0.8 v/v/v)	21.5 ± 4.3	[143]
		Oven dried and ground	Extraction time: 2h		21.3 ± 2.3	
		Oven dried	Extraction time: 2h		14.8 ± 6.8	
		Oven dried and frozen with liquid nitrogen	Extraction time: 2h		20.1 ± 0.5	
		Oven dried and frozen with dry ice	Extraction time: 2h		18.7 ± 3.2	
		Oven dried and ground	Extraction time: 2h	Water-methanol-chloroform-(0.8:2:1 v/v/v)	18.5 ± 0.5	
		No	No		16.4 ± 2.1	
*Chlorella prototheco*ïdes		No	No	Water-methanol-chloroform–(0.8:2:1 v/v/v)	18.5 ± 0.5	
	Fresh	Oven dried		Chloroform-methanol-water (1:2:0.8 v/v/v)	21 ± 1.2	
Strain ACEM 6063	Lyophilized	None	None	Water-methanol-chloroform- (0.8:2:1 v/v/v)	25.9	[153]
		Ultrasound in the solvent	100 W - 2×1 min		26.3	
		None	None	Chloroform-methanol-water (1:2:0.8 v/v/v)	35	
		Ultrasound in the solvent	100 W - 2×1 min		34.3	
		None	None	Chloroform-methanol-water (1:4:0.8 v/v/v)	33.2	
		Ultrasound in the solvent	100 W - 2×1 min	Chloroform-methanol-water (1:4:0.8 v/v/v)	34.6	
Crypthecodinium cohnii	Lyophilized	Ground	No details	Bligh and Dyer	19.9	[203]
		Ground and supercritical CO_2	20 MPa - 313 K	n-Hexane	6.9	
			25 MPa - 313 K		6.3	
			30 MPa - 313 K		5.7	
			20 MPa - 323 K		5.6	
			25 MPa - 323 K		7.1	
			30 MPa - 323 K		8.6	
Scenedesmus obliquus	Lyophilized Pulverization with mortar	None	None	Dichloromethane-methanol (2:1)	17	[260]
		Quartz sand – 0.2 g (10–30 mm) ultrasound – 35 kHz, 80 W, −4°C, 90 min			33.5	
Chlorella sp.	Lyophilized	None	No	6.5 mg EMIM – methanol (1.2:1 w/w)	38	[261]
Duniella sp.		1-ethyl-3-methyl imidazolium, methyl sulfate (EMIM) and a co-solvent	65°C – 18 h – stirring – molar excess of co-solvent of 10 compared to EMIM		8.6	
				6.5 mg EMIM – dimethyl sulfoxide	6.0	
				6.5 mg EMIM – acetic acid	5.6	
				6.5 mg EMIM-acetone	9.2	
				6.5 mg EMIM – chloroform	8.4	
				6.5 mg EMIM – isopropyl alcohol	8.5	
Chlorella sp.		Enzymatic hydrolysis solution 20 g dry/L	Immobilized polyacrylonitrile cellulase	Hexane (28°C, 1–6 h)	53	[184]
		None	None		52	

FAME: fatty acid methyl esters.

stator. Zeiler *et al.* [183] filed a patent for a laser ultra short pulse (USP) producing ultraviolet of short wavelength (less than 400 nm) able to break microalgae cell walls. On the other hand, most of these patents practically did not report any lipid yield.

Biochemical methods

In order to break the link between lipids and microalgae, biological pre-treatments can be used. As an example, using a hydrolysis pre-treatment of microalgae cell wall with cellulase, Fu *et al.* [184] obtained an increase of 70% of microalgae cell wall hydrolysis (measured by monosaccharide analysis) despite the fact that the lipid yield increased only from 52 to 53% (g lipid/g dry biomass). The second problem is that hydrolysis is performed in water phase and lipids must be extracted. In order to break microalgae cell wall, the company Solazyme Inc. (South San Francisco, USA) filed for a patent which mentions using enzymes such as hemicellulase, pectinase, cellulase and driselase, and also infections of microalgae by viruses (e.g. *Paramecium bursaria* chlorella virus) and autolysis (expression of a lysis gene) in order to degrade microalgae cell walls [170]. Another patent was filed using autolysis [185] or enzymes [186] to break the microorganism cell walls of microalgae and recover the lipids with, among other things, an organic solvent.

Lipid purification

When the lipids are extracted, they contain various lipids, which result in a lower biodiesel yield during transesterification rather than the triglycerides whose yield can reach near 100% [187]. As an example, Nagle and Lemke [188] have determined that biodiesel yield for phospholipids and glycolipids could not reach more than 65 and 56% (g FAME/g lipid), respectively. These are important data because microalgae can contain up to 93 wt% of these two components [42]. Furthermore, a study shows that microalgae could contain also added-value compounds such as chlorophyll and carotenoids [58].

According to our best knowledge of the literature, few lab-scale studies have separated the different classes of lipids present in microalgae in order to recover the triglycerides. However, Lin *et al.* [189] filed a patent for a process that allows the selective isolation of some components of microalgae. The process in batch mode could be described as follows: After an extraction and a recovery of lipids with an organic solvent, the organic phase is blended with a mesoporous solid (nanoparticles of mesoporous carbon, activated carbon, nanoparticles of mesoporous silicon (MSN) or silicium gel) and filtration allows the separation of the organic phase. For example, using MSN, the process allows the recovery of 90 wt% of the fatty acid oleate (C18:1) from lipids of the microalga *Neocloris* sp.

Direct transesterification

Direct transesterification is a process in which the lipids are simultaneously extracted and transesterified from microalgae cell walls. Direct transesterification is often used for heterotrophic microalgae [65,146,153,190]. Some authors obtained higher biodiesel yields using direct transesterification than using extraction followed by transesterification [146,187]. This process could have the advantage of reducing the amount of solvent used to transesterify the lipids that could never be extracted. However, most of these studies used an organic co-solvent (hexane, chloroform, etc.) during the direct transesterification in order to increase the biodiesel yield [146,187]. For example, using a homogeneous acid catalyst (H_2SO_4) with methanol, Johnson and Wen [146] transformed lipids of the microalga *Schizochytrium limacinum* into biodiesel with and without a co-solvent (hexane). After 40 min of reaction at a temperature of 90°C, they respectively obtained biodiesel yields of 66 and 7% (g biodiesel/g dry biomass). Then, some studies compared hexane and chloroform as a co-solvent and found out that these two co-solvents had similar biodiesel yields [65,146].

In order to increase the biodiesel yield, some studies tested direct transesterification with physicochemical methods such as microwave and ultrasound. For example, replacing traditional heating with microwave heating, Koberg *et al.* [187] performed a transesterification with strontium oxide (SrO) as a catalyst and obtained an increase of the biodiesel yield from 7 to 37% (g biodiesel/g dry biomass). Despite the advantage of direct transesterification, the energetic costs from the mandatory drying of microalgae make this technique difficult to use at an industrial scale [148].

Transesterification

Reaction

Vegetable oils or microalgae lipids cannot be used directly in diesel engines as their viscosity is relatively high (28–40 mm^2/s) which causes formation of coke deposits in the engine and the injectors [191]. Furthermore, using vegetable crude oils can have negative effects on the biofuel combustion. For example, Altin *et al.* [192], tested the behavior of colza oil at a revolution velocity of 1300 rpm. Despite the fact that they obtained a similar power (97%) compared to petrodiesel, they noticed an increase of (1) the fuel consumption from 246 to 288 g/kWh, (2) the CO concentration from 2225 to 4000 ppmv and (3) the fume density (particular matter) from 29 to 49%. Transesterification of lipids allows reducing oil viscosity to a level similar to that of petrodiesel [191,193]. Despite the fact that most studies consider only a reaction between an oil (triglycerides) and an alcohol, biodiesel can be

Table 3. Studies on biodiesel production from microalgae and the yield obtained.

Microalgae species	Catalyst	Reaction time (min)	Temperature (°C)	Quantitative analysis	Biodiesel yield (% g FAME/ g lipid)	Biodiesel yield (% g FAME/ g dry biomass)	Ref.
Chlorococcum sp.	H_2SO_4/ KCH_3O	120/120	50/55	Gas chromatography (GC)	44	1.5	[156]
Nochlorropsis	Strontium oxide (SrO)	2	60	^{1}H NMR Spectroscopy	99.9	37.1	[187]
Schizochytrium limacinum	H_2SO_4	40	90	GC	73	42	[146]
Chlorella mulleri	HCl NaOH	6	70	Thin layer chromatography (Iatroscan)	68 1.3	Unspecified	[188]
*Chlorella prototheco*ï*des*	H_2SO_4	Unspecified	Unspecified	Elemental analyzer CE-440	68	37.4	[149]
Botrycoccus sp.	KOH/HCl	10/10	75	GC	4.2	1.2	[142]
Chlorella vulgaris					29.6	2.9	
Scenedesmus sp.					19.8	1.9	
Microheterotrophic (strains ACEM 6063)	HCl	60	90	GC	Unspecified	41.3	[153]
Botryococcus braunii	NaOH/bore trifluoride (BF_3)	10/2	Reflux	GC	21.6	2.7	[158]
Nannochloropsis	HCl	Unspecified	Unspecified	GC and ^{1}H NMR spectroscopy	Unspecified	8.4	[143]
Nannochloropsis	H_2SO_4					6.6	
Chlorella prototheocoides	HCl					36.6	
Chlorella prototheocoides	Acetyl chloride					56	
Crypthecodinium cohnii	Acetyl chloride	60	80	GC	Unspecified	19.9	[203]
Scenedesmus obliquus	H_2SO_4	240	70	GC	Unspecified	33.5	[260]
Chlorella sp.	BF_3	15	100	GC	Unspecified	53	[184]
Botryococcus braunii	HCl Acetyl chloride	60 60	90 100	GC	Unspecified	5.5 12.1	[262]
Synechocystis sp.	HCl Acetyl chloride	60 60	90 100			7.3 6.0	
Nochlorropsis occulata	Calcium oxide (CaO)/ aluminium oxide (Al_2O_3) CaO/Al_2O_3	240	50	GC	23 16	Unspecified	[144]
Chlorella pyrenoidosa	H_2SO_4	120	90	GC	93	52	[65]
*Chlorella prototheco*ï*des*	*Candida* sp.	720	Unspecified	GC	98	Unspecified	[145]
*Chlorella prototheco*ï*des*	Lipase (triacylglycerol acylhydrolase)	720	38	GC	98	Unspecified	[194]

FAME: fatty acid methyl esters.

produced from several classes of lipids including glycolipids, phospholipids, FFAs, etc.

Table 3 presents FAME yields and experimental conditions of transesterification of microalgae lipids into biodiesel. According to Table 3, the biodiesel yields (FAME) and the transesterification methods vary. The microalga *Chlorella pyrenoidosa* cultivated in heterotrophic conditions resulted in the highest FAME yield (52% (g FAME/g dry biomass)), which represents a FAME yield of 93% (g FAME/g lipid). Some studies mention biodiesel yields (compared to lipid) of 98% (g FAME/g lipid) using diverse heterotrophic metabolism microalgae [145,194], which can be compared to the yields obtained with vegetable oil transesterification [195].

Alcohol

The most used alcohol for transesterification is methanol because of its lower cost [17], but other alcohols such as ethanol, propanol and butanol can produce similar biodiesel yields [196,197]. Some studies have tested the effect of the methanol-triglyceride molar ratio (between 56:1 and 524:1) for different microalgae on the density of the biodiesel produced [149,198]. For example, by transforming the triglycerides of the microalga *Chlorella* sp. into FAME for 8 h at a reaction temperature of 25°C and increasing the methanol-triglyceride molar ratio from 105:1 to 524:1, Ehimen *et al.* [198] observed a decrease of the density of the biodiesel produced from 889 to 886 kg/m^3. On the other hand, the authors did not specify whether this increase was significant. Methanol-triglyceride molar ratio also has an impact on the biodiesel yield because a molar excess of the alcohol (methanol) reduces the reverse reaction and shifts the equilibrium of FAME production [199]. Performing an acid transesterification (H_2SO_4) of triglycerides of the microalga *Chlorella prototheco*ï*des* at 50°C for 4h, Miao and Wu [149] varied the methanol-triglycerides molar ratio from 25:1 to 84:1 and obtained an optimum methanol-triglycerides molar ratio at 45:1 for a FAME yield of 69% (g FAME/g triglycerides). The molar ratios of methanol-triglyceride used for microalgae lipid transesterification are generally higher than in the case of vegetable oil which is

usually around 6:1 [200] (the stoichiometric molar ratio is 3:1 [201]).

Reaction time

Generally, increasing the reaction time of transesterification has a positive influence on the FAME yield. As an example, using a homogeneous enzymatic transesterification of lipids of the microalga *Chlorella pyrenidosa* at a temperature of 90°C, Li *et al.* [65] obtained a FAME yield ranging from 52 to 94% (g FAME/g lipid) following an increase of the reaction time from 0.5 to 2 h. Reaction time has an influence on the density of the biodiesel. As an example, using a homogeneous acid catalyst (H_2SO_4), a stirring rate of 160 rpm, and a methanol-triglyceride molar ratio of 56:1, Miao and Wu [149] observed a decrease in the density of the biodiesel produced from 0.919 to 0.864 kg/m^3 for respective reaction times of 0 and 4 h.

Homogeneous catalysis

Despite the fact that homogeneous alkali catalysis using NaOH is widely used for vegetable oil transesterification (because it is 4000 times faster than homogeneous acid catalysis), homogeneous alkali transesterification is rarely used for microalgae lipid transesterification. This catalyst gives a biodiesel yield 50 times lower than that of a homogeneous acid catalyst (like HCl) [188]. Sometimes, homogeneous alkali catalysts such as KCH_3O or NaOH are coupled to acid catalysts [142,156,202].

As shown in Table 3, homogeneous acid catalysts are mostly used in the transesterification of microalgae lipids. The main catalysts are HCl [143,149], H_2SO_4 [143,146,202], acetyl chloride (CH_3COCl) [154,203] and bore trifluoride (BF_3) [158,184]. Some studies have shown that CH_3COCl in methanol (5% v/v) is the catalyst which resulted in the highest FAME yield of 56% (g FAME/g dry biomass) for the heterotrophic microalgae *Chlorella protothecoides* compared to a yield of 37% (g FAME/g dry biomass) for HCl [143]. Catalyst concentration also has an effect on the transesterification yield of microalgae lipids. As an example, using four concentrations of H_2SO_4 in methanol (0.56, 1.13, 1.35 and 2.25 mol/L), a temperature of 30°C and a reaction time of 5 h, Miao and Wu [149] mentioned that a catalyst concentration in methanol of 2.25 mol/L produces biodiesel with the lowest density (863 kg/m^3) but with the lowest FAME yield at 38% (g FAME/g dry biomass).

Finally, other techniques that imply the use of catalysts with supercritical solvents such as methanol were tested, but the costs of this technology are relatively high [167]. To our best knowledge of the literature, this technique has not been tested on microalgae lipids.

Heterogeneous catalysis

In order to reduce the amount of chemicals used, some studies consider using heterogeneous catalysts (solids). Moreover, some of these catalysts have less sensitivity to water [204] and can potentially be reused [205]. As for homogeneous catalysts, there are heterogeneous alkali and acid catalysts.

- **Alkali**

Microalgae lipids have been transformed into biodiesel by using heterogeneous alkali catalysts such as calcium oxide (CaO) [144], strontium oxide (SrO) [187] and mixed magnesium-zircone oxides [206]. Some of these catalysts led to high biodiesel yields. As an example, performing a direct transesterification with a heterogeneous catalyst of SrO, Koberg *et al.* [187] achieved a conversion of 100% of *Nannochloropsis* sp. microalgae triglycerides.

Table 4 presents the results of studies for vegetable oil using methanol (as an alcohol) and heterogeneous catalysts.

- **Acid**

Few studies have used acid heterogeneous catalysts to transform microalgae lipid into biodiesel. As an example, Carrero *et al.* [207] compared two types of zeolites (β and ZSM-5) to transform microalgae (*Nannochloropsis gaditana*) lipids into biodiesel. However, at a temperature of 115°C (without specified reaction time, methanol-lipid ratio or catalyst-to-oil ratio) under reflux, they obtained a FAME content of 25% (g FAME/g lipid) compared to 90% (g FAME/g lipid) with H_2SO_4 under the same reaction conditions.

As shown in Table 4, the most utilized heterogeneous acid catalysts used in transesterification studies are zeolites, heteropoly acids, functionalized (sulfate and tungsten) silica or zirconia, ion-exchanging resins, organo-sulfated acids (sulfonic, sulfonates and sulfonamides) functionalized silica [208]. Even if, to our best knowledge of the literature, most of these catalysts have not been tested with microalgae lipids, they show a great potential for microalgae biodiesel. However, heteropoly acids could be interesting as catalysts to transform microalgae lipids (high levels of FFAs) into biodiesel, as these catalysts showed almost no sensitivity to high FFA contents (up to 10 wt%), but are sensitive to water contents (water content higher than 0.1 wt%) [209].

On the other hand, heterogeneous acid catalysts have the disadvantage of ending up in lower lipid conversions or longer reaction times. As an example, by using a catalyst of sulfated zirconium oxide (0.86 wt%) and a molar ratio of 2-ethyl hexanol-dodecanoic acid of 25:1 at a temperature of 80°C, Omota *et al.* [210]

Table 4. Heterogeneous catalysts used for the transesterification of several oils.

Oil	Catalyst	Catalyst/oil (% w/w)	Temperature (°C)	Time (h)	Molar ratio (alcohol/oil)	Biodiesel yield (% g FAME/g oil)	Reference
Cotton seed	Magnesium oxide (MgO)/$MgAl_2O_4$/ γ-Alumina (Al_2O_3)	1	180	15	6:1	36	[263]
	Mg–Al–CO_3 hydrotalcite					98	
	Carbon-based (acid)	0.3	220	3	21	89	[264]
Jatropha curcas	KSF clay	5	160	6	12:1	68	[265]
Palm	Potassium fluoride (KF)/hydrotalcite	3	65	5	12:1	92	[266]
	KF/Ca-Al hydrotalcite	5				98	[267]
	K-Mg-Al hydrotalcite	7	100	6	30:1	87	[268]
	CaO-Zinc oxide (ZnO)	10	60	1	20:1	94	[269]
Rapeseed	Mg–Al-hydrotalcites	1.5	65	4	6:1	91	[270]
Soybean	KF/CaO	3	60–65	1	12:1	90	[271]
	Al-Mg-hydrotalcite	5	180	1	12:1	93	[272]
	MgO					91	[273]
	NaX zeolite/KOH	3	65	8	10:1	86	[274]
	ZnO /strontium nitrate ($Sr(NO_3)_2$)	5	65	1	12:1	87	[275]
	Potassium iodine (KI)/Al_2O_3	2	Reflux	6	15:1	87	[276]
	KI/Zirconium oxide (ZrO_2)					78	
	KI/ZnO					73	
	KI/zeolite NaX					13	
	KI/zeolote KL					18	
	Al-Mg-hydrotalcite	7,5	Reflux	9	15:1	67	[277]
	Potassium nitrate (KNO_3)/Al_2O_3	6,5	Reflux	7	15:1	87	[278]
	CaO/chitosan	13,8	60	3	13.4:1	97	[279]
	CaO/ eggshell calcinated	1	70	5	6.9:1	97	[280]
	MgO	5	70	7	55:1	64	[281]
	Mg-hydrotalcite oxide					14	
	ZnMg-hydrotalcite oxide					7	
	ZnO					0	
	Al_2O_3					0	
	Ca/mesoporous silica	5	60	8	16:1	95	[282]
	KI/mesoporous silica	5	70	8	16:1	90	[283]
	CaO/lanthanum oxide (La_2O_3)	8	65	1	10:1	75	[284]
	KF/Al_2O_3	2	72.7	3	12:1	99	[285]
	Mg –Al- hydrotalcite	5	230	1	13:1	90	[286]
	CaO	0.8	Reflux	2	11.7:1	90	[287]
	CaO/La_2O_3	8	65	3	7.8:1	97	[288]
	CaO/Cerium oxide (CeO_2)	5	58	1	20:1	91	[289]
	La_2O_3/zirconium oxide (ZrO_2)					8.7	
	CaO/La_2O_3					94	
	MgO/$MgAl_2O_4$	3	65	10	15:1	57	[290]
	La/Zeolite β	1.1	60	4	14:5	49	[291]
	CuVOP	3	60	5	6:1	65	[292]
	Calcined sodium silicate	3	60	1	7.5:1	100	[293]
	Phosphazenium hydroxide/SiO_2	4.5	75	12	60:1	90	[294]
Sunflower	Zeolite NaX	10	60	7	6:1	95	[295]
	Al-Si/Potassium carbonate (K_2CO_3)	2	120	1	15:1	97	[296]
	CaO/SBA15	1	60	5	12:1	95	[297]
	Magnesium (Mg)/Calcium (Ca)	2.5	60	3	12:1	92	[298]
	Mg/Aluminium (Al)					66	
Vegetable	KF/CaO	3	65	1	12:1	99.6	[299]
	Aluminium hydrogen sulphate	0.5	220	0.83	16:1	81	[300]
	Carbon-based (acide)	0.2	220	4.5	17	81–95 (FFA)	[301]
Vegetable (ethanol as an alcohol)	None	2	220	5	6.5:1	37	[302]
	MCM-41 (mesoporus silica)					46	
	KIT-41					36	
	SBA-15					39	
	MgO/MCM-41					68	
	MgO/KIT-41					82	
	K/MgO (A)					96	
Zanthoxylum bungeanum	CaO	2.5	95	2.5	11.7:1	96	[303]

FAME: fatty acid methyl esters.

achieved a conversion of dodecanoic acid of 100% for a 70-h reaction time.

Enzymatic catalysis

The main advantage of enzymatic catalysis is the relatively high lipid conversion. A study has shown that a conversion of 98% [194] can be reached by using a lipase (triacylglycerol acylhydrolase, EC 3.1.1.3, 1,200 U, 30 wt%) immobilized on a macroporous resin, a methanol-oil molar ratio of 3:1, a temperature of 38°C, a pH of 7 and a reaction time of 12 h. Despite the fact that some studies used enzymatic catalysis to transesterify heterotrophic microalgae lipids, this type of catalysis has not been tested up to now at industrial scale, perhaps because of the enzymes' high costs [17,211].

Temperature and stirring

An increase of the temperature can have a positive effect on the biodiesel yield. As an example, using an enzymatic transesterification (homogeneous) of the microalga *Chlorella pyrenidosa* lipids, for a reaction time of 2 h, Li *et al.* [65] reached biodiesel yields of 43 to 95% (g FAME/g lipid) at temperatures ranging from 20 to 110°C, respectively. However, for acid homogeneous catalysis, the temperature has less significant effects. As an example, testing temperatures of 30, 50 and 90°C for the acid catalysis of the heterotrophic microalga *Chlorella protothecoïdes*, Miao and Wu [149] obtained biodiesel yields of 56, 58 and 38% (g FAME/g lipid), respectively.

The stirring rate has a positive effect on the quality of the biodiesel produced. As an example, for the homogeneous acid (H_2SO_4) transesterification of microalga *Chlorella* sp. for 2 h at 60°C by using no stirring and stirring at 500 rpm, Ehimen *et al.* [198] observed a decrease of the biodiesel density from 903 et 883 kg/ m^3.

Biodiesel yield and purification

For the microalgae triglyceride transesterification, a conversion of 100% (g FAME/g lipid) can be reached [187], while for other classes of lipids, the yield is lower. For example, phospholipids and glycolipids are converted into biodiesel with yields of 65 and 56% (g FAME/g lipid), respectively [188]. These data are important because some microalgae can have an important amount of phospholipids and glycolipids of 40 and 25% (g/g lipid), respectively [42]. As a consequence, producing biodiesel from microalgae results in a lower biodiesel yield than with vegetable oil (mainly composed of triglycerides), and other compounds have to be separated (chlorophyll, carotenoids etc.) [58].

Biodiesel produced from microalgae must be separated from glycerol, chlorophyll and other by-products. Sometimes, when the process uses organic solvents, water is added to favor the biodiesel separation [146,212], as non-polar solvents are insoluble in water. In cases where there is no organic solvent already present, the main separation processes would be hot water [145], organic solvents such as hexane [156,213] and a blend of water and organic solvents [115,158,203]. Other techniques of purification, based on vegetable oil processes, that could be used for large-scale microalgae biodiesel purification would be: (1) water washing, (2) dry washing and (3) membrane separation.

Production standards and properties

Currently, there are two main biodiesel production standards: American standard (ASTM D6751-10) and European standard (UNE-EN 14214:2010) [214–216]. The following standards are inspired from the previous two standards: Australia (Acte 2000), Brazil (ANP Act No. 42/2003), China (GB/T 2008-2007), India (IS 15607-2005), Indonesia (SNI 04-7182-2006), Japan, Korea, New Zealand (NZS 7500-2005) and Thailand [217]. In Canada, there is a law that allows adding up to 5% (v/v) of biodiesel into petrodiesel [218].

There are important differences between standards UNE-EN 14214:2010 and ASTM D6751-10 used to produce biodiesel from microalgae. The European standard stipulates that the content of linolenic FAME (C18:3) and polyunsaturated FAME (number of double bonds $>$ 3) must not exceed 12 and 1 wt%, respectively [216]. These data are important since microalgae often have a high content of polyunsaturated FAMEs [146,187] at levels that can reach 56 wt% [153]. Some requirements of UNE-EN 14214:2010 are not found in ASTM 6756-10 standards such as the minimum FAME content (96.5% mol/mol min), the density (860–900 kg/m^3, 15°C) and the iodine value (120 g I_2/100 g max). The important biodiesel properties are cetane index, heating value, cold properties, viscosity, oxidation stability and lubricity [219,220].

Cetane index

The cetane index is the capacity for a fuel to flame up during combustion [221]. A higher cetane index means that the shorter time a fuel takes to flame up, the better the quality of the combustion [221,222]. The minimal cetane index for diesel is 40, while the latter for different commercial biodiesels varies between 48 and 65 [223]. For FAME, the cetane index increases with the increase in the number of carbon and decreases with the number of double bonds on the carbon chain. To our best knowledge of the literature, no study has experimentally measured the cetane index of microalgae biodiesel. However, a study has evaluated the cetane index of microalgae biodiesel from their FAME content: varying between 39 and 54 for different species of microalgae such as *Isochrysis galbana* (51.7), *Chaetoceros* sp. (50.6) and *Ondotella weissflogii* (39.3) [224].

According to our knowledge of the literature, no study has tried to increase the cetane number of microalgae biodiesel. On the other hand, some techniques applied to first-generation biodiesel could be used to increase the cetane number of microalgae biodiesel. In addition to hydrogenation, a study has tested nitration of biodiesel with nitric acid (25 wt%) and ethanoic anhydride (63 wt%). Using frying oil, they obtained an increase in the cetane index of the biodiesel from 55 to 61 [225].

Heating value

Heating value is the amount of energy released during biodiesel combustion. For commercial biodiesel, lower heating values vary between 36 and 41 MJ/kg, while

the lower heating value of microalgae biodiesel produced from acid transesterification (H_2SO_4) was measured at 41 MJ/kg by Miao and Wu [149].

Cold properties

Cold properties are defined as the biodiesel behavior when temperature is decreasing. These properties include cloud point and pour point. Cloud point is the temperature at which crystals ($d \geq 0.5$ μm) begin to form, while pour point is the temperature where biodiesel stops flowing [34,226]. As biodiesel has generally poorer cold behavior compared to petrodiesel (higher cloud and poor points), it is recommended to consider these properties for a biodiesel-petrodiesel blend [214]. Based on our knowledge of the literature, no study has measured cloud and poor points for microalgae biodiesel. However, since microalgae contain a high amount of unsaturated FAMEs that can reach 56 wt% [153], microalgae biodiesel would have better cold properties than biodiesel produced from oleaginous vegetable species [34].

Viscosity

Viscosity is a measure of flowing resistance. The lowest viscosity possible is required in order to reduce the flowing resistance. Despite the fact that the transesterification allows a reduction of the viscosity of lipids, the kinematic viscosity of biodiesel (2.8 to 5.7 mm^2/s) is higher than that of petrodiesel (1.8 to 3.8 mm^2/s) [223]. A biodiesel with a high viscosity will cause deposit problems in the combustion chamber and will also increase most pollutant emissions [227,228]. The kinematic viscosity of microalgae biodiesel ranges from about 3.9 to 5.2 mm^2/s [146,149]. Furthermore, like the cetane index, the viscosity increases with the length of the carbon chain and decreases as a function of the unsaturation degree of the FAMEs.

Oxidation stability and biodegradability

Oxidation stability is a very important parameter when biodiesel is stored. Indeed, when the oxygen contained in the air comes into contact with biodiesel, the latter may be transformed into deposits which consist of hydrogen peroxides, aldehydes, acids and oxygenated products [229]. Generally speaking, oxidation increases as a function of the unsaturation degree. Consequently, some authors have evaluated that biodiesel produced from several species of microalgae would have a low oxidation stability because the polyunsaturated FAME content can reach 56 wt% [153], which represents a real problem for storage [224]. Otherwise, if biodiesel is stored for more than a few months, adding antioxidants is recommended to improve its oxidation stability [223,230,231].

Biodegradability is another important factor during storage because microorganisms have the ability to transform biodiesel into end products such as O_2, water and CO_2 [232].

Lubricity

Lubricity of a fuel is defined as the capacity of a fuel to reduce friction between the moving parts of an engine [233–235]. Blending biodiesel with petrodiesel can improve the lubricity inside an engine. As an example, at a temperature of 25°C, Knothe and Steidley [236] obtained friction values (without indicated units) for petrodiesel (without additives) and vegetable based biodiesel of 0.238 and 0.117, respectively. Several factors influence the lubricity of petrodiesel, such as viscosity, acidity, water and sulfur contents [237]. Some studies on biodiesel production from microalgae obtained relatively high sulfur contents (69 ppm) [146] compared to American Society for Testing and Materials (ASTM) standard (15 ppm) [214]. As a consequence, studies on biodiesel lubricity should be performed since, to our best knowledge, no study has measured lubricity of biodiesel produced from microalgae.

Other properties

Concerning the density of biodiesel, that of microalgae biodiesel is higher than petrodiesel. For example, Miao and Wu [149] produced biodiesel from microalgae and measured a biodiesel density of 864 kg/m^3 by performing a direct transesterification of a heterotrophic microalgae (*Chlorella protothecoides*) using methanol and an acid catalyst (H_2SO_4), while the standard for petrodiesel is 0.838 kg/m^3.

By-products to separate and valorize

Among the by-products, glycerol is obtained following the triglyceride transesterification at a mass ratio around 10 times lower than that of FAME [238,239]. Currently, an overproduction of glycerol (a direct consequence of biodiesel production that alone produces 1400 kton (worldwide) of crude glycerol per year) associated with a slight increase of world glycerol consumption from 600 to 870 kton/year from 2003 to 2013 [240,241] led, as a consequence, to a decrease of glycerol prices. From 2004 to 2011, the price of glycerol decreased from 110 to 7.5 US$/ton [238,242]. In order to make biodiesel from microalgae cost effective, glycerol could be transformed into other products (added value products) by chemical [243,244], thermochemical [245] or biological [246] methods. A study suggests that if a microalga contains less than 40 wt% lipids, the anaerobic digestion of microalgae residues is essential to make microalgae biodiesel profitable [247].

Furthermore, microalgae contain non-transesterifiable components (chlorophyll, carotenoids) [58] that can be found in biodiesel after transesterification, which require further purification steps [202].

Cost calculation

Several studies have attempted to determine if a process of biodiesel production from microalgae could be profitable. Some studies calculated the cost of microalgae biomass ranging from 10 to 32 US$/kg biomass based on the initial investment [248,249]. On the other hand, some authors estimated that microalgae would be produced at a lower cost from 3.0 to 3.8 US$/kg biomass for photobioreactors and for 'raceway' pond types, respectively [17]. Nevertheless, in more recent studies (2009–2013), some authors calculated lower biomass production costs for microalgae biomass at 2.7 $US/kg biomass for raceway ponds [250,251], while the cost of microalgae cultivated in photobioreactors would be 7.4 $US/kg biomass. According to the same sources, considering a microalga with a lipid content of 50 wt%, the cost of microalgae cultivated in fermenters would be 1.6$US/kg biomass. It should be noted that 20 to 30% of the costs of biomass are associated with microalgae harvesting [252].

In order to calculate the energy balance from the microalgae biodiesel production process, Lardon *et al.* [148] estimated that with a lipid extraction without previous microalgae biomass drying using a solvent lipid extraction would produce a positive energy balance at 105 MJ/kg biodiesel, while microalgae drying would cause a negative energy balance at −2.6 MJ/kg biodiesel. However, only a process that would use low amounts of nitrogen (concentration non explicit) and an extraction of wet microalgae would consume less energy than biodiesel, which usually contains 37.8 MJ/kg biodiesel [148].

According to Davis *et al.* [253] microalgae biodiesel cost calculation modeling is at an early stage because of missing process data and further research has to be performed because several projects of industrial microalgae culture for biodiesel production process were not successful [254].

Canadian biodiesel production

In 2012, in Canada, petrodiesel consumption represented more than 28% of the total refined oil products, 28 million m^3 [255]. Consequently, in order to meet a demand in biodiesel that would correspond to a blend of biodiesel into petrodiesel of 10 vol% [14], Canada should produce about 2.8 million of m^3 per year of biodiesel. As the density of biodiesel is around 864 kg/m^3 (0.864 kg/L), the biodiesel production should reach 2.4 million tons/year. Considering this production would come from microalgae, a biomass productivity of 0.035 kg/m^2/day (culture in raceway pond) [17], a biodiesel yield of about 37% (g biodiesel/g dry biomass) [187] and a production of 6 months per year (180 days), the surface required for such production would be 1027 million m^2 (1027 km^2).

Biofuels: other applications

Despite the fact that biodiesel is one of the most studied biofuels, microalgae could be used to produce other biofuels such as bioethanol and biomethane [256]. Microalgae could be a raw material for other processes of valorization such as gasification, liquefaction, pyrolysis and hydrogenation [82]. However, these processes are performed at high temperatures and pressures ranging from 300 to 900°C or 7 to 55 MPa, respectively. As an example, researchers have developed patents to transform microalgae biomass by liquefaction using temperatures between 320 and 500°C at pressures between 20 and 55 MPa [257]. Direct combustion could be worked on to produce energy (for example to produce electricity) despite the fact that humidity would be an important factor for the cost efficiency of this process.

Conclusion

The objective of this literature review was to study the possibility of using microalgae as a source of biodiesel: from microalgae culture through biodiesel synthesis to ASTM biodiesel production standards. There are a lot of valuable products contained in microalgae that could be exploited (such as lipid, proteins, sugar, etc.) which could make biodiesel production from microalgae cost effective. Otherwise, the fluctuation of oil prices encourages the government to reduce its oil dependency and reduce CO_2 emissions, which stimulates biofuel production from renewable biomass.

Among the different sources of biomass, microalgae has a high lipid content (up to 75 wt%), which could be used to produce biodiesel. The main advantage of microalgae is based on the fact that microalgae have high culture yields and do not use arable lands. On the other hand, the use of this biomass for industrial biodiesel production requires the improvement of the techniques of extraction, the transesterification of lipids and the purification of biodiesel.

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