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## Production of Biodiesel from Microalgae

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### 1. Introduction

Since the early 70s, several major energy crises have forced the scientific community to find alternative sources of power. In July 2008, the price of crude petroleum reached 145\$US/barrel, the highest price ever achieved in 30 years (BP, 2011; Ervin & Associated, 2011). In July 2011, the price of crude petroleum was still relatively high at 108\$US/barrel (Ervin & Associated, 2011). Moreover, the 2008 world economic crisis encouraged the United States government to develop biofuels in order to blend them with petroleum fuels without engine modifications or distribution process changes (Bindraban *et al.*, 2009; The White House, 2010). Furthermore, in Canada, by 2012, the objective of adding biodiesel into transportation diesel and heating fuels is 2% (v/v) (Natural Ressources Canada, 2011b).

Consequently, in July 2007, a nine-year investment of 1.5 billion CAD\$ (the ecoENERGY for Biofuels Initiative) was announced by the Canadian government to stimulate the production of biofuels in Canada (Natural Ressources Canada, 2011a).

In recent years, most of industrial biodiesels are made from oil (triglycerides) of raw materials (rapeseed, sunflower, soybean, etc.). With the intent to change their physicochemical properties similar to petroleum-based diesel, triglycerides are transesterified into fatty acid alkyl esters, which can be used in a conventional engine without modifications (Knothe, 2010). On the ecological side, in addition to the ability of oleaginous plants to reduce pollutant emissions of greenhouse gases (GHG) by their capacity to trap and use the carbon dioxide (CO<sub>2</sub>), using biodiesel also reduces net emissions of pollutants. Typically, the addition of 20% (v/v) of soybean-based biodiesel in petrodiesel reduces emissions of carbon monoxide (CO), CO<sub>2</sub>, particulate matter (PM) and hydrocarbons (HC) by 11%, 15.5%, 10% and 21%, respectively (Sheehan *et al.*, 1998a; United States Environmental Protection Agency, 2002).

The raw materials are also necessary to feed humans and animals. A large demand for raw materials to produce biodiesel could thus increase their price. Moreover, the culture of conventional vegetable material requires an important amount of water, chemical fertilizers and pesticides, which have a negative impact on the environment (Smith *et al.*, 2009).

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To overcome these problems, researchers are currently exploring a new way of producing biodiesel using microalgae. Indeed, during their growth, photoautotrophic microalgae metabolize inorganic carbon (CO<sub>2</sub>) through photosynthesis (Chen *et al.*, 2011). According to Cadoret & Bernard (2008), microalgae have also the capacity to absorb other pollutants such as phosphates and nitrates. Furthermore, microalgae can accumulate a high amount of fatty acids and have a culture yield per hectare at least 10 times higher than any oily plants (Chisti, 2007).

In this paper, the different sources of energy such as oil, conventional and unconventional biofuels like microalgae based biodiesel will be discussed. Then, the different aspects of the microalgae valorization into biodiesel will be considered. The culture of microalgae, the extraction of lipids, the transesterification process and the biodiesel characteristics will be especially discussed.

## 1.1 Background

Finding new energy resources to compensate the decrease of the world petroleum reserves is an important challenge. The estimation of the world crude oil reserves is a difficult task because it is influenced by political, economic and technological factors (Pirog, 2005). The proven oil reserves represent the amount of petroleum that could be obtained from deposits already discovered with actual technological and economic conditions (Institut Français du Pétrole, 2005; Pirog, 2005). They also include the oil discovered, but that cannot be recovered with current technologies. In 2009, these reserves were estimated to be about 1,376 billion barrels (BP, 2011), which corresponds to a reserve that will last for 35 years (i.e. until 2045) (Shafiee & Topal, 2009).

According to a predictive model from Huber (1956), the proven reserves of oil should have reached a roof value by 2000, as the production of petroleum should begin to fall. In contrast, more recent data show that between 1989 and 2008, the proven oil reserves seem to have increased from 1006 to 1333 billion barrels (BP, 2011). On the other hand, while the world oil consumption was about 86 million barrels/day in 2006, it would reach 107 million barrels/day by 2030. The transportation sector would be responsible for 80% of this increase (U.S. Energy Information Administration, 2009) and would consume 76% of the world oil production by 2030 (International Energy Agency, 2008).

Fossil fuel dependency, mainly in the transportation sector, has encouraged research on biofuels. A recent study shows that microalgae biofuels have the potential to replace 17% of oil imports in the United States used as transportation fuel by 2022 (Wigmosta *et al.*, 2011). Moreover, following the BP oil spill in the Atlantic Ocean, the United States administration is considering reducing his oil imports by 1/3 by 2021 using, among others, biofuels (N. Banerjee, 2011).

## 2. Biofuels as a main alternative

### 2.1 Conventional biofuels

Intending to replace oil-based fuels, many studies have been conducted on non-renewable and renewable energy alternatives.

The researches on non-renewable energy are mainly based on coal and natural gas. In 2009, 89% of the energy produced in Canada (15 exajoules) was coming from non-renewable sources (Statistics Canada, 2011). For example, coal can be converted into syngas (CO, H<sub>2</sub>) by gasification (Naveed *et al.*, 2010), and then into fuel by Fischer-Tropsch reaction, methanol or synthetic natural gas by catalytic processes and hydrogen by water/gas shift reaction (Dry, 1996; Longwell *et al.*, 1995).

Renewable energy sources used in the world in 2006 were mainly biomass and waste (58%), hydropower (31%) and others (12%), including wind, geothermal and solar (International Energy Agency, 2008). Most of renewable energies in Canada was produced by hydroelectricity (89%), while biomass was responsible for 6% of the 81 GWatt of renewable energy produced in 2008 (Nyboer & Grove, 2010). In United States, the energy generated from biomass is expected to increase by 300% between 2009 and 2030 to reach 153 billion of 12 kWatt-h (U.S. Energy Information Administration, 2011). Biomass is a source of energy, which can be used to produce 1<sup>st</sup>, 2<sup>nd</sup> and 3<sup>rd</sup> generation biofuels.

### 2.1.1 First generation

First generation biofuels correspond to those issued from food-based crops (Antizar-Ladislao & Turrion-Gomez, 2008). They mainly correspond to ethanol-based fuels obtained from the fermentation of sugars (corn, beet, sugar cane, etc.), vegetable oil-based fuels (raw oil, biodiesel and renewable diesel produced from catalytic hydrodeoxygenation) (Knothe, 2010; Natural Resources Canada, 2011b) from oleaginous plants (colza, palm, canola, etc.) and biogas emitted from raw material or landfills (Naik *et al.*, 2010).

However, on many levels (environmental, societal), the fact that food resources could be used to produce biofuels shows several limits, as this would create land pollution, a lack of agricultural land (world hunger) and deforestation (Goldemberg & Guardabassi, 2009; National Research Council, 2007). For example, in some European countries such as France, the arable lands available for cultivation of oleaginous plants used for 1<sup>st</sup> generation biofuels production will not be able to support the biofuels demand by 2015, except by saturating the lands in fallow, which would create soil impoverishment problems (Bordet *et al.*, 2006).

### 2.1.2 Second and third generation

Second generation biofuels are the cellulosic-based biofuels obtained from non-food crops materials (wood, leaves, straw, etc.). These biofuels include bioalcohols, bio-oil, 2,5-dimethylfuran (BioDMF), biohydrogen, Fischer-Tropsch diesel, wood diesel (Fatih Demirbas, 2009; Román-Leshkov *et al.*, 2007).

Third generation biofuels are microorganisms (yeast, fungi) biofuels and algae-based fuels like vegetable oils, bio-oil, jet-fuels, biohydrogen, biodiesel, renewable diesel and many others (Fatih Demirbas, 2009; Nigam & Singh, 2011).

Second and 3<sup>rd</sup> generation biofuels are better than 1<sup>st</sup> generation biofuels for sustainable development as they are carbon neutral, or they reduce atmospheric CO<sub>2</sub> as they are carbon negative (Naik *et al.*, 2010). For example, 1<sup>st</sup> generation biodiesel (like soybean) only induces a net reduction of GHG emissions by 41% (Hill *et al.*, 2006). In comparison, for each

ton of microalgal biomass produced, some authors estimate that 1.8 tons of CO<sub>2</sub> would be consumed (180% reduction) (Chisti, 2007).

## 2.2 Microalgae as a source of biofuels

Microalgae can generate diverse biofuels, which are mainly: biomethane produced by anaerobic digestion (Sialve *et al.*, 2009; Spolaore *et al.*, 2006), biohydrogen by photobiological process (Fedorov *et al.*, 2005; Kapdan & Kargi, 2006), bioethanol by fermentation (Choi *et al.*, 2010; Dexter & Pengcheng, 2009), liquid oil by thermal liquefaction (A. Banerjee *et al.*, 2002; Miao & Wu, 2004; Miao *et al.*, 2004; Sawayama *et al.*, 1995) and biodiesel (M. B. Johnson & Wen, 2009; Koberg *et al.*, 2011; Nagle & Lemke, 1990; H. Xu *et al.*, 2006).

Even if industrial scale biofuels from microalgae remain at an early stage, they remain a sustainable solution as a transportation fuel.

## 2.3 Microalgae for biodiesel

Some microalgae species like *Botryococcus braunii* or *Schizochytrium* sp. can contain up to 80% of their dry weight of lipids (Deng *et al.*, 2009). These species can produce a yield of lipids by acre up to 770 times higher than oleaginous plants (colza, sunflower, etc.) and their high scale production allows to consider developing high-yield biodiesel (Chisti, 2007). Another advantage of using microalgae to produce biodiesel is that microalgae can double from 1 to 3 times in 24 hours (Khan *et al.*, 2009). Consequently, the microalgae biomass can be harvested more than once a year. Microalgae can potentially be used also in food, cosmetic, fertilizing and many other industries (Jacob-Lopes & Teixeira Franco, 2010).

### 2.3.1 Sustainable development issues

#### Environmental issues

Microalgae growth is made naturally from sunlight photosynthesis in diverse media (Deng *et al.*, 2009). Replacing 1<sup>st</sup> generation biodiesel with biodiesel from microalgae would reduce the carbon footprint of the process. In fact, producing microalgal biodiesel requires no mechanical seeding of grains, no watering or "harmful" chemical products spreading and no harvesting using heavy engines powered by fossil fuels.

The microalgae production can add value to the gases emitted by coal fired power plants (or other processes) (de Moraes & Costa, 2007) as they are able to absorb CO<sub>2</sub>, nitrogen dioxide (NO<sub>2</sub>) and sulphur dioxide (SO<sub>2</sub>), which are important nutrients for their growth (Malińska & Zabochnicka-Świątek, 2010). Moreover, some studies have shown that some algae can be grown on municipal (*Chlorella* sp.) and industrial wastewaters (*Chlamydomonas globosa*, *Chlorella minutissima* or *Scenedesmus bijuga*) since they also use compounds like phosphorus (P) and metals (Al, Fe, Mg, Mn, Zn, etc.) (Chinnasamy *et al.*, 2010; Wang *et al.*, 2010).

#### Economic and social issues

The production of 1<sup>st</sup> generation biofuels is causing a substantial rise of the world food prices. In order to evaluate the monthly variations for different international food commodities, The FAO Food Price Index can be used. From 2000 to 2011, The FAO Food Price Index (FFPI) and The FAO Cereal Price Index increased from 88 to 238 and from 87 to



245, respectively (FAO, 2011b). In the long term, producing biofuels on arable land could contribute to increasing world hunger. The increase of cereal prices could have an impact on the cost of 1<sup>st</sup> generation biodiesel production as the FAO Oils/Fats Price Index from 2000 to 2011 also increased from 78 to 278 (FAO, 2011b). In North America, the best lands could be used for biodiesel production, because the harvested area for soybean, which is the most used oil for biodiesel production, represents 32% of the 99.5 million ha harvested worldwide (FAO, 2011a).

As microalgae industrial culture does not directly compete with food and wood production, it can represent a great potential economic development. In fact, the use of microalgae for biofuel production would permit to reduce deforestation and preserving the forest heritage. Moreover, the development of valorization of microalgae could favour the energetic autonomy of all countries, including developing countries. Thus, the industrial production of microalgae could be considered as a sustainable solution to energetic, environmental and food problematic.

### 2.3.2 Types of microalgae

Microalgae can be found in a large range of places where light and water are present including ocean, lake, soils, ice, rivers, etc. (Deng *et al.*, 2009). Microalgae demonstrate a great biodiversity (between 200 000 and several millions of species) (Natrah *et al.*, 2007), which can be divided into categories depending on their pigmentation, biological structure and metabolism.

#### Size classification

Microalgae are small organisms, which can be divided into 4 size categories as the microplankton (20 to 1000  $\mu\text{m}$ ), the nanoplankton (2 to 100  $\mu\text{m}$ ), the ultraplankton (0.5 to 15  $\mu\text{m}$ ) and the picoplankton (0.2 to 2  $\mu\text{m}$ ) (Callieri & Stockner, 2002; Gopinathan, 2004). Their small size allows them to do an effective photosynthesis, converting light energy with  $\text{CO}_2$  dissolved in water to produce lipids, carbon hydrates, proteins, etc..

#### Taxonomic groups

Microalgae can be classified into 4 main taxonomic groups: diatoms (*Bacillariophyceae*), green algae (*Chlorophyceae*), cyanobacteria or blue green algae (*Cyanophyceae*) and golden algae (*Chrysophyceae*). However, there are 6 other groups of microalgae composed of yellow-green (*Xanthophyceae*), golden algae (*Chrysophyceae*), red algae (*Rhodophyceae*), brown algae (*Phaeophyceae*), dinoflagellates (*Dinophyceae*), *Prasinophyceae* and *Eustigmatophyceae* (Williams & Laurens, 2010). However, these species are not equally interesting for biodiesel production. As an example, green algae taxonomic group includes the most promising species of microalgae like *Botryococcus* sp., *Dunaliella* sp. and *Chlorella* sp. (Garofalo, 2010) for biodiesel production.

#### Metabolism classification

Microalgae can be separated into 4 main types of metabolism called photoautotrophic, heterotrophic, mixotrophic and photoheterotrophic (Chen *et al.*, 2011).

In photoautotrophic microalgae metabolisms, light (source of energy) converts inorganic carbon ( $\text{CO}_2$ ) and water to biomass by photosynthesis reaction (Cadoret & Bernard, 2008).

Even if photoautotrophic microalgae contain high level of lipids, their biomass productivity in photobioreactors and open ponds is generally lower than heterotrophic microalgae, between 0.117 and 1.54 kg/m<sup>3</sup>/day (Chisti, 2007).

Heterotrophic microalgae need organic carbon as a source of carbon and energy. Their production is carried out in closed bioreactors (fermentors). They are more promising than the photoautotrophic species for the production of biodiesel (Martek, 2008; Xiong *et al.*, 2008; H. Xu *et al.*, 2006). For example, Xiong *et al.* (2008) showed that the biomass productivity obtained from *Chlorella* sp. grew under heterotrophic conditions could reach 7.4 kg/m<sup>3</sup>/day with lipid content ranging from 50 to 58% (g lipid/g dry weight). However, they do not achieve the main goal of producing microalgae, i.e. mitigation of the emissions of CO<sub>2</sub>.

Some microalgae species also have mixotrophic metabolism as they grow in light or dark using both inorganic and organic carbon sources. Growing *Chlorella vulgaris* in the dark, Liang *et al.* (2009) changed CO<sub>2</sub> contained in air for glucose (as a source of carbon) and observed an increase of maximal biomass productivity from 10 to 151 mg/L/day. When they used light and glucose as a source of carbon, the biomass productivity increased to 254 mg/L/day. The use of microalgae with mixotrophic metabolism is relatively rare for biodiesel production (Chen *et al.*, 2011).

Photoheterotrophic metabolism means that microalgae need light as a source of energy and a source of organic carbon (Chen *et al.*, 2011). For example, using glycerol as a source of carbon and a light intensity of 35 μE/m<sup>2</sup>/s<sup>1</sup>, Yang *et al.* (2011) obtained an increase in *Chlorella minutissima* (UTEX2341) biomass concentration from 1.2 to 8.2 g/L after 15 days of culture. As these microalgae need an inexpensive source of organic carbon and are dependant of the sunlight periods, photoheterotrophic metabolism seems less interesting for biodiesel production.

### 2.3.3 Strain promising

Even if microalgae are a large group of microorganisms, not all species are suitable to produce biodiesel. The factors to consider were stated by Grobbelaar (2000): "1-Carbon dioxide tolerance and uptake, 2-Temperature tolerance, 3-Stability for cultivation in specific bioreactors," 4-Secondary valuable products, "5-Specific growth requirements and competing algal, 6-Vulnerability to infection and herbivory potential, 7-Excretion of autoinhibitor, 8-Harvesting and down-stream processing and 9-Manipulation potential of genetic engineering".

### 2.3.4 Lipids yield of microalgae

Intending to increase lipid production, Huntley & Redalje (2007) proposed a method of growth for microalgae in a photobioreactor (PBR) which allows reaching high lipids yield followed by 2 days of nitrogen deficiency in a pond. In general, nutrient deprivation can lead to an increase in lipid content, but not for all species of microalgae. For example, the microalgae *Navicula* (NAVIC1) had the highest lipid content, which raised in exponential phase from 22 to 49% (g lipid/g dry weight) in silicon (Si) deficiency and increased to 58% (g lipid/g dry weight) when nitrogen was limited. On the other hand, nutrient limitation (nitrogen or Si) limitation had less or no significant effect on lipid content of microalgae *Amphora* (AMPHO1) and *Cyclotella* (CYCLO2), respectively (Sheehan *et al.*, 1998b). Other

studies found that P deprivation could have a positive effect on lipid content (Khozin-Goldberg & Cohen, 2006; Xin *et al.*, 2010). For example, increasing the P concentration from 0.14 to 0.37 mg/L, Xin *et al.* (2010) observed a raise of microalgae concentration from 0.14 to 0.37 g/L, while the lipid content decreased from 53 to 23.5% (g lipid/g dry weight).

Finally, an osmotic shock might also stimulate the lipids production. For example, Takagi *et al.* (2006) enhanced the sodium chloride (NaCl) concentration from 3.5 to 7 g/L (0.5 to 1 mol/L) and found an increase in lipid production from 60 to 67% (g lipid/g dry weight). However, these physicochemical treatments could also favour the synthesis of polar lipids like phospholipids or glycolipids associated with cell walls of the microalgae (Cadoret & Bernard, 2008); such lipids are less interesting for biodiesel production (Nagle & Lemke, 1990).

### 3. Production of biodiesel

#### 3.1 Culture of microalgae

The large scale production of microalgae is generally performed with solar energy (photoautotrophic metabolism) in open ponds (raceways), closed systems (photobioreactors) or fermentors.

Open ponds are generally circular with nested loops and are 30 cm deep (Chisti, 2007). However, ponds can have several non neglectable disadvantages. Indeed, as they are open, evaporation and contaminants (protozoa, bacteria or other microalgae) could affect the microalgae productivity (Blanco *et al.*, 2007).

Photobioreactors are continuous culture systems which can achieve concentration of microalgae up to 6.7 g/L (Bai *et al.*, 2011; Chisti, 2007; Ranjbar *et al.*, 2008) in fresh or sea water. Different models of photobioreactors (indoor or outdoor) have been developed including tubular, flate plate, airlift, bubble column and stirred tank (L. Xu *et al.*, 2009). Even if the closed photobioreactor has a higher harvesting efficiency (more biomass) and a good control on culture parameters (temperature, pH, CO<sub>2</sub> concentration etc.) (Suh & Lee, 2003), its capital costs remain higher (around 10 times) than those of open ponds (Carvalho *et al.*, 2006). However, the combination of ponds and photobioreactors can be profitable because microalgae can be grown in open ponds while reducing contamination by undesired species (Huntley & Redalje, 2008). In this culture process, the first step of microalgae production is conducted in a controlled temperature (e.g. by a sea water bath (16-18°C)) photobioreactor. Microalgae are then transferred into an open pond for a 5 days second culture step (Huntley & Redalje, 2007; Huntley & Redalje, 2008).

Fermentors are mainly used to produce heterotrophic microalgae using an organic source of carbon such as glucose, fructose, galactose acetate, glycerol and acetic acid (Cantin, 2010). These bioreactors can reach high biomass concentration (150 g/L) without rheological problems (Wu & Shi, 2008). However, heterotrophic production costs of microalgae in fermentors remains relatively high (Wei *et al.*, 2009).

#### 3.2 Harvesting of microalgae

Microalgae biomass could be harvested by centrifugation, flocculation, gravity sedimentation, filtration, screening, flotation or by electrophoresis techniques (Chen *et al.*,



2011). As microalgae are floating in pond at a concentration less than 0.5 g/L, the harvesting costs can represent 20 to 30% of the industrial microalgae biomass production cost (Carlsson *et al.*, 2007) of 2.95 and 3.80 \$US/kg biomass (photobioreactor and raceway, respectively) (Chisti, 2007).

### 3.3 Extraction techniques

In order to produce biodiesel from microalgae lipids, the later must be priority extracted. The main lipid extraction techniques are the use of chemical solvents, supercritical CO<sub>2</sub>, physicochemical, biochemical and direct transesterification.

#### 3.3.1 Chemical solvents extraction

Chemical solvents method is by far the most commonly used, but less effective when microalgae are still wet (Samorì *et al.*, 2010). Consequently, for laboratory scale extraction of lipids, freeze-drying (J. Lee *et al.*, 2010) is a popular method, but spray-drying (Koberg *et al.*, 2011), oven-drying (Cooney *et al.*, 2009) or vacuum-evaporation (Umdu *et al.*, 2009) have also been used to dry microalgae. However, drying microalgae prior to lipid extraction could require 2.5 times more energy than a process without drying, which makes a process using a prior drying unprofitable (negative balance) (Lardon *et al.*, 2009).

In laboratory scale studies, even if chloroform-methanol blends have been extensively used with high extraction yields up to 83% (g lipid/g dry weight) (Yaguchi *et al.*, 1997), less polar solvent like hexane are often preferred because of their lower toxicity and affinity for non-lipid contaminants (less polar) (Halim *et al.*, 2010). As an example, hexane was used to obtain lipids content up to 55% (g lipid/g dry weight) from a heterotrophic microalgae, *Chlorella protothecoides* (Miao & Wu, 2006). For microalgae lipid extraction on an industrial scale, Soxhlet extraction is not recommended due to high energy requirement (Halim *et al.*, 2010).

Other less toxic solvents like alcohols (ethanol, octanol) or 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) have been tested but the yield of fatty acid methyl ester (FAME) obtained was up to 5 times lower than with n-hexane extraction (Samorì *et al.*, 2010) even if the hydrocarbon (lipid) yield was more than twice higher.

#### 3.3.2 Supercritical carbon dioxide extraction

Supercritical CO<sub>2</sub> (Dhepe *et al.*, 2003) has the advantages of being not toxic, easy to recover and usable at low temperatures (less than 40°C) (Andrich *et al.*, 2005). However, this technique requires expensive equipments (Perrut, 2000) and a huge amount of energy to reach high pressures (Tan & Lee, 2011). Few studies used supercritical CO<sub>2</sub> extraction to recover microalgae lipids and transformed them into biodiesel (Halim *et al.*, 2010) even if some studies obtained lipid content up to 26% (g lipid/g dry weight) from *Nannochloropsis* sp. (Andrich *et al.*, 2005). Using supercritical CO<sub>2</sub> extraction at operating temperature of 60°C and pressure of 30 MPa to extract lipids from *Chlorococcum* sp. microalgae, Halim *et al.* (2010) obtained a higher extraction yield of lipids with supercritical CO<sub>2</sub> than hexane Soxhlet extraction (5.8 and 3.2% (g lipid/g dry weight), respectively). Moreover, using supercritical CO<sub>2</sub> extraction with wet microalgae, Halim *et al.* (2010) obtained a maximum yield of lipids of 7.1% (g lipid/g dry weight) for the same experimental conditions, which was a relatively

low lipid yield compared to other species such as *Botryococcus* sp. (28.6% g lipid/g dry weight) (J. Lee *et al.*, 2010). Consequently, in opposition to chemical solvent extraction, supercritical CO<sub>2</sub> lipid extraction can be stimulated by the presence of water in the blend of microalgae.

### 3.3.3 Physicochemical extraction

Some physicochemical techniques like microwave, autoclaving, osmotic shock, bead-beating, homogenization, freeze-drying, French press, grinding and sonication can be used for microalgae cell disrupting in order to recover lipids (Cooney *et al.*, 2009; J. Lee *et al.*, 2010; S. Lee *et al.*, 1998). Using microwave or bead-beating seems to be the most promising techniques to increase the lipid yield. As an example, J. Lee *et al.* (2010) increased the lipid extraction yield of *Botryococcus* sp. microalgae in water phase from 7.7 to 28.6% (g lipid/g dry weight) using a 5 min microwave pretreatment.

### 3.3.4 Biochemical extraction

Few studies have used biochemical extraction to extract lipids from microalgae. Using a 72 h cellulase hydrolysis pretreatment of the *Chlorella* sp. microalgae, Fu *et al.* (2010) have obtained a hydrolysis yield of sugars of 70% (concentration reducing sugar/concentration total sugar), although the lipids yield has increased only from 52 to 54% (g lipid/g dry weight).

### 3.3.5 Direct (in situ) transesterification

Direct transesterification is a process that blends the microalgae with an alcohol and a catalyst without prior extraction. Number of acid catalysts have been investigated for heterotrophic microalgae biomass including hydrochloric (HCl) or sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) but acetyl chloride (CH<sub>3</sub>COCl) remains the catalyst producing the higher FAME yield of 56% (g FAME /g dry weight) (Cooney *et al.*, 2009). A less polar solvent, like hexane or chloroform, can be added to increase the yield of biodiesel production (M. B. Johnson & Wen, 2009). Direct transesterification using a heterogeneous catalyst could be more effective coupled with microwaves heating. As an example, using microwave with direct transesterification of *Nannochloropsis* in presence of a heterogeneous catalyst (SrO), Koberg *et al.* (2011) reported an increase in the FAME yield from 7 to 37% (g FAME /g dry weight). However, direct transesterification requires a dry biomass, increasing the cost of harvesting.

## 3.4 Transesterification

The direct use of crude vegetable oils in diesel engines is envisageable, but could lead to numerous technical problems. For example, their characteristics (high viscosity, high density, difficulty to vaporize in cold conditions) cause deposits in the combustion chamber, with a risk of fouling and an increase in most emissions (Basha *et al.*, 2009). These drawbacks can be mitigated, but not without some modifications of the diesel engine (Altin *et al.*, 2001). To overcome all these inconveniences, the transformation of microalgae lipids in corresponding esters is essential.

### 3.4.1 Reaction

In the transesterification process, a catalyst and an alcohol are added to a blend of microalgae lipids. The reaction reduces the molecular weight, the viscosity and increases the volatility of microalgae lipids. Different parameters can influence the yield of transesterification like the ratio of alcohol-oil, catalyst types and concentration, reaction time, temperature and agitation rate.

### 3.4.2 Microalgae lipid content

The yield of the transesterification reaction depends on the nature of lipids. For example, monoglycerides like palmitic acid (C16:0) produced a FAME yield of 93% (g FAME /g lipid) while triglycerides like triolein had a yield of 88% (g FAME /g lipid). Furthermore, phospholipids and glycolipids gave a lower yield of 54 to 65% (g FAME /g lipid) and 47 to 56% (g FAME /g lipid), respectively (Nagle & Lemke, 1990). The nature of lipids is an important data for biodiesel production because some microalgae can contain up to 93% (g /g lipid) of phospholipids and glycolipids (Williams & Laurens, 2010). Moreover, some microalgae can also contain lipids such as unsaponifiable lipids carotenoids and other elements (chlorophyll) which are considered as by-products (Bai *et al.*, 2011).

### 3.4.3 Alcohol

Methanol is the most commonly used alcohol because of its low price. However, other alcohols such as ethanol or butanol can also be employed (Chisti, 2007). In traditional alkali-based catalyst transesterification of vegetable oil, the most used methanol to oil molar ratio for transesterification is 6:1 (Marchetti *et al.*, 2007) even if the stoichiometric value is 3:1 for triglycerides (Berriosa & Skelton, 2008). For microalgae lipids transesterification, the optimal methanol to oil ratio is higher. For example, performing a direct transesterification during 8h at 25°C, Ehimen *et al.* (2010) obtained a decrease in the specific gravity (SG) of the biodiesel from 0.8887 to 0.8849 when the molar ratio methanol to oil was increased from 105:1 to 524:1.

### 3.4.4 Catalyst

Catalysts used for transesterification of microalgae lipids are mainly homogenous or heterogeneous. Another method of transesterification using methanol in the supercritical state (without catalyst) has been developed, but the cost of this technology renders its use impossible to date (Tan & Lee, 2011).

Homogenous alkaline catalysts used for transesterification of vegetable oils mainly include sodium or potassium hydroxide (NaOH or KOH) and sodium or potassium methoxide (CH<sub>3</sub>ONa or CH<sub>3</sub>OK) while homogenous acid-catalysts includes H<sub>2</sub>SO<sub>4</sub>, HCl and sulphonic acids (R-SO<sub>3</sub>H) (Helwani *et al.*, 2009). In industrial vegetable oil biodiesel, homogenous alkali-catalysed transesterification is commonly used because homogenous acid-catalyzed transesterification is around 4000 times slower (Chisti, 2007) and these catalysts (NaOH or KOH) are relatively less expensive (Helwani *et al.*, 2009).

However, acidic catalyst is preferable for homogenous transesterification of microalgae lipids because the biodiesel yield obtained by Nagle & Lemke (1990) was more than 50 times

higher using HCl than NaOH (68 and 1.3% (g FAME/g lipids), respectively) as a catalyst for the same conditions of transesterification (0.1h, 70°C). The most used acid catalysts are CH<sub>3</sub>COCl (Cooney *et al.*, 2009), HCl (Tran *et al.*, 2009), or H<sub>2</sub>SO<sub>4</sub> (Miao & Wu, 2006). Using 4 concentrations of H<sub>2</sub>SO<sub>4</sub> (0.56, 1.13, 1.35 or 2.25 mol/L) and a temperature of 90°C, Miao & Wu (2006) found that adding 2.25 mol/L catalyst gave lowest SG (0.863) but a slightly lowest yield of biodiesel (38%, g biodiesel/g lipid).

Different homogenous acid-alkaline (H<sub>2</sub>SO<sub>4</sub>-CH<sub>3</sub>OK, KOH-HCl) catalysts can also be used to transesterify lipids from microalgae (Halim *et al.*, 2010; J. Lee *et al.*, 2010). Using a 1<sup>st</sup> transesterification step of 2 hours with H<sub>2</sub>SO<sub>4</sub> at 50°C and a 2<sup>nd</sup> step of 2 hours with CH<sub>3</sub>OK at 55°C, Halim *et al.* (2010) obtained a maximum biodiesel yield of 44% (g FAME/g lipid). The purpose to add an alkaline catalyst is mainly to increase the FAME yield (transmethylate acylglycerols and phospholipids) (Halim *et al.*, 2010).

Heterogeneous catalyst used for transesterification of triglycerides can be acid, alkali or enzymatic (immobilized enzymes) (Helwani *et al.*, 2009).

Transesterification studies testing heterogeneous catalysts were conducted with *Nannochloropsis* microalgae using alkaline earth oxide base catalyst such as strontium oxide (SrO) (Koberg *et al.*, 2011), calcium oxide (CaO) or magnesium oxide (MgO) (Umdu *et al.*, 2009). As example, performing a direct transesterification, Koberg *et al.* (2011) reported biodiesel yields up to 99.9% (g biodiesel/g lipid) for a time of reaction of 2 min at 60°C for ratio SrO:microalgae of 30% (g catalyst/g microalgae).

Few studies used enzymatic catalysts to transesterify lipids from microalgae. Generally, the most common used enzyme is lipase. A biodiesel yield of 98% (g FAME/g lipid) is reported for heterotrophic microalgae *Chlorella protothecoides* lipids transesterified using lipase (*Candida* sp. 99-125) immobilized on macroporous resins at a concentration of 30% (g/g lipid, 12000 U/g lipid) at 38°C during 12h (Li *et al.*, 2007; Xiong *et al.*, 2008). However, enzymatic biodiesel production is often seen expensive due to the cost of the catalyst from 236 to 836 \$US/ton biodiesel (Sotoft *et al.*, 2010). In comparison, the price of acid catalysts like H<sub>2</sub>SO<sub>4</sub> (98%, w/w) is as low as 100 \$US/ton H<sub>2</sub>SO<sub>4</sub> (Tao & Aden, 2009) for a price of catalyst for biodiesel production from 1.4 to 5.3 \$US/ton biodiesel (1 and 5% (w/w) catalyst, respectively) (Lotero *et al.*, 2005).

### 3.4.5 Reaction time, temperature and stirring

Increasing the reaction time has a positive effect on the SG of the biodiesel produced. For example, performing a direct transesterification of *Chlorella* microalgae at 30°C with H<sub>2</sub>SO<sub>4</sub> as catalyst, Ehimen *et al.* (2010) found that the SG decreased from 0.914 to 0.884 when the reaction time was increased from 0.25 to 12h.

The temperature seems to have less effect on the microalgae biodiesel production than reaction time except for high temperatures. For example, Miao & Wu (2006) used H<sub>2</sub>SO<sub>4</sub> catalyst (2.25 mol/L) and found similar biodiesel yield of 56 and 58% (g biodiesel/g lipid) at temperatures of 30 and 50°C, respectively. At 90°C, the biodiesel yield dropped of about 38% (g biodiesel/g lipid).

Stirring can have a positive effect on the biodiesel quality. For example, Ehimen *et al.* (2010) observed a decrease of the SG from 0.9032 (stirring at 500 rpm) to 0.8831 (no stirring).



### 3.4.6 Purification of biodiesel and by-products

Microalgae biodiesel and by-products must be separated for increasing the biodiesel production. The main separation processes used hot water (50°C) (Li *et al.*, 2007), organic solvents such as hexane (Halim *et al.*, 2010; Wiltshire *et al.*, 2000) and water-organic solvent for a liquid-liquid separation (Couto *et al.*, 2010; Lewis *et al.*, 2000; Samori *et al.*, 2010). When using a non-polar co-solvent for transesterification of lipids, only water is added to separate biodiesel from the by-products (M. B. Johnson & Wen, 2009).

To our present knowledge, there is no study on the purification of biodiesel from microalgae. Based on 1<sup>st</sup> generation biodiesel (Leung *et al.*, 2010), three main means of purification on biodiesel could be applied to microalgae biodiesel purification: "1-water washing 2-dry washing 3-membrane extraction." Based on vegetable oil biodiesel production (Berriosa & Skelton, 2008) and microalgae composition, the main by-products could be unreacted lipids, water, alcohol, chlorophyll, metals and glycerol.

Among the by-products obtained from the biodiesel production, glycerol is the most interesting. Glycerol worldwide consumption remains relatively constant, in recent years, with a consumption of 600 kton/year. Twenty-six percent of glycerol consumption was associated with pharmaceutical, cosmetic and soap industries (Bondioli, 2003). From 2004 to 2011, massive biofuel production created a problem of overproduced glycerol and the price of crude glycerol (80% pure) decreased from 110 to 7.5 \$US/ton (The Jacobsen, 2011; Yazdani & Gonzalez, 2007). Gained glycerol can be transformed into added-value products using many paths including chemical, thermochemical or biological conversion.

Among the chemical added-value products, glycerol can be oxidized or reduced to many compounds like propylene glycol, propionic acid, acrylic acid, propanol, isopropanol, allyl alcohol and acrolein but only some of these products are interesting in terms of market or profitability (D. T. Johnson & Taconi, 2007).

Glycerol can be also converted into Fischer-Tropsch fuel at low temperature (225-300°C) by catalytic processes (Soares *et al.*, 2006) or transformed into hydrogen (H<sub>2</sub>) by catalytic (generally nickel, platinum or ruthenium) or non-catalytic reforming (Vaidya & Rodrigues, 2009).

Biological conversion of glycerol includes fermentation into alcohols (ethanol, butanol, 1,3-propanediol) and other products like H<sub>2</sub>, formate, propionic or succinic acid (Yazdani & Gonzalez, 2007).

Anaerobic digestion of by-products is another possible way to make biodiesel from microalgae cost effective if the lipid content of the microalgae does not exceed 40% (g lipid/g dry weight) (Sialve *et al.*, 2009). For example, Ehimen *et al.* (2010) used anaerobic digestion of microalgae residues issued from a direct transesterification with a constant loading rate of 5 kg volatile solids (VS)/m<sup>3</sup>, temperatures and carbon-to-nitrogen (C/N) mass ratio varying from 25 to 40°C and 5.4 to 24, respectively. For a temperature of 40°C and a C/N mass ratio of 8.53, a maximum methane (CH<sub>4</sub>) concentration of 69% (v/v) with a specific CH<sub>4</sub> yield of 0.308 m<sup>3</sup> CH<sub>4</sub>/kg VS was obtained.

## 4. Characteristics of biodiesel

The physicochemical properties of biodiesel are nearly similar to diesel fuel. The most important properties for biodiesel are cetane number, heat of combustion, viscosity,



oxidative stability, cold flow properties and lubricity (Knothe *et al.*, 2005). Table 1 presents the main properties of microalgae biodiesel compared with diesel and 1<sup>st</sup> generation biodiesel.

1. The cetane number is an indicator of quality of ignition of a fuel which increases with the number of carbon and decreases with the number of unsaturated carbon bounds (Hart Energy Consulting, 2007). Consequently, a higher unsaturated biodiesel like microalgae biodiesel would have a lower cetane number. Based on our present knowledge, no measurement of the cetane number of microalgae biodiesel has been performed. However, some studies approximated the cetane numbers of many species based on their FAME content and found cetane numbers ranging from 39 to 54 (Stansell *et al.*, 2011), while cetane number of petrodiesel fuel are at least between 47 and 51 (ASTM Standard D6751-10, 2010; Knothe, 2006).
2. The heat of combustion shows if a biodiesel is suitable to burn in a diesel engine. The heat of combustion increases with the length of the carbon chain (Knothe, 2005a). Using lipids extracted from heterotrophic microalgae in the presence of H<sub>2</sub>SO<sub>4</sub> in methanol, Miao & Wu (2006) obtained a biodiesel with a heat of combustion of 35.4 MJ/L which is in the range of diesel fuel (36-38 MJ/L).
3. As the cetane number, the viscosity increases with the number of carbon and decreases with the degree of unsaturation (Knothe, 2005b). A higher kinematic viscosity would create engine problems like engine deposits (Knothe & Steidley, 2005a). Transesterification favours a decrease in the viscosity of the oil at values usually between 4 to 6 mm<sup>2</sup>/s (40°C) (National Renewable Energy Laboratory, 2009).
4. Oxidation of biodiesel could happen when the FAME are in contact with oxygen and are transformed into hydrogenperoxides, aldehydes, acids and other oxygenates, which might form deposits (Knothe, 2005). Oxidation of the biodiesel increases as a function of the degree of unsaturation (Hart Energy Consulting, 2007). Oxidation stability of microalgae lipids is therefore a real problem (Stansell *et al.*, 2011) that can be overcome by adding antioxidants if the biodiesel blend is stored more than a few months (National Renewable Energy Laboratory, 2009).
5. Cold flow properties are important parameters for biodiesel production for northern countries like Canada and could be measured by cloud and pour points. The decrease of temperature could lead to the formation of visible crystals ( $d \geq 0.5 \mu\text{m}$ ) in the biodiesel at a limit called cloud point (Knothe, 2005). Cloud point temperature decreases with the mole fraction of unsaturated compounds and slightly increases with the length of the carbon chain (Imahara *et al.*, 2006). Pour point is defined as the temperature at which biodiesel does not flow anymore. Usually, cloud and pour points increase as a function of the molar ratio of biodiesel in diesel fuel from 0 to 100% (National Renewable Energy Laboratory, 2009). A higher level of polyunsaturated compounds in microalgae biodiesel could be a benefit in terms of cold properties (cold and pour points) for a blend microalgae biodiesel/petrodiesel in cold climates.
6. The definition of lubricity for a fuel is "the ability to reduce friction between solid surfaces in relative motion" (Chevron Corporation, 2007; Shumacher, 2005). The lubricity of diesel fuel is influenced by the viscosity, the acidity, the water content and the sulphur compounds (Seregin *et al.*, 1975). Even with additives, the measured friction (no unit reported) of biodiesel (0.114 and 0.117) is lower than the one of petrodiesel

Fuel property	Microalgae biodiesel	References	Diesel	References	Biodiesel	References
Heating value (MJ/L)	35.4	(Miao & Wu, 2006)	36 to 38	(National Renewable Energy Laboratory, 2009)	32 to 36	(Demirbas, 2008; Srivastava & Prasad, 2000)
Cetane Number	39-54 <sup>b</sup>	(Stansell <i>et al.</i> , 2011)	40 to 55	(Lapuerta <i>et al.</i> , 2008)	45 to 70	(Bozbas, 2008; Srivastava & Prasad, 2000)
Kinematic viscosity (at 40°C, mm <sup>2</sup> /s)	3.87-5.2	(M. B. Johnson & Wen, 2009; Miao & Wu, 2006)	1.9 to 3.8	(Bozbas, 2008)	2.8 to 5.7	(Demirbas, 2008; Srivastava & Prasad, 2000)
Specific gravity (15.5°C)	0.864	(Miao & Wu, 2006)	0.81 to 0.86	(Lapuerta <i>et al.</i> , 2008)	0.86 to 0.89	(Alptekin & Canakci, 2008; Srivastava & Prasad, 2000)
Sulfur (wt.%)	0.0069	(M. B. Johnson & Wen, 2009)	0.01 to 0.04	(Bozbas, 2008)	0.0000 to 0.0024	(Bozbas, 2008)
Flash point (°C)	115-204	(M. B. Johnson & Wen, 2009; Miao & Wu, 2006)	60 to 80	(National Renewable Energy Laboratory, 2009)	96 to 190	(Srivastava & Prasad, 2000)
Cloud point (°C)	Not reported		-20 to 0	(Lapuerta <i>et al.</i> , 2008)	-3 to 15	(National Renewable Energy Laboratory, 2009; Srivastava & Prasad, 2000)
Pour point (°C)			-35 to -15	(National Renewable Energy Laboratory, 2009)	-5 to 10	(Tyson, 2001)
Oxidation stability (h)					3 max <sup>c</sup>	(ASTM Standard D6751-10, 2010)
Lubricity (25°C)			0.509 to 0.238 <sup>d</sup>	(Knothe & Steidley, 2005b)	0.114 <sup>d</sup>	(Knothe & Steidley, 2005b)

a Calculated

Table 1. Properties of microalgae biodiesel, diesel and biodiesel

(0.238 and 0.210) for 25 and 60°C (Knothe & Steidley, 2005b). Consequently, a benefit of adding biodiesel in conventional low sulphur diesel fuel is to improve lubricity (Muñoz *et al.*, 2011). For microalgae biodiesel, no lubricity study, to our knowledge, was reported from the literature.

## 5. Standards

European (EN 14214) and American standard (ASTM D6751-10) differ on some levels for biodiesel. For example, EN 14214 stipulates that, polyunsaturated ( $\geq 4$  bonds) ester content and linoleic ester content must be less than 1% (mol/mol) and 12% (mol/mol), respectively (Knothe, 2010). These restrictions are important for biodiesel production from microalgae as the following ester composition is often rich in polyunsaturated content and over these levels (M. B. Johnson & Wen, 2009; Koberg *et al.*, 2011). Some other parameters of EN 14214 are not present in ASTM D6751-10 like esters content, density, iodine value and fatty acid contamination.

EN 14214 is more restrictive for biodiesel from microalgae, as the standard of cetane number is higher (51 vs 47), the maximum viscosity is lower (5 vs 6 mm<sup>2</sup>/s) and the oxidative stability must be higher (6 vs 3 h). Consequently, the ASTM D6751 standard seems to be more adapted to biodiesel from microalgae.

Despite the fact that many studies produced biodiesel from microalgae, few studies ensure that the latter satisfies the official standards (M. B. Johnson & Wen, 2009; Miao & Wu, 2006). Producing biodiesel from *Schizochytrium limacinum*, Johnson & Wen (2009) reported a biodiesel that failed to meet the Grade S15 ASTM standard of water and sediments content of 0.05% (v/v) max (0.1% (v/v)) and sulfur content of 15 ppm max (69 ppm) but satisfied the ASTM acid number of 0.5 mg KOH/g max (0.11 mg KOH/g), free glycerine of 0.020% (w/w) max (0.003% w/w), total glycerine of 0.240% (w/w) max (0.097% w/w), corrosiveness to copper of no. 3 max (1a), kinematic viscosity (40°C) between 1.9-6.0 (3.87 mm<sup>2</sup>/s) and flash point (closed cup) of 93°C min (204°C). In another study, Miao & Wu (2006) satisfied to meet the Grade S15 ASTM standard of flash point (closed cup) (115°C), viscosity (5.2 mm<sup>2</sup>/s, 40°C) and acid number (0.374 mg KOH/g). However, based on our present knowledge, methanol content (EN 14110), sulphated ash (D874), cloud point (D2500), cold soak filterability, P content (D4951), distillation temperature (D1160), sodium and potassium (EN 14538), oxidative stability (EN 15751) have not been measured for microalgae biodiesel, as microalgae biodiesel is relatively new.

## 6. Conclusion

With the increase of the price of crude oil in the late 00s, blending biodiesel with petrodiesel appears a sustainable solution to reduce the dependency on oil producing countries. For now, the 1<sup>st</sup> generation of biofuels currently used could have economic, environmental and social negative consequences. To overcome these problems, producing biodiesel from microalgae lipids seems to be a sustainable solution as microalgae could be used to reduce the CO<sub>2</sub> emissions from coal power plants or wastewater pollution. Researchers are working to engineer super lipids producing microalgae strain in order to increase the yield of biodiesel. Producing biodiesel by transesterification of lipids remains relatively costly compared to 1<sup>st</sup> generation biodiesel. Moreover, the polyunsaturated content of these

biodiesels could be profitable for cold properties, but seems to cause problems with the cetane number and the oxidative stability to meet the American standard (ASTM). Thus, more efforts must be made to reduce the process costs and to increase the biodiesel quality.

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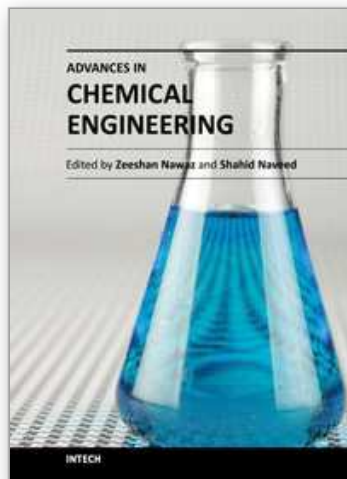


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Chemical engineering applications have been a source of challenging optimization problems in terms of economics and technology. The goal of this book is to enable the reader to get instant information on fundamentals and advancements in chemical engineering. This book addresses ongoing evolutions of chemical engineering and provides overview to the state of the art advancements. Molecular perspective is increasingly important in the refinement of kinetic and thermodynamic modeling. As a result, much of the material was revised on industrial problems and their sophisticated solutions from known scientists around the world. These issues were divided into two sections, fundamental advances and catalysis and reaction engineering. A distinct feature of this text continues to be the emphasis on molecular chemistry, reaction engineering and modeling to achieve rational and robust industrial design. Our perspective is that this background must be made available to undergraduate, graduate and professionals in an integrated manner.

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