Biodiesel production from microalgae

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Abstract

By 2020, according to several government policies like the European Union countries, road transportation fuels must contain at least 10% (v/v) biofuel like biodiesel. Consequently, the world biodiesel production is expected to rise in the next years. However, most biodiesel is produced from vegetable oils, which compete with human food production. Biodiesel from microalgae could help to reach the requested level of biofuel (biodiesel) without endangering the world food supply because microalgae cultivation does not compete with arable land. Nevertheless, the cost of biodiesel production from microalgae must be lowered. One of the main challenges is to extract the lipids from the microalgae and to transform them into biodiesel.

The 1st objective of this study was therefore to compare chloroform-methanol-water and hexane as solvents for *Nannochloropsis Oculata*, *Isochrysis Galbana* and *Pavlova Lutheri* microalgae lipid extraction. The 2nd objective was to transform the lipids into biodiesel by an acid catalysed (acetyl chloride) transesterification. The results obtained demonstrated that a lipid yield of 32% (w/w) could be obtained by an extraction with chloroform-methanol-water without reflux. With hexane reflux, the lipids extracted from the microalgae reached 22% (w/w). The fatty acid methyl ester (FAME) composition was not influenced by the reflux (chloroform-methanol-water) during the solvent extraction. The main FAME weight composition (% wt.) obtained from an acid catalyzed transesterification (100ºC, 1h) were methyl palmitoleate (56-58%), methyl palmitate (12-14%) and methyl eicosapentaenoate (9.6-10.1%).

Keywords: biodiesel, microalgae, extraction, lipids, transesterification, FAME.

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1 Introduction

Oil is a major source of energy. From 1999 to 2009, the world crude oil consumption increased from 75.6 to 84.1 million barrels per day [1]. Consequently, the quest for new petroleum deposits harder to extract increases the risk of environmental disasters like the 2010 oil spill in the United States of America [2]. Moreover, the use of fossil-fuels has a negative impact on the global carbon mass balance.

In order to reduce their fossil-fuel consumption, several governments like European Union countries have formulated policies, which state that petroleum-based fuels must contain at least 10% (v/v) biofuel (bioethanol and biodiesel) by 2020 [3]. Other countries like the United States of America intend to increase the annual biofuel production from 9 to 36 billion gallons between 2008 and 2022 [4]. It is to be noted that the world annual biodiesel production has increased from 1.2 to 4.7 billion gallons between 2005 and 2009 [5]. However, the fact that most biodiesel is produced from oleaginous materials such as soybean cultivated on arable land could lead to land pollution, world hunger and deforestation [6]. Biodiesel from microalgae seems an interesting avenue to replace vegetable-based biodiesel because microalgae culture does not compete with food crops. However, the production costs of microalgae biodiesel are still high, between 2.4 to 6.6 $US/L [7].

Some studies have used direct transesterification (without prior lipid extraction) to produce biodiesel from microalgae [8, 9], but this process requires more energy (up to 2.5 times) for microalgae drying than a process with prior extraction [10].

Intending to replace lipid chloroform-methanol-water extraction, some studies used Soxhlet hexane extraction to extract lipids from *Neochloris oleabundans* microalgae and obtained a high lipid yield of 56% (w/w), compared to the values generally found in the literature (35-65% w/w) [11]. However, Soxhlet extraction requires an important amount of energy [12].

The main goal of this study is to compare the existing extraction methods to determine which is the most suitable to extract lipids from a blend of microalgae (*Nannochloropsis Oculata, Isochrysis Galbana* and *Pavlova Lutheri)*. The 2nd purpose of this study is to determine if the fatty acid methyl ester (FAME) content obtained from an acid transesterification is suitable for biodiesel production.

2 Materials and methods

2.1 Feedstock and solvent

A frozen microalgae blend (*Nannochloropsis Oculata, Isochrysis Galbana* and *Pavlova Lutheri)* was supplied by Nutrocéan Canada (Rimouski, Canada). Table 1 presents the elemental composition of the microalgae. Frozen microalgae were lyophilized at -50ºC using a Virtis specimen freeze drier (model 24DX24, Gardiner, New York, USA) for 5 days and lyophilized microalgae were stored at -20ºC.

Table 1: Elemental composition of frozen microalgae.

	% (w/w)
Moisture	83.9±0.05
Solids	16.1±0.05
Volatile matter	12.8±0.30
Ash	3.3±0.25

Chloroform ($CHCl_3$), methanol (MeOH) and hexane (C_6H_{14}) certified ASC solvents were supplied by Fischer Scientific inc. (Canada) while acetyl chloride (CH_3COCl) (99% pure) was supplied by Fluka (Oakville, USA).

2.2 Lipid extraction

Table 2 summarizes the methodology of all the lipid extraction experiments. Chloroform-methanol-water extraction was performed in experiments 1 and 2 using a modified procedure of Lee *et al.* [13]. Hexane extraction was used in a 3rd experiment. Briefly, in the 1st and 2nd experiments, lyophilized microalgae were dissolved in 100 mL of chloroform-methanol (1:1, v/v). In the 2nd experiment, the blend (solvents-microalgae) was heated under reflux for 5 min and then cooled off, while in the 1st experiment no heating of the blend was applied. For both experiments, the blend was then transferred into a separation funnel and 100 mL of water was added. The separation funnel was shaken for 1 min and the bottom phase (chloroform-lipids) was recovered after settling (1h).

Table 2: Lipids extraction experiments.

Exp.	Solvent treatment	Reflux	Solvent addition	Separation
1	Chloroform-methanol	No	Water	Settling
2	Chloroform-methanol	Yes	Water	Settling
3	Hexane	Yes		Filtration

In the 3rd experiment, the lyophilized biomass was dissolved in 100 mL of hexane and heated under reflux. The blend was cooled off and filtered with a filter paper (Whatman #1).

For all the experiments, the solvent was evaporated under vacuum at 60ºC and the lipids were quantified using an electronic scale (Mettler Toledo AT200). All the lipid extraction results were a mean of 3 replicates and expressed as a function of the dry weight. One-way ANOVA and Turkey tests ($P<0.05$) were used to compare the different treatments.

2.3 Transesterification of lipids and biodiesel recovery

Crude lipids obtained were transesterified using a modified procedure of Lepage and Roy [14]. Four mL of a freshly made methanol-acetyl chloride (100:5, v/v) solution was added to the extracted lipids. The blend was heated at 100ºC (water bath) for 1h (with agitation at 1200 rpm) under a reflux. The excess of methanol was evaporated at 60ºC under a vacuum. Then, 20 mL of hexane was used to

separate biodiesel from other components (mainly glycerol and chlorophyll insoluble in hexane) [12]. Hexane was evaporated at 60ºC under vacuum.

2.4 Analytical methods

The FAME qualitative composition of biodiesel was determined using a Varian-3800 gas chromatograph equipped with a mass spectrometer (Varian Inc, Canada). An external standard of 37 FAMEs (Supelco, 18919-1AMP) was used for FAME identification. One μL of hexane-biodiesel was injected in a DB-225 cyanopropylphenyl-dimethylpolysiloxane capillary column (30m x 0.25mm I.D., 0.25μm film thickness). Helium, used as carrier gas, was set to a flow rate of 2.2 mL/min with a split ratio of 100:1. The oven was heated at 70ºC for 1 min, from 70 to 180ºC at 20ºC/min, from 180 to 220ºC at 3ºC/min and maintained at 220ºC for 1 min. The FAME quantitative composition was determined using a flame ionisation detector (FID), and external standards (Supelco, GLC-10, GLC-50, GLC-80, 18913-1AMP). Pure hydrogen gas (H_2) was used to feed the FID at a temperature of 240°C and nitrogen was used as a make-up gas at a flow rate of 30 mL/min. The FAME composition was an average of 3 replicates. The lipid yields of different treatments were compared using one-way ANOVA and Tukey tests with $P<0.05$.

2.5 Cetane number

The cetane number is an indicator of the quality of a fuel ignition [15]. The cetane number of the biodiesel was estimated from correlations with the FAME weight percentage as follows [16]:

$$CN = 1.068 \cdot \sum (CN_i m_i) - 6.747 \quad (1)$$

where CN is the cetane number, CN_i is the cetane number of $FAME_i$ and m_i is the mass percentage of $FAME_i$. The cetane number of individual FAME (CN_i) was estimated as follows [17]:

$$CN_i = -23.523 + n \cdot (2.366 + 6.299 \cdot e^{-0.411 \cdot db}) \cdot e^{-0.018 \cdot n} \quad (2)$$

where db is the number of double bounds on the acid chain and n is the acid chain length. Table 3 presents the cetane number of individual FAME.

Table 3: Cetane number of different individual FAMEs.

FAME	Cetane number
C14:0	70.8
C16:0	80.4
C16:1	55.0
C18:1	61.6
C18:2	43.3
C18:3	31.2
C20:4	26.5
C20:5	20.7
C22:6	19.4

3 Results and discussion

3.1 Extraction of lipids

Table 4 presents the results for both chloroform-methanol-water and hexane extractions. The lipid content obtained from the microalgae blend varied from 22 to 33% (w/w).

Table 4: Results of chloroform-methanol-water and hexane extraction.

	Lipid content (% w/w)
Chloroform-methanol-water	32.0±1.2
Reflux chloroform-methanol-water	33.0±0.1
Reflux hexane-filtration	22.4±0.6

In a study using water-chloroform-methanol (2:1:1, v/v) to extract lipids from different lyophilized microalgae (*Botryococcus sp., Chlorella vulgaris, Scenedesmus sp.*), Lee *et al.* [13] used several pre-treatments in water phase (autoclave, microwave, sonication, osmotic shock) and obtained a lipid content from 1.8 to 28.6% (w/w). Consequently, the lipid content (32-33% w/w) obtained by chloroform-methanol-water extraction from the blend of microalgae (*Nannochloropsis Oculata, Isochrysis Galbana* and *Pavlova Lutheri*) in the present study seems to be promising for biodiesel production as some *Botryococcus species* like *Botryococcus braunii* used in Lee *et al.* [13] study can contain up to 75% (w/w) lipids.

No significant difference was observed between both chloroform-methanol-water lipid extractions (1st and 2nd experiments). Consequently, chloroform-methanol-water extraction could be used to obtain lipids without reflux.

Both chloroform-methanol-water extractions gave a significant higher lipid yield (32.5±0.5% w/w) than hexane extraction under reflux (22% w/w). The fact that a lower lipid content was obtained with hexane extraction compared to chloroform-methanol-water could be the consequence of the non-polar nature of hexane. In fact, some polar lipids like glycolipids and phospholipids could not be extracted with hexane. It is known that some microalgae like *Nannochloropsis oculata and Isochrysis* species can contain up to 77 and 62% (w/w) of glycolipids and phospholipids (combined), respectively [18]. Some studies used hexane-isopropanol instead of chloroform-methanol-water to extract *Botryococcus braunii* (UTEX 572) lipids and obtained a 1.4 times lower lipid content [19].

3.2 FAME composition

Figure 1 presents the FAME composition of the biodiesel produced for both chloroform-methanol-water. The weight composition remains relatively constant no matter if a reflux was used or not to recover the lipids in microalgae. The

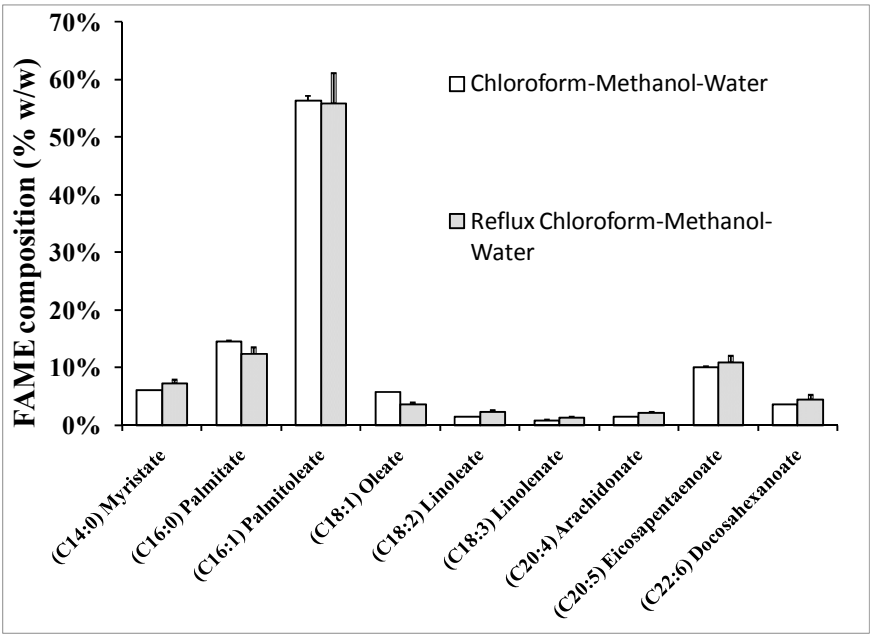

Figure 1: FAME weight composition as a function of the extraction method.

main FAME components were (% wt.): methyl palmitoleate (55.7-58%), methyl palmitate (12.3-14.5%), methyl eicosapentaenoate (9.6-10.9%) and methyl docosahexanoate (6.1-7.3%).

As seen in Figure 1, using a reflux chloroform-methanol-water for microalgae lipid extraction did not influence the FAME composition of the biodiesel. Based on our present knowledge, no study has compared the difference between using a reflux or not in chloroform-methanol-water extraction in terms of FAME composition. However, other studies have found that the nature of the extraction solvent (hexane, alcohol, etc.) does not influence the FAME composition [20].

The fact that around 80% (w/w) of the FAME was composed of monounsaturated (MFA) and saturated (SFA) FAME is interesting for biodiesel production. In fact, the main problem with biodiesel produced from microalgae is polyunsaturated FAME (PFA) (>2 double bounds) content up to 59% (w/w) [9, 16, 21] that causes a low cetane number and poor oxidation stability [16].

The cetane number approximated by calculation was 50.8±0.8, which is over the ASTM standard of 47 [22]. Other studies estimated the cetane number of several microalgae such as *Isochrysis galbana, Odontella weissflogi* and *Chaetoceros* sp. with a similar method and found cetane numbers ranging from 39 to 54 [16].

Based on our present knowledge, there is no model to estimate the oxidation stability, but antioxidants can be added to biodiesel from microalgae to increase the oxidation stability [22].

4 Conclusion

In order to replace conventional oleaginous vegetable-based biodiesel, which is among others a risk for food security, the lipid extraction from a blend of microalgae (*Nannochloropsis Oculata, Isochrysis Galbana* and *Pavlova Lutheri*) was studied. The lipid content obtained was 32% (w/w) for chloroform-methanol-water extraction while hexane reflux extraction conducted to 22% (w/w).

The FAME content of the biodiesel produced after chloroform-methanol-water lipid extractions with and without reflux showed good properties in terms of cetane number (50.8) and oxidation stability as the PFA content was low at around 20% (w/w).

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