Characterization of the Fatty Acid Composition of *Nannochloropsis salina* as a Determinant of Biodiesel Properties

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Nannochloropsis salina was cultured batch-wise to evaluate the potential of the alga to produce biodiesel. The cells were harvested at the end of the exponential growth phase when the concentration was $18 \cdot 10^6$ cells/mL culture. The growth estimated as dry weight from this cell number was (3.8 ± 0.7) mg/L. The lipid and triglyceride contents were 40% and 12% on a dry weight basis, respectively. The amount of the ratio triglycerides/total lipids was approximately 0.3.

The composition of triglyceride fatty acid methyl esters (biodiesel) was analysed by gas-liquid chromatography and identified as: C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:1, and C20:5. The ratio of unsaturated to saturated fatty acid contents was approximately 4.4. Additionally, the characterization of each individual fatty acid ester was discussed with regard to the fuel properties of biodiesel produced by the alga.

Key words: Biodiesel, Lipids, *Nannochloropsis salina*

Introduction

The use of biofuels can play an important role in avoiding excessive dependence on fossil fuels and reducing pollution by greenhouse gases emission (Gouveia and Oliveira, 2009). Biodiesel mainly consists of fatty acid methyl esters (FAMEs), which are produced by the transesterification of biologically derived lipids (Wackett, 2008). Biodiesel has received considerable attention in recent years since it is a renewable and nontoxic fuel. Moreover, biodiesel contributes no net carbon dioxide or sulfur oxides to the atmosphere and emits less gaseous pollutants than normal diesel (Vicente *et al*., 2004).

Vegetable oils of different crops such as soybean, canola, and oil palm are used as a feedstock for the production of biodiesel (Schmidt, 2007).

However, this leads to the consumption of valuable nutritive crops which has been blamed for food price increases, threatening food security and putting tremendous burden on the poor (Boddiger, 2007). Microalgae are characterized by higher biomass production and faster growth compared to other energy crops. Moreover, algal oil is an interesting alternative to popular feedstocks of nutritive crops (Minowa *et al*., 1995), since it does not compete with these traditional foods (Chen *et al*., 2009).

Depending on species, many microalgae have been described as lipid-rich strains for their hydrocarbons and other complex oils. However, not all algal oils are satisfactory for the production of biodiesel (Guschina and Harwood, 2006), due to the development of standards to ensure high product quality. The nature of both the fatty acid and the alcohol in fatty acid esters influences the fuel properties of biodiesel (Knothe, 2005, 2008). Therefore, the evaluation of algal oil fatty acid properties is an important criterion for a substitute of diesel fuel (da Silva *et al*., 2009). Some microalgae have suitable fatty acid profiles and an unsaponifiable fraction allowing the biodiesel production with high oxidation stability (Dote *et al*., 1994; Ginzburg, 1993; Minowa *et al*., 1995). The fuel properties of biodiesel from microalgal oil (*e.g*. density, viscosity, acid value, heating value) are comparable to those of fuel diesel (Miao and Wu, 2006; Rana and Spada, 2007).

In the present study we employed the eustigmatophycean microalga *Nannochloropsis salina* that may fulfill the needs addressed above. The alga is commonly used for lipid generation and can tolerate different environmental conditions (Mohammady *et al*., 2005; Boussiba *et al*., 1987) in comparison to other microalgae. Additionally, it is widely distributed and native to the Mediterrane-

an area (Caron *et al*., 1999). For these reasons, *N. salina* is recommended for commercial purposes. Therefore, in this paper we try to estimate the potential of batch-grown *N. salina* for producing lipids and triglycerides.

The fatty acid ester composition of the biodiesel obtained from the alga was also determined. Although the biodiesel properties were not determined here experimentally, the potential fuel properties resulting from the fatty acid ester composition are discussed.

Material and Methods

Algal strain and culture conditions

Nannochloropsis salina was obtained from the Solar Energy Research Institute Culture Collection in Golden, CO, USA. The algal material was grown axenically in Boussiba's enriched seawater (Boussiba *et al*., 1987). Culture flasks were continuously agitated by bubbling with sterile air, enriched with 0.5% CO_2, at (25 ± 1) °C in a temperature-controlled room. Illumination was provided with an irradiance of 300 μmol/(m^2 s) under a 16 h/8 h light/dark regime.

Growth evaluation

Algal growth was measured by daily counting the cell number under a microscope with a haemocytometer. Dry weight (DW) was determined gravimetrically on 10 mL culture samples, from which cells were harvested, washed, and dried at 90 °C. DW data resulted from the average of a minimum of three representative samples.

Total lipids and triglycerides extraction

At the end of the logarithmic growth phase of algal cultures (day 13), the cells were harvested by centrifugation and divided into two portions. The first portion was used for total lipids determination and the second one for triglycerides determination. For total lipids determination, the cells were extracted with chloroform/methanol (2:1, v/v), according to Bligh and Dyer (1959). The mixtures were transferred into a separatory funnel and shaken for 5 min. The lipid fractions were separated from the funnel and the solvent evaporated by rotary evaporation. For triglycerides (TG) extraction, the second portion of the algal mass was extracted using *n*-hexane (Miao and Wu, 2006), and the microalgal oil was purified by thin layer chromatography (TLC) according to Parrish (1999). The weight of both total lipids and triglycerides obtained was determined, and average weights were calculated.

Fatty acid composition of triglycerides

The triglyceride extract was esterified according to Radwan (1991) and analysed in a Shimadzu gas-liquid chromatograph (Kyoto, Japan), equipped with a flame ionization detector and Hp-5 column material (Agilent, Santa Clara, CA, USA). The carrier gas was nitrogen and the flow rate was 5 mm/min. Identification of the FAMEs was carried out by comparing their retention times with those of standards. Quantification was based on the internal standard method.

Statistical analysis

All analyses were carried out in triplicate, and the standard deviations (SD) were determined.

Results and Discussion

Growth, lipids and triglycerides contents

Algae grew up to a density of 18 · 10^6 cells/mL culture, corresponding to a DW of (3.8 ± 0.7) mg/L. Lipids and triglycerides (TG) contents were 40% and 12% of the DW, respectively. Accordingly, the ratio of triglycerides/total lipids was approximately 0.3 (Table I). Although the data indicated a relatively high amount of lipids, the concentration of the associated triglycerides was relatively small.

Thus, the alga appears to be more suitable for other liquid fuel applications rather than biodiesel.

Table I. Cell density and lipid and triglyceride (TG) contents determined at the end of the exponential growth phase of *N. salina*.

Cell number [· 10^6/mL]	DW [mg/L]	Lipid content (% DW)	TG content (% DW)	TG/lipid ratio
18	3.8 ± 0.7	40	12	0.3

Table II. Characterization of triglyceride fatty acid methyl esters (biodiesel) of *N. salina*.

FAME[a]	Content (%)[b]	b.p. (°C)[c]	Cetane number[d]	Kinematic viscosity[e] [mm^2/s]	HG[f] [kcal/mol]
C14:0 (myristic)	4	250.5	66.2	3.23	2254
C16:0 (palmitic)	6	350	74.5	4.32	2550
C16:1 (palmitoleic)	16.7	286	45	3.67	2657.4
C18:0 (stearic)	7.5	360	86.9	5.85	2696.12
C18:1 (oleic)	12.9	218.5	55	4.51	2828
C18:2 (linoleic)	12.8	215	36	3.65	nr[g]
C18:3 (linolenic)	12	230	28	3.14	nr
C20:1 (gadoleic)	9	265	nr	5.77	3150
C20:5 (EPA)	13.1	nr	nr	nr	nr
Unknowns	6	nr	nr	nr	nr

[a] Fatty acid methyl ester.
[b] Values obtained from three parallel measurements with SD = ± 1 for C14:0 and C16:0, and ± 0.2 for the remaining fatty acids. The ratio of unsaturated to saturated fatty acid contents was approximately 4.4.
[c] Boiling point data from Weast (1986); Gunstone *et al*. (1994); Schenk *et al*. (2008).
[d] Cetane number data from Schenk *et al*. (2008).
[e] Kinematic viscosity data from Knothe (2005); Knothe and Steidley (2005); Gouw *et al.* (1966).
[f] Heat of combustion data from Freedman and Bagby (1989); Weast (1986).
[g] Not reported.

Gouveia and Oliveira (2009) reported more than 28% oil of the DW for *Nannochloropsis* sp. However, when the alga was re-inoculated in N-deficient medium, a large increase in the oil quantity (~50%) was observed. In this respect, manipulating the cultivation conditions of *N. salina* could increase its oil amount.

Triglyceride fatty acid methyl esters

The composition of triglyceride fatty acid methyl esters (biodiesel) of *N. salina* is presented in Table II. Both saturated and unsaturated components were detected, besides some unknowns. The unsaturated fractions were identified as C16:1 (palmitoleic acid methyl ester, 16.7%), C18:1 (oleic acid methyl ester, 12.9%), C18:2 (linoleic acid methyl ester, 12.8%), C18:3 (linolenic acid methyl ester, 12%), C20:1 (gadoleic acid methyl ester, 9%), and C20:5 (eicosapentaenoic acid, EPA, methyl ester, 13.1%). On the other hand, the saturated ones are composed of C14:0 (myristic acid methyl ester, 4%), C16:0 (palmitic acid methyl ester, 6%), and C18:0 (stearic acid methyl ester, 7.5%). The ratio of unsaturated to saturated fatty acids was approximately 4.4.

In this study, the composition of FAMEs of *N. salina* was found to be in agreement with the general distribution pattern of fatty acids in other microalgae (Meng *et al*., 2009). Variation in fatty acid concentrations is determined by many factors including species, culture age, and extraction method (Gouveia and Oliveira, 2009; Tran *et al*., 2009).

Biodiesel quality

Diesel fuel consists mostly of linear and branched alkanes with carbon chain lengths between C_{10} and C_{20} (Whyte *et al*., 1998).

Fatty acids comprising biodiesel are mainly composed of palmitic, stearic, oleic, and linolenic acids (Knothe, 2008). In this study, the chain lengths of *N. salina*'s biodiesel ranged from C_{14} to C_{20} with the composition indicated above. The properties of a biodiesel fuel are mainly characterized by its ignition quality, heat of combustion, cold flow properties, oxidative stability, and kinematic viscosity (Stournas *et al*., 1995). These properties are strongly influenced by the structural features of the fatty acid esters which are based on the type, concentration, and the alcohol moieties (Knothe, 2008).

Here we describe some of the properties of the fatty acid constituents of *N. salina* biodiesel.

Cold flow: Biodiesel fuels containing significant amounts of saturated fatty acids will display higher cold flow properties. On the other hand, higher levels of polyunsaturated fats lower the cold filter plugging point, the temperature at which the fuel starts to form crystals/solidifies and blocks the fuel filters of an engine (Knothe, 2005). The

amount of saturated fatty esters of *N. salina* is about 17.5%. In this correlation, as assumed by Knothe (2008), the cloud point may be well above 0 °C. Consequently, cold flow of *N. salina*'s biodiesel is not considered to be good.

Cetane number: The cetane number is a value describing the combustion quality of diesel fuel during compression ignition. Higher cetane fuels have shorter ignition delay periods than lower cetane fuels. Therefore, it is important to ensure that the cetane number of biodiesel meets the engine cetane rating (Knothe, 2005). The ideal mixture of fatty acids has been suggested to be C16:1, C18:1, and C14:0 in the ratio 5:4:1. Such a biodiesel would have the properties of very low oxidative potential (Schenk *et al*., 2008). In the present work, this ratio is nearly 4:3:1. Accordingly, the concentration of both C16:1 and C18:1 acids of *N. salina* needs to be increased either by means of genetic engineering or manipulating the cultivation conditions. Alternatively, Gouveia and Oliveira (2009) suggested the addition of other oils to the microalgal oil to improve its quality.

Viscosity: Viscosity affects the atomization of a fuel upon injection into the combustion chamber and thereby ultimately the formation of deposits. Viscosity increases with increasing fatty acid chain length and degree of saturation. The higher the viscosity the greater the tendency of the fuel to cause such problems (Knothe, 2005). The viscosity of the methyl esters is lower than that of the ethyl or branched ones. The major advantage for fatty acid estrification by methanol is the lower price compared to other alcohols (Knothe and Steidley, 2007). In this study, the kinematic viscosity of the fatty acid methyl esters of *N. salina* is within the biodiesel standards (1.9–6.0 mm^2/s; Gouw *et al*., 1966).

Heat of combustion: Gross heat of combustion (HG) is a fuel property indicating the suitability of fatty acid esters as diesel fuel. The standard HGs are in the range of 1300–3500 kcal/mol for C_8–C_{22} fatty acid esters (Bridgwater and Maniatis, 2004). However, in this study, the fatty acid chain lengths range from C_{14} to C_{20}, and HGs are in the range of 2254–3150 kcal/mol. Accordingly, in order to improve the HGs of biodiesel, *N. salina*'s oil must be enriched with certain fatty acids.

Overall view of previous studies revealed that cetane number, heat of combustion, melting point, and viscosity of fatty acid methyl esters increase with increasing chain length and decrease with increasing degree of unsaturation (Knothe, 2005). Reducing the saturated fatty acids content of plant oil can improve the cold temperature properties of the biodiesel derived from it (Serdari *et al*., 1999; Stournas *et al*., 1995). Therefore, the proper percentage of saturated and unsaturated fatty acids is very important to the use of microalgae as a biodiesel feedstock (Deng *et al*., 2009). Rashid *et al*. (2008) found that oils with high oleic acid content have a reasonable balance of fuel properties. The addition of methyl oleate has been suggested to improve the oxidative stability and to lower the melting temperature (Knothe, 2008). In this study, a considerable amount of oleic acid has been detected in *N. salina*. However, the algal oil needs to be enriched with some short-chain fatty acids, and the ratio of saturated to unsaturated fatty acids needs to be changed as well.

Conclusions and Future Work

Although *N. salina* is described by many authors as a single cell lipid producer, the content of its oil is relatively low. We assume that the alga is suitable for other liquid fuels rather than biodiesel applications. Therefore, at the moment, biodiesel production by *N. salina* is not practical at the economical level. In order to improve the biodiesel fuel quantity and quality, the algal oil must be enriched with certain fatty acids by up-regulation of fatty acid biosynthesis and/or by down-regulation of β-oxidation. This could be achieved by means of genetic engineering and/or manipulating the cultivation conditions. However, supplementation of *N. salina*'s oil with other short-chain fatty acid esters may be of interest.

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