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A preliminary study on some *Chlorella* spp. for biodiesel production

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Abstract

This paper describes a preliminary analysis of the possibility to use different algae species for biodiesel production. A lab scale cultivation of five *Chlorella* spp. was conducted to evaluate their potential for biodiesel production, with respect to their growth and fatty acids characterization, as an initial step to transferring them into the outdoor open ponds. The results of algal dry wt (mg/l), arranged in descending order, were *Chlorella salina*, 200 ± 0.02; *Chlorella vulgaris*, 192.28 ± 0.00; *Chlorella stigmatophora*, 162 ± 13.06; *Chlorella capsulata*, 101.08 ± 7.54; *Chlorella marina*, 86 ± 6.99, while the growth rates (mg/d) were *Chlorella marina*, 2 ± 0.17; *Chlorella vulgaris*, 1.78 ± 0.14; *Chlorella stigmatophora*, 1.52 ± 0.11; *Chlorella capsulata*, 1.51 ± 0.13; *Chlorella salina*, 1.16 ± 0.09. The highest lipid content (dry wt based) was recorded for *Chlorella capsulata* (446 ± 0.33 mg/g), while *Chlorella vulgaris* showed the lowest content (255 ± 2.5 mg/g). The amounts of the neutral lipids (dry wt and total lipid based) were found in the range of 14-28%, and 60-80%, respectively. Data showed that *Chlorella salina* was the oil-richest species, while *C. capsulata* was the poorest. The extracted oil was also characterized according to its acid and saponification values. Based on the analysis of fatty acid methyl esters (FAMEs), the carbon chain lengths ranged from C_6 to C_{21}, and most of them were of saturated types. The most important fractions for best quality biodiesel (C14 : 0, C16 : 0, and C18 : 0) were detected in all examined microalgae. The distribution patterns of fatty esters in *C. salina*, *C. marina*, and *C. stigmatophora* were the same. C18 : 1 was not present in *C. capsulata*, while C16 : 1 was completely absent from all species. However, no polyunsaturated fatty acids were detected in this study. The relative molecular weight of FAMEs and the percentage of the free fatty acids were also recorded for each microalga. The study was meant not only to enrich the Chlorella database, but it was also concerned with the potential of the three nonnative strains to adapt to the Egyptian habitats to be cultivated under the same conditions. The results of our studies are thus an important achievement.

Key words: biodiesel, *Chlorella*, fatty acids, growth, lipid, oil

Introduction

Utilization of the current petroleum stock is more than 100 times faster than done in nature (Satyanarayana et al., 2011). Continuous petroleum utilization, with a parallel increase in the world population, will have reduced the fossil fuels sources by 2050 (Demirbas, 2009). Moreover, petroleum-based fuels are the main causes of atmospheric pollution. Consequently, a great attention for alternative energy sources is paid to meet both targets of energy demand and environmental pollution (Hossain et al., 2008).

There has been a growing awareness of bioenergy as an alternative energy source. Bioenergy refers to energy biosource-based made available continually. The practice of renewable energies could contribute to solving problems arising from petroleum consumption as well as global warming concerns (Halder, 2011). Biohydrogen, bio-alcohols, and biodiesel all are different forms of bioenergy.

Biodiesel is an energy carrier liquid fuel manufactured, *in situ*, by the esterification process of cellular lipids of any biological system. Chemically, biodiesel is compo-

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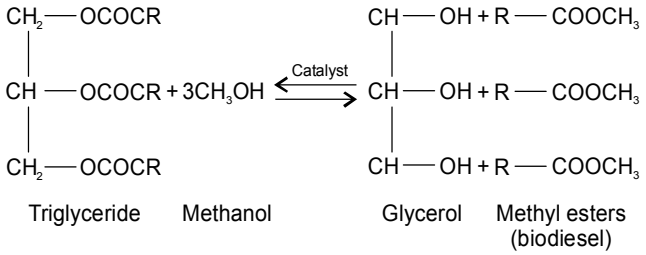

Fig. 1. Chemical reaction of esterification process

sed of fatty acid methyl (ethyl) esters (FAMEs) C16 and C18 (Raj and Kandasamy, 2012; Betiku and Adepoju, 2013). Biodiesel derived from inedible oil sources such as algae is recently recognized to have great benefits (Fig. 1).

Microalgae are organisms researched as a promising source for biodiesel production. The advantages of microalgae over other oleaginous crops (such as olives, palms, peanuts, soya-beans, and sunflowers) are established for a number of reasons (Table 1). Microalgae are easy to cultivate in both fresh and marine waters (Demirbas and Demirbas, 2010), their requirements for nutrients is negligible, and they are characterized by a quick growth rate and a high lipid content (Wu et al., 2012). In addition, algae are a sustainable energy source and can be cost-competitive with other current energy sources (Deng et al., 2009).

Table 1. Oil yield obtained from different oleaginous crops

Crop	Oil yield [l/acre]
* Microalgae	23781-55416
Palm oil	2407
Coconut	1086
Jatropha	764
Canola	480
Soybeans	181
Corn	68

Source: According to Chisti, 2007; * microalgae posited the top of the oleaginous crops list; algal oil content ranges from 30% to 70% of the dry biomass

Oleaginous microalgae can assemble large amounts of triacylglycerols. Moreover, microalgae oil is being viewed as an ideal feedstock for biodiesel manufacture (Hu et al., 2008).

Chlorella is a widespread genus, found in almost all habitats (Wu, 2001). These fast growing microalgae can amass over 20% lipids of their dry wt (Scarsella et al., 2009). Therefore, *Chlorella* possesses great possibilities as a future producer of mechanical biodiesel (Sharma et al., 2012; Yao et al., 2015). But, to prepare *Chlorella* for this purpose, an effort to develop procedures starting from the determination of appropriate strain(s) stage to the production process, to acquire large amounts of biomass and high lipid efficiency, should be undertaken. In this regard, we tried to evaluate the potential of four marine-water strains of *Chlorella* (*C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora* – the last three microalgae were not indigenous Egyptian), and one freshwater *C. vulgaris*) as a preliminary step before transferring the algae into outdoor race ponds (Table 2). In addition, the algae residues, after lipids extraction, were used in aquaculture feeding as described by El-Kassas et al. (2016), and hence considered to be an important value added to the examined *Chlorella* spp.

The objectives were to characterize the microalgal growth, total lipids, and the recovered oily fraction along with its FAMEs contents at the lab scale production.

Materials and methods

Chlorella species

Chlorella marina, *C. capsulata*, and *C. stigmatophora* were collected from Crete, Greece, identified by Dr I. Tzovenis, the National and Kapodistrian University of Athens. *C. salina* was obtained from the marine hatchery of the National Institute of Oceanography and Fisheries in Alexandria, Egypt. The freshwater alga *C. vulgaris* was acquired from the algal collection of Med-algae Lab, the Faculty of Science, Alexandria University. The marine water spp. were grown in the F/2 medium, as described by Guillard and Ryther (1962), while *C. vulgaris* was grown in the freshwater medium as described by Nichols (1973).

Maintenance of Chlorella cultures

Chlorella cultures were grown in batches with a starter inoculum of 1×10^5 cells ml^{-1}. The cultures were grown in 500-ml flasks outfitted with inlet and outlet tubes for air circulation, in a temperature controlled room at $25 \pm 1\,^{\circ}C$, in triplicates. The cultures were dis-

Table 2. Oil yield (%) obtained from different *Chlorella* species cultivated under different conditions

Chlorella name	Oil [%]	Reference
C. sorokiniana	19.3	Rodolfi et al., 2009
C. vulgaris	14-22	Spolaore et al., 2006
C. emersonii	63	Gouveia and Oliveira, 2009
C. vulgaris (CCALA 896)	27.9	Vaičiulytė et al., 2014
C. protothecoides	23-55	Gouveia and Oliveira, 2009
C. vulgaris (CCAP 211/11B)	18	Illman et al., 2000
C. minutissima (UTEX 2341)	31	Illman et al., 2000
C. minutissima	57	Gouveia and Oliveira, 2009
C. sorokiniana (UTEX 1230)	20	Illman et al., 2000
C. emersonii (CCAP 211/11N)	29	Illman et al., 2000
C. protothecoides (CCAP 211/8D)	11	Illman et al., 2000
C. pyrenoidosa	11-26	Nigam et al., 2011
C. sp.	18-57	Gouveia et al., 2009
C. marina	20.3	Muthukumar et al., 2012
C. vulgaris LC8	42.1	Selvarajan et al., 2015
C. sp.	28-32	Kumar and Sharma, 2014
C. fusca	11.67	Del Río et al., 2015
C. zofingiensis	26	Zhu et al., 2013
C. kessleri	51.7	Ota et al., 2016
C. ellipsoidea	37	Purkayastha et al., 2017

turbed with an enriched air with 0.5 CO_2 from a cylinder at 0.4/l/min. Fluorescent lamps with an irradiance of 70 $\mu/m^2/s$ were used to lit the surface of the cultures. Typically, all cultures were grown under a 16 h/8 h light/dark regime. To estimate the *maximum* dry wt and lipid production for each species, on 10^{th} day (late log growth stage), the cells were harvested by centrifugation at 1000 × g for 5 min (using angle rotor centrifuge).

Growth measurements

The growth of *Chlorella* species was growth was monitored every day by measuring the dry wt throughout the growth time of cultures (14 days). To estimate the dry wt, 10-ml culture samples were dried at 105°C for 24 h. The growth rate for each individual species was also calculated.

Gravimetric analysis of total lipid content

Based on the procedure given by Folch and coworkers (1957), 20 ml of algal cultures were harvested and mixed with 10 ml of chloroform:methanol (2 : 1, v/v) for 2 h in a shaking water bath. The homogenate was centrifuged at 1500 g for 10 min. The lipid-containing organic phase was evaporated by a rotary evaporator at 80°C. Cell lipid content was weighted and calculated as percentage of the dry wt.

Recovery of the lipid

Lipid recovery was conducted to obtain the neutral fractions (crud triglycerides). According to the procedure by O'Neil and coworkers (2015), lipid fractions were placed in a porous cellulose thimble and reextracted with *n*-hexane using Soxhlet apparatus for about 18 h. The solvent removal was done by a rotary evaporator, and the neutral lipids (referred as algal oil in this study) were weighed. The amounts of algal oil were calculated as a percentage of the dry wt and total lipid content.

Determination of acid value of oil

About 2-ml oil samples from each strain were dissolved in 50 ml of ethanol. Two drops of phenolphthalein

indicator were appended and the mixtures were titrated against 0.1 N potassium hydroxide solution (KOH). The acid values were determined according to a method by Okpuzor and coworkers (2009).

$$\text{Acid value} = \frac{56.1 \times V \times C}{m}$$

where 56.1 = equivalent weight of KOH; V is the volume of KOH; C is the exact concentration of KOH (0.1 N); and m is the mass of the oil sample. The results are expressed as mg KOH/g of oil.

Determination of the saponification value of oil

According to a procedure by Pearson (1981), a 25-ml ethanolic potassium hydroxide was added to few drops of oil sample and refluxed for 2 h. Successively, 1 ml of phenolphthalein was being added to the mixture and titrated against 0.5 N HCl. The same method was used for blank determination. The saponification value was determined as follows:

$$\text{Saponification value} = \frac{(V_0 - V_1) \times C \times 56.1}{m}$$

where V_0 is the volume of HCl for the blank test; V_1 is the volume of HCl for the sample; C is the concentration of HCl (0.5 N); 56.1 is the equivalent weight of KOH; and m is the mass of the oil sample.

Transestrification of algal oil

About 5 ml of methanolic sulfuric acid and 2 ml of benzene were added to 2 ml of algal oil. The mixture was placed in a water bath at 90 °C for 90 min. After cooling, 8 ml of water and 5 ml of petroleum ether were added, and the solution was stirred vigorously using vortex stirrer for 10 s. The ethereal layers were collected, evaporated to dryness, and then fatty esters were weighed (Radwan, 1991).

Analysis of fatty acid methyl esters

FAMEs were analyzed using gas chromatography (GC Model: HP (Hewlett-Packard) 6890, with FID flame ionization detector) with the detector temperature at 250 °C; injector temperature at 220 °C; injection volume of 3 µl; and split ratio of 50 : 1. Nitrogen was used as the carrier gas with a flow rate of 1 ml/min. The oven temperature was maintained at 150 °C for 2 min, then increased to 200 °C at 10 °C/min, and finally to 250 °C at 5 °C/min and was maintained for 9 min. FAMEs were identified according to their retention times when compared with those of standards (Sigma-Aldrich).

Determination of free fatty acid contents

Free fatty acids (FFA) were determined according to a procedure by Huang and coworkers (2016) from the following equation:

$$\text{FFA\%} = 100 \times \text{AV} \times \text{MW} / 56100$$

where AV is the acid value and MW is the average relative molecular weight of the fatty acids.

Statistical analysis

All experiments were performed in triplicates, and standard deviations (SD) were determined. A statistical analysis was performed using the SPSS software package, version 11.5 (SPSS Inc., Chicago).

Results and discussion

As mentioned earlier, one of the main objectives of this study was to characterize the growth (as one of the most economical criteria for biodiesel production purposes) of the examined strains. Both the daily biomass yield (mg/l) and the growth rate (mg/d) were examined. The growth pattern of the examined *Chlorella* spp. throughout the cultivation period compared with a hypothetical *Chlorella* sourced biodiesel production is shown in Figure 2. Each data point on the curve represents the average of triplicates.

The highest dry wts (mg/l) were recorded as (in descending order): *C. salina* (200 ± 0.02), *C. vulgaris* (192.28 ± 0.00), *C. stigmatophora* (162 ± 13.06), *C. capsulata* (101.08 ± 7.54), and *C. marina* (86 ± 6.99). The growth rates (mg/d) were *C. marina* (2 ± 0.18), *C. vulgaris* (1.78 ± 0.14), *C. stigmatophora* (1.5 ± 0.11), *C. capsulata* (1.51 ± 0.13), and *C. salina* (1.16 ± 0.09). No correlation between the maximum accumulated biomass and growth rate of a specific strain was detected, as shown in Table 3. However, the results of our study are consistent with other reviews by Al-lwayzy et al. (2014) and Reddy et al. (2014) on various *Chlorella* spp. Growth rate is a perspective factor that can be used to assess which algal species have the potential to become a promising possibility for various industrial applications (Araújo and Garcia, 2005). The relatively high growth rate exhibited

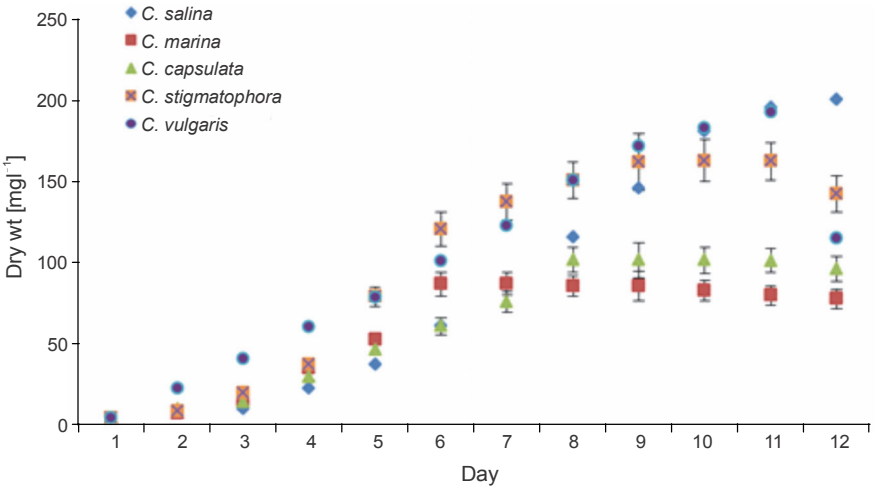

Fig. 2. The growth of tested *Chlorella* species expressed as a dry wt in relation to the time of culture

Table 3. Growth, total lipids, oil content, and characterization of tested *Chlorella* species

	Chlorella spp.				
	C. salina	*C. marina*	*C. capsulata*	*C. stigmatophora*	*C. vulgaris*
Max. biomass concentration [mg/l]	200 ± 0.02	86 ± 0.69	101.08 ± 0.75	162 ± 0.13	192.28 ± 0.00
Growth rate^{-d}	1.16 ± 0.09	2 ± 0.17	1.51 ± 0.13	1.52 ± 0.11	1.78 ± 0.14
Lipid content [mg/g biomass]	268 ± 0.20	267 ± 0.25	446 ± 0.33	355 ± 0.24	255 ± 2.5
Total lipid contents – biomass [%]	26.8 ± 2.08	26.7 ± 2.6	44.6 ± 3.4	25.5 ± 2.5	17.6 ± 2.5
Oil content [mg/g biomass]	280 ± 0.78	182 ± 4.73	137 ± 3.56	151 ± 3.93	195 ± 0.57
Oil-biomass [%]	28 ± 0.73	18 ± 0.47	14 ± 0.36	15 ± 0.39	20 ± 0.52
Oil-total lipid [%]	80 ± 2.08	61 ± 1.59	60 ± 1.56	65 ± 1.69	72 ± 1.87
Acid value	0.393	0.449	0.374	0.224	0.561
Saponification value	50.5	50.5	53.3	39.27	56.1

$^{-d}$ – per day

by the examined algae indicated good adaptation capabilities to the ambient cultivation conditions.

The ability of microalgae to accumulate lipids in appropriate amounts is an important criterion for strain selection as a feedstock for the production of different biofuels, like jet and biodiesel. Determination of quantity and composition of total lipid fractions is an important step to evaluate the algal potential for biodiesel production. Lipids are a large group of heterogeneous compounds containing both neutral and polar fractions. As is well known, total lipids include both structural and storage fractions. Oil (storage lipids) is mainly composed of triglycerides which is the main source of good quality biodiesel production. High triglycerides production levels reflect high biodiesel amount expected to be manufactured.

The main purpose of reextraction of total lipids with *n*-hexane (lipid recovery) was to obtain the neutral lipids fractions (mainly triglycerides that are referred to in this study as algal oil), which constitute the raw material for biodiesel production. The amount and characterization of lipid fractions are presented in Table 3. Lipid generation (dry wt based) was performed from more than 17% to 44%. *C. capsulata* was the most promising lipid producer (446 ± 0.33 mg/g). Both *C. salina* and *C. marina* accumulated a similar lipid amount (26.8 ± 2.08% and 26.7 ± 2.6%, respectively), trailed by *C. stigmatophora* (25.5 ± 2.5%), *C. vulgaris* being the last. The presented

Table 4. FAMEs composition and abundance in tested *Chlorella* species

FAMEs	*C. salina* [mg/g (%)]	*C. marina* [mg/g (%)]	*C. capsulata* [mg/g (%)]	*C. stigmatophora* [mg/g (%)]	*C. vulgaris* [mg/g (%)]
Saturated FAs					
C6 : 0 (caproic)	0.01 (0.1)	0.01 (0.1)	0.01 (0.1)	0.01 (0.1)	0.03 (1.2)
C8 : 0 (caprylic)	0.06 (1.1)	0.02 (0.5)	0.01 (0.3)	0.01 (0.3)	0.05 (1.7)
C10 : 0 (capric)	0.12 (2.1)	0.13 (2.9)	0.05 (1.2)	0.04 (1.1)	0.07 (2.2)
C11 : 0 (undecylic)	0.02 (0.3)	0.02 (0.4)	0.01 (0.3)	0.01 (0.3)	0.01 (0.2)
C12 : 0 (lauric)	0.29 (4.8)	0.12 (2.6)	0.15 (3.7)	0.14 (3.3)	0.13 (4.3)
C13 : 0 (tridecanoic)	0.65 (11.0)	0.25 (5.5)	0.33 (7.9)	0.30 (7.2)	0.25 (8.2)
C14 : 0 (myristic)	1.54 (25.9)	0.69 (15.0)	0.85 (20.4)	0.77 (18.6)	0.60 (19.5)
C15 : 0 (pentadecanoic)	1.36 (22.9)	0.60 (13.0)	0.70 (16.9)	0.63 (15.3)	0.36 (11.6)
C16 : 0 (palmitic)	0.65 (10.9)	1.39 (30.3)	1.17 (28.4)	1.10 (25.8)	0.83 (26.8)
C17 : 0 (margaric)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.11 (3.5)
C18 : 0 (stearic)	0.11 (1.9)	0.43 (9.4)	0.43 (10.3)	0.39 (9.4)	0.07 (2.2)
C20 : 0 (arachidic)	0.06 (1.0)	0.18 (4.0)	0.00 (0.0)	0.07 (1.8)	0.04 (1.3)
C21 : 0 (heneicosanoic)	0.16 (2.7)	0.34 (7.5)	0.00 (0.0)	0.17 (4.1)	0.05 (1.7)
Monounsaturated FAs					
C14 : 1 (myristoleic)	0.22 (3.7)	0.10 (2.1)	0.10 (2.5)	0.10 (2.2)	0.12 (3.7)
C15 : 1(*cis*-10-pentadecenoic)	0.66 (11.1)	0.28 (6.1)	0.33 (8.0)	0.30 (7.3)	0.27 (9.0)
C17 : 1(*cis*-10-heptadecenoic)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.00 (0.0)	0.05 (1.7)
C18 : 1 (oleic)	0.03 (0.5)	0.60 (0.6)	0.00 (0.0)	0.13 (3.2)	0.04 (1.2)
∑SFA	5.03 (84.7)	4.18 (92.2)	3.71 (89.5)	3.64 (87.3)	2.6 (84.4)
∑MUFA	0.91 (15.3)	0.41 (8.8)	0.43 (10.5)	0.53 (12.7)	0.48 (15.6)
SFA/MUFA	5.5	10.19	8.63	6.86	5.4
Relative MW	667.694	667.694	930.255	667.694	688.721
FFA%	0.467	0.534	0.620	0.266	0.688

Values in parentheses are percentage of total FAMEs; abbreviations: SFA – saturated fatty acids, MUFA – monounsaturated, MW – molecular weight, FFA – free fatty acids

results are in agreement with the results obtained by other researchers (Muthukumar et al., 2012; Sudha et al., 2013). The variation in the amounts of lipids produced by different algae species may be attributed to the difference of the metabolic flux created from photosynthesis (Feng et al., 2011), due to (the) cell development (Ilavarasi et al., 2011).

Chlorella salina was the richest oil-bearing species (28%), while *C. capsulata* produced the smallest amounts (14%). A study performed by Muthukumar and coworkers (2012) reported 10-80% of oil content in microalgae, on dry wt bases. In *Chlorella* sp., the reported amounts were in the range of 20-35%, depending on the cultivation conditions (Chisti, 2007). However, oil/lipid percentages ranged among the investigated algae from 60 to 80%. Chen and coworkers (2012) studies showed that these results were relatively high, compared to that recorded for *C. sorokiniana* (51.7%).

Acid value of oil is a measure of mineral acids and FFA found in a fuel sample. It is expressed in mg KOH required to neutralize 1 g of oil (indicated as mg KOH/g oil). The acid value for biodiesel should be <0.50 mg KOH/g, according to the American Society of Testing Materials (ASTM D6751) (Renita et al., 2014). Similarly, the saponification value expressed by mg KOH required 1 g of oil. The importance of both acid and saponification

values reflect the corrosive nature of the fuel (Renita et al., 2014; Li et al., 2016). In this study, acid values recorded for *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora*, and *C. vulgaris* were 0.393, 0.449, 0.374, 0.224, and 0.561, respectively, while the saponification values were 50.5, 50.5, 53.3, 39.27 and 56.1, as noted earlier. *C. stigmatophora* was found to have the lowest acid and saponification values. Data given here are consistent with the results of other studies for microalgae *Scenedesmus* sp. and *Nannochloropsis* sp. (Chen et al., 2012). Saponification values of all the examined *Chlorella*'s oil are relatively low compared with the study conducted by Li and coworkers (2016) on *Scenedesmus* sp.

The quality of fuel depends on the profiles of the fatty acids comprising biodiesel. Both fatty acid chain lengths and degree of saturation do affect the biodiesel properties. In this study FAMEs were analyzed and identified by gas chromatography (GC), and details are presented in Table 4. The data showed the presence of a variety of fractions, which ranged according to their chain length from C_6 to C_{21}. The most diverse strain with regard to FAMEs was *C. vulgaris*, followed by *C salina*, *C. marina*, and *C. stigmatophora*. But, the smaller abundance of FAMEs was found in *C. capsulata*. Typically, the same distribution pattern of fatty acids was observed in *C salina*, *C. marina*, and *C. stigmatophora.* Whyte and coworkers (1998) postulated that the perfect chain length for the production of quality biodiesel is C_{10}-C_{18}; in our study FAMEs chain lengths exceeded this proportion. Furthermore, the majority of the fractions were of saturated types. The proportions of saturated fractions detected in *C. salina*, *C. marina*, *C. capsulata, C. stigmatophora*, and *C. vulgaris* were 84.7%, 92.2%, 89.5%, 87.3%, and 84.4%, respectively. According to Rasoul-Amini and coworkers (2011) a high saturation degree of FAMEs contributes to biodiesel quality. High amounts of saturated fatty esters result in a higher stability of fuel, but with poor cold flow properties of biodiesel (Sharma et al., 2015). Importantly, no polyunsaturated fatty acids were detected in the study. It is well known that polyunsaturated FAMEs affect biodiesel quality negatively. Knothe (2005) indicated that molecular characteristics of fatty acids, such as the length of the carbon chain and degree of unsaturation, significantly influence the oxidative stability and ignition quality. Consequently, our results support the choice of the studied microalgae as good candidates for large-scale biodiesel production. According to Rasoul-Amini and coworkers (2011), palmitic (C16 : 0) and stearic (C18 : 0) acids are the most important fatty ester fractions for biodiesel quality. In the present study, *Chlorella* created these fractions in appropriate amounts. The amounts of palmitic acid were 10.9%, 30.3%, 28.4%, 25.8%, and 26.8% for *C. salina*, *C. marina*, *C. capsulata*, *C. stigmatophora*, and *C. vulgaris*, respectively; the amounts of stearic acid were 1.9%, 9.4%, 10.3%, 9.4%, and 2.2%, respectively. Oleic acid (C18 : 1) was not detected in *C. capsulata*; however, it was found in small amounts in other tested species. Both C17 : 0 and C17 :1 fractions were detected only in *C. vulgaris*. Importantly, they were detected by Yao and coworkers (2015) in *Chlorella* sp. The relative molecular weight (MW) of fatty esters and the percentage of associated FFA were also recorded in this study. The amounts of FFA ranged from 0.27% to 0.69% for *C. stigmatophora* and *C. vulgaris,* respectively, that were comparable with the amounts determined in the study of Yao and coworkers (2015) on *Chlorella* sp. and other microalgae.

Conclusions

In this paper, we attempted to characterize five *Chlorella* species originating from different regions of the world, with respect to their growth, lipid, and fatty acids contents, as biodiesel producers, at the lab scale cultivation prior to transferring the algae into race open ponds. Data showed that whether the alga was freshwater or marine cultivated, native or nonnative, there were some similarities between the target species. But, each strain showed particular specifications that distinguished it from the others. For example, *C. salina* showed the maximum content (200 ± 0.02 mg/l), and oil (280 ± 0.78 mg/l) yield. *C. marina* showed the fastest growth rate (2 ± 0.17), although it accumulated the smallest amounts of dry wt (86 ± 0.69 mg/l). *C. capsulata* was characterized to possess the highest lipids production (446 ± 0.33 mg/l), with the lowest amount of oil (137 ± 3.56 mg/l). However, *C. vulgaris* was described as the least lipid containing species (255 ± 2.5 mg/l), along with a unique presence of both C17 : 0 and C17 : 1 fatty esters.

The outcomes given in this study are in general promising as a preliminary step for a commercial cultivation. Further optimizations of the cultivation systems could contribute to improving the algae to be more developed for biodiesel practice.

Acknowledgments

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References

Al-lwayzy S., Yusaf T., Al-Juboori R. (2014) *Biofuels from the freshwater microalgae Chlorella vulgaris (FWM-CV) for diesel engines*. Energies 7(3): 1829-1851.

Araújo S., Garcia V. (2005) *Growth and biochemical composition of the diatom Chaetoceros cf. wighamii bright well under different temperature, salinity and carbon dioxide levels. I. Protein, carbohydrates and lipids*. Aquaculture 246(1): 405-412.

Betiku E., Adepoju T. (2013) *Methanolysis optimization of sesame (Sesamum indicum) oil to biodiesel and fuel quality characterization*. Int. J. Energ. Env. Eng. 4: 9-16.

Chen L., Liu T., Zhang W., Chen X., Wang J. (2012) *Biodiesel production from algae oil high in free fatty acids by two-step catalytic conversion*. Bioresource Technol. 111: 208-214.

Chisti Y. (2007) *Biodiesel from microalgae*. Biotechnol. Adv. 25: 294-306.

Del Río E., Armendáriz A., García-Gómez E., Guerrero G. (2015) *Continuous culture methodology for the screening of microalgae for oil*. J. Biotechnol. 195: 103-107.

Demirbas A. (2009) *Progress and recent trends in biodiesel fuels*. Energy Convers. Manage. 50(1): 14-34.

Demirbas A., Demirbas M. (2010) *Algae energy: algae as a new source of biodiesel. Green energy and technology*. London: Springer-Verlag pp 204.

Deng X., Li Y., Fei X. (2009) *Microalgae: a promising feedstock for biodiesel*. Afr. J. Microbiol. Res. 3(13):1008-1014.

H.Y. El-Kassas H. Y., Mohammady N. G.-E., H.S. El-Sayed H. S., EL-Sherbiny B. A. (2016) *Growth and biochemical variability of complete and lipid extracted Chlorella species (application for Artemia franciscana feeding).* Rend. Fis. Acc. Lincei. 27: 761-774.

Feng Y., Li C., Zhang D. (2011) *Lipid production of Chlorella vulgaris cultured in artificial wastewater medium*. Bioresource Technol. 102(1): 101-105.

Folch J., Lees M., Sloane-Stanley G. (1957) *A simple method for the isolation and purification of total lipids from animal tissues*. J. Biochem. 226(1): 497-509.

Gouveia L., Oliveira A. (2009) *Microalgae as a raw material for biofuels production*. Ind. Microbiol. Biotechnol. 36: 269-274.

Gouveia L., Marques A., da Silva T., Reis A. (2009) *Neochloris oleabundans UTEX #1185: a suitable renewable lipid source for biofuel production*. Ind. Microbiol. Biotechnol. 36: 821e6.

Guillard R., Ryther J. (1962) *Studies of marine planktonic diatoms: I. Cyclotella nanaHustedt, and detonulaconfervacea (cleve) gran*. Can. J. Microbiol. 8(2): 229-239.

Halder P. (2011) *Bioenergy knowledge, perceptions, and attitudes among young citizens – from cross-national surveys to conceptual model*. Dissertationes Forestales 135. 39 p. Available at: http://www.metla.fi/dissertationes/df135.htm

Hossain S., Salleh A., Boyce A., Chowdhury P., Naqiuddin M. (2008) *Biodiesel fuel production from algae as renewable energy*. Am. J. Biochem. Biotech. 4(3): 250-254.

Hu Q., Sommerfeld M., Jarvis E., Ghirardi M., Posewitz M., Seibert M., Darzins A. (2008) *Microalgal triacylglycerols as feedstock for biofuel production: Perspectives and advances*. Plant J. 54: 621-639.

Huang Y., Zhang D., Xue S., Wang M., Cong W. (2016) *The potential of microalgae lipids for edible oil production*. Appl. Biochem. Biotechnol. 180: 438-451; doi: 10.1007/s12010-016-2108-6.

Ilavarasi A., Mubarakali D., Praveenkumar R., Baldev E., Thajuddin N. (2011) *Optimization of various growth media to freshwater microalgae for biomass production*. Biotechnology 10: 540-545.

Illman A., Scragg A., Shales S. (2000) *Increase in Chlorella strains calorific values when grown in low nitrogen medium*. Enzyme Microb. Technol. 27: 631-635.

Knothe G. (2005) *Dependence of biodiesel fuel properties on the structure of fatty acid alkyl esters*. Fuel Proces. Technol. 86(10): 1059-1070.

Kumar M., Sharma M. (2014) *Potential assessment of microalgal oils for biodiesel production: A review*. J. Mater. Environ. Sci. 5(3): 757-766.

Li T., Xu J., Wu H., Wang G., Dai S., Fan J., He H., Xiang W. (2016) *A saponification method for chlorophyll removal from microalgae biomass as oil feedstock*. Mar. Drugs 14: 162-181; doi: 10.3390/md14090162.

Muthukumar A., Elayaraja S., Ajithkumar T., Kumaresan S., Balasubramanian T. (2012) *Biodiesel production from marine microalgae Chlorella marina and Nannochloropsis salina*. J. Petrol. Technol. Altern. Fuels 3: 58-62.

Nichols H. (1973) *Growth media – freshwater*. [in:] *Handbook of phycological methods: culture methods and growth measurements*. Ed. Stein J.R., Cambridge University Press: pp. 7-24.

Nigam S., Rai M., Sharma R. (2011) *Effect of nitrogen on growth and lipid content of Chlorella pyrenoidosa*. Am. J. Biochem. Biotechnol. 7(3): 124e9.

Okpuzor J., Okochi V., Ogbunuga A., Ogbonnia S., Fagbayi T., Obidiegwu C. (2009) *Estimation of cholesterol level in different brands of vegetable oils*. Pak. J. Nutr. 8(1): 57-62.

O'Neil G., Culler A., Williams J., Burlow N., Gilbert G., Carmichael C., Nelson R., Swarthout R., Reddy C. (2015) *Production of jet fuel range hydrocarbons as a co-product of algal biodiesel by butenolysis of long-chain alkenones*. Energ. Fuel 29: 922-930.

Ota S., Oshima K., Yamazaki T., Kim S., Yu Z., Yoshihara1 M., (2016) *Highly efficient lipid production in the green alga Parachlorella kessleri: draft genome and transcriptome endorsed by whole-cell 3D ultrastructure*. Biotechnol. Biofuels 9: 13; doi: 10.1186/s13068-016-0424-2.

Pearson D. (1981) *The chemical analysis of food*. Churchill Publishing, London: 580-581.

Purkayastha J., Bora A., Gogoi H., Singh L. (2017) *Growth of high oil yielding green alga Chlorella ellipsoidea in diverse autotrophic media, effect on its constituents*. Algal Res. 21: 81-88.

Radwan S. (1991) *Sources of C20-polyunsaturated fatty acids for biotechnological use*. Appl. Microbiol. Biotechnol. 35: 421-430.

Raj M., Kandasamy M. (2012) *Tamanu oil, an alternative fuel for variable compression ratio engine*. Int. J. Energy. Env. Eng. 3: 18-25.

Rasoul-Amini S., Montazeri-Najafabady N., Mobasher M., Hoseini-Alhashemi S., Ghasemi Y. (2011) *Chlorella sp.: a new strain with highly saturated fatty acids for biodiesel production in bubble-column photobioreactor*. Appl. Energ. 88(10): 3354-3356.

Reddy H., Muppaneni T., Sun Y., Li Y., Ponnusamy S., Patil P., Cooke P. (2014) *Subcritical water extraction of lipids from wet algae for biodiesel production*. Fuel 133: 73-81.

Renita A., Sreedhar N., Peter M. (2014) *Optimization of algal methyl esters using RSM and evaluation of biodiesel storage characteristics*. Bioresour. Bioprocess. 1: 1-19.

Rodolfi L., Zittelli G., Bassi N., Padovani G., Biondi N., Bonini G., et al. (2009) *Microalgae for oil: strain selection, induction of lipid synthesis and outdoor mass cultivation in a low-cost photobioreactor*. Biotechnol. Bioeng. 102(1): 100-112.

Satyanarayana K., Mariano A., Vargas J. (2011) *A review on microalgae, a versatile source for sustainable energy and materials*. Int. J. Energ. Res. 35: 291-311.

Scarsella M., Parisi P., D'Urso A., De Filippis P., Opoka J., Bravi M., (2009) *Achievements and perspectives in hetero- and mixotrophic culturing of microalgae*. Chem. Eng. Transac. 17: 1065-1070.

Selvarajan R., Felföldi T., Tauber T., Sanniyasi E. (2015) *Screening and evaluation of some green algal strains (Chlorophyceae) isolated from freshwater and soda lakes for biofuel production*. Energies 8: 7502-7521; doi: 10.3390/en8077502.

Sharma A., Sahoo P., Singhal S. (2015) *Feasibility of biodiesel production from Chlorella vulgaris grown in flat plate photobioreactor under outdoor conditions*. Int. J. Chem. Tech. Res. 8(6): 671-678.

Sharma K., Schuhmann H., Schenk P. (2012) *High lipid induction in microalgae for biodiesel production*. Energies 5(5): 1532-1553.

Spolaore P., Joannis-Cassan C., Duran E., Isambert A. (2006) *Commercial applications of microalgae*. J. Biosci. Bioeng. 101(2): 87-96.

Sudha K., Shalma S., Naveena B., Prakash S. (2013) *Effect of nitrogen concentration on growth and lipid content of Chlorella marina and Dunelliallasalina for biodiesel production*. Int. J. Int. Sci. Inn. Tech. 2(3): 28-32.

Vaičiulytė S., Padovani G., Kostkevičienė J., Carlozzi P. (2014) *Batch growth of Chlorella vulgaris CCALA 896 versus semi-continuous regimen for enhancing oil-rich biomass productivity*. Energies 7: 3840-3857; doi: 10.3390/en7063840.

Whyte L., Hawari J., Zhou E., Bourbonniere L., Inniss W., Greer C. (1998) *Biodegradation of variable-chain length alkanes at low temperatures by a psychrotrophic Rhodococcus sp*. Appl. Environ. Microbiol. 64(7): 2578-2584.

Wu L., Chen P., Huang A., Lee C. (2012) *The feasibility of biodiesel production by microalgae using industrial wastewater*. Bioresource Technol. 113: 14-18.

Wu H. (2001) *Identification of Chlorella spp. isolates using ribosomal DNA sequences*. Bot. Bull. Acad. Sinica 42: 115-121.

Yao L., Gerde J., Lee S-L., Wang T., Harrata K. (2015) *Microalgae lipid characterization*. J. Agric. Food. Chem. 63: 1773-1787.

Zhu L.,Takala J., Hiltunen E., Wang Z. (2013) *Recycling harvest water to cultivate Chlorella zofingiensis under nutrient limitation for biodiesel production*. Bioresour. Technol. 144: 14-20.