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IMPACT OF NITROGEN REGIME ON FATTY ACID PROFILES OF *DESMODESMUS QUADRICAUDATUS* AND *CHLORELLA* SP. AND ABILITY TO PRODUCE BIOFUEL

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Microalgae have emerged as one of the most promising sources for fatty acid production. Since the various fatty acid profiles (chain length, degree of unsaturation, and branching of the chain) of the different sources influence biodiesel fuel properties, it is important to possess data on how the presence of $NaNO_3$ as nitrogen source can influence the profile of produced fatty acids from algae. The fatty acid profiles of *Desmodesmus quadricaudatus* and *Chlorella* sp. were detected in pure batch cultures experiments. BG-11 nitrogen free medium and the medium contained 1.5 g $NaNO_3$ l^{-1} were used in this investigation. At late stationary growth phase in nitrogen free medium, *Chlorella* sp. produced 58.39% saturated fatty acids and 41.60% unsaturated fatty acids. While in medium contained 1.5 g $NaNO_3$ l^{-1} *Chlorella* sp. produced 62.08% saturated fatty acids and 37.92% unsaturated fatty acids. In nitrogen free medium *D. quadricaudatus* produced 66.92% saturated fatty acids and 33.07% unsaturated fatty acids. While in cultures contained 1.5 g $NaNO_3$ l^{-1} *D. quadricaudatus* produced 51.62% saturated fatty acids and 48.37% unsaturated fatty acids.

The fatty acid profile of *Chlorella* sp. and *D. quadricaudatus* that isolated from Egyptian water body and grown in nitrogen free medium may be suitable for biodiesel production. The results discussed and compared to fatty acid profiles produced by other algal species.

Key words: batch cultures, *Chlorella* sp., *Desmodesmus quadricaudatus*, fatty acid, nitrogen regime, biodiesel

INTRODUCTION

Microalgae have significant environmental and commercial importance. They are not only sources of food for humans and animals, but are also the sources of a wide range of chemical compounds used in industry, food tech-

nology and pharmaceuticals. They are microscopic, photosynthetic renewable resources with the potential to produce large quantities of lipids (fats and oils). Hossain *et al.* (2008) stated that it is very simple to extract oil from algae.

Many microalgae have high photosynthetic efficiency, fast growth rate, high biomass productivities and highest CO_2 fixation and O_2 production rate. Also, it can be grown in variable climates, non-arable land including marginal areas unsuitable for agricultural purpose, no seasonal production, thrive in non-portable water, use less water and do not compete with food crop culture. Many microalgae are capable of accumulating a large amount of lipids in the cells (Sheehan *et al*. 1998). On average, the lipid contents typically range from 10% to 30% of dry weight. Algae grown to late logarithmic growth phase typically contain 30–40% proteins, 10–20% lipids and 5–15% carbohydrates (Barsanti and Gualtieri 2006). Mata *et al*. (2010) showed that depending on the specific algae species and their cultivation conditions, however, microalgal lipid production might range widely from 2% to 75%. In some extreme cases, it can reach 70–90% of dry weight (Chisti 2007, Li *et al.* 2008). Nitrate and silicate stress has effect on the lipid content in a variety of phytoplankton (Shifrin and Chisholm 1981). Illman *et al*. (2000) studied five strains of the green alga *Chlorella*, and reported an increase in lipid content in all five strains when grown in low-nitrogen media. In the case of *Chlorella emersonii* and *C. minutissima*, 63% and 56% oil were obtained in low-nitrogen media compared to 29% and 31% in high-nitrogen media, respectively. Scragg *et al*. (2002) supported these findings by also finding that the lipid content increased in low-N medium for *Chlorella* strains.

Dayananda *et al*. (2007) illustrated that the content of lipid, carbohydrate and proteins varies from species to species. Most common algae like *Botryococcus braunii*, *Chlorella*, *Crypthecodinium*, *Cylindrotheca*, *Dunaliella*, *Isochrysis*, *Nannochloris*, *Nannochloropsis*, *Neochloris*, *Nitzschia*, *Phaeodactylum*, *Porphyridium*, *Schizochytrium*, *Scenedesmus* and *Tetraselmis* species have oil levels between 20% and 50%, but higher productivities can be reached. Under adverse growth conditions, such as nitrogen limitation, low temperature, high light intensity, high salt concentration and high iron concentration the lipid content in some of micro-algae increased (Hsieh and Wu 2009). This may be due to alteration in their lipid biosynthesis pathways towards the formation and accumulation of neutral lipids (Hu *et al.* 2008). Lipid accumulation was up to 80% of dry cell weight and mainly in the form of tri-acyl-glycerol due to the shift in metabolism from membrane lipid synthesis to the storage of neutral lipids tri-acyl-glycerides (TAG). Paulson and Ginder (2007) concluded that algal lipids occur in cells predominantly as either polar lipids (mostly in membranes) or lipid bodies, typically in the form of tri-acyl-glycerides (TAG). The latter are accumulated in large amounts during photosynthesis as a mechanism to endure adverse environmental conditions. Polar lipids usually contain polyunsaturated fatty acids (PUFA), which are long-chained, but have

good fluidity properties. TAG in lipid storage bodies typically contain mostly saturated fatty acids (SFA) that have high-energy contents, but, depending on the fatty acid profile of the algal strain, may lack fluidity under cold conditions. Provided the algal oil is low enough in moisture and free fatty acids, biodiesel is typically produced from TAG with methanol using base-catalysed transesterification (Schuchard *et al.* 1998). Fatty acids are converted as source of energy. The idea of using microalgae as a source of fatty acids, which may be changed to fuel is not new (Kapdan and Kargi 2006, Meier 1955). Changing of fatty acids to fatty acid alkyl esters, fatty acid methyl esters (FAME), or long-chain mono alkyl esters are the main key for biodiesel production from algae (Thomas 2006). Triglycerides in oil are transesterificated with a lower alcohol in the presence of an acidic or a basic catalyst into the corresponding long-chain fatty acid alkyl esters (Tyagi *et al.* 2010).

The accumulation of lipid begins as the cells enter stationary phase and cell division ceases; the timing of this event would be different for individual cells within a population. Nutrient limitation, generally nitrate or silica, can trigger lipid accumulation in microalgae. Nutrient deprivation can cause a decrease in cell division, which presumably results in "targeting" of excess fixed carbon into storage lipids. In green algae, lipid accumulation is induced among others by N starvation. N is a component of many cellular molecules, and N limitation would induce a complex response, affecting photosynthesis, protein and nucleic acid synthesis, and other biochemical processes (Sheehan *et al*. 1998). Shafik (1991, 2003) and Kenesi *et al*. (2009) studied the effect of nitrogen forms and its concentration on growth rate, cell composition and morphology of some green and prokaryotic algae.

This study aimed to show the effects of absence and presence of nitrogen on fatty acid profiles produced by *Chlorella* sp. and *Desmodesmus quadricaudatus* isolated from Egyptian water. Moreover, to compare the fatty acids profiles of the studied species to that used for biofuel production in some literature.

MATERIALS AND METHODS

Isolation and identification of algal strains

Microalgae samples were collected from variety of freshwater bodies at Port-Said City in summer 2009. *Chlorella* sp. and *Desmodesmus quadricaudatus* (Turpin) Hegewald (this name is currently regarded as a synonym of *Scenedesmus quadricauda* (Turpin) Brébisson) were isolated from these samples. Initial strain isolations were performed by streaking out samples onto 1.5% agar plates containing BG-11 medium. Each strain was examined using binocular light microscopy (SME-F4D, Rating: 85 V to 265 V, 50/60 Hz, Halogen lamp:

60 V 20 W, Delay-action fuse: 1 A) to look for morphological differences and to confirm identification of isolated algal strains according to Smith (2010).

Cultivation and experiment design

The used BG-11 medium contained (g l^{-1}), $NaNO_3$, 1.5; $K_2HPO_4 \cdot 3\ H_2O$, 0.04; $MgSO_4 \cdot 7\ H_2O$, 0.075; $CaCl_2 \cdot 2\ H_2O$, 0.036; citric acid, 0.006, ferric ammonium citrate, 0.006; Na_2EDTA, 0.001; Na_2CO_3, 0.02. In addition to 1 ml of trace metal solution (including H_3BO_3, 2.86 g; $MnCl_2 \cdot 4\ H_2O$, 1.81 g; $ZnSO_4 \cdot 7\ H_2O$, 0.222 g; $Na_2MoO_4 \cdot 2\ H_2O$, 0.390 g; $CuSO_4 \cdot 5\ H_2O$, 79 mg and $Co(NO_3)_2 \cdot 6\ H_2O$, 49.4 mg l^{-1}) was used for growth and fatty acid production of both species. However, $NaNO_3$ was omitted from the medium in case of N free medium experiments. The pH of all cultures was 7.4.

All cultures were incubated at room temperature of 27±1 °C and continuous light using two fluorescent tubes of TL-D 18 W (snow white extreme cool day light E9, made in Poland) and bubbled with sterilised air.

A colony from the agar cultures of *Chlorella* sp. and *D. quadricaudatus* transferred to fresh BG-11 liquid medium contained $NaNO_3$ and leave to grow under the above growth conditions. This culture used as stock culture in next experiments.

Three cultures for each species were grown in fresh BG-11 medium contained 1.5 g $NaNO_3$ l^{-1} to measure growth as chlorophyll-a. Specific growth and the maximum growth rates calculated by Equation 1:

$$\text{specific growth rate; } \mu = \ln(N1–N2)/(t1–t2),$$

where *N1* and *N2* are biomass at time *t1* and *t2*, respectively.

To determine fatty acid composition, certain volumes of the stock culture were inoculated to the fresh medium contained 1.5 g $NaNO_3$ l^{-1}. Chlorophyll-a, turbidity and nitrate were measured each three days. After depletion of nitrate from culture suspension, the culture was divided into two new culture flasks. One flask contained fresh medium with 1.5 g $NaNO_3$ l^{-1} (diluted culture with N) and the other was diluted with $NaNO_3$ free medium (diluted culture without N). The algal biomasses were harvested and fatty acid profiles determined at late stationary growth phase (the end of the experiments). All cultures were pure but non-axenic and ran in three replicates.

Measurement of growth and estimation of algal biomass

The growth of algae measured as chlorophyll-a. Chlorophyll-a measured according to Iwamura *et al*. (1970) at 653, 666 and 750 nm using 6800 Double beam UV/Visible Spectrophotometer, Jenway, made in England.

Growth rate (μ, d^{-1}) were calculated according to Equation 1 (Andersen 2005).

Nitrate concentration determination

Nitrate concentration in the algal suspension determined according to Cataldo *et al.* (1975) using spectrophotometer (6405 UV/Visible Spectrophotometer, Jenway, England).

Biomass collection

The colonies of *Desmodesmus quadricaudatus* were self-settled by stopped culture aeration for 2 hours then the settled biomass was collected and dried at 30 °C for 2 days.

For settling of *Chlorella* cells, alum (Aluminum Sulphate octadecahydrate; $Al_2(SO_4)_3 \cdot 18\ H_2O$, molar mass 666.42 g mol^{-1}), as a chemical flocculent was added to the culture of *Chlorella* sp. for settling then the biomass was harvested and dried at 30 °C for 2 days. The lowest alum concentration that makes the best precipitation of *Chlorella* sp. was determined by adding 0, 1.8, 3.0, 4.8, 6.0, 7.8, 9.0 and 10.8 m mol alum l^{-1} to the culture and mixed for 2 min thereafter left to settling.

Oil extraction

Dried algal biomass (2 g) were extracted all night in hexane/ether solution (1/1, V/V) to extract oil. The mixture was kept for 24 h for settling. This step repeated until the extract became hyaline (Basova 2005). Then the extract was evaporated in vacuum to release hexane and ether solutions (Hossain *et al.* 2008) using rotary evaporator (Diagonal Condenser-RE300, PTFE/glass liquid pathway for chemical inertness, sparkles induction motor, long life graphite impregnated PTFE vacuum seal, efficient flask and vapour tube ejection system, speed range 20 to 190 rpm, Vacuum 1 mm Hg, made in UK).

Fatty acid analysis

The extracted oil was converted to fatty acid methyl ester according to Luddy *et al.* (1960) and measured using gas chromatography (Perkin Elmer Auto system XL) equipped with flame ionization detector (FID), fused silica capillary column DB-5 (60 m × 0.32 mm i.d.). The oven temperature was maintained initially at 150 °C and programmed from 150 °C to 240 °C at rate 3 °C/min,

then held at 240 °C for 30 min. The injector temperature was 230 °C. Detector temperature was 250 °C and carrier gas was Helium with flow rate of 1 ml min^{-1}). The 3-Nonanone used as standard.

RESULTS

A strain of *Chlorella* sp. and *Desmodesmus quadricaudatus* were isolated, identified and grown in BG-11 medium. The growth of both species was investigated as chlorophyll-a concentration. The cultures entered stationary growth phase after 72 h. The maximum growth rates (μ_{max}) were 3.02 and 2.96 d^{-1} for *Chlorella* sp. and *D. quadricaudatus*, respectively (Fig. 1).

In the experiments for fatty acids production, *Chlorella* sp. and *D. quadricaudatus* were grown in culture medium contained 1.5 g $NaNO_3$ l^{-1} till nitrate undetected in the culture suspension. Nitrate concentration was undetected in culture suspension by day 6 and day 12 for *Chlorella* sp. and *D. quadricaudatus*, respectively (Fig. 2). Then the cultures divided to two equal volumes and diluted by fresh medium. One culture group was grown in culture medium containing 1.5 g $NaNO_3$ l^{-1} and the other group in nitrate free medium.

Table 1 recorded the growth of all cultures biomass measured as chlorophyll-a and the changing of growth rates of both species.

Chlorella reached a maximum chlorophyll-a concentration of 10650±54 μg l^{-1} after 12 days of dilution in diluted cultures with 1.5 g $NaNO_3$ l^{-1}. While *D. quadricaudatus* reached a maximum chlorophyll-a concentration of 2207±68 μg l^{-1} after 3 days of dilution (Table 1).

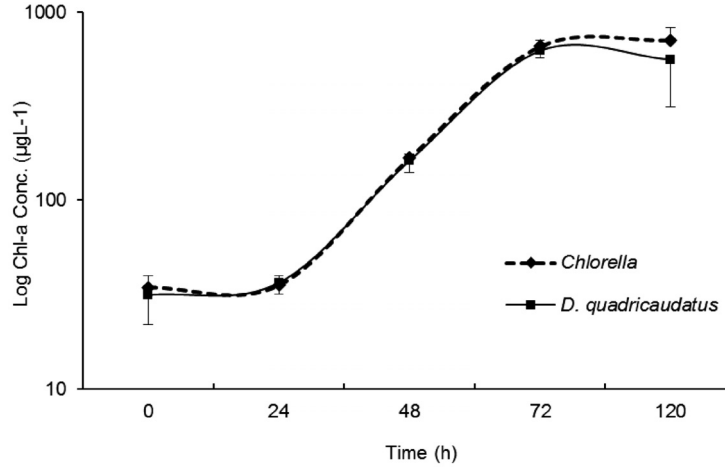

Fig. 1. Growth curve of *Chlorella* sp. and *Desmodesmus quadricaudatus* measured as chlorophyll-a at room temperature of 27±1 °C, with continuous light. Standard error bars for *Chlorella* sp. shows in plus direction and for *D. quadricaudatus* in minus direction

Table 1
Growth of *Chlorella* sp. and *Desmodesmus quadricaudatus* measured as chlorophyll-a (μg l^{-1}) at room temperature of 27±1 °C, with continuous light

Algal species	Time (days)	Diluted culture with N		Diluted culture without N	
		Chlorophyll-a (μg l^{-1})	Growth rate (d^{-1})	Chlorophyll-a (μg l^{-1})	Growth rate (d^{-1})
Chlorella sp.	0	1038.8±0.0		1034.5±10.5	
	3	5520.8±94.5	0.56	2152.8±19.2	0.24
	6	8714.2±47.7	0.15	3085.3±35.0	0.12
	9	9770.5±54.4	0.04	3238.2±35.5	0.02
	12	10650.5±53.9	0.03	847.8±9.2	–0.45
	15	10268.3±11.9	–0.01	838.8±9.5	0.0
D. quadricaudatus	0	628.6±48.8		630.8±0.5	
	3	2207.3±68.0	0.42	682.9±0.8	0.03
	6	2149.7±71.3	–0.01	713.0±0.5	0.01
	9	1715.2±11.9	–0.08	638.7±19.1	–0.04
	12	1639.5±10.5	–0.02	1342.9±34.0	0.25

In diluted cultures of nitrate free medium, *Chlorella* reach a maximum chlorophyll-a concentration of 3238±35.5 μg l^{-1} after 9 days of dilution (Table 1). While *D. quadricaudatus* reach a maximum chlorophyll-a concentration of 1343±34 μg l^{-1} only after 12 days of dilution (Table 1). At late stationary phase (last day of the experiments), samples were harvested from all cultures for fatty acids analysis.

The specific growth rates (μ) of both algae were calculated as chlorophyll-a concentration in all cultures. The specific growth rates (after 3 days)

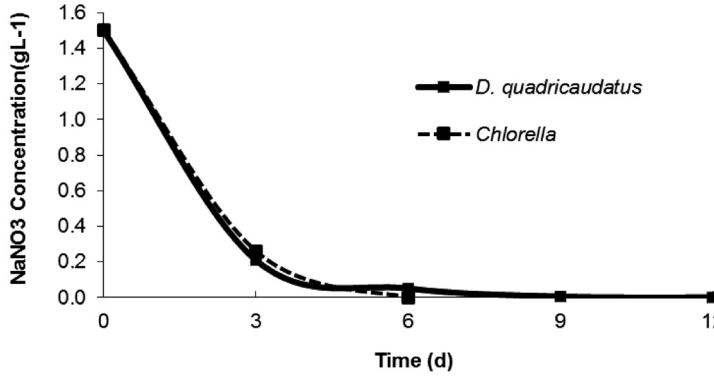

Fig. 2. Nitrate concentration in *Chlorella* sp. and *Desmodesmus quadricaudatus* cultures measured at room temperature of 27±1 °C, with continuous light

Table 2
Fatty acid profile (% of total fatty acids) of *Chlorella* sp. and *Desmodesmus quadricaudatus* measured by GC

Strain	Media		Saturated fatty acids (%)					Unsaturated fatty acids (%)					Total fatty acids (%)
		I.S.*	14.0	16.0	18.0	Total	16.1	18.1	18.2	18.3	20.5	Total	
Chlorella sp.	1.5 g $NaNO_3$ l^{-1}	N*	–	14.39	47.69	62.08	–	–	22.22	–	15.70	37.92	100
	Nitrogen free medium	0.01	1.04	50.03	7.32	58.39	1.65	12.79	15.57	8.79	2.80	41.60	100
D. quadricaudatus	1.5 g $NaNO_3$ l^{-1}	0.01	–	18.19	33.43	51.62	–	35.02	–	–	13.35	48.37	100
	Nitrogen free medium	0.01	–	60.15	6.77	66.92	5.42	6.65	21.00	–	–	33.07	100

I.S.* = internal standard (3-Nonanone); N* = not detected

of *Chlorella* were 0.56 and 0.24 d^{-1} existing $NaNO_3$ and $NaNO_3$ free medium cultures, respectively. While specific growths rates of *D. quadricaudatus* were 0.42 and 0.25 d^{-1} in diluted cultures existing $NaNO_3$ and $NaNO_3$ free medium, respectively (Table 1). The low chlorophyll-a concentration for *Chlorella* and *D. quadricaudatus* from the cultures $NaNO_3$ free medium may be consequence of lost viability of some cells under severe combined nitrogen starvation at the last period of the experiments (stationary phase) (Table 1).

The yield of both algae were harvested by precipitation of algal cells at the end of experiments. *Chlorella* cells were not self-precipitate. Therefore, different concentrations of alum were tested to detect the minimum alum concentration for complete precipitation of *Chlorella* cells. This concentration was 1.8 m mol alum after 10 minutes mixing, while *D. quadricaudatus* colonies were self-precipitated.

Analyses of fatty acids show that *Chlorella* sp. produced 58.39% saturated fatty acids, which were myristic acid (C14.0), palmitic acid (C16.0) and stearic acid (C18.0) and 41.60% unsaturated fatty acids, which were palmitoleic acid (C16.1), oleic acid (C18.1), linoleic acid (C18.2), linolenic acid (C18.3) and eicosapentaenoic acid (C20.5) in the $NaNO_3$ free medium (Table 2). While in medium containing $NaNO_3$ *Chlorella* sp. produced 62.08% saturated fatty acids (palmitic acid, C16.0 and stearic acid, C18.0) and 37.92% unsaturated fatty acids "linoleic acid, C18.2 and eicosapentaenoic acid, C20.5" (Table 2).

Desmodesmus quadricaudatus produced 66.92% saturated fatty acids (palmitic acid, C16.0 and stearic acid, C18.0) and 33.07%

unsaturated fatty acids (palmitoleic acid, C16.1, oleic acid, C18.1 and linoleic acid, C18.2) in $NaNO_3$ free medium. While in medium containing $NaNO_3$ produced 51.62% saturated fatty acids (palmitic acid, C16.0 and stearic acid, C18.0) and 48.37% unsaturated fatty acids "oleic acid, C18.1 and eicosapentaenoic acid, C20.5" (Table 2). So the results in Table 3 show that the type and amount of fatty acids more or less affected by the mode of nitrogen supply.

Figure 3 shows the fatty acid profile as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) of *Chlorella* sp. and *D. quadricaudatus* grown in media with or without $NaNO_3$.

DISCUSSION

The choice of microalgae for fatty acids profiles and for biofuel production requires a balance between species that grow quickly against those, which produce oil in large quantities. Therefore, *Chlorella* sp. and *Desmodesmus quadricaudatus* were chosen in this investigation, where both species have high maximum growth rate of 3.02 and 2.96 d^{-1}, respectively. These maximum growth rates are significant factor for algal mass production. Shafik (1991) recorded a maximum growth rate for *Scenedesmus spinosus* (currently, *Desmodes-*

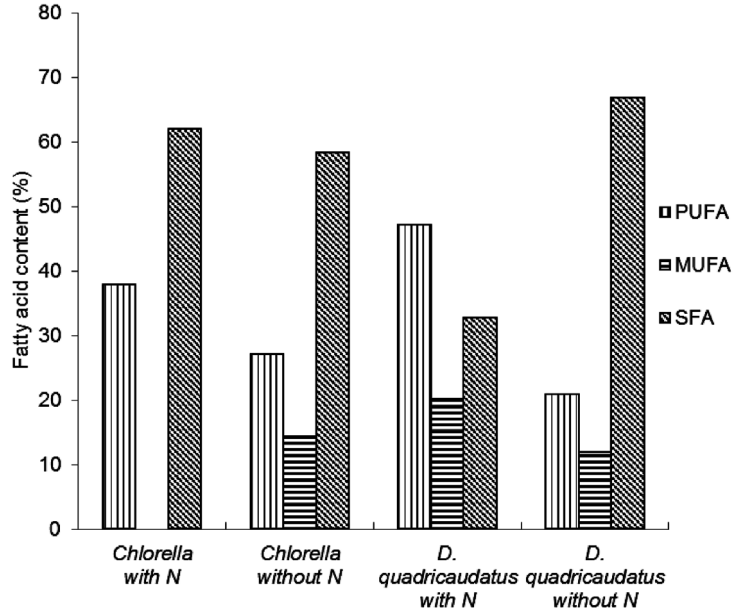

Fig. 3. Fatty acid profile of *Desmodesmus quadricaudatus* and *Chlorella* sp. grown in BG-11 medium contained 1.5 g $NaNO_3$ l^{-1} or in nitrogen free medium. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

mus spinosus) of 3.0 d^{-1}, which is close to the obtained data. High maximum growth rate is an indication for favourable growth conditions. Jena *et al*. (2012) obtained that the specific growth rate for both *Chlorella* and *Scenedesmus* sp. was 0.38 d^{-1}. Further, given that most oil is produced during a "starvation" or "stress" phase, successful fatty acid production will require species that can be reliably manipulated, is tolerant of a range of environmental perturbations – natural and operator induced – and is able to "recover" from these alterations and continue growing (Jena *et al*. 2012). It is important to possess data on how the nitrogen starvation can influence the profile of produced fatty acids so the various fatty acid profiles of the different sources can be used for production, such as biodiesel fuel.

One of the most important processes for studying the fatty acid profiles is the harvesting of algal yield. Algae can be harvested using micro-screens, by centrifugation, or by flocculation. Froth flotation is another method to harvest algae. Interrupting the carbon dioxide supply to an algal culture system could cause algae to flocculate on its own, which is called "autoflocculation". Harvesting by sedimentation (bioflocculation) was really promising, but it was strain specific and was increased by N limitation (Sheehan *et al*. 1998). Harvesting capability is an important feature of microalgae fatty acids analysis (Christenson and Sims 2011, Scholz *et al.* 2011). Park *et al*. (2011) showed that many microalgae settle under adverse conditions, and this could be tested under small-scale conditions. Some strains of *Chlorella* did not settle (Sheehan *et al.* 1998). Here the tested *Chlorella* sp. was not self-precipitated. Therefore, different concentrations of alum were used to detect the lowest alum concentration that precipitate most of *Chlorella* cells. This concentration was 1.8 m mol $Al_2(SO_4)_3 \cdot 18\ H_2O$. However, *D. quadricaudatus* was self-precipitated and easily harvested.

Basova (2005) showed the major fatty acids of different algal groups and reported that the lipids of many microalgae species are rich in polyunsaturated fatty acids (PUFAs).

Jena *et al*. (2012) obtained that *Scenedesmus* sp. produced 36.5% saturated fatty acid and 63.5% unsaturated fatty acid. *Scenedesmus* sp. contained high amount of palmitic acid (16:0, 30.3%) and the unsaturated fatty acids were represented by linoleic acid (C18:2, 21.1%) and oleic acid (18:1, 17.5%). Other long-chain PUFAs are present in small amount. These properties make *Scenedesmus* sp. suitable for biodiesel production. Rodolfi *et al*. (2009) concluded that culturing and environmental conditions affect the productivity of algae, lipid yield and fatty acid compositions. In a pilot study of *Chlorella* sp. the high growth rates could be achieved by increasing nitrogen concentrations, while lipid accumulation could be achieved by nitrogen starvation. Basova (2005) reported that *Chlorella* sp. produced 34.0% saturated fatty acid and 66.0% un-

Table 3
Comparison of fatty acid composition (% of total fatty acids) of some microalgal strain grown at stationary phase

Fatty acids	*C1*	*S1*	*C2*+N	*C3*–N	*D1*+N	*D2*–N
12.0	0.3	–	–	–	–	–
14.0	0.9	0.7	–	1.04	–	–
15.0	0.3	0.3	–	–	–	–
16.0	24.5	30.3	14.39	50.03	18.19	60.15
16.1	4.9	6.5	–	1.65	–	5.42
16.2	1.3	5.7	–	–	–	–
16.3	–	–	–	–	–	–
16.4	–	–	–	–	–	–
17.0	2.1	2.6	–	–	–	–
17.1	10.0	2.7	–	–	–	–
18.0	2.5	1.2	47.69	7.32	33.43	6.77
18.1	15.1	17.5	–	12.79	35.02	6.65
18.2	5.7	21.1	22.22	15.57	–	21.0
18.3	26.3	9.2	–	8.79	–	–
18.4	–	–	–	–	–	–
20.0	0.4	0.1	–	–	–	–
20.1	0.4	–	–	–	–	–
20.2	0.3	0.3	–	–	–	–
20.3	0.3	0.1	–	–	–	–
20.4	–	–	–	–	–	–
20.5	1.1	0.8	15.70	2.80	13.35	–
21.0	1.1	–	–	–	–	–
22.0	1.1	0.6	–	–	–	–
22.1	0.6	–	–	–	–	–
24.0	0.8	0.3	–	–	–	–
SFA	34.0	36.5	62.08	58.39	51.62	66.92
USFA	66.0	63.5	37.92	41.60	48.37	33.07

C1, S1 presented *Chlorella* sp. and *Scenedesmus* sp. after Jena *et al*. (2012), in the present study, *C2*+N indicates *Chlorella* sp. grown in culture contain 1.5 g $NaNO_3$ l^{-1}, *C3*–N indicates *Chlorella* sp. grown in nitrogen free medium, *D1*+N indicates *Desmodesmus quadricaudatus* grown in culture contain 1.5 g $NaNO_3$ l^{-1} and *D2*–N indicates *D. quadricaudatus* grown in nitrogen free medium

saturated fatty acid, higher amount of UFA was present among which PUFA content is 35%. The unsaturated fatty acids, linolenic acid (18.3) was the most dominant fatty acid (26.3%) besides the PUFA, oleic acid (18:1, 15.1%) and palmitic acid (16:0, 24.5%) were the principal fatty acids in *Chlorella* sp. (Jena *et al*. 2012) (Table 3).

Thomas *et al*. (1984) recorded that at low concentration of nitrogen *Chlorella* synthesised saturated (16:0) and monounsaturated (18:1) fatty acids, whereas at high nitrogen concentrations the 16:2, 16:3, 16:4 and 18:2 fatty acids predominated. *Chlorella* sp. is an oleaginous alga, that has a potential application, is currently used at pilot experimental level for biodiesel production, is commercially produced and available in large quantities.

Lee *et al*. (2011) stated that saturated and unsaturated fatty acids like palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3) are common fatty acids for biodiesel production. Thomas *et al*. (1984) reported that significant fatty acids used for biodiesel production, include saturated fatty acids and polyunsaturated fatty acids (PUFAs), such as C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3 fatty acids. In our results, *Chlorella* sp. in $NaNO_3$ free media tended to produce a diverse fatty acid pattern consisting of saturated (C14.0, C16.0 and C18.0) and unsaturated fatty acids (C16.1, C18.1, C18.2, C18.3 and C20.5). *D. quadricaudatus* produced unsaturated fatty acids (C16.1, C18.1 and C18.2) and saturated fatty acids, which were represented by (C16.0 and C18.0) in $NaNO_3$ free media (Tables 2, 3). Accordingly, both species that isolated from Egyptian water body may be suitable for biodiesel production, where it contains all these types of fatty acids.

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