IMPACT OF NITROGEN REGIME ON FATTY ACID PROFILES THAT PRODUCED BY DESMODESMUS QUADRICAUDATUS AND CHLORELLA SP.

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Abstract

Microalgae have emerged as one of the most promising sources for fatty acids 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 batch cultures experiments. BG-11 nitrogen free medium or the medium contained 1.5g $NaNO_3 l^{-1}$ 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 1.5g $NaNO_3$ l^{-1} produced 62.08% saturated fatty acids and 37.92% unsaturated fatty acids. In nitrogen free media *D. quadricaudatus* produced 66.92% saturated fatty acids and 33.07% unsaturated fatty acids. While in cultures contained 1.5g $NaNO_3$ l^{-1} produced 51.62% saturated fatty acids and 48.37% unsaturated fatty acids. The fatty acid profile of *Chlorella* sp. that isolated from Egyptian water body and grown in nitrogen free media may suitable for biodiesel production. The results discussed and compared to fatty acids profiles produce by other algal species.

***Keywords*:** Batch cultures, *Chlorella* sp., *Desmodesmus quadricaudatus*, Fatty acid, Nitrogen regime.

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 technology

(ISSN: 1110-8649)

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.

As demonstrated (**Gouveia and Oliveira, 2009)** 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 **(U. S. Department of Energy, 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, micro-algal 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 **(U. S. Department of Energy, 1998)**. **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 *Chlorella 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 *Chlorella*, *Crypthecodinium*, *Cylindrotheca*, *Dunaliella*, *Isochrysis*, *Nannochloris*, *Nannochloropsis*, *Neochloris*, *Nitzschia*, *Phaeodactylum*, *Porphyridium*, *Schizochytrium*, *Tetraselmis*, *Botryococcus braunii* and *Scenedesmus* 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 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 triacylglycerol 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 a 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-catalyzed 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 change 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 **(U. S. Department of Energy, 1998)**. **Shafik (2003)** and **Kenesi *et al*. (2009)** studied the effect of nitrogen forms and its concentration on growth rate, cell composition and morphology.

This study aimed to show the effects of absent and presence of nitrogen on fatty acid profiles produced by *Desmodesmus quadricaudatus* and *Chlorella* sp. isolated from Egyptian water. Moreover, compere the fatty acids profiles of studded 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) "Bréb." (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 species identify according to **Smith (2010)**.

Cultivation and experiments design

The used BG-11 medium **(Barsanti and Gualtieri, 2006)** contained (gl^{-1}), $NaNO_3$, 1.5; $K_2HPO_4 \cdot 3H_2O$, 0.04; $MgSO_4 \cdot 7H_2O$, 0.075; $CaCl_2 \cdot 2H_2O$, 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 $H3BO_3$; 2.86 g, $MnCl_2 \cdot 4H_2O$; 1.81 g, $ZnSO_4 \cdot 7H_2O$; 0.222 g, $Na_2MoO_4 \cdot 2H_2O$; 0.390 g, $CuSO_4 \cdot 5H_2O$; 79 mg and Co $(NO_3)_2 \cdot 6H_2O$; 49.4 mg l^{-1}) was used to determine the growth and fatty acid production. 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 sterilized air.

Cultures of both strains was started by transferring of a colony of the species in question with the help of platinum wire, respectively into 50, 100, 250, 500, and 1000 ml flasks containing BG-11 medium with 1.5 g $NaNO_3$ l^{-1}. This culture used as stock culture in experiments.

New cultures for each species ran in fresh BG-11 medium contained 1.5g $NaNO_3$ l^{-1}. Samples were daily harvested to measure growth and maximum growth rates as turbidity and chlorophyll-a content.

To determine fatty acid composition, a certain volume of the stock culture were inoculated to fresh medium containing 1.5 g $NaNO_3$ l^{-1} (starter culture). After depletion of nitrate from the culture suspension, the culture was divided into two new culture flasks. One of the flask contained fresh medium with 1.5g $NaNO_3$ l^{-1} (diluted culture with N), while the other was diluted with combined nitrogen free medium (diluted culture without N). At late stationary growth phase, the cells were harvested and processed to determine fatty acid profiles. Chlorophyll-a, turbidity and nitrate were measured for the new cultures. All cultures ran in three replicates.

Measurement of growth and estimation of algal biomass

Turbidity at 750 nm and chlorophyll a were used for measurement and calculation of algal growth and growth rate. Chlorophyll a content was measured according to **Iwamura *et al*. (1970)** at 653, 666 and 750nm using 6800 Double beam UV/Visible Spectrophotometer, Jenway, made in England.

Growth rate (μ, d-1) were calculated according to Eq. (1) **(Andersen, 2005)**:

$$\text{specific growth rate; } \mu = \ln(N1 - N2)/(t1 - t2) \qquad \text{Eq. (1)}$$

Where N1 and N2 are biomass at time $t1$ and $t2$, respectively.

Nitrate concentration determination

The concentrations of nitrate in the media were determined with spectrophotometer (6405 UV/Visible Spectrophotometer, Jenway, made in England) according to **Cataldo *et al*. (1975)**.

Biomass collection

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

Oil extraction

Dried algae (2g) 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)**.

Evaporation

The extract was evaporated in vacuum to release hexane and ether solutions using rotary evaporator (Diagonal Condenser-RE300, PTFE/glass liquid pathway for chemical inertness, sparkless induction motor, long life graphite impregnated PTFE vacuum seal, efficient flask and vapor tube ejection system, speed range 20 to 190 rpm, Vacuum 1 mm Hg, made in U.K.) according to **(Hossain *et al*., 2008)**.

Fatty Acid Analysis

The extracted oil was methyled according to **(Luddy *et al*., 1960)**. The fatty acids were measured using Gas Chromatography (Perkin Elmer Auto system XL) equipped with Flame Ionization Detector (FID), fused silica capillary column DB-5 (60m * 0.32mm i.d.), oven temperature was maintained initially at 150°C and programmed from 150°C to 240°C at rate 3°C/min, held at 240°C for 30 min. Injector temperature was 230°C. Detector temperature was 250°C and carrier gas was Helium with flow rate of 1ml/min). The used standard was 3-Nonanone.

Results

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

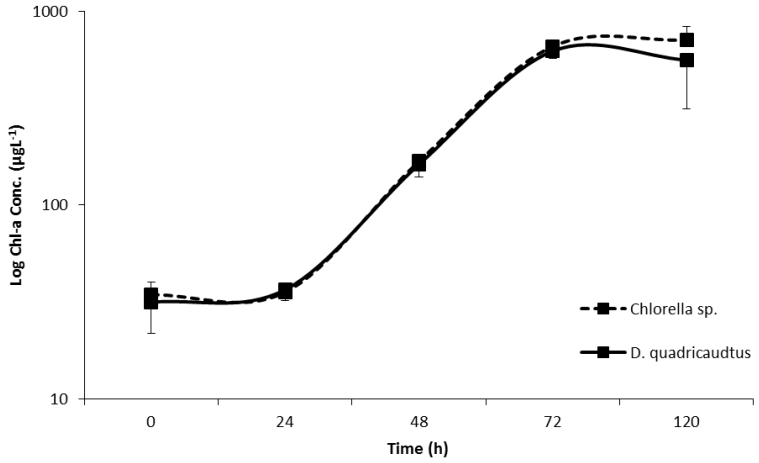

Figure (1): Growth curve of *Chlorella* sp. and *D. quadricaudatus* measured as Chlorophyll a at room temperature of 27±1°C, with continuous light. Standard error bars for *Chlorella* sp. shows in plus direction and for *D. quadricaudatus* in minus direction.

Growth of *Chlorella* sp. as turbidity and chlorophyll-a concentration in the started culture, which contained 1.5g $NaNO_3$ l^{-1} until day 6 of the experiment is shown in Figs. 2 and 3. On day 6, nitrate concentration was undetected in culture suspension (Fig. 6), and then the culture was divided to two equal volumes and diluted by fresh medium (diluted culture). One culture group was grown in culture medium containing $NaNO_3$ and the other in nitrogen free medium. The growth of algae is shown in Fig 2 and 3.

In case of *D. quadricaudatus*, nitrogen was undetected in the culture suspension of starter culture on day 12 (Fig. 6). At that day, the same procedure was done as same as the culture of *Chlorella* sp.

In diluted cultures with 1.5g $NaNO_3$ l^{-1}, *Chlorella* sp. reach a maximum chlorophyll a concentration of 10650 ± 54 µg l^{-1} and turbidity was 1.25 ± 0.003 after 12 days of dilution. While *D. quadricaudatus* reach a maximum chlorophyll-a concentration of 2207 ± 68 µg l^{-1} and turbidity of 1.01 ± 0.006 after 3 and 6 days of dilution for chlorophyll a and turbidity, respectively (Figs. 4 and 5).

In diluted cultures of N free medium, *Chlorella* sp. reach a maximum chlorophyll-a concentration of 3288 ± 35.45 µg l^{-1} and the turbidity was 0.50 ± 0.008 after 9 days of dilution (Figs. 2 and 3). While *D. quadricaudatus* reach a maximum chlorophyll-a concentration of 1342 ± 34 µg l^{-1} and turbidity of 0.50 ± 0.008 after 12 days of dilution (Figs. 4 and 5).

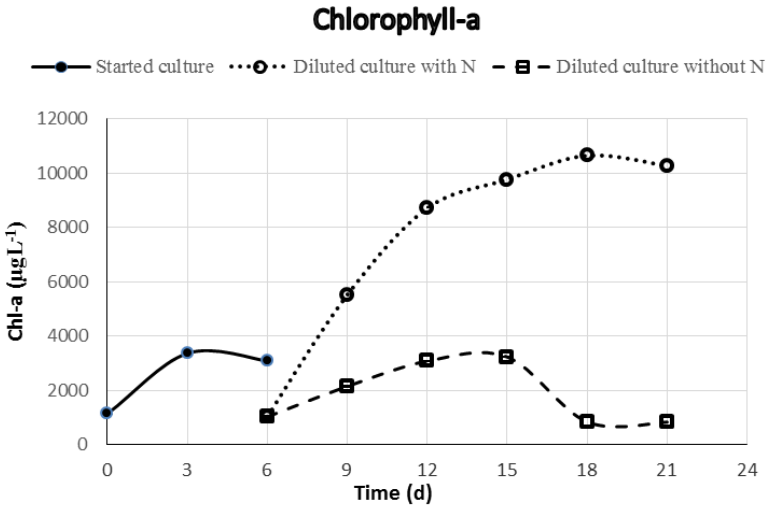

Figure (2): Growth of *Chlorella* sp. measured as Chlorophyll a at room temperature of 27±1°C, with continuous light.

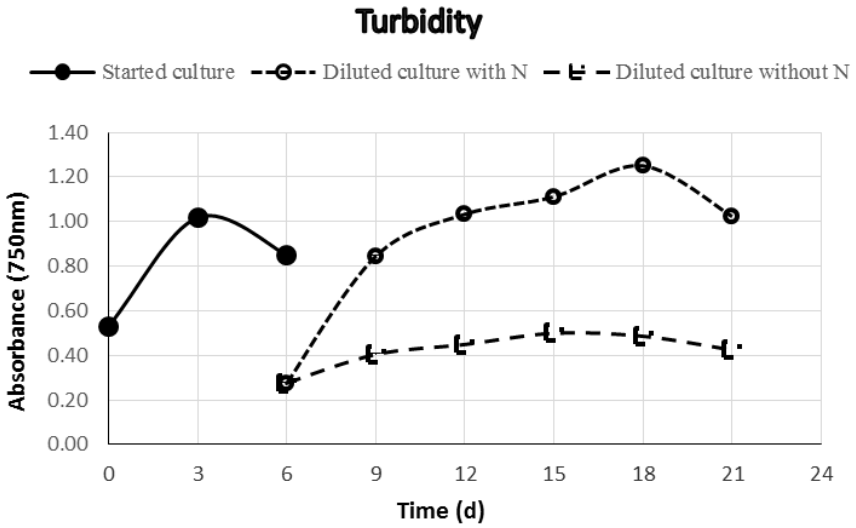

Figure (3): Growth of *Chlorella* sp. measured as Turbidity at room temperature of 27±1°C, with continuous light.

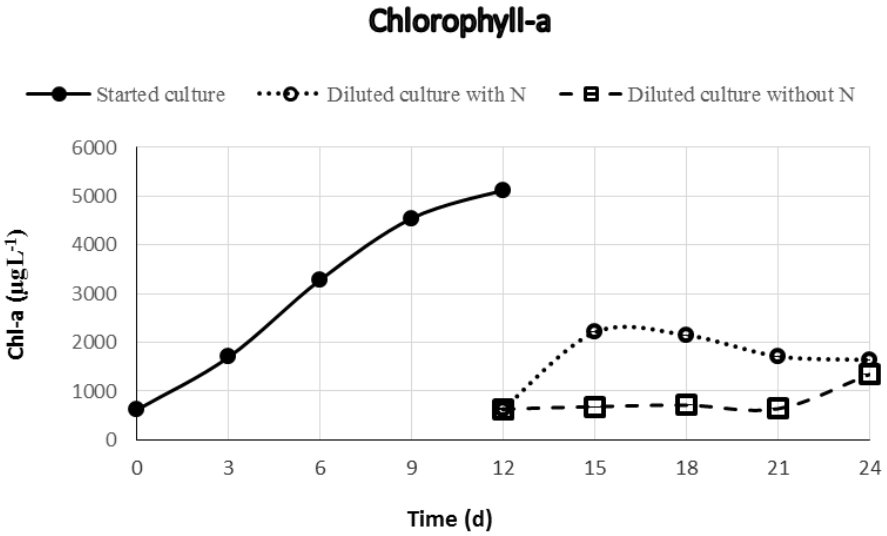

Figure (4): Growth of *D. quadricaudatus* measured as Chlorophyll a at room temperature of 27±1°C, with continuous light.

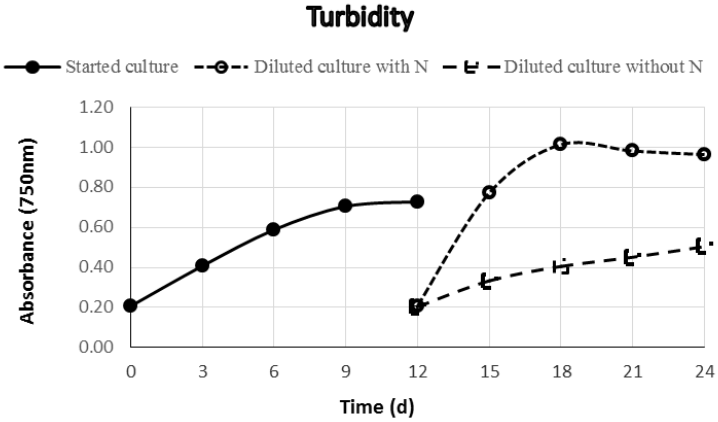

Figure (5): Growth of *D. quadricaudatus* measured as Turbidity at room temperature of 27±1°C, with continuous light.

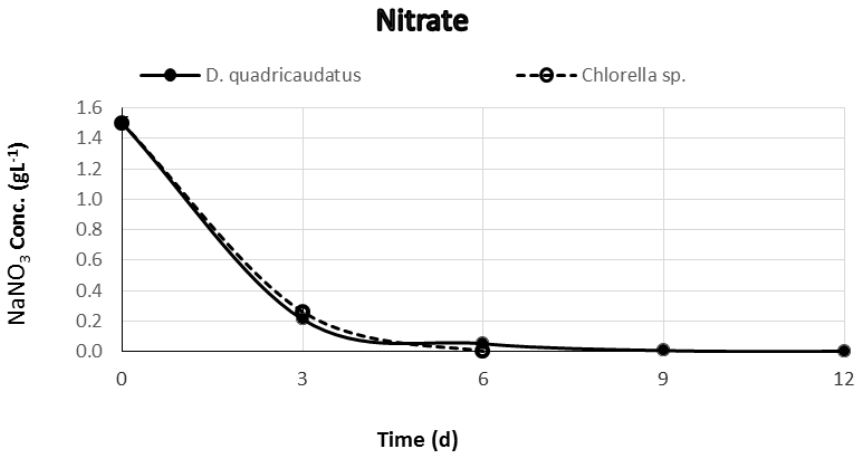

Figure (6): Sodium Nitrate concentration in *Chlorella* sp. and *D. quadricaudatus* cultures measured at room temperature of 27±1°C, with continuous light.

At late stationary phase, samples were harvested from each culture for fatty acids analysis.

Different concentrations of alum were tested to detect the minimum alum concentration for complete precipitation of *Chlorella* cells. This concentration was 1.8 mmol alum after 10 minutes mixing. *D. quadricaudatus* colonies were self-precipitated.

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 nitrogen free media (Table 1). While in medium contained1.5g $NaNO_3$ l^{-1} *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 1".

D. 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 nitrogen free media. While in medium contained 1.5g $NaNO_3$ l^{-1} 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 1).

Table 1 summarize the changes of fatty acids profiles of *Chlorella* sp. and *D. quadricaudatus* with nitrogen regime and the percent of fatty acid composition for both species. So, the type and amount of fatty acids more or less affected by mode of nitrogen supply. Fig. 7 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 present of nitrogen or in nitrogen free media.

Table 1. Fatty acid profile (% of total fatty acids) of *Chlorella* sp. and *D. quadricaudatus* measured by GC.

Strain	Media	I.S.*	Saturated fatty acids (%)				Unsaturated fatty acids (%)						Total fatty acids (%)
			14.0	16.0	18.0	Total	16.1	18.1	18.2	18.3	20.5	Total	
Chlorella sp.	1.5g $NaNO_3L^{-1}$	N*	----	14.39	47.69	62.08	----	----	22.22	----	15.70	37.92	100
	Nitrogen-free media	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	1.5g $NaNO_3L^{-1}$	0.01	----	18.19	33.43	51.62	----	35.02	----	----	13.35	48.37	100
	Nitrogen-free media	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

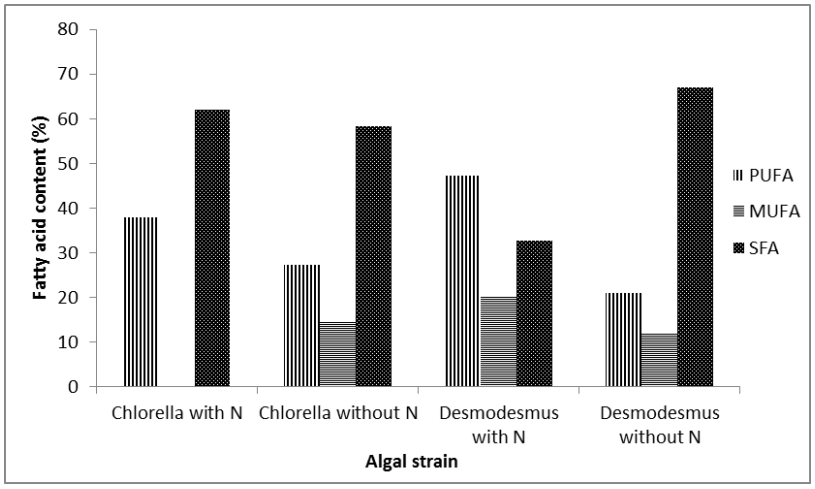

Figure (7): Fatty acid profile of *D. quadricaudatus* and *Chlorella* sp. grown in BG-11 medium contended 1.5g $NaNO_3l^{-1}$ or in nitrogen free medium. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

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 *D. quadricaudatus* were choice 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)**. **Shafik (1991)** recorded a maximum growth rate for *Scenedesmus spinosus* (newly, *Desmodesmus spinosus*) of 3.0 d^{-1} which is close to the obtained data. High maximum growth rate is an indication for favor 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 acids production will require species that can be reliably manipulated, are tolerant of a range of environmental perturbations - natural and operator induced - and are able to "recover" from these alterations and continue growing **(Jiang *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 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) has promise, but was strain specific and was increased by N limitation **(U. S. Department of Energy, 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 **(U. S. Department of Energy, 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 mmol $Al_2(SO4)_3.18H_2O$. However, *D. quadricaudatus* was self-precipitated and easily harvested.

Basova (2005) showed the major fatty acids of different algal groups. **Basova (2005)** 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. contains high amount of palmitic acid (16:0, 30.3%) and the unsaturated fatty acid represented by linoleic acid (C18:2, 21.1%) and oleic acid (18:1, 17.5%). Other long chain

PUFA are present in small amount. These properties make *Scenedesmus* sp. suitable for biodiesel production. **Basova (2005)** also, reported that *Chlorella* sp. produced 34.0% saturated fatty acid and 66.0% unsaturated fatty acid, higher amount of UFA was present among which PUFA content is 35%. Among unsaturated fatty acids, linolenic acid was the most dominant fatty acid (26.3%) Besides the PUFA, oleic acid (18:1, 15.1%) and palmitic acid (16:0, 24.5%) are principal fatty acids (Table 2).

Table 2. Comparison of fatty acid composition (% of total fatty acids) of some microalgal strain grow 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

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. **Thomas *et al*. (1984)** recorded that at low concentration of nitrogen *Chlorella* synthesized 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 nitrogen free media tended to produce the fatty acid pattern is a diverse, 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 are represented by (C16.0 and C18.0) in nitrogen free media (Tables 1, 2). Accordingly, both species that isolated from Egyptian water body may be suitable for biodiesel production, where it contains all these types of fatty acids.

In Table 2: C1, S1 presented *Chlorella* sp. and *Scenedesmus* sp. after **Jena *et al*. (2012)**. In the present study, C2 indicates *Chlorella* sp. grown in culture content 1.5g $NaNO_3$ l^{-1}, C3 indicates *Chlorella* sp. grown in nitrogen free media, D1 indicates *D. quadricaudatus* grown in culture content 1.5g $NaNO_3$ l^{-1} and D2 indicates *D. quadricaudatus* grown in nitrogen free media.

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تأثير النيتروجين على صورة الحمض الدهني المنتج من طحلب ديسموديسمس كوادريكودس و طحلب الكلوريلا

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ظهرت الطحالب الدقيقة باعتبارها واحدة من أكثر المصادر الواعدة لإنتاج الأحماض الدهنية. يعتبر الأختلاف فى أشكال الأحماض الدهنية (طول السلسلة، ودرجة التشبع، وتفرع السلسلة) من مصادر مختلفة مؤثر فى خصائص وقود الديزل الحيوي|. من الأهمية بمكان أن نمتلك بيانات حول كيفية وجود نترات الصوديم كمصدر للنتروجين و الذى يمكن أن يؤثر فى تركيب و انتاجية الأحماض الدهنية من الطحالب. تم تحديد تركيب الحمض الدهني لطحلب ديسموديسمس كوادريكودس (*Desmodesmus quadricaudatus*) و نوع من طحلب الكلوريلا (*Chlorella* sp.) باستخدام العديد من التجارب لزراعة تلك الطحالب. استخدم فى هذه الدراسة وسط النمو BG-11 الخالى من النيتروجين و وسط النمو المحتوى على 1.5 جرام من نترات الصوديوم. في أواخر مرحلة الثبات فى النمو فى الوسط الخالى من النيتروجين ، أنتج طحلب الكلوريلا 58.39٪ من الأحماض الدهنية المشبعة و41.60٪ من الأحماض الدهنية غير المشبعة. بينما في وسط النمو المحتوى على 1.5 جرام من نترات الصوديوم أنتج نفس الطحلب 62.08٪ من الأحماض الدهنية المشبعة و37.92٪ من الأحماض الدهنية غير المشبعة. في الوسط الخالى من النيتروجين أنتج طحلب ديسموديسمس كوادريكودس 66.92٪ من الأحماض الدهنية المشبعة و33.07٪ من الأحماض الدهنية غير المشبعة. بينما في وسط النمو المحتوى على 1.5 جرام من نترات الصوديوم أنتج ذلك الطحلب 51.62٪ من الأحماض الدهنية المشبعة و48.37٪ من الأحماض الدهنية غير المشبعة. و بلمحة عن الحمض الدهني لنوع طحلب الكلوريلا المعزول من من المسطحات المائية المصرية و النامى على وسط نمو خالي من النيتروجين قد يكون مناسب لإنتاج وقود الديزل الحيوي. تمت مناقشة النتائج و مقارنتها بالصور المختلفة للأحماض الدهنية التى تنتج بواسطة أنواع الطحالب الأخرى.