



Enhancement of Oil Accumulation in Microalga *Scenedesmus obliquus*

Farouk K. El-Baz^{1*}, Sayeda, M. Abdo², Gamila H. Ali²

¹Plant Biochemistry Depart., National Research Centre, 33 El Bohouth st. (former El Tahrir st.)-Dokki, Giza, Egypt.

²Water pollution Research Depart., National Research Centre, 33 El Bohouth st. (former El Tahrir st.)-Dokki, Giza, Egypt.

*Corresponding author's E-mail: fa_elbaz@hotmail.com

Accepted on: 18-10-2015; Finalized on: 30-11-2015.

ABSTRACT

This work aims to examine the possibility of enhancing the oil productivity of microalga *Scenedesmus obliquus* by modifying the nitrogen content of the medium and method of cultivation. Nitrogen deficiency increased oil content from 10% (in control culture) to 32% (in nitrogen deficient culture). Also, the cultivation method affects the oil content of *Scenedesmus obliquus*. Since the transfer of the alga cells from batch system cultivation technique to a semi-continuous system increased the oil content of *Scenedesmus obliquus* from 32% to 40% under nitrogen deficiency. The saturated and unsaturated fatty acids methyl ester was identified. The results showed that *Scenedesmus obliquus* possesses a favorable fatty acids profile that can be utilized for biodiesel production.

Keywords: *Scenedesmus obliquus*, cultivation system, nitrogen deficiency, lipid content, saturated fatty acids, unsaturated fatty acids.

INTRODUCTION

Recently, liquid biofuels from microalgae have received wide attention since they are made from non-toxic, biodegradable, and renewable resources, and their use can lead to a decrease in the emission of harmful air pollutants.^{1,2} Among the microalgae, *Scenedesmus* and *Chlorella* species have the most desirable features for efficient and economic combination of CO₂ fixation, wastewater treatment and lipid synthesis toward biodiesel production.^{3,4} However, the economic feasibility for algae mass culture for biodiesel production greatly depends on high biomass productivity and lipid yield.⁵ Several strategies have been applied to improve microalgae growth and lipid content. These include optimization of the medium compositions (e.g., type of carbon source, nitrogen, phosphorus, vitamins and salts)⁶, physical parameters (e.g., pH, temperature and light intensity)⁷ and type of metabolism (e.g., phototrophic, heterotrophic, mixotrophic and photoheterotrophic growth). The utilization of appropriate cultivation conditions and medium composition (especially nitrogen source) are of a great importance.⁸ The same authors added that the experimental design strategy combined with the use of the desirability function for the optimization of nitrogen, phosphorus and vitamin showed to be a successful tool for maximizing cell and oil production simultaneously. They concluded that to enhance the economic feasibility of using algal oil for biodiesel production, the micro algal biomass productivity, lipid cell content, and overall lipid productivity are the three key parameters that need to be enhanced.

The lipid content of microalgae could be increased by various cultivation strategies, such as nitrogen depletion,

phosphate limitation, high salinity, high iron concentration and high light intensity.⁹⁻¹²

The first step in developing an economic algal process is to choose the suited algal species and strains.¹³ Fast growth promotes high biomass productivity which consequently increases yield per harvest volume in a certain period (productivity) and decreases cost.¹⁴ *Scenedesmus obliquus* is a freshwater microalga that can grow in industrial wastewaters of different origins showing good adaptation ability.¹⁵⁻¹⁶ It is also considered one of the best candidates for biodiesel production among several microalgae species^{17,18}, with a lipid content ranging between 18.8 and 29.3 % dwt for a nutrient-replete medium and up to 42 % dwt for a nutrient-deficient medium.¹⁹ Another important finding is the possibility of doubling the biomass productivity by operating the cultivation and harvesting in continuous mode instead of batch as described by different authors.¹⁹⁻²¹ The aim of the current work is to enhance oil production in *Scenedesmus obliquus* and to assess its potential use as a biodiesel feedstock.

MATERIALS AND METHODS

Isolation, Purification and Identification of *Scenedesmus obliquus*

Scenedesmus obliquus (Figure 1) was isolated from River Nile water by using BG11 media for algal isolation and purification.²² *Scenedesmus* identification has been done according to the keys of identification.²³ The strain was isolated by spreading 0.1ml of water samples into petri dishes containing BG11 with 1.5% agar for solidification. Single colonies of algae were then re-cultivated as non-axenic batch cultures (50ml) at 25±2°C and 24hr with continuous white fluorescent lamp intensity ≈2500Lux.





Figure 1: *Scenedesmus obliquus* microalgae.

BG11 Nutrient Composition

Nutrient Composition of BG11 was used²² for growing *Scenedesmus obliquus* (Table 1).

Cultivation and Starvation of *Scenedesmus obliquus*

The organism was grown in conical flask 5 liter containing 2 L of media with 1.5 g/L NaNO₃ concentration for two weeks.

The algal biomass after the growth phase was harvested and inoculated in BG11 media with 0.025 g/L NaNO₃ for another two weeks to investigate the effect of decreasing nitrate concentration on total lipid content of *Scenedesmus obliquus*.

In addition, the biomass was harvested and inoculated in an open pond with capacity of 25L (Figure 2) and cultured with 22 liter of BG11 media with NaNO₃ 0.025 g/L in order to determine the variation of lipid content when the microalgae transferred to semi pilot scale.

Table 1: BG11 nutrient composition.

Macronutrients	mg/L
NaNO ₃	1500.00
K ₂ HPO ₄	40.00
MgSO ₄ .7H ₂ O	75.00
CaCl ₂ .2H ₂ O	36.00
Citric acid	6.00
Na ₂ CO ₃	20.00
Na ₂ EDTA	1.00
Ferric ammonium citrate	6.00
Micronutrients	mg/L
H ₃ BO ₃	2.86
MnCl ₂ .4H ₂ O	1.81
ZnSO ₄ .7H ₂ O	0.222
Na ₂ MoO ₄ .2H ₂ O	0.39
CuSO ₄ .5H ₂ O	0.079
Co(NO ₃) ₂ .6H ₂ O	0.0494

- Add 1ml/L into the culture medium from the micronutrient.
- After autoclaving and cooling pH of medium is about 7.

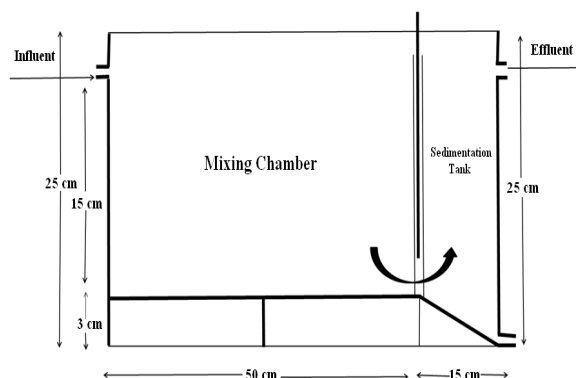


Figure 2: Schematic Diagram of the Continuous Flow Tank - semi pilot scale (Side View).

Lipid Extraction

Modified method of Bligh and Dyer²⁴ was used. The biomass of microalgae was dried and ground into homogenous fine powder. The dry cells were mixed with methanol-chloroform (1:1, v/v) as a co-solvent using homogenizer for minutes at 800 rpm in a proportion of 1 gm in 75 ml of solvent mixture. The homogenate mixture was subjected to a magnetic stirrer at 30°C for 2hrs. Cell residue was removed by filtering. The filtrate was transferred into a separating funnel and sufficient water was added to induce biphasic layering. After settling the solvent mixture was partitioned into two distinct phases, top light green aqueous methanol layer containing most of the co-extracted non-lipids and bottom dark green chloroform layer containing most of the extracted lipids. The chloroform layer was collected in a pre-weighted flask and evaporated using a rotary evaporator.

Preparation of fatty acids

The lipid samples were methylated by heating under reflux using methanolic HCL (5%) at 60°C. The fatty acids were extracted with petroleum ether 40-60 °C. The ether extract was washed three times with distilled water then dried over anhydrous sodium sulfate, and filtered off.²⁵

Gas chromatography/mass spectrometry (GC/MS)

The GC/MS analysis was performed using a Thermo Scientific, Trace GC Ultra/ISQ Single Quadruple MS, TG-5MS fused silica capillary column (30m, 0.251mm, 0.1mm film thickness). For GC/MS detection, an electron ionization system with ionization energy of 70eV was used. Helium gas was used as the carrier gas at a constant flow rate of 1ml/min. The injector and MS transfer line temperature was set at 280°C. The oven temperature was programmed at an initial temperature 150°C (hold 4 min) to 280°C as a final temperature at an increasing rate of 5°C/min (hold 4min).

The quantification of all the identified components was investigated using a percent relative peak area. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system.

RESULTS AND DISCUSSION

Effect of nitrogen deficiency on oil content of *Scenedesmus obliquus*

The results in table (2) showed that the total lipid content in the control sample of *Scenedesmus obliquus* (1.5g/L NaNO₃) was 10%±0.1. Abdo²⁵ found that in a comparison study *Scenedesmus quadricauda* has total lipid content of 16% while *Scenedesmus obliquus* has total lipid 10%, although the two species belong to the same algal group. After decreasing nitrate concentration and growing on 5 L conical flasks (as a batch system) the total lipid percentage reached 32.1%±0.3. El-Sheekh¹⁴ showed that nitrogen deficiency revealed increase in fatty acid content of *Scenedesmus obliquus* by 54% over control but fatty acid productivity was decreased as a result of growth decline. Furthermore, Abo El-Enin²⁶ stated that *Chlamydomonas variabilis* recorded decreasing in lipid accumulation with nitrate depletion. In the present study when growing *Scenedesmus obliquus* on semi pilot scale under the same nitrate concentration, the total lipid content was increased and reached 40%±0.5. The increase in total lipid content of *Scenedesmus obliquus* is mainly due to decreasing nitrate concentration in the media. These results are in agreement with the results of Griffiths and Harrison¹³ which showed that, the lipid content at 0.15 g/L NaNO₃ was higher than that at 1.5 g/L. The potential use of *Hematococcus pluvialis* as a biodiesel feedstock was studied by Damiani.²⁷ The results showed that total lipid content of the control cells was 15.6%, whereas that of cells exposed to continuous high light intensity with nitrogen sufficient and nitrogen deficient medium was 34.85% and 32.99%, respectively. Also, the results of Subhasha²⁸ showed that as decreasing the nitrate concentration in the culture medium, the lipid content increased.

In the present study oil content was drastically varied under normal and stress conditions. About 10% of oil content was found in *Scenedesmus obliquus* grown under normal condition. As conditions became stress (nitrogen starvation) oil content was increased and reached 32% and 40% in *Scenedesmus obliquus* grown under different cultivation conditions. Lardon²⁹ found that the control of nitrogen stress during growth led to maximum oil production from *Chlorella vulgaris*.

However oil content of 20-30% is easy to induce in several microalgae species.³⁰ Exceptionally an oil content of 86% was reported in *Botryococcus brauna*.³¹ However the major obstacle in focusing *B. brauna* is its poor growth rate and low micro algal biomass productivity.³²

Additionally, Rodolfi³³, added that a two phase strategy will require before the cultures is N- starved for oil synthesis, enough biomass (to be used as inoculums) is produced under N-sufficiency followed by a second stage for efficient lipid accumulation under N-deprivation. These results may be explained by the fact that under stress condition, all carbon produced are used for the

production of oil and other substances that played an important role in algal tolerance in defense mechanisms. In addition, Sheehan³⁴ suggested that the reasons for the increase oil content was that under nutrient stress starvation, the rate of production of all cell compound is lower but oil production remains higher leading to an accumulation of oil in the cells.

Gouveia and oliveira¹⁷ reported more than 28% oil of the DW for *Nannochloropsis*. However, when the algae were re- inoculated in N-deficient medium, a large increase in the oil quantity (50%) was observed. Also, Mandal and Mallick³⁵ found that the lipid content of *Scenedesmus obliquus* reached 43% when grown under N-deficiency compared to 12.8% in control treatment. They added that species that are high salt tolerant and are able to grow and reproduce under nutritional scarcity by altering their metabolic pathways efficiently found most promising in this regard. On the other hand, other stress conditions including high salinity or high temperature and variation in pH are also found to be controlling factors that govern the channeling of metabolism from starch to oil.³⁶ In conclusion, the data of the present investigation illustrated that under nitrogen starvation, oil content of *Scenedesmus obliquus* was highly increased by subculturing in semi pilot scale from 32.1% to 40%. This result indicate that culturing system may affect the fatty acids content in the sample even it has higher lipid content.

Fatty acids composition of *Scenedesmus obliquus*

Fatty acids methyl ester of *Scenedesmus obliquus* identified by GC/MS analysis are illustrated in (Figures 3-4) and Table (3). Both saturated and unsaturated fatty acids were detected. The saturated fatty acids were identified mainly as C16:0, palmitic acid methyl ester. On the other hand the unsaturated fraction was detected as palmitoleic methyl ester C16:1, 18:1 oleic acid methyl esters C18:2 linoleic acid. The most commonly synthesized fatty acids have chain length ranged from C16 to C18.

The results also showed that hexadecanoic acid methyl ester (palmitic acid) was detected in all samples (Figure 5) but with higher percentage in samples collected batch culture (48.84%), while it decrease in samples collected from semi pilot scale to 40.89%.

The GC/MS chromatograph of *Scenedesmus obliquus* oil individual FAME are shown in Figure 5 and Table 4. It demonstrates that *Scenedesmus obliquus* grown under N-deficiency contains mainly saturated fatty acids (~80-85% of total acyl methyl esters) which confirm its high oxidative stability.

While esters from other fatty acid such as palmitoleic and oleic acids were of 3.61-12.95 and 2.22-14.40%, respectively. Thus, *Scenedesmus obliquus* could be considered as a potential source for biodiesel production.³⁵



Table 2: Total lipid content of *Scenedesmus obliquus* grown under nitrogen starvation and different cultivation systems.

Cultivation system Lipid content	Control culture 1.5g/L NaNO ₃	Batch culture 0.025g/L NaNO ₃	Semi pilot scale 0.025g/L NaNO ₃
Total lipid %	10%±0.1 (100%)	32.1%±0.3 (320%)	40%±0.5 (400%)

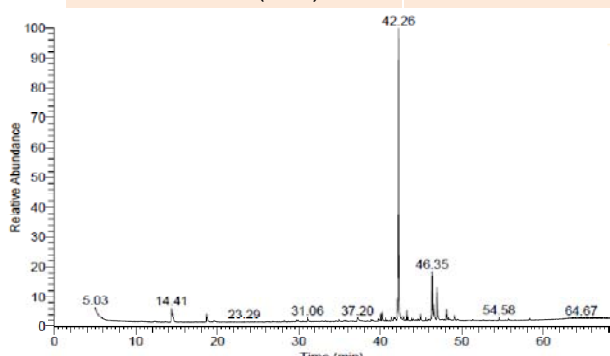
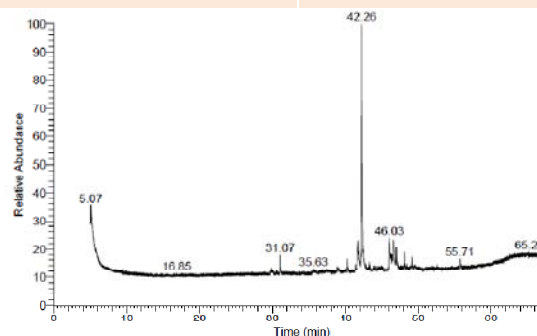
Table 3: GC/MS analysis of fatty acids methyl ester of *Scenedesmus obliquus* grown in different cultivation systems under N-starvation.

RT ^a	Compound name	Molecular formula	Area %		
			1	2	3
5.07	Butanoic acid, 2aminooxy	C4H9NO3	-	-	3.48
18.70	7,10,13Eicosatrienoic acid, methyl ester	C21H36O2	4.80	1.91	-
37.20	Cyclopentanetridecanoic acid, methyl ester (CAS)	C19H36O2	-	1.24	-
39.79	Pentadecanoic acid, methyl ester	C16H32O2	-	0.65	-
40.28	4-Piperidineacetic acid, methyl ester derivative	C23H32N2O4	-	-	2.52
42.12	9-Hexadecenoic acid-palmitoleic	C16H30O2	0.80	2.18	6.24
42.26	Hexadecanoic acid, methyl ester- palmitic acid	C17H34O2	11.40	48.84	40.89
46.22	6,9,Octadecadienoic acid, methyl ester-linoleic acid	C19H34O2	5	0.69	-
46.35	8-Octadecenoic acid, methyl ester-oleic acid	C19H36O2	4.90	8.70	1.07
46.50	2-Hexadecenoic acid, 2,3 dimethyl, methyl ester	C19H36O2	-	0.30	-
46.61	10-Octadecenoic acid, methyl ester	C19H36O2	-	2.34	-
46.96	Octadecanoic acid, methyl ester stearic acid	C19H38O2	14.80	6.27	-
46.99	Heptadecanoic acid, 14 methyl, methyl ester,	C19H38O2	-	-	2.85
49.12	Octadecanoic acid, 9,10 dichloro, methyl ester-stearic derivative	C19H36Cl2O2	-	1.47	-
49.43	9,12,15,Octadecatrienoic acid - ALA	C27H52O4Si2	3.40	0.40	-

Where, RT: retention time, 1: Control treatment 2: Batch culture 3: Semi pilot scale

Table 4: FAME of *Scenedesmus obliquus* and their relative quantities grown under different cultivation systems under nitrogen deficiency.

Fatty acids	Relative%		
	Control	Batch culture	Semi pilot scale
Palmitic acid (C16:0)	51.6	80.90	84.83
Palmitoleic acid (C16:1)	3.6	3.61	12.95
Oleic acid (C18:1)	22.2	14.40	2.22
Linoleic acid (C18:2)	22.6	1.14	-

**Figure 3:** GC-MS spectrum of FAME of *Scenedesmus obliquus* grown in batch culture under nitrogen starvation**Figure 4:** GC-MS spectrum of FAME of *Scenedesmus obliquus* grown in semi pilot scale under nitrogen starvation

Methyl palmitate

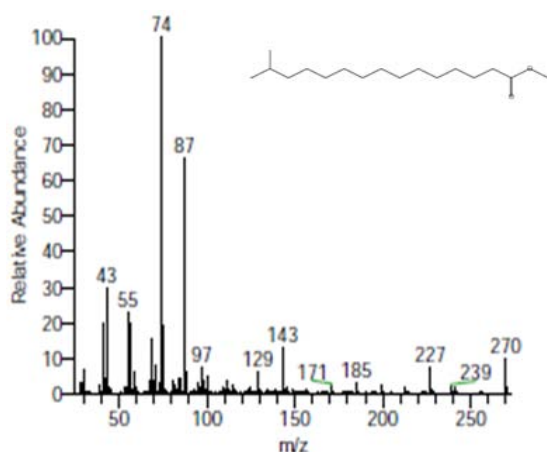


Figure 5: Hexadecanoic acid, methyl ester (CAS) Formula C17H34O2, MW 270, CAS# Methyl palmitate.

Oils with high oleic acid and palmitic acids content have been reported to have a reasonable balance of fuel including their ignition quality, combustion heat, cold filter, plugging point, oxidative stability, viscosity and lubricity which are determined by the structure of fatty acids.³⁷ In this study, the fatty acids composition of *Scenedesmus obliquus* was found to be in agreement with the general profile of fatty acids in other microalgae.³⁸

Fatty acids methyl esters with 4 and more double bonds are susceptible to oxidation and this reduces their acceptability for use in biodiesel. The results of the present study indicated that fatty acids with 4 or more double bonds are not detected. The microalgal possesses a favorable fatty acids profile that can be utilized for biodiesel production with high oxidation stability.³⁹

The European standard (En 14214) limits linolenic acid methyl ester (C18:3) content in biodiesel for vehicle use to 12%. However, the results of the present study showed that Linolenic acid (C18:3) content in *Scenedesmus obliquus* reached 0.40%. In view of the composition of many microalgal oils, most of them are unlikely to comply with the European biodiesel standards, but this need not be a significant limitation.

The extent of insaturation of microalgal oil and its content of fatty acids with more than 4 double bonds can be reduced easily by partial catalytic hydrogenation of the oil, the same technology that is commonly used in making margarine from vegetable oil.^{40,41}

CONCLUSION

The enhancement of oil production in *Scenedesmus obliquus* indicated the potential of this microalga as a biodiesel feedstock. However, cultivation conditions still need improvement to achieve adequate energy balance in mass culture of the algae.

Acknowledgement: This work was supported and funded by the project entitled "Biodiesel production from algae as a renewable energy source". Funding organization:

Research Development and Innovation (RDI), Funding Program: EU-Egypt Innovation Fund, 2014-2016.

REFERENCES

1. Ho SH, Chen CY, Chang JS, Effect of Light Intensity and Nitrogen Starvation on CO₂ Fixation and Lipid/Carbohydrate Production of an Indigenous Microalga *Scenedesmus obliquus* CNW-N. *Bioresource Technology*, 113, 2012, 244-252.
2. Hakalin NLS, Paz AP, Aranda DAG, Moraes LMP, Enhancement of cell growth and lipid content of a freshwater microalga *Scenedesmus sp.* by optimizing nitrogen, phosphorus and vitamin concentrations for biodiesel production. *Natural Science*, 6, 2014, 1044-1054.
3. Blerch DM, Kangas PC, Mulbry WW, Turbulence and nutrient interactions that control benthic algal production in an engineered cultivation raceway. *Algal Research*, 2, 2013, 107-112.
4. Xin L, Ying HH, Ke G, Xue SY, Effects of different nitrogen and phosphorus concentrations on the growth, nutrient uptake, and lipid accumulation of a freshwater microalga *Scenedesmus sp.* *Bioresource Technology*, 101, 2010, 5494-5500.
5. Liu J, Huang JC, Fan KW, Jiang Y, Zhong YJ, Sun Z, Chen F, Production potential of *Chlorella zofingienensis* as a feedstock for biodiesel. *Bioresource Technology*, 101, 2010, 8658-8663.
6. Mata TM, Martins AA, Caetano NS, Microalgae for biodiesel production and other applications: A Review. *Renewable and Sustainable Energy Reviews*, 14, 2010, 217-232.
7. Rawat I, Ranjith KR, Mutanda, T, Bux F, Biodiesel from microalgae: A critical evaluation from laboratory to large scale production. *Applied Energy*, 103, 2013, 444-467.
8. Yeh KL, Chang JS, Effects of cultivation conditions and media composition on cell growth and lipid productivity of indigenous microalga *Chlorella vulgaris* ESP-31. *Bioresource Technology*, 105, 2012, 120-127.
9. Liu ZY, Wang GC, Zhou BC, Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresources Technology*, 99, 2008, 4717-4722.
10. Reitan KI, Rainuzzo JR, Olsen Y, Effect of nutrient limitation on fatty acid and lipid content of marine microalgae. *Journal of Phycology*, 30, 1994, 972-979.
11. Rao AR, Dayananda C, Sarada R, Shamala TR, Ravishankar GA, Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. *Bioresource Technology*, 98, 2007, 560-564.
12. Gordillo FJ, Goutx M, Figueroa FL, Niell FX, Effects of light intensity, CO₂ and nitrogen supply on lipid class composition of *Dunaliella viridis*. *Journal of Applied Phycology*, 10, 1998, 135-144.
13. Griffiths MJ, Harrison ST, Lipid productivity as a key characteristic for choosing algal species for biodiesel production. *Journal of Applied Phycology*, 21, 2009, 493-507.
14. El-Sheekh M, Abomohra A, Hanelt D, Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production.

- World Journal of Microbiology and Biotechnology, 29, 2013, 915-922.
15. Hodaifa G, Martínez ME, Sánchez S, Use of industrial wastewater from olive-oil extraction for biomass production of *Scenedesmus obliquus*. Bioresource Technology, 99, 2008, 1111-1117.
 16. Hodaifa G, Martínez ME, Sánchez S, Daily doses of light in relation to the growth of *Scenedesmus obliquus* in diluted three-phase olive mill wastewater. Journal of Chemical Technology and Biotechnology, 84, 2009, 1550-1558.
 17. Gouveia L, Oliveira AC, Microalgae as a raw material for biofuels production. Journal of Industrial Microbiology and Biotechnology, 36, 2009, 269-274.
 18. Miranda JR, Passarinho PC, Gouveia L, Bioethanol production from *Scenedesmus obliquus* sugars: The influence of photobioreactors and culture conditions on biomass production. Applied Microbiology and Biotechnology. 96, 2012, 555-564.
 19. Ruiz J, Álvarez-Díaz PD, Arbib Z, Garrido-Pérez C, Barragán J, Perales JA, Performance of a flat panel reactor in the continuous culture of microalgae in urban wastewater: Prediction from a batch experiment. Bioresource Technology, 127, 2013, 456-463.
 20. McGinn PJ, Dickinson KE, Park KC, Whitney CG, MacQuarrie SP, Black FJ, Assessment of the bioenergy and bioremediation potentials of the microalga *Scenedesmus* sp. AMDD cultivated in municipal wastewater effluent in batch and continuous mode, Algal Research, 1, 2012, 155-165.
 21. Ruiz-Marin A, Mendoza-Espinosa LG, Stephenson T, Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresource Technology, 101, 2010, 58-64.
 22. Stanier RY, Kunisawa MM, Cohn- Bazire G, Purification and properties of unicellular blue green algae (order chroococcales). Bacteriology review, 35, 1971, 171-201.
 23. Streble H, Krauter D, Das Leben im Wassertropfen, Mikroflora und Mikrofauna des Süßwassers. Franckh'sche Verlagshandlung, Stuttgart, 1978.
 24. Bligh EG, Dyer WJA, Rapid Method of Total Lipid Extraction and Purification. Canadian Journal of Biochemistry and Physiology, 37, 1959, 911-917.
 25. Abdo SM, Entesar Ahmed, Abo El-Enin S, El Din RS, El Diwani G, Ali G, Growth rate and fatty acids profile of 19 microalgal strains isolated from River Nile for biodiesel production. Journal of Algal Biomass Utilization, 4(4), 2013, 51-59.
 26. Abo El-Enin S, Ahmed E, Abdo SM, El Din RS, El-Diwani G, Ali G, Investigation of factors affecting lipid accumulation in fresh water microalgae for enhancement of biofuel production. ChemXpress, 5(2), 2014, 48-55.
 27. Damiani MC, Popovich CA, Constenla D, Leonardi PI, Lipid analysis in *Haematococcus pluvialis* to assess its potential use as biodiesel feedstock. Bioresource Technology, 101, 2010, 3801-3807.
 28. Subhasha N, Prakash, RM, Rupali S, Effect of nitrogen on growth and lipid content of *Chlorella pyrenoidosa*. American Journal of Biochemistry and Biotechnology, 7(3), 2011, 124-129.
 29. Lardon L, Helias, AQ, Sialve B, Steyer J-P, Bernard O, 2009, Life-cycle assesment of biodiesel production from microalgae. Environmental Science & Technology, 3, 1-6.
 30. Chisti Y, Biodiesel from microalgae. Biotechnology Advances, 25, 2007, 249-306.
 31. Brown AC, Knrchts BA, Coruwny E, Hydrocarbon content and its relationship to physiological state in the green alga *Eotryoeoccus braunii*. Phytochemistry, 8, 1969, 543-547.
 32. Li X, Hu HY, Gan K, Yang J, Growth and nutrients removal properties of a freshwater microalga *Scenedesmus* sp. LX1 under different kinds of nitrogen sources. Ecological Engineering, 36, 2010, 379-381.
 33. Rodolfi L, Chini ZG, Bassi N, Padovani G, Biondi N, Bonini G, Tredici MR, Microalgae for Oil: Strain Selection, Induction of Lipid Synthesis and Outdoor Mass Cultivation in a Low-Cost Photobioreactor. Biotechnology and Bioengineering, 102, 2009, 100-112.
 34. Sheehan J, Dunahay T, Benemann J, Roessler P, A look back at the U.S. Department of Energy's Aquatic Species Program – Biodiesel From Algae, Golden, CO, National Renewable Energy Institute, NREL/TP-580-24190, 1998, 328 pp.
 35. Mandal S, Mallick N, Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. Applied Microbiology and Biotechnology, 84, 2009, 281-191.
 36. Takagi M, Yoshida KT, Effect of salt concentration on intracellular accumulation of lipids and triacylglyceride in marine microalgae *Dunaliella* cells. Journal of Bioscience and Bioengineering, 101, 3, 2006, 223-226.
 37. Prabakaran P, Ravindran AD, *Scenedesmus* as a potential source of biodiesel among selected microalgae. Current Science, 102, 4, 2012, 616-620.
 38. Rasoul-Amini S, Montazeri-Najafabady N, Mobasher MA, Hoseini-Alhashemi, S., Ghasemi Y, *Chlorella* sp.: A new strain with highly saturated fatty acids for biodiesel production in bubble-column photobioreactor. Applied Energy, 88, 10, 2011, 3354-3356.
 39. Miao X, Wu Q, Biodiesel production from hetrtrophic microalgal oil. Bioresource Technology, 97, 2006, 841-846.
 40. Jang E, Jung, M, Min DB, Hydrogenation for low trans and high conjugation fatty acids. Comprehensive Reviews in Food Science and Food Safety, 4, 2005, 22-30.
 41. Dijkstra, AJ, Revisiting the formation of trans isomers during partial hydrogenation of tricylaytion oils. European Journal of Lipid Science and Technology, 108(3), 2006, 249-64.

Source of Support: Nil, Conflict of Interest: None.

