Mar. Sci. Tech. Bull. (2022) 11(2): 194-201
e–ISSN: 2147–9666
info@masteb.com

dergipark.org.tr/en/pub/masteb
www.masteb.com
DOI: 10.33714/masteb.1100624

Marine Science and Technology Bulletin

RESEARCH ARTICLE

Effects of nitrogen and phosphorus concentrations on the growth and lipid accumulation of microalgae *Scenedesmus obliquus*

Leyla Uslu[1*] • Oya Işık[1] • Yasemin Barış[1] • Selin Sayın[2]

[1] *Çukurova University, Fisheries Faculty, Basic Science Department, 01330, Adana, Turkey*
[2] *İskenderun Technical University, Faculty of Marine Sciences and Technology İskenderun, 31200, Hatay, Turkey*

ARTICLE INFO

Article History:
Received: 08.04.2022
Received in revised form: 18.05.2022
Accepted: 19.05.2022
Available online: 20.06.2022

Keywords:
Growth
Lipid
Scenedesmus obliquus
Nitrogen concentration
Phosphorus concentration

ABSTRACT

In the study, *Scenedesmus obliquus* green algae was cultivated under laboratory conditions at 21±2°C, 16:8 (light:dark) photoperiod and continuous aeration in different nitrogen and phosphorus ratio nutrient medium and its growth was determined. Dry weight, cell density (optical density) and chlorophyll *a* and *b* were used to determine the growth of the algae. The best growth was determined in the group consisting of 30 ml $NaNO_3$+10 ml PO_4. The amount of biomass obtained was determined as 1.549 gL^{-1} in this group. The lowest values were the group containing 5 ml $NaNO_3$+5 ml PO_4. With the decrease in the amount of nitrogen in the medium, an increase in the amount of carotene and a decrease in the amount of chl *a* and *b* were detected. The highest lipid values were determined as 36.7% in the group containing 5 ml $NaNO_3$+5 ml PO_4 and 36.2% in the group containing 5 ml $NaNO_3$+10 ml PO_4.

Please cite this paper as follows:

Uslu, L., Işık, O., Barış, Y., & Sayın, S. (2022). Effects of nitrogen and phosphorus concentrations on the growth and lipid accumulation of microalgae *Scenedesmus obliquus*. *Marine Science and Technology Bulletin*, *11*(2), 194-201. https://doi.org/10.33714/masteb.1100624

Introduction

It is important to cultivate microalgae species that have commercial value in the field of algae biotechnology due to the metabolites they produce. High quality vitamins, essential unsaturated fatty acids and amino acids can be synthesized from microalgae groups. Nutrient elements, light, temperature and pH have important roles in the growth of algae. However, it is known that the most important factor in the lipid production of microalgae is the nutritional regime, especially such as nitrogen (N) and phosphorus (P) limitations (Sugimoto et al., 2008). Microalgae cells increase some of their metabolites

* Corresponding author
E-mail address: hizarcil@cu.edu.tr (L. Uslu)

under stress. The N atom influences production and accumulation carbohydrates and lipids in the cell. N restriction is associated with cellular fatty acids and growth, causing a decrease in cell number and chl-*a* amounts, and an increase in organic carbon compounds such as lipids. One of the renewable, non-toxic biodiesel fuel sources as an energy source is microalgae biomass. *Scenedesmus obliquus* is a freshwater species and is considered as a biodiesel source (Mandal & Mallick, 2009). In addition to having high biomass, it has been reported in the literature that it contains high lipid (Mandal & Mallick, 2009).

Hundreds of microalgae strains with high lipid content have been screened and their lipid production metabolism has been characterized and reported (Sheehan et al., 1998). There are many studies dealing with the amount and quality of lipids in the cell can be altered because of changes in growth conditions such as temperature and light intensity or nutrient medium properties, N concentration, phosphates and iron (Illman et al., 2000; Liu et al., 2008).

Nutrient concentration is a key factor in the coupling system of biodiesel production. In this article, the effects of N and P nutrient concentrations on the growth and lipid accumulation characteristics of *S. obliquus* were examined.

Material and Methods

Microalgae Cultivation Conditions

Scenedesmus obliquus (UTEX # 393), a member of the green algae (Chlorophyceae) group, was used in this study. The experiment was set up in the Algal Biotechnology Laboratory with constant light (40 µmol photon $m^{-2}s^{-1}$) and a 16:8-hour light and dark cycle and a constant temperature (21±2°C) using the semi-continuous culture method. Illumination was measured with a radiation sensor LI-COR (LI-250). The experimental study was carried out in Bold Basal medium (BBM), in 2L glass flasks and with 10% inoculation intensities, and the culture was continuously stirred by air. In the experiment, two main elements (N and P) that affect the growth of algae but contribute to lipid production were selected and used at various concentrations. A total of 6 groups were formed with the combinations of the different ratios of N and P concentrations (Table 1) (Concentration in Final Medium: 10 ml $NaNO_3$=2.94*10^{-3}mM, 10 ml K_2HPO_4=4.31*10^{-4}mM, 10 ml KH_2PO_4=1.29*10^{-3}mM).

Table 1. Trial groups and quantities used in the study

Trial Groups	Quantity Used
1. Trial group	10 ml $NaNO_3$, 10 ml PO_4
2. Trial group	5 ml $NaNO_3$, 5 ml PO_4
3. Trial group	30 ml $NaNO_3$, 10 ml PO_4
4. Trial group	30 ml $NaNO_3$, 5 ml PO_4
5. Trial group	10 ml $NaNO_3$, 5 ml PO_4
6. Trial group	5 ml $NaNO_3$, 10 ml PO_4

Note: All applications were made in triplicate.

Cell Density (OD) and Dry Weight

Cell density (OD) was checked daily by a visible spectrophotometer UV-Vis SP-3000 nano at 665 nm (Kaewkannetra et al., 2012). Regression equation was created according to the regression curve formed between dry weight and OD and the amount of dry weight was calculated according to equation 1 (Yue & Chen, 2005).

$$Dry\ weight\ (gL^{-1})x = 1.22OD_{665} + 0.0001 \quad (1)$$

Chlorophyll and Carotene Analysis

The chlorophyll and carotene were measured at three days intervals. Five milliliters of each *S. obliquus* culture were taken and filtered using Whatman GF/CTM, 1.2 µm, UK. For chlorophyll extraction, the filters were put in 15 mL glass tubes to which 10 mL of 95% ethanol /water mixture was added on it, and left in the refrigerator (+4°C) in the dark for 24 hours. At the end of the extraction period, the upper clear part was removed and absorption values were measured at 470, 649 and 665 nm in the visible spectrophotometer. Using the formula below, chl-*a*, chl-*b* and total carotene amounts were calculated (Sartory & Grobbelaar, 1984).

$$Chl\ a = 13.7 \times OD_{665} - 5.76 \times OD_{649} \quad (2)$$

$$Chl\ b = 23.96 * OD_{649} - 7.32 * OD_{665} \quad (3)$$

$$Carotene = \frac{(1.00 \times OD470 - 2.05 \times Chl\ a)}{245} \quad (4)$$

Lipid and Protein Analysis

At the end of the experiment, all of the remaining cultures were harvested for 10 minutes with the help of refrigerated centrifuge (Heraeus, Suprafuge 22) at 7500 rpm rotation speed.

The biomass obtained was dried at 55°C for protein and total lipid analysis. Protein analysis was performed according to the Kjeldahl method (Williams, 1984). Total lipid values were calculated according to the percentage of dry biomass. Total lipid analysis was made according to the method applied by Bligh & Dyer (1959).

Statistical Analysis

The data were analyzed by statistical analysis using IBM SPSS-12 and the graphs were drawn by Microsoft Excel (2010 Microsoft Corporation, USA) program. Two-way analysis of variance (ANOVA) was used to test the effects of N and P limitation of the culture on lipid, protein, cell density OD, chl *a* and *b*, carotene and dry weight. When differences were found in two-way ANOVA, Duncan multiple comparison test (HSD), (SPSS) of one-way ANOVA (Version 12.0, SPSS, Chicago, IL) was used (Zar, 1999).

Results

In the study, *S. obliquus* was cultured under laboratory conditions and their growth were determined by performing continuous aeration in nutrient media containing different ratios of N and P at 21±2°C, and 16:8 (light: dark) photo period. Growth and pigment production (chlorophyll *a*, *b* and carotenoid) of *S. obliquus* were followed over time.

The growth of the *S. obliquus* was followed over a period of three weeks (Figure 1). In the experiment, the initial cell density OD values of all groups were statistically similar with value of 0.294±0.003. Considering the cell density OD values of the groups on the last day, the best result was obtained in the group containing 30ml $NaNO_3$+10ml PO_4 and 30ml $NaNO_3$+5ml PO_4. The results were statistically significant ($P<0.05$) compared to other groups. The last day cell density OD in these groups were 1.297±0.005 and 1.270±0.3, respectively. The lowest cell density OD values were found for group containing 5ml $NaNO_3$+5ml PO_4, and this value was determined as 1.119±0.008. Cell density OD of the treatment groups was shown below (Figure 1). Likewise, the highest amount of dry matter was determined in groups containing 30 ml of $NaNO_3$ ($P<0.05$) (Table 2). As can be seen from the Table 2, the best growth was observed in the group containing 30ml $NaNO_3$+10ml PO_4. The lowest biomass values were in the group containing 5ml $NaNO_3$+5ml PO_4. The highest biomass was determined as 1.583 and 1.549 gL^{-1} in the groups containing 30ml $NaNO_3$. The lowest biomass was determined as 1.366 gL^{-1} in the group containing 5ml $NaNO_3$+5ml PO_4 ($P<0.05$).

The last day biomass, cell density OD, chl *a*, chl *b* and carotene amounts of *S. obliquus* at 40 $\mu molm^{-2}s^{-1}$ photon light intensity and different ratios of nutrients are summarized in Table 2. It was determined that the amount of carotene increased and the amount of chlorophyll decreased with the decrease of N in the nutrient medium. The highest amount of carotene was determined in the group including 5ml $NaNO_3$+ 5ml PO_4 with the lowest biomass and the lowest chlorophyll value.

Nutrient limitation leads to an increase in the content of lipids but decrease in the content of protein in cells of *S. obliquus*. Reducing the concentration of the N resulted in an approximately 2.5-fold increase in the cellular lipid content of cells. However, P deficiency in the growth medium did not cause an increase in lipid content. Protein and lipid content of the experimental groups were summarized in Figure 2 and Figure 3. As indicated in Table 2, the highest biomass and

Table 2. Last day biomass, optical density, chlorophyll *a*, *b* and carotene values of *S. obliquus* in different N and P ratios

Trial Groups	Biomass (gL^{-1})	Cell density OD	Chl *a* (μgmL^{-1})	Chl *b* (μgmL^{-1})	Carotene (μgmL^{-1})
10 ml $NaNO_3$, 10 ml PO_4	1.404±0.1^{b}	1.151±0.2^{b}	2.152±0.02^{b}	0.301±0.02^{b}	1.089±0.04^{b}
5 ml $NaNO_3$, 5 ml PO_4	1.366±0.2^{c}	1.119±0.3^{c}	0.953.5±0.02^{d}	0.102±0.004^{d}	1.203±0.03^{a}
30 ml $NaNO_3$, 10 ml PO_4	1.583±0.2^{a}	1.297±0.2^{a}	2.410.5±0.03^{a}	0.357±0.002^{a}	0.651±0.02^{c}
30 ml $NaNO_3$, 5 ml PO_4	1.549±0.3^{a}	1.270±0.3^{a}	2.145±0.001^{b}	0.318±0.001^{b}	0.649±0.001^{c}
10 ml $NaNO_3$, 5 ml PO_4	1.405±0.2^{b}	1.152±0.1^{b}	1.028±0,01^{c}	0.153±0.002^{c}	1.094±0.03^{b}
5 ml $NaNO_3$, 10 ml PO_4	1.401±0.1^{b}	1.148±0.1^{b}	1.017±0.02^{c}	0.168±0.001^{c}	1.181±0.002^{a}

Note: Values are means (n=3). Means in a row without a common superscript letter differ ($p<0.05$) as analyzed by one-way ANOVA and the Duncan test.

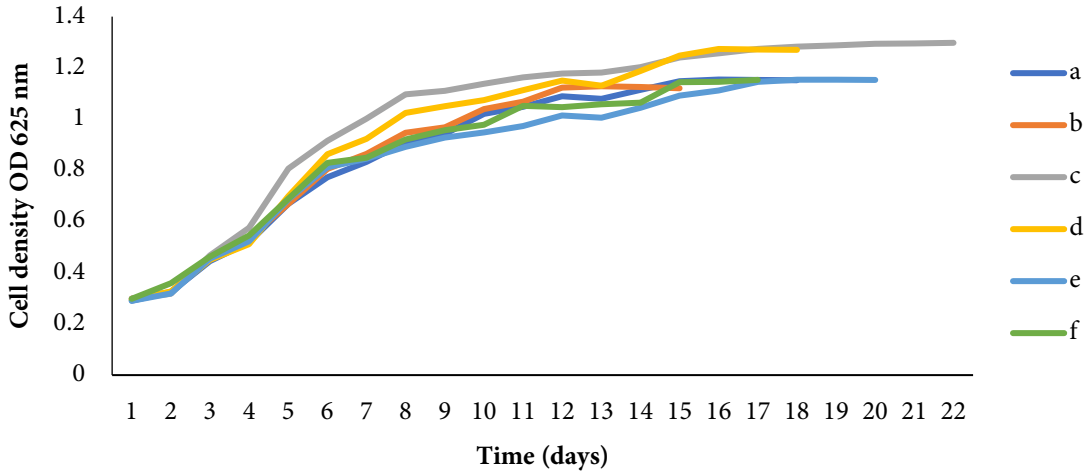

Figure 1. Optical density values of treatment groups. **a:**10 ml $NaNO_3$, 10 ml PO_4, **b:** 5 ml $NaNO_3$, 5 ml PO_4, **c**: 30 ml $NaNO_3$, 10 ml PO_4, **d**: 30 ml $NaNO_3$, 5 ml PO_4, **e:**10 ml $NaNO_3$, 5 ml PO_4, **f:** 5 ml $NaNO_3$, 10 ml PO_4 (mean values, n=3).

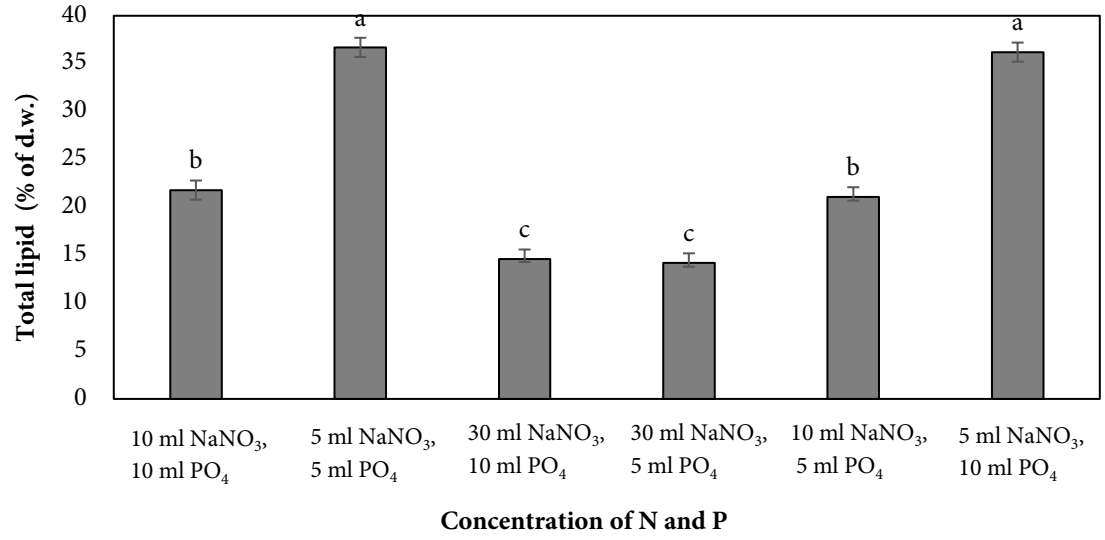

Figure 2. Total lipid production of *S. obliquus* in response to different N and P concentrations. Each error bar represents mean±standard error triplicates (n=3). Mean values different letters (a-c) are significantly different at P<0.05, one-way ANOVA.

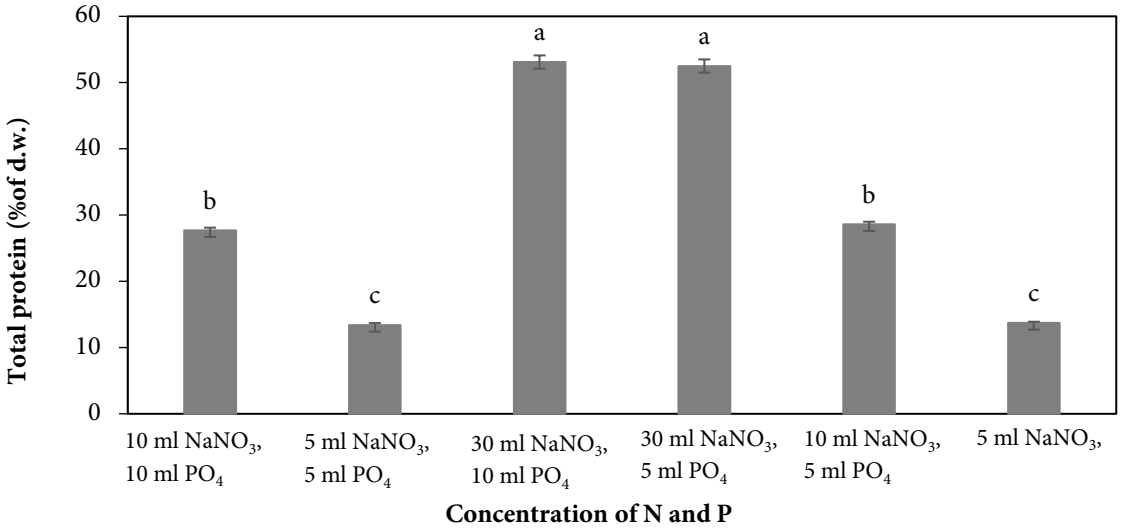

Figure 3. Total protein production of *S. obliquus* in response to different N and P concentration. Each error bar represents mean±standard error triplicates (n=3). Mean values different letters (a-c) are significantly different at P<0.05, one-way ANOVA.

optical density was obtained in the groups containing 30ml $NaNO_3$. As seen in Figure 2 and Figure 3, the amount of lipid and protein varied depending on the N amount in the culture medium which caused the lipid ratio to increase as the N amount decreased. The highest lipid values were determined as 36.7% in the group containing 5ml $NaNO_3$+5ml PO_4 and 36.2% in the group containing 5ml $NaNO_3$+10ml PO_4 ($P<0.05$). Protein contents were found to be the lowest in the groups with the highest lipid values. The highest protein contents were found in the groups containing 30ml $NaNO_3$ ($P<0.05$).

Discussion

Despite advances in algal biotechnology, various difficulties are encountered in the culture of microalgae species. The main purpose in the production of phototrophic organisms is generally to provide a continuous culture at optimal cell density. During the cultivation of an algae species in the outdoor environment, various environmental factors change greatly both daily and seasonally, requiring the cells in the culture to constantly react to these conditions. Biochemical composition of biomass depends on growth conditions such as environmental factors, nutrient environment, temperature, salinity, pH, light (Sukenik, 1991).

Microalgae *S. obliquus* is an excellent species for biodiesel production in terms of their important fatty acids (Abd El Baky et al., 2012; Gouveia & Oliveira, 2009). Limiting elements such as N and P that are necessary for algae growth can increase lipid content (Abd El Baky et al., 2012; El-Sheekh et al., 2013; Ho et al., 2012; Mandal & Mallick, 2009). The nitrogen element, which plays a role in the structure of many macromolecules, especially proteins, is one of the most important elements for microalgae. Limiting N in the growing medium causes a decrease in some metabolites such as protein, while it also causes an increase in some metabolites such as lipid and carbohydrate. It also slows down growth (Simionato et al., 2013, Li et al., 2012). In this study, it was determined that N depletion caused significant changes in the lipid content of *S. obliquus* ($P<0.05$), but P deficiency had no effect. Similar results have been observed in many microalgal species such as *Chlorella* (Illman et al., 2000) and *Botryococcus braunii* (Dayananda et al., 2007). Lipid contents increased in *Nannochloropsis oculata*, *Chlorella vulgaris* and *Chaetoceros muelleri* in N-deficient culture media (McGinnis et al., 1997; Mutlu et al., 2011). Damiani et al. (2010) cultured *Haematococcus pluvialis* under different stress conditions (high light and high light-N deficiency) and stated that the total lipid increased from 15% to 32.99% in N deficiency and high light. Anand & Arumugam (2015) reported that they observed a 2.27- fold increase in lipid for *Scenedesmus quadricauda* in the N deficient growth medium. Kamalanathan et al. (2016) reported that physiological changes are more pronounced in N deficiency in *Chlamydomonas reinhardtii*. They also reported that the photosynthetic performance of *C. reinhardtii* showed large changes under N limitation but relatively did not change with P starvation. They reported that lipid concentrations per cell were at least 2.4 times higher in groups with N deficiency and the amount of protein was lower than in the control group. Mutlu et al. (2011) cultured *C. vulgaris* in a medium containing nitrogen and phosphorus at different concentrations in their study. The lipid value was highest in the group with N deficiency, the amount of lipid did not increase much in the group with P deficiency. They also reported the highest amount of protein in the control group. Pancha et al. (2014) cultured *Scenedesmus* sp. in a nutrient medium containing different levels of nitrogen. They reported that the lipid content of *Scenedesmus* sp. was much higher in N-free environment than in BG-11 medium. Uslu et al. (2011) cultured *Spriulina* in nutrient medium containing different amounts of N. They reported the highest lipid (17.05%) in the group cultured under N deficiency and the highest protein (67.4%) in the control group.

Overall, they found that N deficiency was more effective on lipid and protein levels than P deficiency. In this study, as in many studies, N restriction increased the cellular lipid ratio by decreasing the growth rate. However, in groups with N deficiency, the biomass amounts are not very low. Groups with increased lipid content can be evaluated economically.

Microalgal biomass is very important in lipid studies. The aim of the studies is to keep both lipid and biomass amounts of algae at high levels. In this study, the decrease in the amount of N in the nutrient medium caused an increase in cellular lipid and a decrease in biomass. In the present study, the highest biomass was found in the groups containing 30ml $NaNO_3$, while the lowest lipid ratio was determined in these groups. N deficiencies stated that the amount of biomass decreases, which was consistent with the results of many studies (Adenan et al. 2016, Uslu et al. 2011, Mutlu et al. 2011). Adenan et al. (2016) reported that in *Chlorella* sp. and *Chaetoceros* sp., when N deficiency was applied, the lipid ratio increased, but the amount of biomass, protein and carbohydrate decreased.

In many studies investigating the effects of N restriction on metabolites in microalgae cultures, it was observed that the

ratio of organic carbon compounds such as lipids increased, while decreases in cell number and chl a were found. However, yellowing of culture colors has been observed due to increases in carotene content (Shifrin & Chisholm 1981; Sukenik et al. 1989).

Uslu et al. (2020) cultured *Isochrysis affinis galbana* in nutrient medium containing different amounts of N in tubular and panel systems. In the study, they reported that the chlorophyll content decreased and the total carotene content increased in 50% N (-) cultures. The highest OD values were found in control group, whilst the lowest OD was obtained in the 50% N (-) group. It was concluded that the OD was lower in the N deficient groups as in other parameters. In this study, chlorophyll content decreased in cultures with N deficiency and total carotene content increased. Likewise, the highest OD was determined in the groups with the highest N content.

Conclusion

The first studies in the field of algal biotechnology were generally carried out with species with high protein content and easily cultivable. However, the discovery of algae that produces valuable metabolites other than protein has also continued. *Scenedesmus obliquus* is one of the important species in the field of microalgal biotechnology due to its metabolites accumulated in the cell. In our study, the best growth and protein content was found in the group containing 30 ml of $NaNO_3$, while the lipid ratio was found in the groups containing 5 ml of $NaNO_3$. If *S. obliquus* is to be used as a source of biodiesel, it is recommended to use groups containing 5 ml of $NaNO_3$ with a lipid ratio of 36%. As a result of the study, we can say that *S. obliquus* is a potential algae species to be used for biodiesel purposes.

Acknowledgements

We gratefully acknowledge the research funding provided for this project (Project No: FBA-2018-11155) by the Scientific Research Projects Unit at Cukurova University for their financial support.

Compliance With Ethical Standards

Authors' Contributions

LU planned, analyzed data, conducted experiments and wrote all parts of manuscript, OI helped in finalizing research theme and objectives, YB guided experiments, SS helped in writing results and discussion.

Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical Approval

For this type of study, formal consent is not required.

References

Abd El Baky, H. H., El-Baroty, G. S., Bouaid, A., Martinez, M., & Aracil, J. (2012). Enhancement of lipid accumulation in *Scenedesmus obliquus* by optimizing CO_2 and Fe^{3+} levels for biodiesel production. *Bioresource Technology*, *119*, 429-432. https://doi.org/10.1016/j.biortech.2012.05.104

Adenan, N. S., Yusoff, F. M., Medipally, S. R., & Shariff, M. (2016). Enhancement of lipid production in two marine microalgae under different levels of nitrogen and phosphorus deficiency. *Journal of Environmental Biology*, *37*(4 Spec No), 669-676.

Anand, J., & Arumugam, M. (2015). Enhanced lipid accumulation and biomass yield of *Scenedesmus quadricauda* under nitrogen starved condition. *Bioresource Technology*, *188*, 190-194. https://doi.org/10.1016/j.biortech.2014.12.097

Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. *Canadian Journal of Biochemistry and Physiology*, *37*(8), 911-917. https://doi.org/10.1139/o59-099

Damiani, M. C., Popovich, C. A., Constenla, D., & Leonardi, P. I. (2010). Lipid analysis in *Haematococcus pluvialis* to assess its potential use as a biodiesel feedstock. *Bioresource Technology*, *101*(11), 3801-3807. https://doi.org/10.1016/j.biortech.2009.12.136

Dayananda, C., Sarada, R., Kumar, V., & Ravishankar, G. A. (2007). Isolation and characterization of hydrocarbon producing green alga *Botryococcus braunii* from Indian freshwater bodies. *Electronic Journal of Biotechnology*, *10*(1), 78-91.

El-Sheekh, M., Abomohra, A. E. F., & Hanelt, D. (2013). Optimization of biomass and fatty acid productivity of *Scenedesmus obliquus* as a promising microalga for biodiesel production. *World Journal of Microbiology and Biotechnology*, *29*(5), 915-922. https://doi.org/10.1007/s11274-012-1248-2

Gouveia, L., & Oliveira, A. C. (2009). Microalgae as a raw material for biofuels production. *Journal of Industrial Microbiology and Biotechnology*, *36*(2), 269-274. https://doi.org/10.1007/s10295-008-0495-6

Ho, S. H., Chen, C. Y., & Chang, J. S. (2012). Effect of light intensity and nitrogen starvation on CO_2 fixation and lipid/carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW-N. *Bioresource Technology*, *113*, 244-252. https://doi.org/10.1016/j.biortech.2011.11.133

Illman, A. M., Scragg, A. H., & Shales, S. W. (2000). Increase in Chlorella strains calorific values when grown in low nitrogen medium. *Enzyme and Microbial Technology*, *27*(8), 631-635. https://doi.org/10.1016/S0141-0229(00)00266-0

Kaewkannetra, P., Enmak, P., & Chiu, T. (2012). The effect of CO_2 and salinity on the cultivation of *Scenedesmus obliquus* for biodiesel production. *Biotechnology and Bioprocess Engineering*, *17*(3), 591-597. https://doi.org/10.1007/s12257-011-0533-5

Kamalanathan, M., Pierangelini, M., Shearman, L. A., Gleadow, R., & Beardall, J. (2016). Impacts of nitrogen and phosphorus starvation on the physiology of *Chlamydomonas reinhardtii*. *Journal of Applied Phycology*, *28*(3), 1509-1520. https://doi.org/10.1007/s10811-015-0726-y

Li, Y., Fei, X., & Deng, X. (2012). Novel molecular insights into nitrogen starvation-induced triacylglycerols accumulation revealed by differential gene expression analysis in green algae *Micractinium pusillum*. *Biomass and Bioenergy*, *42*, 199-211. https://doi.org/10.1016/j.biombioe.2012.03.010

Liu, Z. Y., Wang, G. C., & Zhou, B. C. (2008). Effect of iron on growth and lipid accumulation in *Chlorella vulgaris*. *Bioresource Technology*, *99*(11), 4717-4722. https://doi.org/10.1016/j.biortech.2007.09.073

Mandal, S., & Mallick, N. (2009). Microalga *Scenedesmus obliquus* as a potential source for biodiesel production. *Applied Microbiology and Biotechnology*, *84*(2), 281-291. https://doi.org/10.1007/s00253-009-1935-6

McGinnis, K. M., Dempster, T. A., & Sommerfeld, M. R. (1997). Characterization of the growth and lipid content of the diatom *Chaetoceros muelleri*. *Journal of Applied Phycology*, *9*(1), 19-24. https://doi.org/10.1023/A:1007972214462

Mutlu, Y. B., Işık, O., Uslu, L., Koç, K., & Durmaz, Y. (2011). The effects of nitrogen and phosphorus deficiencies and nitrite addition on the lipid content of *Chlorella vulgaris* (Chlorophyceae). *African Journal of Biotechnology*, *10*(3), 453-456.

Pancha, I., Chokshi, K., George, B., Ghosh, T., Paliwal, C., Maurya, R., & Mishra, S. (2014). Nitrogen stress triggered biochemical and morphological changes in the microalgae *Scenedesmus* sp. CCNM 1077. *Bioresource Technology*, *156*, 146-154. https://doi.org/10.1016/j.biortech.2014.01.025

Sartory, D. P., & Grobbelaar, J. U. (1984). Extraction of chlorophyll *a* from freshwater phytoplankton for spectrophotometric analysis. *Hydrobiologia*, *114*(3), 177-187. https://doi.org/10.1007/BF00031869

Sheehan, J., Dunahay, T., Benemann, J., & Roessler, P. (1998). *Look back at the US department of energy's aquatic species program: biodiesel from algae; close-out report* (No. NREL/TP-580-24190). National Renewable Energy Lab., Golden, CO.(US). https://doi.org/10.2172/15003040

Shifrin, N. S., & Chisholm, S. W. (1981). Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. *Journal of Phycology*, *17*(4), 374-384. https://doi.org/10.1111/j.1529-8817.1981.tb00865.x

Simionato, D., Block, M. A., La Rocca, N., Jouhet, J., Maréchal, E., Finazzi, G., & Morosinotto, T. (2013). The response of *Nannochloropsis gaditana* to nitrogen starvation includes de novo biosynthesis of triacylglycerols, a decrease of chloroplast galactolipids, and reorganization of the photosynthetic apparatus. *Eukaryotic Cell*, *12*(5), 665-676. https://doi.org/10.1128/EC.00363-12

Sugimoto, K., Midorikawa, T., Tsuzuki, M., & Sato, N. (2008). Upregulation of PG synthesis on sulfur-starvation for PS I in *Chlamydomonas*. *Biochemical and Biophysical Research Communications*, *369*(2), 660-665. https://doi.org/10.1016/j.bbrc.2008.02.058

Sukenik, A. (1991). Ecophysiological considerations in the optimization of eicosapentaenoic acid production by *Nannochloropsis* sp. (Eustigmatophyceae). *Bioresource Technology*, *35*(3), 263-269. https://doi.org/10.1016/0960-8524(91)90123-2

Sukenik, A., Carmeli, Y., & Berner, T. (1989). Regulation of fatty acid composition by irradiance level in the eustigmatophyte *Nannochloropsis* sp. *Journal of Phycology*, *25*(4), 686-692. https://doi.org/10.1111/j.0022-3646.1989.00686.x

Uslu, L., Işık, O., Koç, K., & Göksan, T. (2011). The effects of nitrogen deficiencies on the lipid and protein contents of *Spirulina platensis*. *African Journal of Biotechnology*, *10*(3), 386-389.

Uslu, L., Işık, O. & Cimen, B. A. (2020). The effect of nitrogen deficiency on the growth and lipid content of *Isochrysis affinis galbana* in two photobioreactor systems (PBR): Tubular and flat panel. *Journal of Agricultural Sciences*, *26*(3), 282-289. https://doi.org/10.15832/ankutbd.526989

Williams, S. (1984). *Official methods of analysis* (No. 630.24 A8 1984). Association of Official Analytical Chemists.

Yue, L., & Chen, W. (2005). Isolation and determination of cultural characteristics of a new highly CO_2 tolerant fresh water microalgae. *Energy Conversion and Management*, *46*(11-12), 1868-1876. https://doi.org/10.1016/j.enconman.2004.10.010

Zar, J. H. (1999). *Biostatistical Analysis*. 4th ed. Prentice Hall.