Ciência Rural, Santa Maria, v.54:09, e20230269, 2024 http://doi.org/10.1590/0103-8478cr20230269
ISSNe 1678-4596
BIOLOGY

Growth performance and biochemical composition of *Tetradesmus obliquus* (Turpin) M.J. Wynne in media with different nitrogen concentrations

Rıza Akgül[1*]

[1]Burdur Food, Agriculture and Livestock Vocational High School, Burdur Mehmet Akif Ersoy University, 15030, Burdur, Türkiye. E-mail: rizaakgul@gmail.com. *Corresponding author.

ABSTRACT: Microalgae have a great potential to change their metabolism pathway under stress condition, and nitrogen deficiency is an effective technique for maximizing lipids synthesis. This study focus on the growth performance, and biochemical composition of *Tetradesmus obliquus* (Turpin) M.J. Wynne cultured in different nitrogen concentrations (BG 11-Blue-Green Medium, 50% N+, 50% N-, 75% N+, 75% N-). Specific growth rate and protein content of *T. obliquus* decreased with nitrogen deficiency, but its lipid content was increased. Maximum specific growth rate (0.20-0.21) and protein content (47.82%, 47.62% DW-dried weight) values were obtained in 75% N+, 50% N+ medium. The highest lipid percentage was obtained in 50% N- medium. The biochemical composition of *T. obliquus* changes based on nitrogen concentration. Polyunsaturated fatty acids (PUFAs) are more abundant (49.69%) in this microalga under deficient nitrogen. To obtain more lipid and PUFAs in *T. obliquus*, N deficiency is an effective method, but it is not applicable to protein content. *T. obliquus* can be used as an alternative nutritional supplement with its high PUFAs content. Since different results are obtained from species to others in such studies, culture conditions should be determined for each species and according to the desired item.
Key words: biochemical composition, *Tetradesmus obliquus*, microalgae culture, nitrogen.

Desempenho do crescimento e composição bioquímica de *Tetradesmus obliquus* (Turpin) M.J. Wynne em meio com diferentes concentrações de nitrogênio

RESUMO: As microalgas têm um grande potencial para alterar sua via metabólica sob condição de estresse, e a deficiência de nitrogênio é uma técnica eficaz para maximizar a síntese de lipídios. Este estudo enfoca o desempenho do crescimento e a composição bioquímica de *Tetradesmus obliquus* (Turpin) M.J. Wynne cultivado em diferentes concentrações de nitrogênio (BG 11-Blue-Green Medium, 50% N+, 50% N-, 75% N+, 75% N-). A taxa de crescimento específico e o teor de proteína de *T. obliquus* diminuíram com a deficiência de nitrogênio, mas seu teor de lipídeos aumentou. Os valores máximos de taxa de crescimento específico (0,20-0,21) e teor de proteína (47,82%, 47,62% DW-peso seco) foram obtidos em meio 75% N+, 50% N+. O maior percentual lipídico foi obtido em meio 50% N-. A composição bioquímica de *T. obliquus* muda com base na concentração de nitrogênio. Os ácidos graxos poliinsaturados (PUFAs) são mais abundantes (49,69%) nesta microalga sob nitrogênio deficiente. Para obter mais lipídios e PUFAs em *T. obliquus*, a deficiência de N é um método eficaz, mas não é aplicável ao teor de proteína. *T. obliquus* pode ser usado como um suplemento nutricional alternativo com seu alto teor de PUFAs. Como são obtidos resultados diferentes de uma espécie para outra nesses estudos, as condições de cultivo devem ser determinadas para cada espécie e de acordo com o item desejado.
Palavras-chave: composição bioquímica, *Tetradesmus obliquus,* cultivo de microalgas, nitrogênio.

INTRODUCTION

With the increasing world population, the amount of energy needed by humanity is increasing and scientists are working on alternative energy sources and food. Microalgae, which are single-celled aquatic microorganisms, can be grown in fresh water, salt water and wastewater as they are tolerant to environmental stresses. This has caught the attention of scientists.

Different growing conditions, affects the microalgae growth rates, biomass yields, production of lipids and fatty acids in different ways to their nutritional values (ZARRINMEHR et al., 2019; ZIENKIEWICZ et al., 2020).

They can change their biochemical composition under different stress conditions and easily synthesize the target molecules (YAAKOB et al., 2021). Previous studies showed that changes in concentrations of nutrients, especially nitrogen (N) and phosphorus (P) create remarkable changes in microalgae growth, lipid and protein content, amino acids and fatty acid methyl esters (FAMEs) composition (ZHUANG et al., 2018).

Culturing the microalgae in N deficiency medium is one of the most efficient factors for increasing the lipid concentration of microalgae and many studies have been done for this purpose (NAYAK et al., 2019). N deficiency increases the amount of lipid, changes biomass productivity and

Received 05.15.23 Approved 12.13.23 Returned by the author 02.28.24
CR-2023-0269.R1
Editors: Leandro Souza da Silva Corina L. V. A. F. Carranca

growth performance may not result in parallel. If the lipid yield is decreased by reducing the amount of nitrogen, the number of cells may not increase sufficiently and this may cause the lipid yield to decrease (SHARMA et al., 2019). Recent studies are needed to clarify this issue and to understand the metabolic mechanism by working with different species under different conditions. Also, it should be determined which medium is more effective for increasing C16/C18 fatty acids that can improve the combustion performance of biodiesel and saturated fatty acids which are important for oil and biodiesel production (AN et al., 2020).

We had an accession number for *Tetradesmus obliquus* from NCBI and we cultured this microalga easily before like a preliminary study; so, in this research it was chosen as material. Beside this, there are lots of novel study with *Tetradesmus* that aim the same question of this research and had attractive results (KIM et al., 2019).

The fact that microalgae change their biochemical composition under varying environmental conditions, the number of studies that aim to obtain more lipid and protein and change the amino acid and fatty acid composition via exposing to nutrient stress is increasing. Based on this idea, in the present research, *T. obliquus* was cultured in media with different N concentrations, and the effects of N amount on growth parameters of the culture and biochemical composition of *T. obliquus* were investigated. With this study, a species that has not been studied in this field before will be brought to the literature.

MATERIALS AND METHODS

Isolation and culturing of microalgae

T. obliquus was isolated from Ergene River Basin (Turkey) and characterized by molecular approach phylogenetically as *Acutodesmus obliquus* (NCBI Accession Number: KF470790) in 2014. But in 2015, Wynne reclassified this microalga as *T. obliquus* (Turpin) M.J. Wynne, so we used the current name in this study (WYNNE & HALLAN, 2015). It was obtained from the Algal Biotechnology Laboratory, Mehmet Akif Ersoy University (Burdur, Turkey). Light and scanning electron micrographs of *T. obliquus* are shown in figure 1.

For cultivation, the first step was to culture the microalgae in BG11 (Blue-green medium) (RIPPKA et al., 1979), until it reaches its exponential phase under 16/8h light/dark photoperiod (100 µmolphoton $m^{-2}s^{-1}$), at 24±3 °C and pH 7.5.

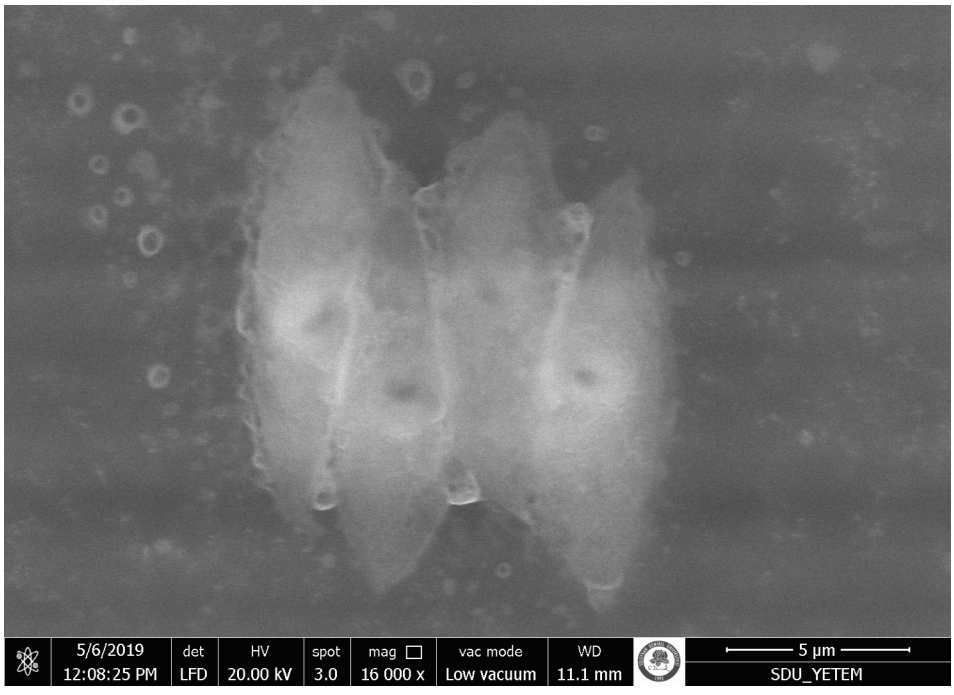

Figure 1 - Scanning electron microscope of *T. obliquus* (B).

The second step entails harvesting and transferring microalgae into media with different N concentrations (BG 11 (Control), 50% N+, 50% N-, 75% N+, 75% N-), the BG 11 that is control medium has 1.5 g/L ($NaNO_3$) N concentration (Table 1) [N+: contains more nitrogen than BG11; N-: contains less nitrogen than BG11].

For this purpose, the cells were inoculated into cultures in 2000 mL flasks under the conditions mentioned above. The cultured flasks were bubbled with air gas (1 L/minute). For adjusting the initial cell number value as $3.6x10^5$ cell/mL; optical density (OD, 680 nm) of all cultures was measured about 0.09.

Growth performance analysis

Growth performance was analyzed by the number of whole cells in colonies (CN, cell/mL) via a thoma hemocytometer; the OD value was measured with spectrophotometer (Shimadzu UV-1650) at 680 nm on the same day when the culture was sampled (WANG et al., 2009).

Dried weight (DW) values were measured by harvested of culture (30 mL) through glass fiber filter (Whatman GF/C, 1.2 µm, UK), which were left to dry in oven at 105 °C for two hours (BOUSSIBA et al., 1992). Harvesting was made by centrifuging the cultures while in their stationary phase flowed by drying.

For calculating the specific growth rate of each replicate, cell number and DW values during exponential phase (exp.) were used (GUILLARD, 1973).

Specific growth rate (μ)= $\ln (N_t/N_0)/T_t\text{-}T_0$

N_t: CN at the end of exp. phase; $N_{0:}$ CN at the start of exp. phase; Tt: the last day of exp. phase; T_0: initial day of exp. phase.

Table 1 - Recipe of BG 11 medium (RIPPKA et al., 1979).

Compound	Amount (mg $L^{-1}H_2O$)
Na_2CO_3	20
Na_2 EDTA	1
$NaNO_3$	1500
K_2HPO_4	40
$MgSO_4.7H_2O$	75
$CaCl.2H_2O$	36
Citric acid	6
Ferric ammonium citrate	6
Trace metal mix[a]	1 mL L^{-1}

[a]H_3BO_3, 2.86 g/L; $MnCl_2.4H_2O$, 1.81 g/L; $ZnSO_4.7H_2O$, 0.222 g/L; $Na_2MoO_4.2H_2O$, 0.39 g/L; $CuSO_4 \cdot 5H_2O$, 0.079 g/L; $Co(NO_3)_2 \cdot 6H_2O$, 0.049 g/L.

Doubling time: Tt = 0.6931/µ

Biomass productivity (BP): as the dry biomass produced per day (g L^{-1} day^{-1}).

Volumetric BP $P_{Biomass}$ was calculated by $P_{Biomass}(gL^{-1}day^{-1})=(X_2\text{-}X_1).(t_2\text{-}t_1)^{-1}$. X_1: DW (gL^{-1}) of t_1 (starting point of cultivation); X_2: DW (gL^{-1}) of t_2 (endpoint of cultivation).

Detection of protein and lipid content, amino acids and fatty acids profiles

The total N content of microalgae was measured according to Dumas method (SHEA & WATTS, 1939). For crude protein amount, this equation was used: "protein amount=nitrogen content x 4.44" (LOPEZ et al., 2010). The calculations were given as percentage of dried weight.

Amino acid compositions were specified by using HPLC method (KÖSE et al., 2011). For detecting crude lipid content Bligh and Dyer's method was used with 100 mg dried biomass (BLIGH & DYER, 1959). Fatty acid methyl esters (FAMEs) were analyzed chromatographically using the sample preparing method (METCALF et al., 1966).

Statistical analysis

All experiment groups replicated 3 times (n = 3). The reported values are the mean ± standard deviation (SD) of three values. Data were analyzed using one-way analysis of variance (ANOVA) using Minitab Statistical Software 2016 2 (Microsoft, USA). A difference was considered significant at the level of $P < 0.001$.

RESULTS AND DISCUSSION

In this research, cell number (CN), optical density (OD) and dried weight (DW) values were used for detecting *T. obliquus* growth. Figure 2 shows the CN; figure 3 shows OD values and figure 4 represents DW of *T. obliquus* in different culture media.

Maximum cell number value ($2433x10^4$ cell/mL) was obtained in the control medium at the 40th day and OD value (1.0 nm) and DW value (1.32 g/L) were obtained at the same day. The highest CN values were 50% N+, 75% N-, 75% N+, 50% N-, respectively. Also, it was observed that other data indicating growth performance such as OD, DW and specific growth rate values are in parallel with CN values.

R^2 correlation values between optical density-cell number and optical density-dry cell weight is given in table 2. Specific growth rate, doubling time and biomass productivity in media with different N concentrations is given in table 3.

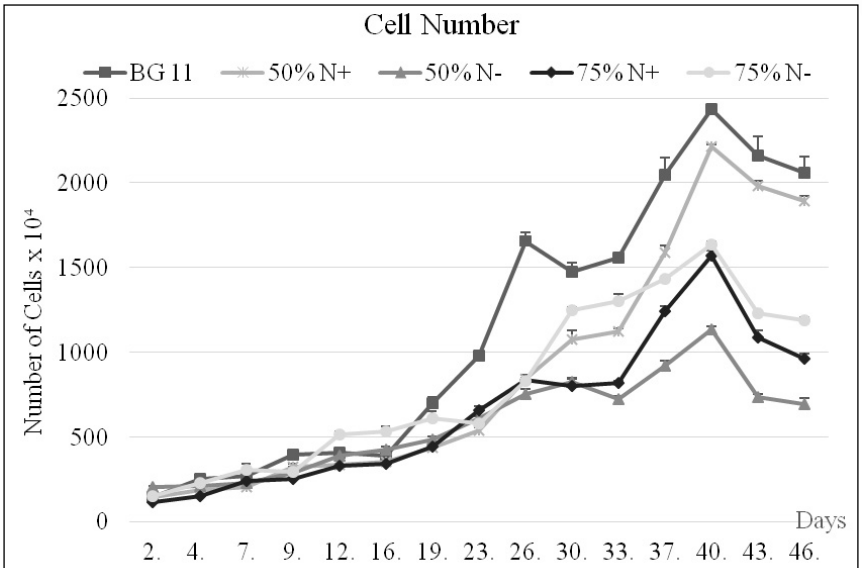

Figure 2 - Cell numbers (CN) of *T. obliquus* in media with different N concentrations. Error bars show the SD of three replicates for each N concentration (n = 3).

In this study, total protein and lipid content were showed in figure 5. As for protein amounts, from much to less, it is as follows: 50% N+ (47.82%); 75% N+ (47.62%), 75% N- (45.69%), 50% N- (44.31%); Control (42.03%).

Results of amino acid compositions and amounts (Table 4) show that essential amino acids (EAAs) -expect arginine- are higher in the control medium, generally. Arginine (47.95 mg/g DW) is the most important amino acid found in 75% N- medium. Other EAAs such as leucine (16.2 mg/g DW) and isoleucine (2.80 mg/g DW) in 75% N+ medium; histidine (3.32 mg/g DW) and lycne (5.22 mg/g DW) in 50% N+ medium; phenyl alanine (8.41 mg/g DW)

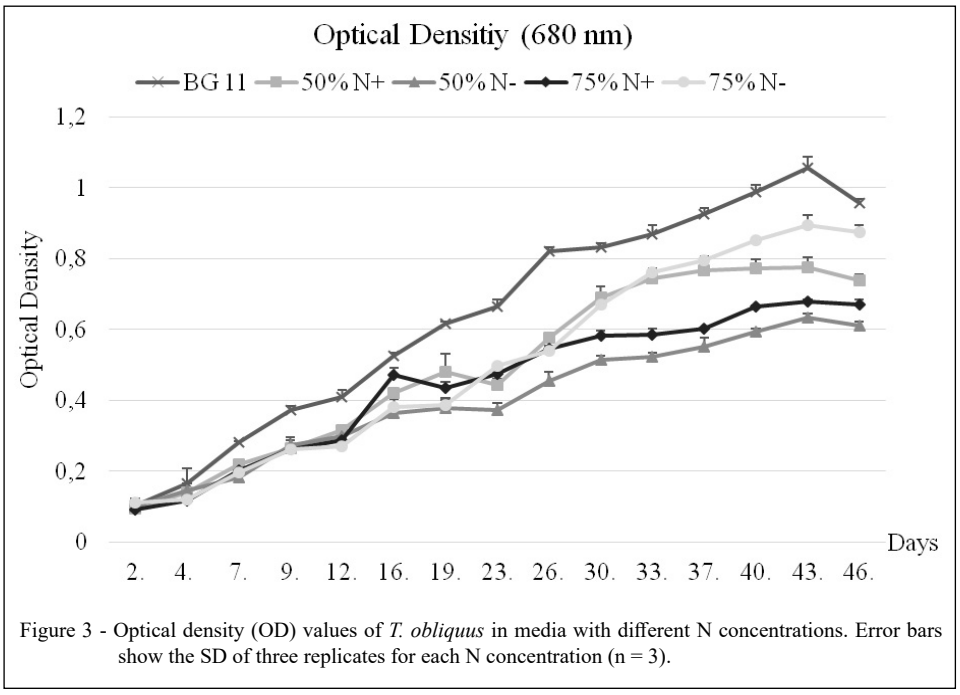

Figure 3 - Optical density (OD) values of *T. obliquus* in media with different N concentrations. Error bars show the SD of three replicates for each N concentration (n = 3).

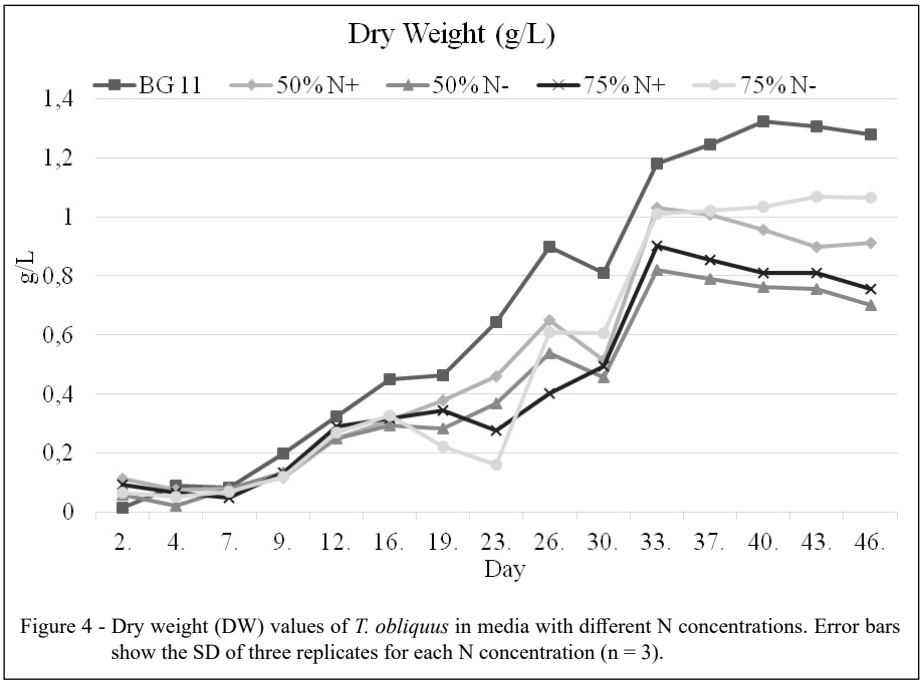

Figure 4 - Dry weight (DW) values of *T. obliquus* in media with different N concentrations. Error bars show the SD of three replicates for each N concentration (n = 3).

in Control medium have the highest amount. Besides these, valine (19.23 mg/g DW) has the max. amount in 75% N+ medium. Arginine, leucine and valine are the dominant amino acids in *T. obliquus*.

Fatty acid results are shown in table 5; they are grouped as Saturated fatty acids (SFAs), Monounsaturated fatty acids (MUFAs), Polyunsaturated fatty acids (PUFAs). The highest level of total SFAs (23.33%) and palmitic acid (C16:0) (19.95%) values are in 75% N+ medium. The maximum total MUFAs (20.0%) and oleic acid (C18:1) (13.41%) amounts are in 75% N-. The highest percentage of PUFAs (49.69%) and linolenic acid (C18:3) (37.67%) are observed in 50% N- medium.

All these results showed that high nitrogen concentration could accelerate the algal growth because nitrogen is a macro element for cell metabolism. Besides this, N is the most important component for chlorophyll and photosynthesis, cellular respiration, proteins and all enzymes. Similar to our results, XIE et al. (2017) reported that low N amount caused low cell growth of *Chlorella vulgaris*. ZHU et al. (2014) specified specific growth rate and BP values of *Chlorella zofingiensis* under N starvation/repletion; they stated that the values in the presence of nitrogen were almost twice the values in the absence of nitrogen. SONKAR & MALLICK (2017) stated that the algae density increased with increasing nitrogen. Similar to these studies, it is mentioned in many studies that high N concentration resulted in higher algal biomass (YODSUWAN et al., 2017).

Table 2 - R^2 correlation values between optical density-cell number and optical density-dry cell weight.

Media	OD-CN	OD-DW
BG 11	0.949	0.9715
50% N+	0.895	0.9461
50% N-	0.899	0.9435
75% N+	0.885	0.8804
75% N-	0.955	0.9498

Beside this result, some studies show that high nitrogen causes toxic affect because of the activity of nitrate reductase. This activity converts nitrate into nitrite and ammonia and these molecules create toxicity to reduce biomass production (JEANFILS et al., 1993). Our results suppored this item because the growth performance in the control medium is higher than 50% N+ and 75% N+ media.

Conversely, in our result, the second highest OD value was recorded in 75% N- medium. This situation could be explained by the increase in lipid accumulation and cell size in N deficiency. Anyway, the relation between CN and OD depends on culture media and cell age etc declared that (FAKHRY & MAGHRABY, 2015).

Table 3 - Specific growth rate, doubling time and biomass productivity of *T. obliquus* in media with different N concentrations.

Media	Specific growth Rate (μ)	Doubling Time (Tt)	Biomass productivity $gL^{-1}day^{-1}$
BG 11 (Control)	0.2135 ± 0.001	3.2455 ± 0.009	0.00089 ± 0.00001
50% N+	0.2115 ± 0.009	3.2771 ± 0.010	0.00051 ± 0.00001
50% N-	0.1897 ± 0.007	3.6541 ± 0.006	0.00046 ± 0.00001
75% N+	0.2016 ± 0.008	3.4372 ± 0.010	0.00047 ± 0.00001
75% N-	0.2014 ± 0.001	3.4413 ± 0.014	0.00065 ± 0.00001

Data were expressed as mean ± SD, n=3.

With the increasing population and energy demand, the search for raw materials that can be used for energy production has also accelerated. For this purpose, microalgae have begun to draw attention because of their ability to produce lipid and fatty acids that can be used in biodiesel production.

A lot of microalgae species when exposed to stress are known to accumulate large amount of lipid that causes high yields of oil (YADAVALLI et al., 2012). Microalgae have a defense mechanism under stress. Accumulating polysaccharides and/ or neutral lipids in this way leads to the production of various metabolites such as carotenoids, neutral lipids, important FAMEs (YU et al., 2015).

So, nutrient starvation is the most effective and preferred way for this purpose. But it is seen in previous studies that under stress conditions growth performance rates are lower usually in the time that obtaining high lipid content (HUERLIMANN et al., 2010).

Beside this; in some species nutrient deficiency does not accelerate lipid accumulation. For example, under nutrient starvation *Dunaliella salina* decreased lipid content from 25 to 9% but increased carbohydrate from 16 to 56% (SPOLAORE et al., 2006). So, each species can react differently to the same conditions.

Microalgae are an important protein source with their high protein and amino acid synthesis capacity and they can be used as alternative/ supportive nutrients and not only protein and amino acids; especially polyunsaturated fatty acids are so valuable as a supplement nutrient (KHAN et al., 2018). As they can be used as a nutritional supplement; it is important to detect the biochemical profiles will change depending on the change of culture conditions.

Results in our own study are not surprising because nitrogen is the basic building block of proteins. So, the amount of protein showed a direct proportion to the amount of nitrogen. BAJWA et al. (2018) in *Chlorella*, *Scenedesmus*, *Nannochloropsis* and *Chlorococcum* algal strains and MUTLU et al. (2011) found high protein content in N and P rich media for *Chlorella vulgaris*.

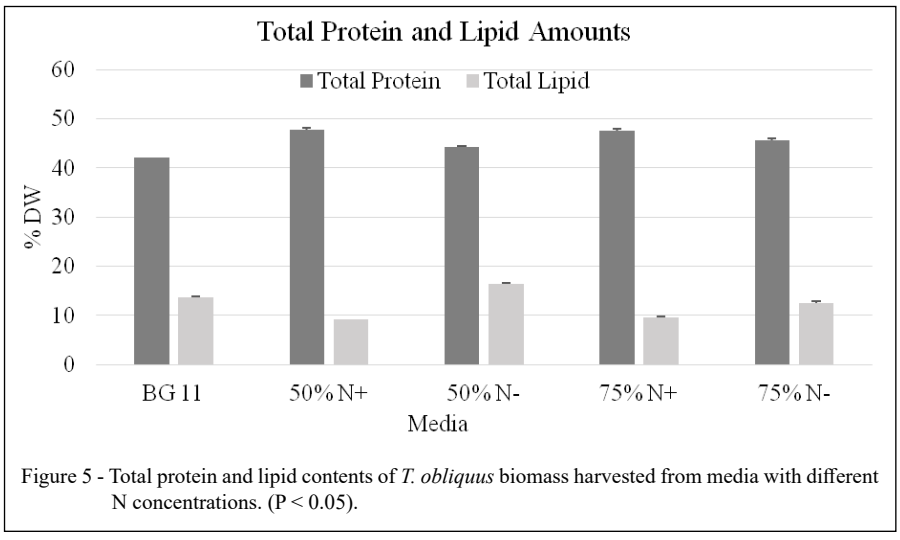

Figure 5 - Total protein and lipid contents of *T. obliquus* biomass harvested from media with different N concentrations. ($P < 0.05$).

Table 4 - Amino acids profiles and amounts (mg/g DW) of *T. obliquus* biomass harvested from media with different N concentrations.

Amino acids	BG11	%50 N+	%50 N-	%75 N+	%75 N-
Arginine	15.09	15.11	14.23	11.11	47.95
Serine	20.48	0.242	0.263	0.339	0.246
Glycine	10.57	0.381	0.448	0.425	0.17
Alanine	10.82	0.497	0.808	0.52	0.402
Proline	24.35	0.673	1.851	1.253	0.148
Valine	8.982	1.443	5.567	19.23	2.411
Threoline	2.209	2.081	3.52	3.432	1.264
Methionine	4.214	1.491	2.286	3.251	0.359
Isoleucine	16.46	1.261	1.337	2.802	0.316
Leucine	26.11	7.078	11.23	16.2	0.411
Phenylalanine	8.415	1.728	5.421	3.095	0.121
Tyrosine	3.301	0.148	0.518	1.086	0.115
Aspartic acid	0.361	1.4	0.352	0.514	0.862
Glutamic acid	3.799	3.087	0.155	0.148	0.97
Histidine	10.09	3.328	0.124	0.252	0.785
Lysine	10.05	5.227	0.129	0.177	1.364
Total	175.301	45.175	48.239	63.834	57.894

These results showed that N deficiency increased arginine amount and excess N increased valine amount. It could be understood from these results that N concentration is quite effective in modifying the amino acid composition. *T. obliquus* can be used as animal feed, as it contains most of the EAAs at high rates (LIM et al., 2018). HEMPEL et al. (2012) found the similar results for *Chlorella vulgaris* 132 to ours as 4.5% DW (Arginine).

WHO (2007) declared that lysine, leucine, isoleucine, valine, threonine, aromatic amino acids (phenylalanine and tyrosine), tryptophan, sulphur amino acids (methionine and cysteine) and histidine as indispensable amino acids. These amino acids are abundant in our samples as shown in the results which culture conditions increase these amino acids.

Maximum total lipid content (16.46 %DW) was detected in 50% N- medium and then

Table 5 - FAMEs percentages (% of total fatty acids) of *T. obliquus* biomass harvested from media with different N concentrations.

Name of Fatty Acid	BG 11	50% N+	50% N-	75% N+	75% N-
----------Saturated fatty acids (SFAs)----------					
C14:0 (myristic acid)	2.208	2.118	2.097	2.277	2.185
C16:0 (palmitic acid)	19.342	18.555	18.371	19.953	19.141
C18:0 (stearic acid)	1.073	1.029	1.019	1.107	1.062
Total SFAs	22.623	21.702	21.487	23.337	22.388
----------Monounsaturated fatty acids (MUFAs)----------					
C16:1 (9-hexenoic acid)	2.156	2.068	2.048	2.224	2.134
C18:1 (oleic acid)	12.513	13.004	11.885	12.908	13.415
C18:1 t10, t11e 12	4.498	4.315	5.111	4.64	4.451
Total MUFAs	19.167	19.387	19.044	19.772	20.00
----------Polyunsaturated fatty acids (PUFAs)----------					
C18:2 (linoleic acid, c9 c12)	12.348	11.845	12.015	11.234	12.219
C18:3 (linolenic acid, c9, c12, c15)	36.291	36.678	37.678	36.121	37.061
Total PUFAs	48.639	48.523	49.693	47.355	49.28
Others	7.789	7.473	7.40	8.033	7.707
Total FAMEs Amounts	98.218	97.085	97.624	98.497	99.375

13.71%, 12.55%, 9.60% and 9.18% in Control, 75% N-, 75% N+, 50% N+, respectively. This is clear that N deficiency triggered lipid accumulation and it is compatible with many studies that stated N deficiency changed lipid metabolism and converted membrane lipids to neutral lipid storage (DOMINGUEZ et al., 2015). Physical and chemical parameters such as salinity, temperature, pH and nutrient levels are quite effective on fatty acid composition. Especially N concentration leads to obtain different results of same species (CHEN et al., 2011).

These results showed that N deficiency leads to increase in MUFAs and PUFAs amounts. Similar to our results, the amount of MUFAs increased because of nitrogen deficiency in some studies (SHIFRIN & CHISHOLM, 1981). DHUP & DHAWAN (2014) found that the percentage of MUFAs is much higher in the lowest N concentration medium in *Monoraphidium* sp.. Also, high cetane number indicates that biodiesel quality is associated with high amount of SFAs and MUFAs. According to our results, 75% N- medium with highest MUFAs (20.0%) is suitable for obtaining high cetane number.

Total lipid and FAMEs profiles could be used to estimate cetane number to determine the quality of biodiesel (ISLAM et al., 2013). So, studies that investigate the total lipid amount and fatty acid composition in varying culture conditions are very important in this regard. PUFAs are essential fatty acids, are important in animal and human nutrition and play serious role in cardiovascular health and baby growth. Thus, high PUFAs make algae more valuable (VOIGT et al., 2000). Some microalgae can produce a huge quantity of PUFA such as C22:5 + C22:6 (39.4%) in *Schizochytrium limacinum*, and C20:5 (25%) in *Porphyridium cruentum* (SAJJADI et al., 2018). Present study show that N deficiency causes an increase in PUFAs. In contrast to our results, ÖRDÖG et al. (2016) detected in their study with *Chlorella* species that higher SFAs and MUFAs values are 1% N medium and high PUFAs values are 10% N medium. It was declared that a marine diatom *Phaeodactylum tricornutum* cultured with higher nitrogen concentration accumulated more PUFA (YODSUWAN et al., 2017).

These results vary from species to species and other conditions of culture except the studied factor. So, to obtain clearer results on species basis, more studies should be done for this aim on this subject.

Lipid of microalgae contains SFAs, MUFAs and PUFAs; SFAs and MUFAs are more suitable for producing biodiesel and PUFAs are important in human and animal nutrition (OCHSENREITHER et al., 2016). In this study, the percentage of PUFAs is much higher than those of SFAs and MUFAs. Similar to our results, ABBA et al. (2017) reported that the ratios of SFAs, MUFAs and PUFAs were 38.7%, 16.7% and 44.9%, respectively.

Besides this, in a lot of studies SFAs concentrations were much higher than those of PUFAs (RINNA et al., 2017). Also, GONZALEZ et al. (2019) found high SFAs and total lipid value at low nitrate concentration in optimum temperature for *Chaetoceros gracilis*.

The proportions of myristic acid, palmitic acid, palmitic acid, and stearic acid in SFAs are very important for biodiesel production. Percentages of palmitic acid are between 18.37%-19.95% in our results. YASIN et al. (2019) found that palmitic acid amount is 27.21% and 23.84%, respectively, but higher results could be found in literature (YASIN et al., 2019). It means that *T. obliquus* is not suitable for biodiesel production. This microalga can best be used in nutrition because of its high PUFAs value.

CONCLUSION

The maximum population growth rate of *T. obliquus* was found in the control medium and then 50% N+ medium. High nitrogen concentration speeds up cell division and number. Maximum protein content was detected in 50% N+ and then 75% N+. Our results showed that the nitrogen is the basic molecule for synthesizing protein, and it causes an increase in protein content. The highest percentage of lipid was seen in 50% N-, Control, 75% N-, respectively. It can be understood that N deficiency causes an increase in lipid storage. And amino acid and FAMEs compositions change according to nitrogen amount. PUFAs concentration increase because of N deficiency.

From our study, to obtain beneficial biomass by modifying nutrient concentration from *T. obliquus*, nitrogen is a good choice for this aim. Due to high protein content in all media and high PUFAs concentration in N deficiency, this microalga can be used as a nutrition supplement for humans and animals. This study is also very important in terms of providing data for unstudied species and future studies in this field.

ACKNOWLEDGEMENT

This research was funded by Mehmet Akif Ersoy University, grant number 0455-MP-17.

REFERENCES

ABBA, Z. et al. Fatty acids composition of microalga Botryococcus sp. cultured in synthetic medium. **Journal of Science and Technology**, v.9, n.4, p.117-121, 2017. Available from: <https://publisher.uthm.edu.my/ojs/index.php/JST/article/download/2200/1376/7578>. Accessed: May, 15, 2023.

AN, M. et al. Effects of nitrogen forms and supply mode on lipid production of microalga Scenedesmus obliquus. **Energies**, v.13, n.3, p.697-710, 2020. Available from: <https://doi.org/10.3390/en13030697>. Accessed: May, 10, 2023. doi: 10.3390/en13030697.

BAJWA, K. et al. Evaluation of nutrient stress (nitrogen, phosphorus regimes) on physio-biochemical parameters of oleaginous microalgal strains and SEM study under nutrient stress. **International Journal of Environmental Sciences & Natural Resources**, v.10, n.1, p.1-7, 2018. Accessed: May, 10, 2023. doi: 10.19080/IJESNR.2018.10.555776.

BLIGH, E. G.; DYER, W. J. A rapid method of total lipid extraction and purification. **Canadian Journal of Biochemistry and Physiology**, v.37, p.911-917, 1959. Available from: <https://doi.org/10.1139/o59-099>. Accessed: May, 10, 2023.

BOUSSIBA, S. et al. Enchancement and determination of astaxanthin accumulation in green alga Haematococcus pluvialis. **Methods in Enzymology**, v.213, p.386-391, 1992. Available from: <https://doi.org/10.1016/0076-6879(92)13140-S>. Accessed: May, 10, 2023.

CHEN, M. et al. Effect of nutrients on growth and lipid accumulation in the green algae Dunaliella tertiolecta. **Bioresource Technology**, v.102, n.2, p.1649-1655, 2011. Available from: <https://doi.org/10.1016/j.biortech.2010.09.062>. Accessed: May, 15, 2023.

DHUP, S.; DHAWAN, V. Effect of nitrogen concentration on lipid productivity and fatty acid composition of Monoraphidium sp.. **Bioresource Technology**, v.152, p.572-575, 2014. Available from: <https://doi.org/10.1016/j.biortech.2013.11.068>. Accessed: May, 15, 2023. doi: 10.1016/j.biortech.2013.11.068.

DOMINGUEZ, M. C. R. et al. Lipid accumulation and antioxidant activity in the eukaryotic acidophilic microalga Coccomyxa sp. (strain onubensis) under nutrient starvation. **Journal of Applied Phycology**, v.27, p.1099-1108, 2015. Available from: <https://doi.org/10.1007/s10811-014-0403-6>. Accessed: May, 12, 2023.

FAKHRY, E. M.; MAGHRABY, D. M. E. Lipid accumulation in response to nitrogen limitation and variation of temperature in Nannochloropsis salina. **Botanical Studies**, v.56, n.6, p.1-8, 2015. Available from: <https://doi.org/10.1186%2Fs40529-015-0085-7>. Accessed: May, 10, 2023. doi: 10.1186/s40529-015-0085-7.

GONZALEZ, F. et al. Effect of nitrate and irradiance on fatty acid production in microalgae cultivated for feeding larvae and broodstock conditioning in batch culture. **Revista de Biologia Marina Oceanografia**, v.54, p.91-106, 2019. Available from: <https://doi.org/10.1016/j.jwpe.2017.06.006>. Accessed: May, 15, 2023.

GUILLARD, R. R. L. Division Rates. In: Stein, J. R. (Ed.), **Handbook of phycological methods: culture methods and growth measurements**, Cambridge University Press, London, 1973, pp.289-311.

HEMPEL, N. et al. Biomass productivity and productivity of fatty acids and amino acids of microalgae strains as key characteristics of suitability for biodiesel production. **Journal of Applied Phycology**, v.24, p.1407-1418, 2012. Available from: <https://doi.org/10.1007/s10811-012-9795-3>. Accessed: May, 10, 2023.

HUERLIMANN, R. et al. Growth, lipid content, productivity and fatty acid composition of tropical microalgae for scale-up production. **Biotechnology and Bioengineering**, v.107, n.2, p.245-257, 2010. Available from: <https://doi.org/10.1002/bit.22809>. Accessed: May, 10, 2023. doi: 10.1002/bit.22809.

ISLAM, M. A. et al. Microalgal species selection for biodiesel production based on fuel Properties derived from fatty acid profiles. **Energies**, v.6, n.11, p.5676-5702, 2013. Available from: <http://dx.doi.org/10.3390/en6115676>. Accessed: May, 15, 2023. doi:10.3390/en6115676.

JEANFILS, J. et al. Effect of high nitrate concentrations on growth and nitrate uptake by free living and immobilized Chlorella vulgaris cells**. Journal of Applied Phycology**, v.5, p.369-374, 1993. Available from: <https://doi.org/10.1007/BF02186240>. Accessed: May, 10, 2023.

KHAN, M. I. et al. The promising future of microalgae: Current status, challenges and optimization of a sustainable and renewable industry for biofuels, feed and other products. **Microbial Cell Factories**, v.17, n.36, 2018. Available from: <https://www.doi.org/10.1186/S12934-018-0879-X>. Accessed: May, 10, 2023. doi: 10.1186/s12934-018-0879-x.

KIM, Y. et al. Characterization of fatty acid components from Tetradesmus obliquus KNUA019 (Chlorophyta, Scenedesmaceae) for a resource of biofuel production. **Brazilian Journal of Botany**, v.42, n.3 p.431-439, 2019. Accessed: May, 10, 2023. doi: 10.1007/s40415-019-00556-9.

KÖSE, S. et al. Commercial test kits and the determination of histamine in traditional (ethnic) fish products-evaluation against an EU accepted HPLC method. **Food Chemistry**, v.125, p.1490-1497, 2011. Available from: <https://doi.org/10.1016/j.foodchem.2010.10.069>. Accessed: May, 10, 2023.

LIM, A. S. et al. Amino acids profiles of six dinoflagellate species belonging to diverse families: Possible use as animal feeds in aquaculture. **Algae**, v.33, n.3, p.279-290, 2018. Available from: <https://doi.org/10.4490/algae.2018.33.9.10>. Accessed: May, 10, 2023.

LOPEZ, C. V. G. et al. Protein measurements of microalgal and cyanobacterial biomass. **Bioresource Technology**, v.101, n.19, p.7587-7591, 2010. Available from: <https://doi.org/10.1016/j.biortech.2010.04.077>. Accessed: May, 10, 2023.

METCALF, L. D. et al. Rapid preparation of fatty acid esters from Lipids for gas chromatographic. **Analitical Chemistry**, v.38, n.3, p.514-515, 1966. Available from: <https://doi.org/10.1021/ac60235a044>. Accessed: May, 10, 2023.

MUTLU, Y. B. et al. The effects of nitrogen and phosphorus deficiencies and nitrite addition on the lipid content of Chlorella vulgaris (Chlorophyceae). **African Journal of Biotechnology**, v.10, n.3, p.453-456, 2011. Accessed: May, 10, 2023. doi: 10.5897/AJB10.1390.

NAYAK, M. et al. Exploration of two-stage cultivation strategies using nitrogen starvation to maximize the lipid productivity in Chlorella sp. HS2. **Bioresource Technology**, v.276, p.110-118, 2019. Available from: <https://doi.org/10.1016/j.biortech.2018.12.111>. Accessed: May, 10, 2023. doi: 10.1016/j.biortech.2018.12.111.

OCHSENREITHER, K. et al. Production strategies and applications of microbial single cell oils. **Frontiers in Microbiology**, v.7, p.1539, 2016. Available from: <https://doi.

org/10.3389/fmicb.2016.01539>. Accessed: May, 15, 2023. doi: 10.3389/fmicb.2016.01539.

ÖRDÖG, V. et al. Effect of temperature and nitrogen concentration on lipid productivity and fatty acid composition in three Chlorella strains. **Algal Research**, v.16, p.141-149, 2016. Available from: <https://doi.org/10.1016/j.algal.2016.03.001>. Accessed: May, 15, 2023.

RINNA, F. et al. Wastewater treatment by microalgae can generate high quality biodiesel feedstock. **Journal of Water Process Engineering**, v.18, p.144-1149, 2017. Available from: <https://doi.org/10.1016/j.jwpe.2017.06.006>. Accessed: May, 15, 2023.

RIPPKA, R. et al. Generic assignments, strain histories and properties of pure cultures of cyanobacteria. **Microbiology**, v.111, n.1, p.1-61, 1979. Available from: <https://doi.org/10.1099/00221287-111-1-1>. Accessed: May, 10, 2023.

SAJJADI, B. et al. Microalgae lipid and biomass for biofuel production: A comprehensive Review on lipid enhancement strategies and their effects on fatty acid composition. **Renewable and Sustainable Energy Reviews**, v.97, p.200-232, 2018. Available from: <http://dx.doi.org/10.1016/j.rser.2018.07.050>. Accessed: May, 15, 2023. doi: 10.1016/j.rser.2018.07.050.

SHARMA, K. K. et al. High lipid induction in microalgae for biodiesel production. **Energies**, v.5, n.5, p.1532-1553, 2012. Available from: <https://doi.org/10.3390/en5051532>. Accessed: May, 10, 2023.

SHEA, F.; WATTS, C. E. Dumas method for organic nitrogen. **Industrial and Engineering Chemistry, Analytical Edition**, v.11, n.6, p.333-334, 1939. Available from: <https://doi.org/10.1021/ac50134a013>. Accessed: May, 10, 2023.

SHIFRIN, N. S.; CHISHOLM, S. W.. Phytoplankton lipids: interspecific differences and effects of nitrate, silicate and light-dark cycles. **Journal of Phycology**, v.17, n.4, p.374-384, 1981. Available from: <https://doi.org/10.1111/j.1529-8817.1981.tb00865.x>. Accessed: May, 15, 2023.

SONKAR, S.; MALLICK, N. Development of a single-phase nitrate feeding strategy for enhanced lipid productivity from green microalgae for biodiesel production. **Environmental Progress & Sustainable Energy**, v.36, n.1, p.222-231, 2017. Available from: <https://doi.org/10.1002/ep.12443>. Accessed: May, 10, 2023.

SPOLAORE, P. et al. Commercial applications of microalgae. **Journal of Bioscience and Bioengineering**, v.101, n.2, p.87-96, 2006. Available from: <https://doi.org/10.1263/jbb.101.87>. Accessed: May, 10, 2023. doi: 10.1263/jbb.101.87.

VOIGT, R. G. et al. Relationship between omega-3 long-chain polyunsaturated fatty acid status during early infancy and neuro developmental status at 1 year of age. **Journal of Human Nutrition and Dietics**, v.15, n.2, p.111-120, 2000. Available from: <https://doi.org/10.1046/j.1365-277x.2002.00341.x>. Accessed: May, 15, 2023. doi: 10.1046/j.1365-277x.2002.00341.x.

WANG, L. et al. Cultivation of green algae Chlorella sp. in different wastewaters from municipal wastewater treatment plant. **Applied Biochemistry and Biotechnology**, v.162, n.4, p.1174-1186, 2009. Available from: <https://doi.org/10.1007/s12010-009-8866-7>. Accessed: May, 10, 2023.

WHO - World Health Organization, Food and Agriculture Organization of the United Nations, United Nations University. **Protein and Amino Acid Requirements in Human Nutrition. Report of a Joint FAO/WHO/UNU Expert Consultation** (WHO Technical Report Series 935), 2007, ISBN:92 4 120935 6.

WYNNE, M. J.; HALLAN, J. K. Reinstatement of Tetradesmus G. M. Smith (Sphaeropleales, Chlorophyta). **Feddes Repertorium**, v.126, n.3-4, p.83-86, 2015. Available from: <https://doi.org/10.1002/fedr.201500021>. Accessed: May, 10, 2023.

XIE, T. et al. Nitrate concentration-shift cultivation to enhance protein content of heterotrophic microalga Chlorella vulgaris: Over-compensation strategy. **Bioresource Technology**, v.233, p.247-255, 2017. Available from: <https://doi.org/10.1016/j.biortech.2017.02.099>. Accessed: May, 10, 2023. doi: 10.1016/j.biortech.2017.02.099.

YAAKOB, M. A. et al. Influence of nitrogen and phosphorus on microalgal growth, biomass, lipid, and fatty acid production: An overview. **Cells**, v.10, n.2, p.393, 2021. Available from: <https://doi.org/10.3390/cells10020393>. Accessed: May, 08, 2023. doi: 10.3390/cells10020393.

YADAVALLI, R. et al. Lipid accumilation studies in Chlorella pyreniodosa using customized photobioreactor-effect of nitrogen source, light intensity and mode of operation. **International Journal of Engineering Research and Application**, v.2, n.3, p.2446-2453, 2012. Available from: <https://www.researchgate.net/publication/230562205_Lipid_Accumulation_Studies_In_Chlorella_Pyrenoidosa_Using_Customized_Photobioreactor_Effect_of_Nitrogen_Source_Light_Intensity_and_Mode_of_Operation>. Accessed: May, 10, 2023.

YASIN, N. H. M. et al. The effect of microalgae harvesting on lipid for biodiesel production. **Materials Today: Proceedings**, v.19, n.4, p.1582-1590, 2019. Available from: <https://doi.org/10.1016/j.matpr.2019.11.186>. Accessed: May, 15, 2023.

YODSUWAN, N. et al. Effect of nitrogen concentration on growth, lipid production and fatty acid profiles of the marine diatom Phaeodactylum tricornutum. **Agriculture and Natural Resources**, v.51, n.2, p.190-197, 2017. Available from: <https://doi.org/10.1016/j.anres.2017.02.004>. Accessed: Dec. 27, 2023.

YU, N., et al. Optimization of process configuration and strain selection for microalgae-based biodiesel production. **Bioresource Technology**, v.193, p.25-34, 2015. Available from: <https://doi.org/10.1016/j.biortech.2015.05.101>. Accessed: May, 10, 2023.

ZARRINMEHR, M. J. et al. Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, Isochrysis galbana. **Egypt J. Aquat. Res.**, v.46, n.2, p.153-158, 2019. Available from: <https://doi.org/10.1016/j.ejar.2019.11.003>. Accessed: May, 08, 2023. doi: 10.1016/j.ejar.2019.11.003.

ZHU, S. et al. Metabolic changes of starch and lipid triggered by nitrogen starvation in the microalga Chlorella zofingiensis. **Bioresource Technology**, v.152, p.292-298, 2014. Available from: <https://doi.org/10.1016/j.biortech.2013.10.092>. Accessed: May, 10, 2023. doi: 10.1016/j.biortech.2013.10.092.

ZHUANG, L. L. et al. Effects of nitrogen and phosphorus concentrations on the growth of microalgae Scenedesmus. LX1 in suspended-solid phase photobioreactors (ssPBR). **Biomass & Bioenergy**, v.109, p.47-53, 2018. Available from: <https://doi.org/10.1016/j.biombioe.2017.12.017>. Accessed: May, 10, 2023.

ZIENKIEWICZ, A., et al. The microalga Nannochloropsis during transition from quiescence to qutotrophy in response to nitrogen availability. **Plant Physiol.**, v.182, n.2, p.819-839, 2020. Available from: <https://doi.org/10.1104%2Fpp.19.00854>. Accessed: May, 08, 2023. doi: 10.1104/pp.19.00854.