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Research article

Effect of phosphorus and silicon limitation on lipid production of *Phaeodactylum tricornutum* as a potential source of biodiesel

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Abstract: This study was carried out to determine the effects of different ratios of phosphorus (P) and Silicon (Si) on the growth, lipid, protein, and fatty acid production of *Phaeodactylum tricornutum* and to source renewable, non-toxic biofuels. While the highest lipid was determined as 34.9% in the group with 50% P restriction, it was observed that 20% and 50% Si restriction did not have a positive effect on lipid production. In *P. tricornutum* biomass, total saturated fatty acids (ΣSFA) were generally low and total unsaturated fatty acids (ΣUFA) were quite high in all groups. The highest polyunsaturated fatty acid (PUFA) values were determined in the 50%P&50%Si and 50%P&20Si restriction groups. Although EPA, one of the most important fatty acids in the PUFA group, was observed at high values in all groups, the highest value was found to be 12.28% in the 50%P&50%Si restriction group.

Keywords: fatty acids, P limitation, *Phaeodactylum tricornotum*, protein, Si limitation, total lipid.

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Introduction

Industrialization and population growth in the world a long time has led to rapid increases in the use of non-renewable energy resources. When this situation is examined, it has been seen that the highest energy demand worldwide is fuels. Research on fuel demand has led to an emphasis on biofuel development studies (Schenk et al., 2008).

One of the most important factors affecting the growth of algae is nutritive elements, and the two most important elements are Nitrogen (N) and Phosphor (P). In addition, due to the skeletal structure of the cell wall of diatoms, Silicon (Si) is also a very important element. Diatoms are essential organisms for the diet of marine life as they produce large amounts of polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid (EPA). The survival and reproductive success of many sea creatures are due to the amount and composition of lipids in phytoplankton. Abiotic factors such as temperature and low P level can affect the lipid composition of diatoms (Rousch et al., 2003).

Diatoms are an important source of bioactive compounds. In addition to bioactive compounds within diatoms, it can produce a wide variety of lipids. On average, diatoms can reach a lipid content of up to 25% of dry weight. However, the lipid content may increase under adverse environmental conditions such as high temperature, nutrient availability or nutrient starvation. Diatoms have a high value for lipid production in the biotechnology industry (Yi et al., 2017). P is an important nutrient for algae and a component of molecules such as phospholipids and nucleic acids. In plants, P plays an important role in many critical metabolic processes, especially photosynthesis and respiration (Plaxton and Tran, 2011).

Si does not enter into the basic structure of living matter like other nutritional elements. But it forms the skeleton of many marine forms (siliceous algae, sponges, diatoms). Although many elements are abundant on earth, their concentration in sea water is quite low. Apart from dissolved silica, seawater also contains suspended particles of cosmic terrestrial and biological origin rich in silica.

However, this special Si cannot be directly assimilated by living organisms and therefore does not form a nutrient element (Kocataş, 1993).

While the majority of reported studies have shown the effects of N on the biodiesel productivity of *P. tricornutum*, only a limited number of studies have discussed the effects of P and Si. Therefore, the aim of this study was to study the effects of P and Si for *P. tricornutum* as a source for biodiesel production.

Material and Methods

Inoculum source and cultivation conditions

The diatom *P. tricornutum* (UTEX-646) was obtained from Algal Biotechnology Laboratory, Fisheries Faculty, Cukurova University, Turkey. The culture medium was natural seawater+f/2-Si (Guillard, 1973). The stock solution $NaNO_3$, $NaH_2PO_4H_2O$, $Na_2SiO_39H_2O$, nutrient mediums, and microelement were autoclaved at 121°C for 30 min and stored at 4°C.

Phaeodactylum tricornutum was cultivated in 5 L glass flasks with a 20% inoculation rate. In the laboratory where the experiment was carried out, the temperature values were kept at 20±2°C by using the air conditioning device. During the experiment, 80 µmol photon m^2s^{-1} light intensity was used in cultures. In addition, a 16:8 hours light-dark period was applied. The applied light intensity was measured with a light meter (Licor, LI-250). Fluorescent lamps were used as the light source. In the experiment, P and Si limitations were tried in Si/F2 medium at ‰30 salinity. Nutrient media consisting of 50%Si, 50%P, 20%Si, 50%Si&50%P, and 20%Si&50%P limitaion (-) were prepared and the experiment was set up.

The *P. tricornutum* strain was pre-cultured and prepared for experimentation. The cultures were harvested in the stagnation phase at different times according to the treatments applied. In order to determine the growth of the cultures, OD, dry matter content and, chl *a* content was determined by taking samples from the cultures daily. At the end of the experiment, protein, lipid, and fatty acids were determined.

Biomass determination

In order to determine the amount of dry matter, 20 mL of microalgae cultures were taken from each balloon daily. *Phaeodactylum tricornutum* cells were separated from the medium and concentrated by means of a vacuum created from Whatman GF/C filter papers with 0.45 µ mesh opening, which was previously kept in an oven at 105°C for 1 hour, cooled in a desiccator and tared (W0) Afterwards, the samples, which were dried in an oven at 105°C for 2 hours, were weighed (W1) on a precision balance after cooling in the desiccator and the dry matter amounts were calculated. Biomass was determined based on the weight difference between W0 and W1 (Vonshak, 1988).

Chlorophyll a (Chl a) Analysis

Chl *a* analysis was made according to Kulkarni and Nikolov (2018) and calculated according to the formula below.

$$Chl\ a = 13.36 * Abs665nm - 5.19 * Abs649nm \quad (1)$$

Optical Density (OD)

In order to measure OD, after the cultures were mixed homogeneously daily, 3 ml of sample was taken with the help of a pipette. The samples taken from the tubes were placed in quartz cuvettes and the visible spectrophotometer was read at a wavelength of 750 nm (Lu et al., 2017).

Protein Analysis

Total crude protein was made in the Kjeldahl distillation unit according to the Kjeldahl method (AOAC, 1998).

The amount of protein was calculated according to the formula in the following equation (Eq. 2)

$$\%N = \frac{14.01 * (A - B) * M}{g * 10} * 100$$

$$\%Protein = \%N * 6.25 \quad (2)$$

A: The amount of HCl consumed for the sample

B: The amount of HCl consumed for the blind

M: Acid molarity

g: Sample quantity

Lipid Analysis

Lipid analysis was performed according to the method applied by Bligh and Dyer (1959). The dry biomass was kept overnight for lipid extraction in a Chloroform:methanol (2:1, v/v) mixture. Approximately 120 ml of solvent per gram of dry sample was used in each extraction step. The solid phase was carefully separated twice using filter paper (Advantec filter paper, no. 1, Japan) to ensure complete separation. The solvent phase was evaporated on a rotary

evaporator under vacuum at 60°C. this procedure was repeated three times until all lipid was extracted.

Fatty acid (FA) analyses

Fatty acid methyl esters were made from the extracted lipid according to the method of Ichihara et al., (1996). 4mL of 2M KOH and 2mL of n-heptane were added to 25 mg of extracted lipid sample. Then, it was vortexed for 2 minutes at room temperature and centrifuged at 4000 rpm for 10 minutes, and the heptane layer was analyzed by gas chromatography (GC).

Gas chromatographic conditions

Fatty acid content was analyzed using a GC Clarus 500 instrument (Perkin–Elmer, USA), a flame ionization detector (FID), and an SGE (60mx0.32mm ID BPX70x0.25 µm, USA) column. Injector and detector temperatures were adjusted to 260°C and 230°C, respectively. In the meantime, the oven temperature was kept at 140°C for 8 minutes, then it was increased by 4oC every minute until 220°C, from 220°C to 230°C by 4°C every minute and kept at 230°C for 15 minutes, the analysis was completed after a total of 45.5 minutes. By controlling the sample size at 1 µl and the carrier gas at 16ps, the split current ratio of 40.0mL/min (1:40) was used. Fatty acids were determined depending on the arrival times of the FAME mixture consisting of standard 37 components (Supelco 37 F.A.M.E. Mix C4-C24 Component, Catalogue No. 18919).

Statistical analyses

For data analysis, all experiments were performed in triplicate and analysed by ANOVA using SPSS version 19 software (SPSS, Chicago, IL, USA). Duncan's multiple range test was performed to identify significant differences. The values given represent the mean and standard deviation for three samples. Significant differences were defined as $P < 0.05$.

Results and Discussion

Algal growth profiles

The growth profiles of *P. tricornutum* in different media treatments at the end of the experimental period are shown in Figure 1. The biomass concentration in control, %50P-, %50Si-, %20Si-, %50P-&%50Si- and %50P&%20Si- treatments were 0.820, 0.799, 0.580, 0.674, 0.703, and 0.580 g/L, respectively. The biomass productivities of all treatment groups were compared to the control group. The lowest growth was observed in the group of 50%P-&20%Si- and 50%Si-.

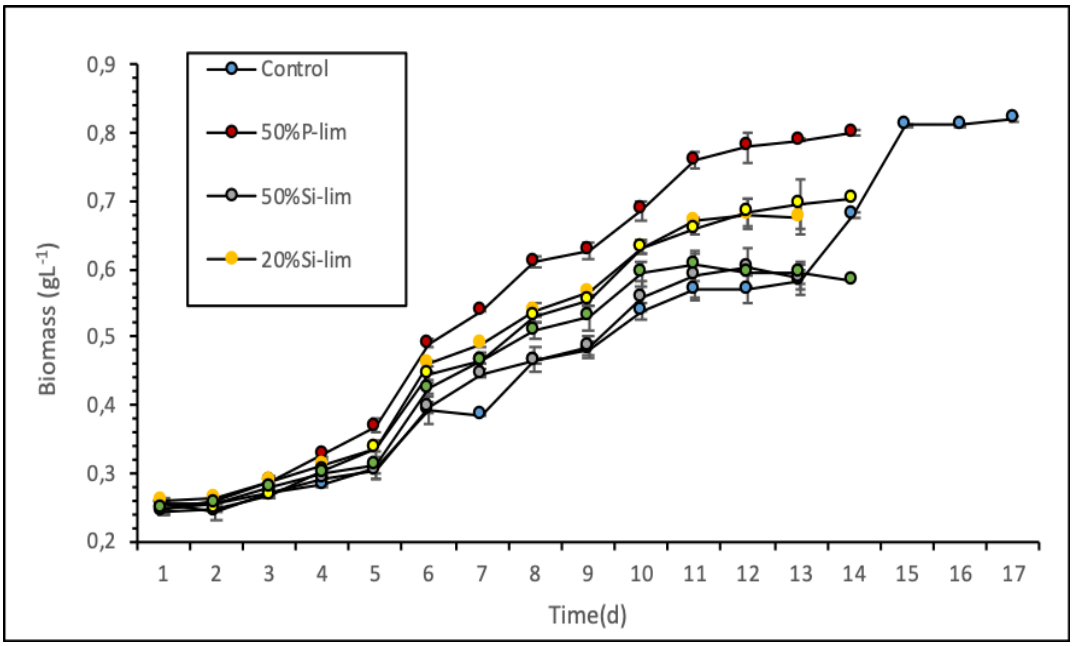

Figure 1. Biomass productivities of *Phaeodactylum tricornutum* cultured in different nutrient medium.

The table summarizing the OD values of all treatments is given in Table 1. Considering the last day values among the groups with similar initial cell densities, the best growth was in the group with 50%P- compared to the other groups. The lower growths were observed in the cultures of 50%P-&20%Si- and 50%Si- than in the other groups.

Table 1. The OD values of *Phaeodactylum tricornutum* on the different treatment groups

Treatments	OD_{first}	OD_{last}
Control	0.246±0.01	0.762±0.003[a]
50%P-lim	0.242±0.01	0.743±0.001[a]
50%Si-lim	0.249±0.01	0.551±0.03[c]
20%Si-lim	0.250±0.01	0.629±0.05[b]
50%P-&50%Si-lim	0.237±0.01	0.656±0.04[b]
50%P-&20%Si-lim	0.241±0.01	0.544±0.02[c]

There is a statistical difference between the means of lines symbolized by the letters [a, b, c] (P<0.05). Values are presented as mean ± SD of three determinations.

Chl a, Protein and Lipid values

Chl *a,* protein, and lipid contents of *P. tricornutum* were summarized in Table 2. Initial Chl *a* values were found to be similar to each other, and the mean Chl *a* values of all groups were determined as 271±2 μgL^{-1}. Trials of different treatments were completed on different days and Chl *a* values on the last day are given in Table 2. The highest Chl *a* values were found in the 50%P- group. The lowest Chl *a* content was determined to 50%P-&20%Si- culture.

Total protein values were determined using dry biomass harvested on the last day. The highest protein content was reported for the %20Si- group after the control group. Total lipid values were determined from the dry biomass obtained on the last day. The highest lipid value was determined in 50%P- treated and the closest values were determined for the cultures of 50%P-&50%Si- and 50%Si- (Table 2 and Figure 2).

Table 2. Chl *a* (μgL^{-1}), protein (%) and lipid (%) contents of *Phaeodactylum tricornutum* cultured in different nutrient medium.

Treatments	Chl *a* (μgL^{-1}) $_{last}$	Protein (%)$_{last}$	Lipid (%)$_{last}$
Control	721±11[a]	30.6±0.3[a]	17.3±0.1[e]
50%P-lim	719±7[a]	16,0±0.1[c]	34.9±0.4[a]
50%Si-lim	217±12[e]	16.4±0.1[c]	33.8±0.3[b]
20%Si-lim	457±13[c]	29.4±0.2[ab]	18.2±0.1[d]
50%P-&50%Si-lim	498±6[b]	15.3±0.1[d]	34.5±0.3[a]
50%P-&20%Si-lim	285±10[d]	28.2±0.3[b]	27.1±0.2[c]

There is a statistical difference between the means of lines symbolized by the letters [a, b, c, d, e] (P<0.05). Values are presented as mean ± SD of three determinations.

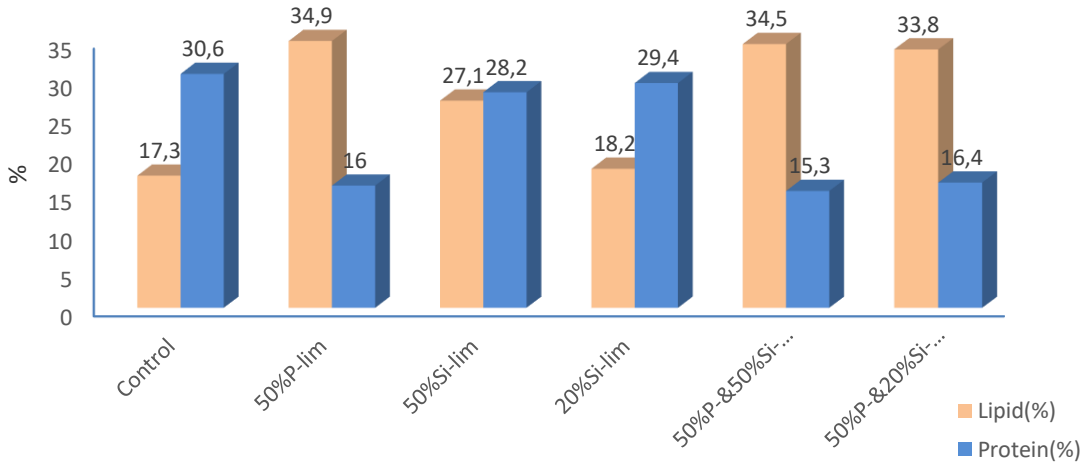

Figure 2. Protein-lipid graph of the groups

FA Composition

The fatty acid changes of the different nutrient limitation groups are given in Table 3. It was observed that saturated fatty acids (ΣSFA) were generally low and unsaturated fatty acids (ΣUFA) were quite high in *P. tricornutum* cultured in sea water with a salinity of ‰30 and in which some nutritional changes were made. Although the observation values in terms of ΣSFA between the groups seemed to be close to each other, there was a statistical difference between them ($P<0.05$). The highest SFA was found in the

50%P- and 20% Si- groups. The most dominant fatty acid in SFA was C16:0 palmitic acid in all groups and the highest was detected in the 20%Si- group. Although monounsaturated fatty acids (ΣMUFA) were high in all groups, they were highest in 50%P- and 20%Si- groups. Palmitoleic acid (C16:1) was the predominant in MUFA, with 39.84% being the highest in the 20%Si- group. The other dominant fatty acid (C18:1ω9) is oleic acid, which was highest in the control group and lowest in the group containing 20%Si-. Polyunsaturated fatty acids (PUFA) were found the highest in 50%P-&50%Si- and 50%P-&20Si- groups. The most important fatty acid in the PUFA group is EPA (C20:5ω3). Although EPA was high in all groups, it was found to be 12.28% in the 50%P-&50%Si- group and 9.36% in the lowest control group. At the end of the experiment, total UFA was found to be high in the groups other than the control group, and a statistical difference was determined between the groups (*P<0.05*).

Table 3. FA profile of *Phaeodactylum tricornutum* biomass after the cultivation under different P and Si treatments (% dry weight of biomass)

	Control	**50%P-lim**	**50%Si-lim**	**20%Si-lim**	**50%P-&50%Si-lim**	**50%P-&20%Si-**
C6:0	0.83±0.02	0.225±0.02	0.21±0.001	0.39±0.001	0.29±0.001	0.88±0.01
C8:0	0.55±0.001	0.46±0.04	0.4±0.001	0.43±0.001	0.64±0.002	0.51±0.02
C10:0	0.75±0.001	0.655±0.02	0.6±0.002	0.6±0.001	0.945±0.007	0.71±0.04
C12:0	0.8±0.002	0.69±0.01	0.63±0.001	0.65±0.002	1.025±0.007	0.795±0.02
C14:0	0.7±0.002	0.61±0.04	0.535±0.007	0.56±0.001	0.885±0.007	0.66±0.01
C16:0	16.45±0.01	18.705±0.81	17.705±0.06	19.11±0.01	15±0.07	16.09±0.04
C17:0	0.67±0.002	0.615±0.02	0.74±0.001	0.66±0.002	0.84±0.001	0.755±0.007
C18:0	1.16±0.001	0.81±0.04	0.89±0.002	0.69±0.001	0.895±0.007	0.9±0.01
C20:0	0.38±0.001	0.275±0.02	0.265±0.007	0.31±0.002	0.26±0.01	0.26±0.04
C22:0	0.09±0.001	0.09±0.001	0.09±0.001	0.09±0.001	0.11±0.002	0.11±0.002
C24:0	0.02±0.001	0.02±0.001	0.02±0.001	0.02±0.001	0.025±0.002	0.03±0.001
∑SFA	**22.4^{b}**	**23.155^{a}**	**22.085^{b}**	**23.51^{a}**	**20.118^{d}**	**21.7^{c}**
C14:1	4.4±0.01	4.42±0.15	4.765±0.02	4.36±0.13	4.13±0.01	3.805±0.06
C16:1	31.02±1.3	38.5±1.47	37.2±1.2	39.84±0.4	34.975±0.2	35.735±0.5
C17:1	1.83±0.001	1.77±0.18	2.2±0.01	1.64±0.02	2.135±0.14	2.31±0.09
C18:1ω9	7.42±0.02	5.2±0.2	4.995±0.02	4.46±0.01	5.235±0.007	4.655±0.03
C18:1ω7	1.64±0.02	1.29±0.01	1.36±0.02	1.2±0.01	1.52±0.04	1.585±0.06
∑MUFA	**46.31^{d}**	**51.18^{a}**	**50.52^{b}**	**51.5^{a}**	**47.995^{c}**	**48.09^{c}**
C18:2ω6c	1.54±0.02	1.775±0.06	1.7±0.01	1.81±0.01	1.62±0.01	1.665±0.02
C18:3ω6	0.64±0.02	0.175±0.07	0.13±0.02	0.15±0.01	0.27±0.001	0.265±0.02
C18:3ω3	2.17±0.02	0.685±0.07	0.58±0.01	0.69±0.01	0.75±0.01	0.85±0.02
C20:2	0.19±0.01	0.02±0.002	0.24±0.01	0.02±0.01	0.21±0.02	0.21±0.01
C20:4ω6	0.51±0.03	0.55±0.01	0.65±0.02	0.45±0.01	0.53±0.01	0.575±0.02
C20:3ω6	0.05±0.01	0.05±0.002	0.055±0.007	0.05±0.01	0.06±0.02	0.06±0.01
C20:5ω3 (EPA)	9.36±0.01	10.45±0.09	11.125±0.03	9.88±0.02	12.28±0.01	11.95±0.25
C22:6ω3 (DHA)	0.84±0.02	0.86±0.01	0.93±0.01	0.81±0.01	1.09±0.02	1.10±0.01
∑PUFA	**15.3^{b}**	**14.565^{c}**	**15.41^{b}**	**13.86^{d}**	**16.81^{a}**	**16.85^{a}**
∑UFA	**61.61^{d}**	**65.745^{a}**	**65.93^{a}**	**65.36^{b}**	**64.805^{c}**	**64.94^{c}**
Defined	**84.01**	**88.9**	**88.015**	**88.87**	**84.923**	**86.64**

There is a statistical difference between the means of the columns symbolized by the letters $^{a, b, c, d, e}$ (*P<0.05*). Values are presented as mean ± SD of three determinations.

DISCUSSION

In this study, the effects of P and Si deficiency on the lipid content of *P. tricornotum* belonging to the class Bacillariophyceae total protein, lipid, Chl *a*, and fatty acids were investigated. The findings obtained from the research were tried to be discussed and necessary conclusions were drawn according to the subjects examined.

P is an important nutrient for algae and plays an important role in many critical metabolic processes, especially photosynthesis and respiration (Plaxton and Tran,

2011). Like N, Si is known to be strongly associated with microalgae growth and cell metabolism, especially since it is the main component of diatom cell walls (Coombs et al., 1967). In our study, a medium containing all the necessary nutrients for *P. tricornutum* was prepared and defined as the control group. While the amount of dry matter determined on the last day of the experiment in the control group was 0.820 gL^{-1}, 0.799 gL^{-1} in the culture with 50%P-limitation, 0.703 gL^{-1} in the culture with 50%P-&50%Si- application, lower dry matter amounts were determined as 0.674 gL^{-1} in the 20%Si- applied group, 0.588 gL^{-1} in 50%Si- application and 0.580 gL^{-1} in 50%P-&20%Si- deficiency. The highest lipid content was determined between 34.9% and 50%P-. It is seen that the dry matter productivity, in other words, the growth, of the 50%P- applied group was the best after the control group. The fact that the 50%P- applied group was closest to the control group in terms of biomass efficiency can be accepted as an indicator of its applicability as a stress factor for lipid production.

The Chl *a* pigment was found to be the highest (719 μgL^{-1}) in the culture treated with 50%P- compared to the other groups. In the control group, 721 μgL^{-1} Chl *a* was determined. While the highest protein rate was 30.6% in the control group compared to the other groups in which nutrient deficiency was applied, the lowest protein rate was 16% in the 50%P- limitation group, in which the highest lipid content was determined. It is observed that 20% and 50%Si- has no effect on lipid increase in *P. tricornutum* cells. Looking at the dry matter amounts, similarly, the lowest dry matter amounts are observed in the 20% and 50%Si- applied groups.

The lowest growth was observed in the groups treated with 50%P-&20%Si- and 50%Si-. Alipanah *et al.* (2018) reported in their study that P restriction causes some decreases in cell density, as well as decreases in the amount of Chl *a.*

In the study carried out, the application of Si deficiency in the nutrient medium caused a decrease in the amount of dry matter. The decrease in growth in Si-deficient cultures may be associated with a reduced ability of cells to divide. The concentration of nutrients, especially Si for diatoms, is the most important element required for carbohydrate and lipid biosynthesis (Lari et al., 2016). In our study, the highest lipid content was found in all groups with P reduction. The lipid values in the group made 50%P- and 50%P-&50%Si- were around 34% and were similar. Both P and P&Si limitations increased lipid production. It has been reported that Si limitation causes lipid accumulation in some diatoms (*Chaetoceros gracilis* and *Nitzschia* spp.) (Jiang et al., 2015). However, in our study, only Si deficiency did not cause any lipid increase. The contribution of P- to the lipid increase was high.

It has been reported that the highest lipid content in *I. galbana* cultured in F/2 medium containing different levels of P (0-150%) was detected in the groups cultured in medium containing 25% and lower levels of P (Roopnarain et al., 2014). *Nephrochlamys yushanlensis* control was cultured with N, N&P, and P deficiency and lipid contents were determined. Total lipid contents were found to be 31% control, 58.6% N-, 34% P-, and 49% N&P-, respectively (Maltsev et al., 2021). The lipid content of Diatom *Navicula pelliculosa* has been reported to increase by approximately 60% during a 14-hour silica starvation period (Coombs et al., 1967). P restriction has been reported to increase lipid accumulation in several microalgae species (Siron et al., 1989). This also applies to *P. tricornutum*, where P restriction triggers an increase in lipid content (Alipanah et al., 2018; Yang et al., 2014). In our study, P limitation increased the amount of lipids.

The concentrations of the elements used in the medium, especially the deficiency of elements such as N and P, which are effective in the growth of algae, increase the lipid ratio in many algae species, while causing a decrease in the protein ratio. In our study, while the lowest protein content was found in all groups with P reduction, it was found to be lower but close to the control in only Si- limitation groups. Encinas-Arzate *et al.* (2020) reported that the protein content in the groups with Si reduction in *Navicula* was higher than the groups with N reduction, and higher than the control group.

In addition to environmental factors such as light, temperature and salinity, the presence or absence of nutritive elements can affect the lipid ratio as well as the ratio of fatty acids (Takagi, 2006). The degree of unsaturation of fatty acids is also an important parameter in the adaptation of algae to environmental conditions (Ben-Amotz et al., 1984). Although the total saturated fatty acids (SFA) were relatively close to each other in our study, they were found to be around 23% in the groups treated with the highest 50%P- and 20%Si-. Unsaturated fatty acids, on the

other hand, were higher in the other groups except for the control group. This shows that *P. tricornutum* adapts well to the environment. Yang *et al.* (2014) found that the main fatty acids C16:0, C16:1 and C20:5n-3 was high in *P. tricornutum*, while C18 fatty acids were quite low. resulting in C16:0 and C16:1 content and a relatively lower content of C18:1, C18:3, C20:5n-3 and C22:6n-3; They noted a noticeable reduction in the presence of a particularly important component, C20:5n-3 (eicosapentaenoic acid, EPA). In our study, the most dominant fatty acids were C16:0, C16:1 and EPA values were found to be slightly higher in the groups with P deficiency than in the control group. Most likely, stress factors may be directed to the formation of short-chain fatty acids (such as C16 and C16:1), which are their intermediate substrates for lipid synthesis. Spilling *et al.* (2021), in their study, found EPA in *T. baltica* in the group with the highest Si reduction in dry matter. In our study, EPA was found to be higher in groups with Si reduction. The fatty acids used in the production of biodiesel and determining its quality are palmitic acid (C16:0) and oleic acid (C18:1), which are also found in vegetable oils (Kaur et al., 2012). In terms of biodiesel, C16-C18 fatty acids can provide the best relationship between oxidative stability and cold flow properties (Knothe et al., 2009), these values were found to be high in our study. In our study, palmitic acid was found to be high especially in the groups with 50%P and 20%Si limitation. SFA and MUFA rates were around 23% and 50%, respectively, with SFA (26.52%), MUFA (21.91%) (Branco-Vieira et al., 2017) are in agreement with other studies. These findings show that *P. tricornutum* lipids can achieve high cetane number and low iodine value, which complies with the requirements of European (EN 14214) and USA (ASTM D6751) standards (Hoekman et al., 2012).

PUFA is an important structural acid group in aquaculture and is very important for the high reproduction and larval growth rate of organisms. Since PUFA is important for humans and animals, PUFA supplementation is needed and the most important source of PUFA is marine fish. In fact, fish do not produce PUFA, they only consume PUFA-rich microalgae and take PUFA into their body. *P tricornutum* is one of the algae rich in PUFA. In our study, the amount of PUFA was not very high in all groups, with an average of 15.45%. In studies, *P. tricornutum* PUFA contents were found to be around 32.02% (Branco-Vieira et al., 2017), 60% (Rodolfi et al., 2017). In these studies, it was stated that the results were higher because the amount of nutrient element was increased, temperature, light intensity and outdoor conditions. However, Reitan et al., (1994) stated that the amount of PUFA decreased in nutrient-limited environments, which is consistent with our results.

As a result, 50%P and 50%Si deficiency in terms of UFA, the most important nutritional element affecting the amount of fatty acids; In terms of SFA, 50%P and 20%Si deficiencies were observed in the groups.

Reducing the concentrations of the main elements (N and P) in the medium causes a decrease in cell density and thus a decrease in the amount of protein. This situation causes a decrease in the amount of Chl *a* due to the lack of production of ATP and NADPH energy molecules that enable chlorophyll synthesis. This situation causes an increase in organic compounds such as lipids and carbohydrates in the algae (Roopnarain *et al*, 2014). In this study, decreases in the amount of Chl *a* and protein in the groups treated with P deficiency; Increases in lipid content were observed.

CONCLUSIONS

This study was carried out to determine how the growth of *P. tricornutum* in culture medium prepared with different ratios of nutrient elements is affected and also what changes occur in its biochemical structure.

If the results obtained in the study are summarized; changing the ratios of nutritional elements caused changes in cell densities, chlorophyll, dry matter, protein, lipid and fatty acids. While the best growth was in the control group, the closest 50% was found in the P- applied group. In terms of protein, approximately 30% protein was obtained in the 20%Si- applied groups. The best groups in terms of lipid were all groups that received 50%P-. It would be more appropriate to do 50%P- for the studies to be carried out for this purpose. Because the lipid ratio is close to each other in both 50%P and 50%P&20%Si and 50%P&50%Si limitations, there is no need for Si reduction in the environment. At the same time, the aim of lipid studies is to increase the lipid ratio while keeping the biomass ratio high. In the environment where 50%P- is applied, the amount of biomass is very close to the control. Since the fatty acids palmitic and oleic acids used in biodiesel production and determining the quality of biodiesel are found at high levels

in *P. tricornutum*, we can say that this species can be a source of biodiesel.

In conclusion, as a result of the study conducted to increase the growth and valuable metabolites of *Phaeodactylum tricornutum*, we can say that the adaptation ability of this species is very good. We can suggest that this species should be evaluated as a raw material source in lipid and biodiesel studies, since it easily survives in different nutritional deficiencies and increases lipid at a significant level.

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Ethical Approval

No need to ethical approval for this study.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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