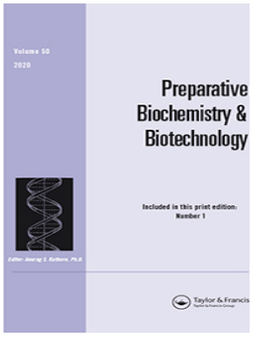

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Effects of nitrogen concentration on growth, biomass, and biochemical composition of *Desmodesmus communis* (E. Hegewald) E. Hegewald

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ABSTRACT

Nitrogen, being one of the building blocks of biomacromolecules, is an important nutrient for microalgae growth. Nitrogen availability alters the growth and biochemical composition of microalgae. We investigated the effects of different nitrogen concentrations on specific growth rate (SGR), biomass productivity (BP), total protein and lipid content and amino acid and fatty acid composition of *Desmodesmus communis*. Nitrogen deficiency increased algal growth and changed the lipid amount and composition. The maximum growth and BP were detected in 75% N−medium. The highest total protein and lipid amount were detected in 50% N− and 75% N−media, respectively. Amino acid and fatty acid compositions of samples varied widely depending on the nutrient concentrations. The amount of unsaturated fatty acid (USFAs) was higher than saturated fatty acid (SFAs) and Linolenic acid percentage is higher than the limit of European standards in all media. The data reported here provide important contributions how nitrogen scarcity and abundance affect the growth and biochemical content of microalgae and this information can further be utilized in culture optimization in studies aimed at microalgae production for biofuels.

KEYWORDS

Biochemical composition; *Desmodesmus communis*; growth rate; nitrogen deprivation

Introduction

Microalgae are photosynthetic microorganisms capable of producing the most valuable biomolecules such as pigments and fatty acids. These bioactive molecules are used in many areas such as cosmetics, agriculture, pharmaceutical industry, food and feed, and wastewater treatment. Due to its economic value, microalgae culture and isolation of the various bioactive molecules have gained great importance.[1–4] In recent years, algal biotechnological studies have emphasized on the cultivation of microalgae in modified culture media and the variation of these bioactive molecules and their contents.

Desmodesmus/*Scenedesmus* spp. are freshwater green microalgae, commonly studied due to their resistance to stress conditions and high growth rate.[5,6] These microalgae are attractive research subjects due to the large number of metabolites that they produce.[7,8] Many studies with *Desmodesmus communis* have found that different culture media alters the biochemical composition.[9,10] Additionally, biomass productivity and biochemical composition change by different culture conditions,[11,12] especially, nutrient concentrations.[13–17] The carbohydrate and lipid variety increase by changing the cultivation conditions.[9,18–23] Nutrient limitation is the most effective strategy for production and accumulation of biomolecules.[21,24]

Nitrogen is an important component of several biomolecules such as proteins, nucleic acids, and chlorophyll. Nitrogen deficiency slows down the cell growth and development, disrupts the biochemical structure of the cell. The amounts of nitrogen in the culture media where microalgae are grown strongly affect the specific growth rate and the biochemical composition of microalgae. Many studies show that nitrogen deficiency slows down the growth of microalgae, increases the number of lipids and carbohydrates and accelerates protein synthesis. Nitrogen affects the lipid metabolism and composition of fatty acids and amino acids.[24] When the nitrogen is limited, the proportion of saturated fatty acids (SFAs) and monounsaturated fatty acids (MUFAs) increase and the proportion of polyunsaturated fatty acids (PUFAs) decreases with respect to total lipids.[25]

Material and methods

Microalgae and cultivation conditions

The microalgae *D. communis* (Accession number: KF470792) was obtained from the Algal Biotechnology Laboratory, Mehmet Akif Ersoy University (Burdur, Turkey). Figure 1 shows the scanning electron micrographs of *D. communis*. The cells were maintained in BG11 medium at 23 °C, under 12 hr light/12 hr dark photoperiod. The cells were centrifuged, washed with deionized water and

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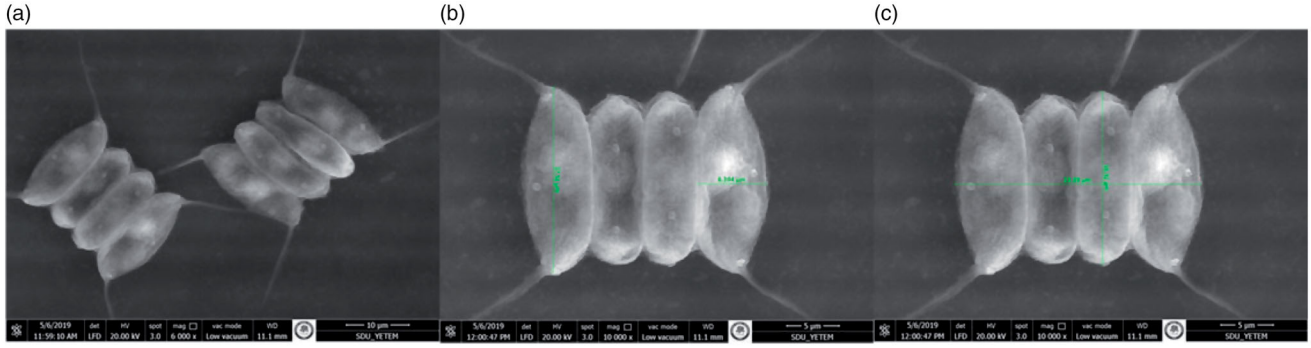

Figure 1. Scanning electron micrographs of *D. communis*; general view (a), scale bar: 10 µm; width and length of a cell (b) and colony (c), scale bar: 5 µm.

Table 1. Composition of BG 11 medium.[26]

Compound	Amount (mg/L H_2O)
Na_2CO_3	20
Na_2 EDTA	1
$NaNO_3$	1500
K_2HPO_4	40
$MgSO4.7H_2O$	75
$CaCl.2H_2O$	36
Citric acid	6
Ferric ammonium citrate	6
Trace metal mix[a]	1 mL/L

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

inoculated to cultures (10% v/v); the cultures were incubated in 2000 mL Erlenmeyer flasks. All cultures were initiated with an Optical Density (OD) of about 0.09 and 3.6×10^5 cells/mL, at 680 nm. The cultures were grown at the following conditions: 28 °C, 16 hr light/8 hr dark photoperiod, illumination 70 µmol/m^2/s and initial pH 7.5. The culture was semi-continuously aerated with air at a rate of 1 L/min.

Nitrogen limitation

The microalgae were studied for growth and biochemical composition in different concentrations of nitrate. The BG11 medium's nitrogen concentration is 1.5 g/L, $NaNO_3$ (Table 1) and it was the (1) control group Medium. Other media were (2) Medium (50% N+); (3) Medium (50% N−); (4) Medium (75% N+); (5) Medium (75% N−) [N+: more N concentration than the control group; N−: less N concentration than the control group].

Cell growth analysis

Microalgae growth was observed in one-day interval by calculating the OD at 680 nm with UV/visible spectrophotometer (Shimadzu UV-1650, Kyoto, Japan). The cell counting (cell/mL) was also performed through a hemocytometer at the time when OD was measured. Dried cell weight (DCW) was detected by filtering 10 mL of microalgae culture through glass fiber filter (Whatman GF/C, 1.2 µm, UK) and drying the biomass on the filter at 105 °C for 2 hr.[27] At the stationary phase of culture for harvesting the microalgal biomass, the cultures were centrifuged and dried to detect DCW.

The following growth parameters of each replicate were estimated by using cell count during log phase of growth and DCW values.

Specific growth rate (μ): $\mu = \ln (N_t/N_0)/T_t - T_0$ where N_t is the number of cells at the end of log phase, N_0 is the number of cells at the start of log phase, Tt is the final day of log phase and T_0 is starting day of log phase.

Doubling time: $Tt = 0.6931/\mu$.

Biomass productivity (BP): as the dry biomass produced per day (g/L/day).

Volumetric biomass productivity P_{Biomass} was calculated by the following equation:

$$P_{\text{Biomass}}(\text{g/L/day}) = (X_2 - X_1)\ (t_2 - t_1) \quad (1)$$

Where X_1 and X_2 were the DCW concentrations (g/L) on days t_1 (starting point of cultivation) and t_2 (endpoint of cultivation), respectively.

Determination of biochemical composition

For crude protein calculation, the Dumas method (Gerhardt Dumatherm Elemental Analyser) was used for measuring the total nitrogen content of microalgae and it was calibrated using acetanilide as a reference standard.[28] For estimating the protein amount of the samples, the following equation was used: "protein amount = nitrogen content × 4.44".[29] The results were expressed as percent of dry weight. For detecting the amino acid profiles in dried *D. communis* samples, using HPLC method (Shimadzu Prominence HPLC, Kyoto, Japan) according to Köse et al. [30]

Atleast 100 mg dried biomass was used for lipid extraction according to Bligh and Dyer's method.[31] The fatty acids were identified using gas chromatography (AGILENT 5975C, AGILENT 7890A GC, Agilent, Santa Clara, CA) equipped with column DB WAX (50 × 0.20 mm, 0.20 µm). The operating conditions of the device were determined according to Seçilmiş and Bardakçı.[32]

Statistical analysis

Three biological replicates were established for each culture condition. Values were reported as the mean ± standard

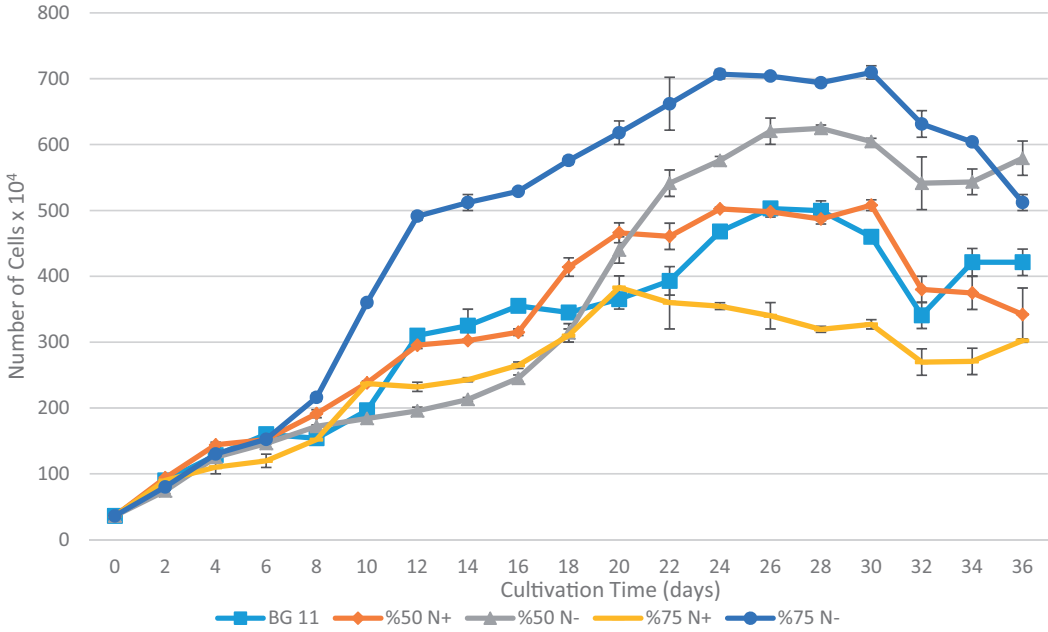

Figure 2. Cell number dynamics of *D. communis* in five different culture media. Error bars represent the standard deviation between the three replicates of each culture condition ($n = 3$).

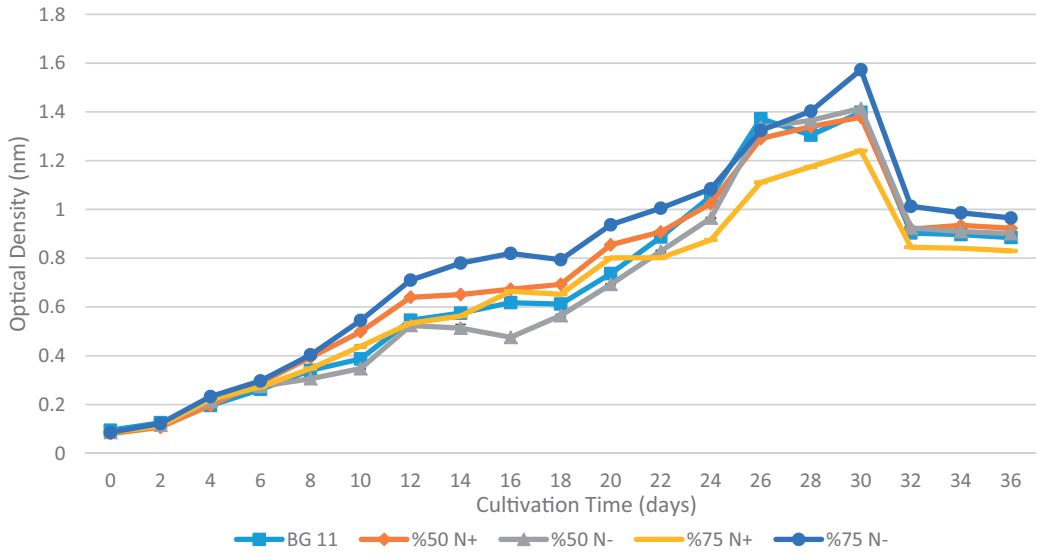

Figure 3. Optical density (OD) dynamics of *D. communis* growth in five culture media. Error bars represent the standard deviation between the three replicates of each culture condition ($n = 3$).

deviation of three replicates. Data were analyzed by one-way analysis of variance (ANOVA) using Microsoft Office Excel 2007 (Microsoft, Redmond, WA).

Significant test results were at the level of $p \leq 0.05$.

Results and discussion

Effects of nitrogen limitation on cell number, dry cell weight (DCW), biomass productivity, and specific growth rate

The growth of the microalgae is affected by the alterations of the nutrient concentrations of the culture medium and conditions. Determination of the optimum growth conditions is very important for commercial algal biomass production. Nitrogen is an important element for microalgae growth since it is present in many biomolecules, such as proteins and nucleic acids. Nitrogen limitation causes to increase in lipid and protein amounts.[33,34]

Table 2. Linear relationship (R^2 values) between optical density (OD) and cell number (CN); OD and Dry cell weight (DCW).

Media	OD-CN	OD-DCW
BG 11	0.947	0.878
50% N+	0.948	0.940
50% N−	0.955	0.906
75% N+	0.879	0.901
75% N−	0.949	0.924

The effects of the nitrogen concentrations on the growth of microalgae were recorded by cell count (cell number/L),

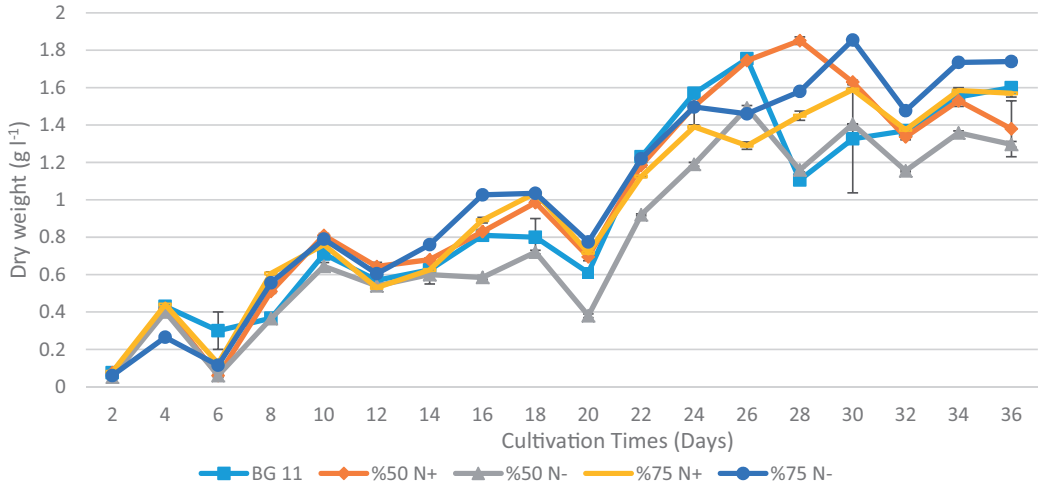

Figure 4. Dried cell weight (DCW) values of *D. communis* in five culture media. Error bars represent the standard deviation between the three replicates of each culture condition ($n = 3$).

Table 3. Growth kinetics and biomass, biomass productivity, *D. communis* in five culture media.

Media	Specific growth rate (μ)	Doubling time (Tt)	Biomass productivity (g/L/day)
BG 11	0.251 ± 0.001	2.765 ± 0.013	0.041 ± 0.0008
50% N+	0.273 ± 0.001	2.536 ± 0.009	0.040 ± 0.001
50% N−	0.262 ± 0.001	2.641 ± 0.01	0.036 ± 0.001
75% N+	0.315 ± 0.0007	2.197 ± 0.005	0.042 ± 0.006
75% N−	0.293 ± 0.001	2.369 ± 0.009	0.047 ± 0.001

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

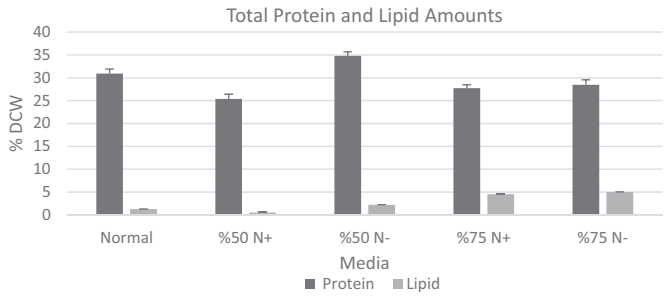

Figure 5. Total protein and lipid contents of *D. communis* biomass harvested from five different media. A difference was considered significant at the level of $p < 0.05$.

OD and DCW every other day. CN and DCW data were supported by OD measurements. Strong correlation values were found between OD-CN and OD-DCW.

The effects of nitrate restriction on cell number (Figure 2), and on OD at 680 nm (Figure 3), of *D. communis* are shown. The maximum cell number (709.6×10^4 cell/mL) and OD (1.57 at A680) were observed in 75% N − medium on 30th day of culture and the lowest cell number (269.6×10^4 cell/mL) and lowest OD value (0.84 at A680) were recorded in 75% N + medium on 32nd day of culture. Fakhry and El Maghraby reported similar results to ours.[35]

Figure 4 shows the nitrogen effects on DCW; maximum DCW (1.855 g/L) was recorded in 75% N − medium on the 30th day and minimum DCW value (1.32 g/L) in BG11 medium on the same day. And the lowest DCW value (0.38 g/L) in the exponential phase was recorded in 50% N − medium on the 20th day. Biomass productivity (BP) values were correlated to DCW data; maximum BP (0.047) was recorded in 75% N − medium and minimum BP (0.036) was in 50% N − medium (Table 3). These results are consistent with previous reports.[36,37]

The maximum specific growth rate (0.315) value was recorded in 75% N + and then 75% N − media (0.293); the minimum specific growth rate (0.251) was estimated for the BG 11 medium. The highest doubling time (2.765) was recorded in BG 11 medium and the lowest one (2.197) is in 75% N + medium (Table 3). The low doubling time indicates a high specific growth rate[38] and our results are compatible with this.

The highest cell number, OD, DCW, and biomass productivity values were recorded in 75% N − medium, and otherwise, the lowest cell number and OD values were recorded in 75% N + medium; the lowest DCW and biomass productivity were recorded in 50% N − medium. All results show that nitrogen deficiency increases microalgal growth. Total lipid results (Figure 5) supported our cell growth results; maximum lipid was recorded in 75% N − medium, which means that the maximum cell number causes production of maximum lipid-containing cell membranes. But we have some conflict in our results that cannot be explained only by the amount of nitrogen. A living organism may react differently to the same changes. Other physical conditions such as temperature, light regimes, pH, etc. should be studied to obtain more accurate and indisputable results for microalgal growth.[36,39–45]

Effects of nitrogen limitation on biochemical composition of Desmodesmus communis

Figure 5 shows the effects of nitrogen limitation on lipid and crude protein amounts of *D. communis*. The maximum

Table 4. Amino acids compositions and amounts (mg/g DCW) of *D. communis* biomass harvested from five different media.

Aminoacids/media	BG 11	50% N+	50% N−	75% N+	75% N−
Arginine	40.8 ± 0.2	42.14 ± 0.02	0.559 ± 0.1	3.911 ± 0.2	27.87 ± 2
Serine	0.644 ± 0.002	0.5 ± 0.2	1.883 ± 0.2	2.149 ± 0.1	1.64 ± 0.2
Glycine	0.54 ± 0.02	0.539 ± 0.002	0.181 ± 0.02	1.391 ± 0.2	1.8 ± 0.2
Alanine	0.926 ± 0.002	0.881 ± 0.001	1.83 ± 0.2	1.996 ± 0.2	1.849 ± 0.2
Proline	0.121 ± 0.001	0.325 ± 0.002	1.976 ± 0.4	0.226 ± 0.02	7.175 ± 0.1
Valine	2.371 ± 0.002	0.328 ± 0.002	0.653 ± 0.2	0.2 ± 0.1	0.599 ± 0.2
Methionine	0.489 ± 0.002	0.609 ± 0.003	0.457 ± 0.04	0.229 ± 0.02	0.963 ± 0.2
Isoleucine	0.658 ± 0.002	0.4 ± 0.1	0.313 ± 0.01	0.163 ± 0.02	1.911 ± 0.15
Leucine	0.777 ± 0.002	1.107 ± 0.002	6.927 ± 0.4	0.399 ± 0.2	2.452 ± 0.2
Phenylalanine	0.244 ± 0.002	0.162 ± 0.002	25.29 ± 3	0.254 ± 0.01	0.704 ± 0.002
Tyrosine	0.243 ± 0.002	0.171 ± 0.002	8.774 ± 0.21	0.107 ± 0.002	0.149 ± 0.02
Aspertic acid	0.748 ± 0.002	1.597 ± 0.002	0.182 ± 0.02	0.554 ± 0.2	0.213 ± 0.1
Glutamic acid	0.436 ± 0.002	0.463 ± 0.002	0.121 ± 0.02	1.274 ± 0.02	0.123 ± 0.02
Histidine	0.25 ± 0.002	1.792 ± 0.002	0.284 ± 0.02	1.981 ± 0.2	0.13 ± 0.02
Lysine	0.337 ± 0.002	5.779 ± 0.002	0.181 ± 0.02	3.106 ± 0.1	0.268 ± 0.02
Total	49.584 ± 0.23	56.793 ± 0.06	49.611 ± 4.82	17.94 ± 1.39	47.846 ± 3.19

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

Table 5. Fatty acids compositions and percentage (% of total fatty acids) of *D. communis* biomass harvested from five different media.

Name of fatty acid	BG 11	50% N+	50% N−	75% N+	75% N−
Saturated fatty acids (SFA)					
C14:0 (myristic acid)	2.054 ± 0.2	2.349 ± 0.2	2.254 ± 0.2	2.184 ± 0.1	2.324 ± 0.2
C16:0 (palmitic acid)	18 ± 2	18.223 ± 0.2	19.015 ± 1	19.139 ± 1	20.366 ± 0.2
C18:0 (stearic acid)	0.99 ± 0.128	1.142 ± 0.1	1.095 ± 0.1	1.062 ± 0.1	1.13 ± 0.1
Total SFAs	21.052 ± 2.08	21.714 ± 0.5	22.364 ± 1.1	22.385 ± 1.2	23.82 ± 0.3
Monounsaturated fatty acids (MUFA)					
C16:1 (palmitoleic acid)	2.006 ± 1	2.294 ± 0.2	2.201 ± 0.2	2.133 ± 0.1	2.27 ± 0.1
C18:1 (oleic acid)	11.645 ± 0.2	13.316 ± 0.3	13.838 ± 0.2	12.381 ± 0.2	13.17 ± 60.1
C18:1 t10, t11e 12	5.008 ± 0.002	4.786 ± 0.2	3.822 ± 0.2	4.45 ± 0.2	4.736 ± 0.2
Total MUFAs	18.659 ± 0.798	20.396 ± 0.7	19.861 ± 0.6	18.964 ± 0.5	20.182 ± 0.4
Polyunsaturated fatty acids (PUFA)					
C18:2 (linoleic acid, c9 c12)	11.772 ± 0.2	11.589 ± 0.45	12.605 ± 0.2	12.218 ± 0.2	11.467 ± 0.2
C18:3 (linolenic acid, c9, c12, c15)	36.917 ± 0.21	37.262 ± 0.2	36.632 ± 0.19	35.909 ± 0.2	36.011 ± 0.99
Total PUFAs	48.689 ± 0.41	48.851 ± 0.65	49.237 ± 0.0006	48.127 ± 0.4	47.478 ± 1.19
Others	5.528 ± 0.2	6.326 ± 0.2	6.066 ± 0.2	5.879 ± 0.2	6.257 ± 0.15
Total FAME Amounts	93.928 ± 2.27	97.287 ± 2.05	97.528 ± 1.5	95.355 ± 2.3	97.737 ± 2.04

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

crude protein amount (34.77%DCW) was measured in 50% N − medium, the second-highest value (30.91%DCW) was measured in BG 11 medium and the lowest value (25.36%DCW) in the 50% N + medium. Our results show that N deficiency cause a reduction protein amount and some studies are parallel to our results.[46–49]

The maximum total amino acid value (57.483 mg/g DCW) was recorded for 50% N + medium and the lowest one (17.94 mg/g DCW) was for 75% N+ (Table 4). The medium that has minimum protein amount has maximum amino acid content. Arginine is the most abundant amino acid in BG 11 medium, 50% N+, 75% N + and 75% N − media; Phenylalanine is the most abundant in 50% N + and other amino acid's profile and amounts of the media vary (Table 4). The physical conditions of culture or sources of the microalgal culture cause differentiation in the amino acid profiles.[50,51] On the other hand; not only nitrogen concentration but the type of nitrogen affects the amount and composition of amino acids.[52,53]

The highest lipid amount (5.024%DCW) was recorded in the 75% N − medium and the lowest value (0.522%DCW) in the 50% N + medium (Figure 5). Nitrogen limitation in algal culture enhances synthesis and storage of lipids.[54–56]

75% N − media has the maximum total FAME amounts than the 50% N−, 50% N+, 75% N+, and BG 11 media, respectively (Table 5). This result supports that N limitation increases FAME concentration.

When the biomass obtained from 5 different media is examined in terms of fatty acid content, the following fatty acids were higher than the control BG11 media: Palmitic acid, oleic acid, linoleic acid, linolenic acid (Table 5).

USFAs and SFAs constitute the majority of the lipid content of microalgae grown in nitrogen-limited media.[57,58] In all the biomasses obtained from microalgae grown in five different media, the USFAs content is considerably higher than the SFAs content. The highest SFAs content (23.82%) was obtained in the 75% N − medium and the lowest one (21.052%) in the BG 11 medium. The highest amount of MUFAs (20.396%) was obtained in the 50% N + medium and the lowest one (18.659%) in BG11 medium. On the other hand; the highest amount of PUFAs (49.237%) was obtained in the 50% N − medium and the lowest one (47.478%) in 75%N − medium (Table 5).

High levels of SFAs in *D. communis* grown in N limitation medium could increase oxidative stability and cetane number of the biodiesel produced. Also, a high USFA

percentage is an advantage for the cold flow properties of biodiesel.[59]

Palmitic acid is the most abundant of SFAs. The highest amount (20.366%) was found in the 75% N − medium.

Linolenic acid (omega 3-ω 3) is the most abundant of USFAs. The highest amount (37.262%) was found in 50% N + medium. The second most USFA type is oleic acid (Omega 9-ω 9; 13.838%) in 50% N − medium and the third most USFA is linoleic acid (Omega 6-ω 6; 12.605%) in 50% N − medium.

European standards (EN14214) have stated the limits of linolenic acid (C18:3) as 12% in biodiesel. The high linolenic acid reduces biodiesel stability due to oxidation[59] The results of this study show that *D. communis* is an appropriate microalgae species for biodiesel production because of its high linolenic acid content (37.262% in 50% N + medium).

When all these results were evaluated, nitrogen limitation increases the amount of SFAs but does not show the same effect on the amount of USFAs. This difference can be related to the oxidative damage of USFAs.[60] Additionally, USFAs are defenseless to changes culture conditions such as nutrient content, CO_2, light, and temperature.[61]

However, there is no statistically significant difference between the highest (71.23% in 50%N+) and lowest (68.92% in 75% N+) amounts of USFAs. But it is useful to specify that more USFAs were detected in the biomass obtained from nitrogen-limiting media than those obtained from BG 11 medium, we can conclude that the limitation of nitrogen increases the amount of USFAs.

Conclusions

When all the data of the present study were evaluated; freshwater microalgae *D. communis* (Accession number: KF470792) is able to reproduce more rapid in nitrogen limitation media. The highest biomass productivity (0.0465 g/L/day) and specific growth rate ($\mu = 0.29277$) were detected in 75% N − medium. Besides; nitrogen limitation causes an increase in the amount of crude protein and lipid. FAME analysis of microalgae for biodiesel production is crucial, the desired level of linolenic acid percentage shows that *D. communis* can be utilized for biodiesel production. From this perspective, this study is very important in understanding how nitrogen scarcity and abundance affect microalgae growth and microalgae biochemical content. In addition, the observations from this study can be utilized to optimize the culture conditions for biofuel production from microalgae.

Funding

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