TÜBİTAK

Turkish Journal of Botany

http://journals.tubitak.gov.tr/botany/

Research Article

Turk J Bot
(2019) 43: 458-467

doi:10.3906/bot-1811-17

Two pronounced *Chlorella* strains for efficient biodiesel feedstock production

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Received: 13.11.2018 • **Accepted/Published Online:** 03.04.2019 • **Final Version:** 08.07.2019

Abstract: In this study, two indigenous *Chlorella* strains were evaluated for triacylglycerol (TAG) production and related parameters during 10 days of N-deprivation. The strains were identified based on morphological characteristics and genomic information, named as *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17. Time-dependent reduction of growth was accompanied by decreased chlorophyll content, reduced oxygen evolution ratio, and elevated carotenoid content of both strains cultivated in N-free Bold's basal medium. Nitrogen deprivation induced total lipid and neutral lipid content in both strains, supported by FTIR measurement of TAGs. *Chlorella* sp. IMU17 showed higher production of TAGs as a response to N-deprivation. Strikingly, relative polysaccharide content showed a rapid increase on the first days and a noticeable reduction was recorded especially after 5 days of N-deprivation while TAG production showed a gradual increase during the whole period of N-deprivation in both strains. Starch might be a predominant form of carbon storage in the short term and the continuous increase of TAG production might be supported by degradation of starch in a longer period of N-deprivation. FAME analysis of lipids showed that the saturation level of the fatty acids of both strains meets the European standard EN 14214 requirements. Lastly, N-deprivation stimulated a significant reduction of PUFA production in *Chlorella* sp. IMU17. Thus, *Chlorella* sp. IMU17 might stand as a promising candidate for biodiesel feedstock production with its low PUFA content and a concomitant induction of saturation of fatty acids as a clear response to N-deprivation.

Key words: *Chlorella*, triacylglycerol, neutral lipid, fatty acid methyl ester, nitrogen deprivation

1. Introduction

Algae are among the top eukaryotic organisms that rapidly undergo changes in response to fluctuations in their environment. Rapid adaptation of algae has fueled numerous studies for induction of algal lipid production as a source of biofuel. At the core of these studies microalgae lie as the main test subjects since they are more efficient at converting solar energy to produce a variety of metabolites than macroalgae and higher plants (Juneja et al., 2013). Several microalgae have been used for biodiesel production with the most common being *Chlorella* species (Safi et al., 2014). *Chlorella* specimens are generally well known for their rapid growth rate, carbon dioxide fixation ability, and high capacity of lipid production (Gors et al., 2010).

Production of biofuel from renewable sources is widely considered the most sustainable alternative to fossil fuels and a viable means for environmental and economic sustainability (Chisti, 2008). Although much research has been dedicated to exploiting the responsive nature of microalgae to produce lipids, mass production of the algae itself is impeded due to this very responsive nature; as the algae are subjected to stress factors (limitation of nutrients, water, carbon dioxide, etc.), they respond by rapidly producing high amounts of essential molecules (proteins, carbohydrates, fatty acids), but at the same time their growth rate is limited (Huang et al., 2010). Hence, the goals of research in algal biotechnology include finding ways to increase the reproductive rate, improve metabolism of inputs, and enhance the production of desired products in useful species (Grima et al., 2003). Productive strains able to produce cells with simultaneously high growth rate and lipid content are required. As Pulz and Gross (2004) highlighted, successful algal biotechnology mainly depends on choosing the right alga with relevant properties for specific culture conditions and products. In the current study, two indigenous *Chlorella* strains were evaluated for potential use in triacylglycerol production.

2. Materials and methods

2.1. Strain and culturing conditions

The strain *Chlorella* sp. IMU12 was isolated from Nemrut Crater Lake (42°15′25″N, 38°31′11″E), the largest volcanic

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lake in Turkey, located at the base of the western part of the caldera of the Nemrut stratovolcano. A few hot steams and gas outlets that have no evident source are found in the lake and a few more active ones are at the base of the caldera. The other species chosen for the study was named as *Chlorella* sp. IMU17. This strain was isolated from Lake Narlıgöl (38°20′50″N, 34°29′28″E), a crater lake with a diameter of 500 m and depth of 50 m, located in the Çiftlik district of Niğde Province, Turkey. The lake is freshwater, mainly fed by rain and weak spring water resources from surrounding lakes. There are also gas outlets and springs at the bottom of the lake. The strains were identified based on morphological characteristics (Borowitzka and Borowitzka, 1988) and genomic information. The strains have been cultured and are maintained in the İstanbul Medeniyet University Microalgae Culture Collection, İstanbul Medeniyet University, Turkey. The specimens were identified using sequence analysis of the 18S rRNA gene. For this aim, the genomic DNA fragment was amplified by PCR, sequenced, and analyzed as described previously (Hoham et al., 2002) . The primers used for amplification of the DNA fragment containing a partial 18S rRNA gene were as follows: forward: 5'-ATTGGAGGGCAAGTCTGGT-3' and reverse: 5'-ACTAAGAACGGCCATGCAC-3'. The same primers were used for Sanger sequencing. Sequence comparisons of the 18S rRNA genes between the *Chlorella* specimens and related species were performed using the NCBI databases with BLASTn search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and the BioEdit graphical biological sequence editor v7.0.9.

For experimentation, the *Chlorella* strains were grown in 200 mL of modified Bold's basal medium (BBM) in 500-mL flasks under continuous light intensity of 150 µmol photons m^{-2} s^{-1} as measured at the external glass wall of the flasks (light source: Philips TL-D 18W/840 recyclable cool daylight) in a temperature-controlled orbital shaker (Sartorius, Certomat BS-T) with 120 rpm speed at 25 °C temperature. Starting cell density was approximately 3×10^4 cells/mL for all groups. For experimentation, cells from the stock cultures were centrifuged at 900 × *g* for 3 min, and the pellets were washed two times using N-free BBM medium. The pellets were then resuspended in N-free BBM medium and the cells were grown under constant light exposure on a rotary shaker with 120 rpm speed and incubated as stated above. Microalgae from at least three biological replicates of each experimental group were cultivated under the defined conditions. Growth responses of the strains were followed during 25 days of incubation by following changes in absorbance value as measured at a wavelength of 720 nm. For experimental analysis, microalgae were harvested on the 1st, 3rd, 5th, 7th, and 10th days of incubation.

2.2. Oxygen evolution

Net oxygen evolution activities of the *Chlorella* strains were measured using a Clark-type oxygen electrode system (Hansatech Oxytherm, Hansatech Ins. Ltd., Norfolk, UK) as described previously (Kaliamurthi et al., 2016). Two milliliters of cell culture with adjusted absorbance value of $A_{680} = 2.0$ with respective solutions was inoculated into the reaction vessels and continuously stirred at 25 °C. Cells were first left in dark conditions for 2 min, and then the oxygen evolution rate was measured under dark and illuminated conditions. Each process was recorded for 5 min. The intensity of the illumination in the vessel was 480 µmol photons m^{-2} s^{-1}. Net oxygen evolution was calculated by subtracting the consumed oxygen rate from the light-saturated rate of oxygen evolution.

2.3. Quantification of total chlorophyll and carotenoids

Chlorophyll and carotenoids were determined using a spectrophotometric method modified from Jeffrey and Humphrey (1975). A 250-mg frozen microalgal pellet was resuspended with 500 mL of 90% acetone, incubated by mixing for 15 min, and centrifuged at 12,000 × *g* for 5 min at room temperature. The supernatant was then loaded in a 96-well plate. The absorbances were collected at 470, 630, 647, 664, and 750 nm and ratios were calculated using the equations given by Jeffrey and Humphrey (1975). Total chlorophyll results were presented as a sum of chlorophyll *a* and *b*.

2.4. Fourier transform infrared spectroscopy (FTIR) measurement of triacylglycerols (TAGs) and polysaccharides

Approximately 50 mg of wet biomass was obtained by means of absorbance calculation ($A_{680} = 4$) and centrifugation at 2000 × *g* for 3 min at 4 °C. Samples were then vacuum-dried at 40 °C for 1 h and the dried algae samples were pelleted and placed on the sampler module. Infrared spectra were recorded over a wavenumber range of 4000 to 400 cm^{-1} with 128 scans on a Fourier transform infrared spectroscope (PerkinElmer-L160000A, USA) equipped with an ATR module. The bands were assigned to specific molecular groups on the basis of biochemical standards and published studies as previously described (Mairet et al., 2011). FTIR peak values attributed to ester group (C=O) vibration of triglycerides (1744 cm^{-1}), C–O stretching frequencies coupled with C–O bending frequencies of the C–OH groups of polysaccharides (1045 cm^{-1}), and amide I absorption (1652 cm^{-1}) were used for calculation of changes in triglyceride and polysaccharide levels. The deviation of the FTIR spectrum level of the amide I band was not more than 14% in any group so the amide I band was taken as a reference for FTIR spectra normalization and ratio determination. Relative TAG and polysaccharide contents were determined by calculating the ratio of the TAG (1744 cm^{-1}) or polysaccharide (1045 cm^{-1}) band to

the amide I (1652 cm^{-1}) band as described before (Dean et al., 2010; Elibol Cakmak et al., 2014). For each time point, ratios of the TAG to amide I or polysaccharide to amide I of control samples were arbitrarily assigned a value of 1; thus, increases in relative TAG or polysaccharide content of N-starved and replete *Chlorella* strains were displayed as "-fold increase" in the ratio of the TAG or polysaccharide to amide I band.

2.5. Estimation of total lipids and neutral lipids

Total lipid levels of microalgae were measured gravimetrically as described by Bligh and Dyer (1959). Spectrofluorometric quantification of neutral lipids was achieved by using the fluorescent Nile Red staining method as described by Elsey et al. (2007). Relative fluorescence intensity of Nile Red staining was quantified on a fluorescence spectrometer (Shimadzu RF-6000, Japan) using 530-nm excitation and 570-nm emission wavelengths.

2.6. Fatty acid methyl ester (FAME) quantitation

FAME quantitation was performed as suggested by Praveenkumar et al. (2012). Extraction buffer (300 µL MeOH containing 2% H_2SO_4, v/v) was added to approximately 20 mg of lyophilized algal sample including 30 µg of nonadecanoic acid (Sigma-Aldrich, USA) as an internal standard. Samples were incubated for 2 h at 80 °C and 750 rpm. Then samples were cooled to room temperature, supplemented with 300 µL of NaCl and 300 µL of hexane, vortexed, centrifuged, and put on a rack at room temperature for phase formation. Then the hexane layer on the upper phase was transferred to a glass insert vial for gas chromatography (Thermo, TRACE 1310) analysis. One microliter of each sample was injected into a FAMEWAX column (Restek, USA) (30 m × 32 mm ID × 25 µm film thickness). The temperature program was set to an initial 120 °C with 10-min hold and 5 °C increment up to 230 °C with 5-min hold. Column flow was adjusted to 22.2 mL/min. The instrument conditions were as follows: carrier gas, nitrogen; FID set to 260 °C; and a split ratio of 10:1. Each sample was analyzed in triplicate, and FAME identification was done by comparison with a standard certificate, Supelco FAME mix C8-C24 (Bellefonte, PA, USA).

2.7. Statistical analysis

Each experiment was repeated twice with three biological replicates. Thus, final data in this article are the mean values of at least three separate samples collected at two different times (n = 6). Means of averages with standard errors are presented throughout the manuscript and data evaluation was done by using t-tests (two tails, pair type) with significance criterion of 0.05 to assess the significance between different groups evaluated for the same time point.

3. Results

3.1. Growth response

Changes in growth of *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17 were recorded during 25 days of nitrogen deprivation (Figures 1a and 1b). Both strains did not show remarkable decrease in growth by day 5 of nitrogen deprivation. Decrease in growth was recorded as 23% and 28% on day 5 and reached its maximum decrease of 46% and 52% on day 25 in nitrogen-deprived *Chlorella* sp. IMU12 (Figure 1a) and *Chlorella* sp. IMU17 (Figure 1b), respectively.

3.2. Changes in chlorophyll and carotenoid content

There was a decrease in total chlorophyll content accompanied by an increase in carotenoid levels as a response to nitrogen deprivation in both *Chlorella*

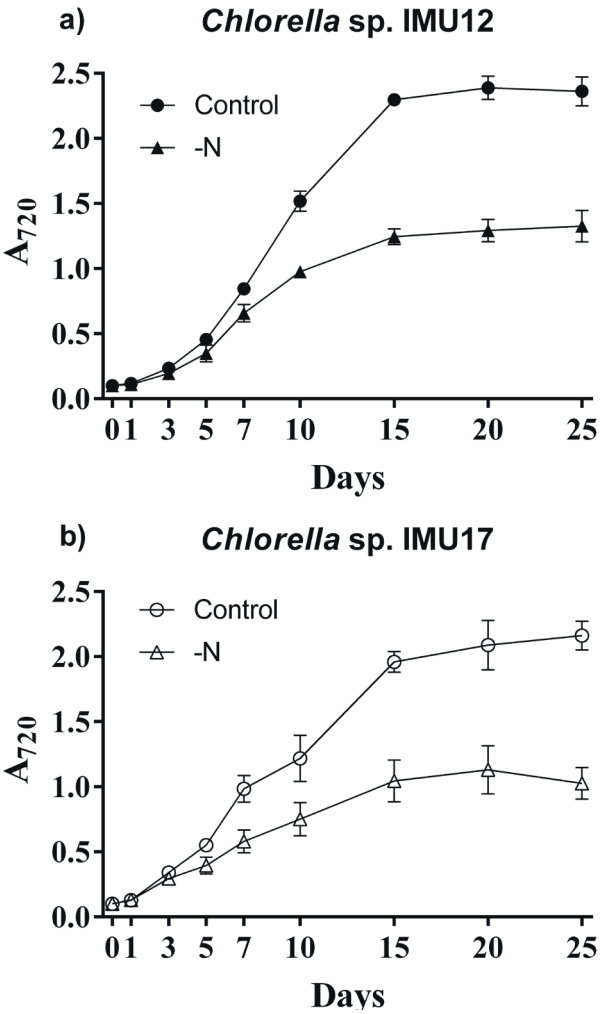

Figure 1. Growth analysis of *Chlorella* sp. IMU12 (a) and *Chlorella* sp. IMU17 (b) incubated in N-free growth medium. For all datasets, each point represents the mean (±SD) of at least six replicate culture flasks.

strains. In *Chlorella* sp. IMU12, total chlorophyll content decreased significantly by 19% by day 5 and ended with a 22% decrease on day 10 of N-deprivation (Figure 2a). Likewise, in *Chlorella* sp. IMU17, total chlorophyll content decreased significantly by 15% by day 3 and ended with a 17% decrease on day 10 of N-deprivation (Figure 2b).

Both *Chlorella* strains expressed increased carotenoid levels as a response to N-deprivation. In *Chlorella* sp. IMU12, carotenoids levels increased significantly by 32% by day 7 and ended with a 22% increase on day 10 of N-deprivation (Figure 2c). In *Chlorella* sp. IMU17, carotenoids levels increased significantly by 17% by day 5 and ended with a 26% increase on day 10 of N-deprivation (Figure 2d). As a result, the Chl/Car ratios of both *Chlorella* strains decreased significantly in response to N-deprivation. Decreased Chl/Car ratios in *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17 were recorded as 29% and 31% by day 5 and 36% and 34% on the 10th day of N-deprivation (Figures 2e and 2f).

3.3. Oxygen evolution

The net oxygen evolution rate of both strains did not significantly change during 3 days of N-deprivation (Figure 3). However, starting from the 5th day, the oxygen evolution rate gradually decreased, ending with 29% and 20% lower ratios in *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17, respectively (Figures 3a and 3b).

3.4. Changes in neutral lipid, TAG, and polysaccharide content

Nile Red staining of neutral lipids showed that there was a 1.26- and 1.12-fold increase on the 3rd day, gradually reaching a 1.88- and 2.28-fold increase in *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17, respectively, at the end of 10 days of N-deprivation (Figure 4a). Following spectrofluorometric detection of neutral lipid content, FTIR measurement was performed to detect changes in TAG and polysaccharide contents of the *Chlorella* strains in response to inorganic nitrogen availability. As suggested by FTIR results, TAG levels of both strains showed a rapid and gradual increase starting from the first day of N-deprivation (Figure 4b). Starting with a 1.21-fold increase in TAG level, N-deprivation of *Chlorella* sp. IMU12 stimulated 2.48-fold TAG production at the end of 10 days of incubation. Likewise, TAG production increased 1.14-fold on the first day and gradually reached 2.71-fold increased TAG levels in N-deprived *Chlorella* sp. IMU17 as observed at the end of the 10th day of incubation. Furthermore, both strains showed aggressive increase in polysaccharide levels on the first 3 days followed by a gradual decrease for the following 7 days of N-deprivation (Figure 4c). Relative polysaccharide level increased 1.6- and 2.25-fold on the 1st and 3rd days, and then a gradual decrease of 1.5-fold was observed at the end of the 10 days in *Chlorella* sp. IMU12. The strain *Chlorella* sp. IMU17 expressed approximately a 1.75-fold increase in relative polysaccharide content on the 1st day and a 2.2-fold increase as the maximum increase on the 5th day of N-deprivation; however, a significant drop of 1.4-fold was recorded after this time point up to the end of the 10 days of incubation.

3.5. Total lipid and FAME analysis

The total lipid content of both strains increased as a response to N-deprivation (Figure 5). The total lipid content of *Chlorella* sp. IMU12 showed a dramatic increase of 53% on the 3rd day up to 83% on the 10th day of N-deprivation (Figure 5a). *Chlorella* sp. IMU17 expressed milder changes under N-deprivation. The total lipid content did not change during the first 3 days. However, starting from the 5th day of N-deprivation, there was a 17% increase, leading up to a 68% increase at the end of the 10 days of N-deprivation (Figure 5b).

The FAME profile of both strains were analyzed from the 5th and 10th days of N-deprivation. There were no significant changes in saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) levels but there was a 11% and 15% decrease in polyunsaturated fatty acid (PUFA) levels in *Chlorella* sp. IMU12 on the 5th and 10th day of N-deprivation, respectively (Figure 5c). The differentiation of FAME content was more remarkable in *Chlorella* sp. IMU17. The SFA and MUFA levels increased, and PUFA levels decreased. Increases in SFA levels were recorded as 10% and 11% and increases in MUFA levels were recorded as 18% and 21%, while 27% and 34% decreased PUFA levels were recorded on the 5th and 10th days of incubation (Figure 5d).

4. Discussion

Among mineral nutrients, nitrogen is required in the largest amount, representing 7%–20% of microalgal dry weight depending on the physiological state of the microalgae (Qiang, 2013). Availability of nitrogen is a major limiting factor for microalgal growth as it is a structural component of amino acids, nucleic acids, and a variety of secondary metabolites. In the present study, nitrogen deprivation caused a significant decrease in the growth of both strains starting from the 5th day of deprivation (Figure 1). Nitrogen-related arrest of microalgal growth has been reported as a common response as it is a major macroelement that necessitates growth in several aspects (Zhu, 2015). Also in the current study, a decrease in growth was accompanied by decreased chlorophyll content (Figures 2a and 2b) and a simultaneous increase in carotenoid levels (Figures 2c and 2d). In order to facilitate efficient carbon utilization under stress conditions, microalgae need to keep chlorophyll and carotenoid levels at a balance. The chlorophyll/carotenoid ratio was around 1.5 in both strains at the onset of N-deprivation. However,

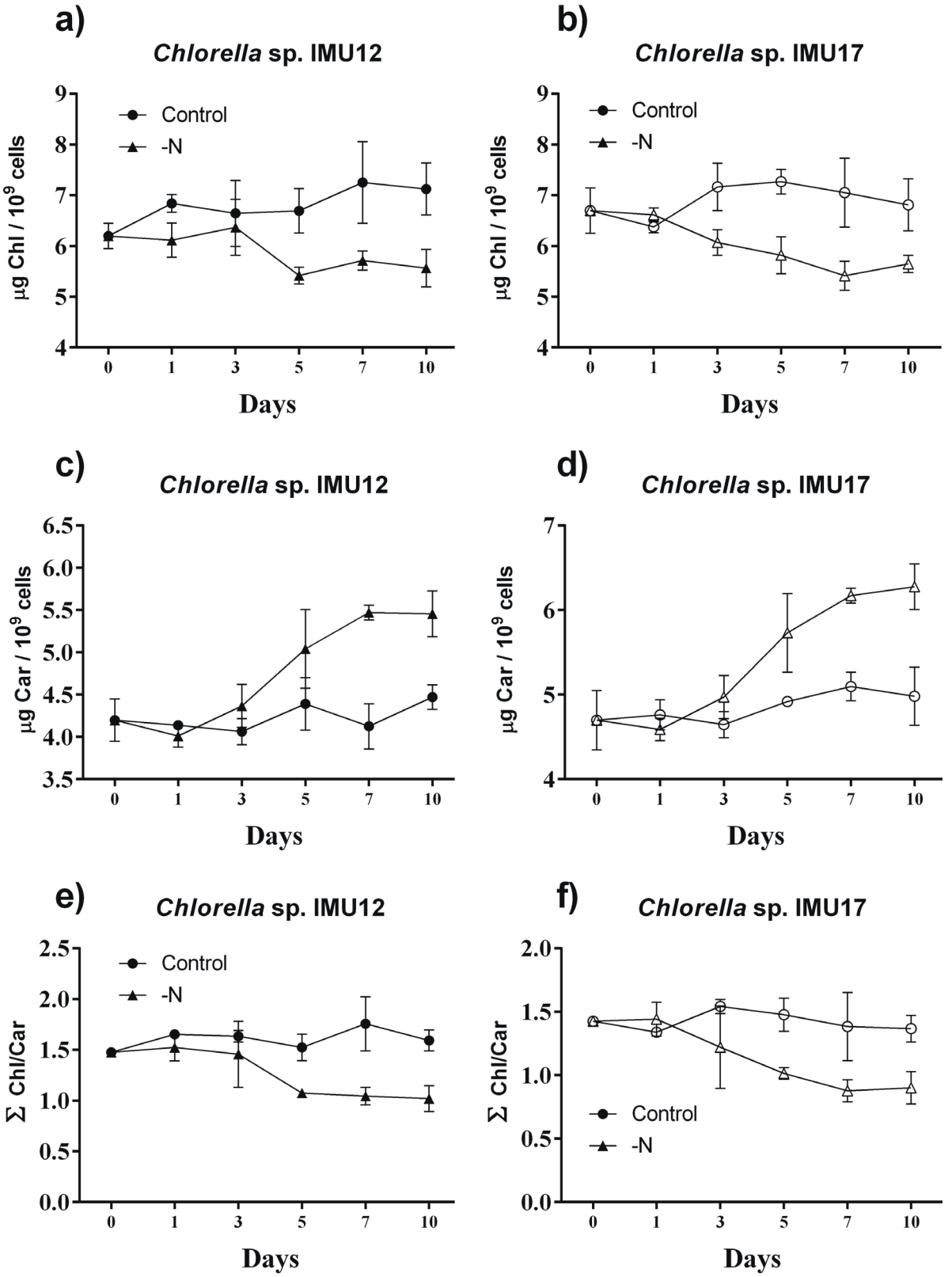

Figure 2. Changes in total chlorophyll, total carotenoid, and Chl a/b ratio of *Chlorella* sp. IMU12 (a, c, e) and *Chlorella* sp. IMU17 (b, d, f) in response to N-deprivation during 10 days of incubation. For all datasets, each point represents the mean (±SD) of at least six replicate culture flasks.

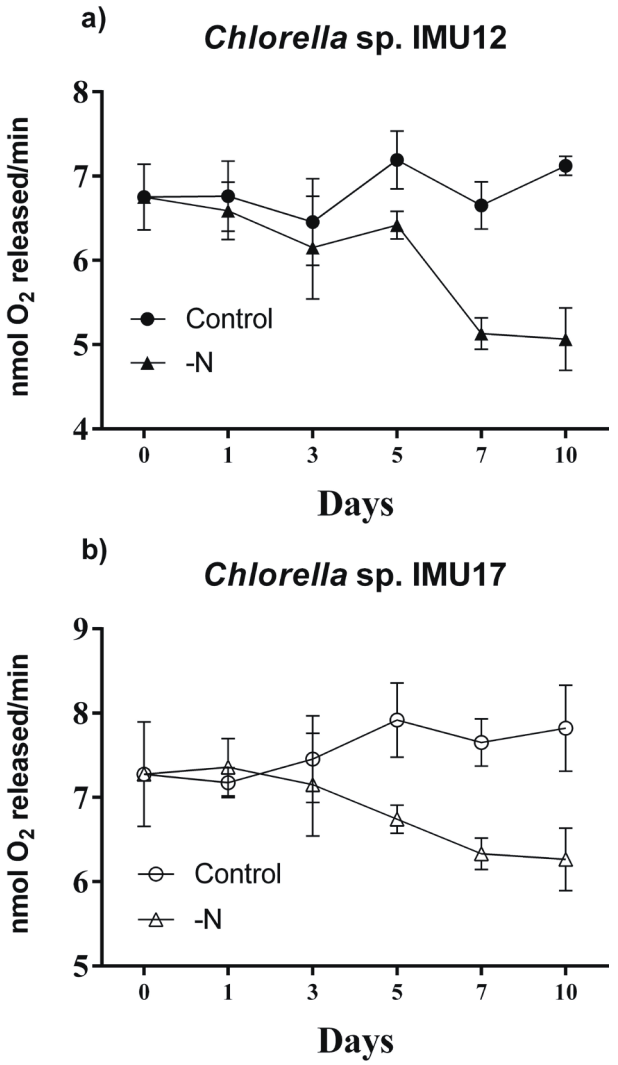

Figure 3. Changes in net oxygen evolution ratio of *Chlorella* sp. IMU12 (a) and *Chlorella* sp. IMU17 (b) in response to N-deprivation during 10 days of incubation. For all datasets, each point represents the mean (±SD) of at least six replicate culture flasks.

5 days of N-deprivation stimulated a 30% decrease in the Chl/Car ratio in both strains (Figures 2e and 2f). The decrease in chlorophyll content is defined as a general response, decelerating overall photosynthetic activity as a response to N-deprivation (; Young and Beardall, 2003; Ikaran et al., 2015). Degradation of chlorophylls might be regarded as a first stage of autophagy, a response to N-deprivation in *Chlorella* strains (Zhang et al., 2018). On the other hand, carotenoid production was reported to increase in N-deprived microalgae in order to protect the photosynthetic device from damage by light (Ledford and Niyogi, 2005).

The oxygen evolution rate of microalgae refers to changes in photosynthetic activity. In the current study, net oxygen evolution rates of both strains showed a gradual decrease after 5 days of N-deprivation (Figure 3). Nitrogen-dependent reduction of photosynthetic activity of microalgae has been examined thoroughly in some valuable studies. Recently, it was reported that N-deprivation of *Chlorella sorokiniana* induced high-energy accumulation via reduction of photosynthetic activity and chlorophyll levels as well as a shift in metabolism leading to the accumulation of oils (Negi et al., 2016). Berges et al. (1996) showed that the impact of N-limitation to photosynthesis in microalgae is mainly related to photosystem II quantum yield. Supportively, Young and Beardall (2003) showed that nitrogen limitation leads to accumulation of inorganic carbon via reduced photosynthetic activity in another green alga, *Dunaliella tertiolecta*.

Microalgal lipids can be categorized as structural and storage lipids. Structural lipids have high contents of PUFA and they play important roles in maintaining specific membrane functions and undertake physiological roles to balance dynamic responses to changing environmental conditions (Sharma et al., 2012). Storage lipids are mainly composed of neutral lipids, which can be easily catabolized to produce metabolic energy. In this study, the fluorescent Nile Red staining of neutral lipids showed that a gradual increase in production of neutral lipids started from the 3rd day of N-deprivation in both strains (Figure 4a). Neutral lipids generally present in the form of TAGs are composed mainly of SFAs and MUFAs, which can be trans-esterified to produce biodiesel. The FTIR results suggest that a gradual increase in TAG production in both strains might be stimulated even from the first day of N-deprivation (Figure 4b). The strain *Chlorella* sp. IMU17 showed higher performance to produce TAGs as a response to N-deprivation. Biochemical synthesis of TAG is induced in algae when energy consumption is lower than the energy input. Ikaran et al. (2015) reported that reduction of nitrogen-rich molecules was accompanied by increased carbohydrate contents for the first days and a longer period of N-deprivation favored TAG production in *Chlorella vulgaris*. Our results showed that both strains expressed more polysaccharide production on the first days followed by a linear decrease while TAG production showed a gradual increase up to the end of the 10 days of N-deprivation (Figure 4c). These results may suggest that surplus energy might be primarily stored as carbohydrates on the first days of N-deprivation and longer starvation favors TAGs production via degradation of polysaccharides in the *Chlorella* strains examined in this study. Likewise, Li et al. (2015) stated that lipid production is largely dependent on starch degradation in *Chlorella sorokiniana*.

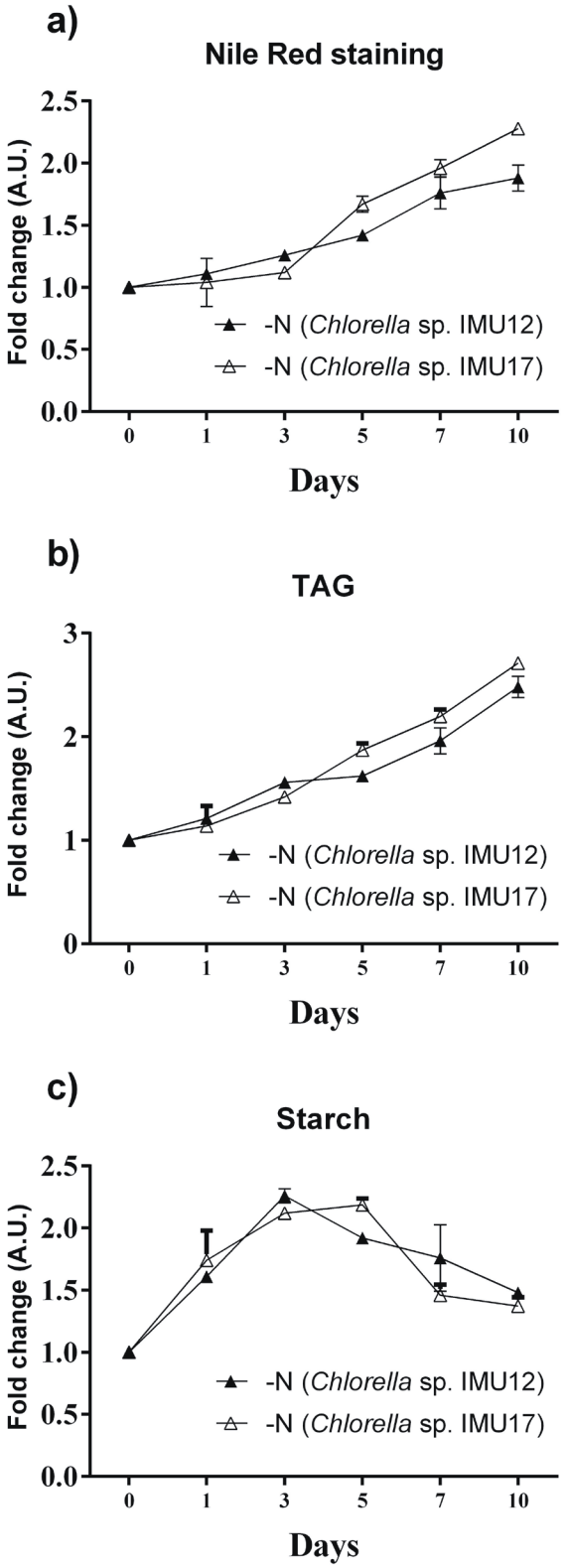

Figure 4. Changes in neutral lipid content (a), TAG:amide I ratio (b), and polysaccharide:amide I ratio (c) of *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17. For all datasets, each point represents the mean (±SD) of at least six replicate culture flasks.

Reduced growth rate refers to production of storage metabolites in the form of carbohydrates and lipids in several microalgae species (Spolaore et al., 2006; Sharma et al., 2012). This study shows that total lipid content of microalgae tends to increase along with a simultaneous decrease in the growth. At the start of the experiment, the total lipid contents of *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17 were recorded as 23.1% and 26.3% based on dry weight. Nitrogen deprivation stimulated remarkable increases in total lipid content in both strains. The total lipid content of *Chlorella* sp. IMU12 and *Chlorella* sp. IMU17 were recorded as 44.6% and 47.6% at the end of 10 days of N-deprivation (Figures 5a and 5b). Depending on algal strains, lipid content of the cells can reach up to 60% of total dry biomass under favorable conditions (Siegler et al., 2012). Illman et al. (2000) reported significant increases in total lipid content of *Chlorella vulgaris* (from 18% to 40%), *C. emersonii* (from 29% to 63%), *C. sorokiniana* (from 20% to 22%), and *C. minutissima* (from 31% to 57%) as a response to N-limitation. Supportively, Shuba and Kifle (2018) reported that *Chlorella* strains show great diversity in their total lipid content, ranging from 2% to 56%, which was attributed to *C. pyrenoidosa* (2%), *C. sorokiniana* (23%), *C. emersonii* (29%), *C. minutissima* (31%), *C. protothecoides* (55%), and *C. vulgaris* (14-56%). Thereby, the abundance and induction of total lipids of the strains used in this study might represent them as considerable candidates for microalgal lipid production.

FAME analysis of microalgal lipids showed that *Chlorella* sp. IMU12 did not show remarkable variation in response to N-deprivation. There was a slight decrease of PUFA levels as observed by FAME analysis (Figure 5c). On the other hand, the FAME profile of *Chlorella* sp. IMU17 showed dramatic changes in response to N-deprivation. The SFA and MUFA contents showed significant increases while 27% and 34% decreases of PUFA content were recorded on the 5th and 10th days of N-deprivation, respectively (Figure 5d). Notably, *Chlorella* sp. IMU12 showed rapid time-based increase of total lipids while N-deprivation induced substantial lipid remodeling along with stress induced growth impairment in *Chlorella* sp. IMU17. SFAs and MUFAs are the most abundant fatty acids in microalgae with palmitic acid (C16:0) and oleic acid (C18:1) as the major fatty acids (Kumar et al., 2016). Moreover, algae are able to synthesize significant amounts of different PUFAs to cope with biotic and abiotic stressors (Cagliari et al., 2011). However, we noted a significant decrease of PUFA content in response to N-deprivation in *Chlorella* sp. IMU17. The PUFA content was around 29% and N-deprivation resulted in decreased PUFA content to 22% on the 5th day and to 18% on the 10th day of incubation (Figure 5d). Supportively, Guarnieri et al. (2011) reported that N-deprivation caused a significant decrease of PUFA

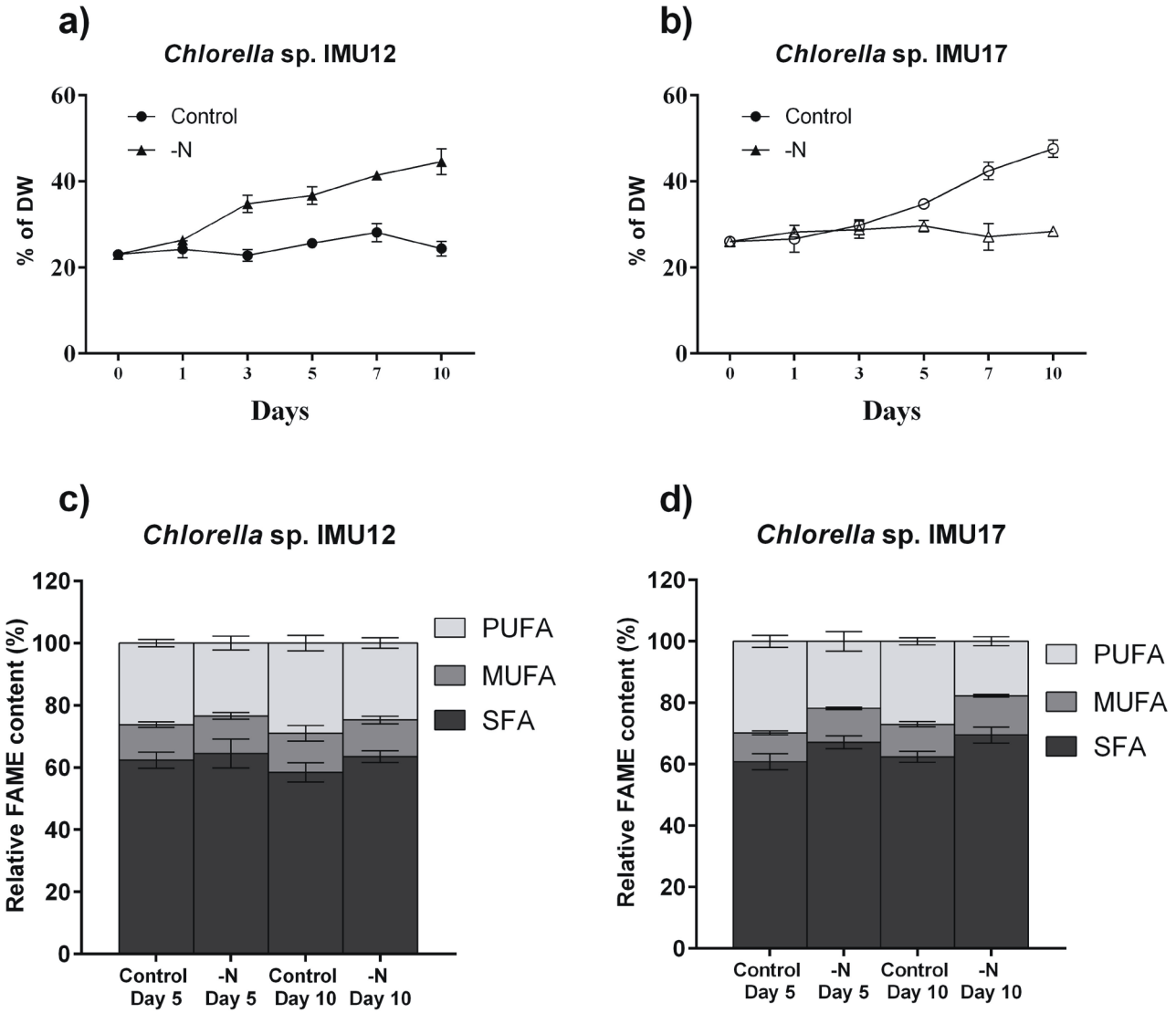

Figure 5. Changes in total lipid content and FAME profile of *Chlorella* sp. IMU12 (a, c) and *Chlorella* sp. IMU17 (b, d). For all datasets, each point represents the mean (±SD) of at least six replicate culture flasks.

as accompanied by increased MUFA content in *Chlorella vulgaris*. Biodiesel properties such as lubricity, viscosity, density, NOx emissions, cetane number, and oxidative stability are basically dependent on FAME profile of lipids (Francisco et al., 2010). PUFAs are prone to oxidation, which results in increased NOx emission of the biodiesel, while SFAs, and to a certain extent MUFAs, lower the cetane number, increasing the stability of the produced biodiesel (Saraf and Thomas, 2007). Thus, overall, the strain *Chlorella* sp. IMU17 represented a higher increase of TAGs and a more dynamic regulation of FAMEs with increased saturation of fatty acids.

To conclude, two indigenous *Chlorella* strains isolated from two different volcanic lakes expressed considerable increases in TAG production as a response to N-deprivation. A time-dependent degradation of starch might contribute effectively to the gradual increase of TAG accumulation in both strains. With their lower PUFA contents, both strains might be employed for studies with large-scale cultivation for biodiesel feedstock production. Ultimately, *Chlorella* sp. IMU17 might stand as a promising candidate for biodiesel feedstock production with its lower PUFA content and a concomitant induction of saturation of fatty acids as a clear response to N-deprivation.

Acknowledgment
This study was supported by grants from the Scientific and Technological Research Council of Turkey (TÜBİTAK, Project # 112Y029) and the Research Fund of İstanbul Medeniyet University (Project # FBA-2012-185).

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