Research Article

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Screening of fatty acid composition in *Nitzschia* sp.

*Nitzschia* sp. türünde yağ asidi kompozisyonunun taranması

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Abstract

**Introduction:** The main target of this study was to compare the effects of nitrogen limitation and light intensities on cell growth, lipid content and fatty acid profile of *Nitzschia* sp.

**Methods:** F/2 medium and N-free F/2 medium were both tested at two different light intensities of 11 and 56 μE m$^{-2}$ s$^{-1}$ in the internal loop air-lift photobioreactor for *Nitzschia* sp.

**Results:** The presence of nitrogen led to more cells grown efficiently. Furthermore, the increase in chlorophyll content went parallel to the increase in dry weight. The most abundant saturated and monounsaturated fatty acids were pentadecanoic acid (C15:0) and palmitoleic acid (C16:1) which constituted 17%–42% and 15%–48% of total fatty acids for all growth conditions, respectively. It was recorded that palmitoleic acid was present at higher concentrations than palmitic acid.

**Discussion and conclusion:** The noteworthy finding was that the lipid content increased with increasing the growth rate of *Nitzschia* sp. under phototrophic conditions.

**Keywords:** Airlift bioreactors; Fatty acid; Lipid; *Nitzschia* sp.; Microalgae.

Özet

**Giriş ve amaç:** Bu çalışmadaki ana hedef *Nitzschia* sp. türünün büyüme hızı, lipit içeriği ve yağ asidi profilini üzerine nitrojen kısıtlamasının ve ışık şiddetinin etkilerinin karşılaştırılmasıdır.

**Yöntem ve gereçler:** Hava kaldırılmış fotobiyoreaktörde *Nitzschia* sp. türü için F/2 ve azotsuz F/2 kültür ortamları iki farklı ışık şiddetinde (11 ve 56 μE m$^{-2}$ s$^{-1}$) test edilmiştir.

**Bulgular:** Nitrojen varlığı daha fazla hücrenin üremesine sebep olmuştur. Ayrıca, klorofil içeriğindeki artış kuru ağırlıktan paralel gitmiştir. Doymuş yağ asitlerinden pentadekanoik asit (C15:0, 17%–42%) ve palmitoleik asit (C16:1, 15%–48%) en yüksek miktarlarda bulunmuştur. Palmitoleik asidin palmitik asitden daha yüksek konsantrasyonlarda bulunduğu kaydedilmiştir.

**Tartışma ve sonuç:** Fototrofik koşullarda altında *Nitzschia* sp. türünün büyüme hızı arttıkça lipit içeriği artmıştır.

**Anahtar Kelimeler:** Hava kaldırılmış Biyoreaktör; Yağ asidi; Lipit; Mikroalg; *Nitzschia* sp.

Introduction

Microalgae have a rapid growth rate, strong adaptive capacity to the surrounding environment, and high lipid content. Lipid accumulation in microalgae is indeed well known as stated by many examples of their uses in aquaculture for fish or mollusks feeding [1, 2]. Chisti [3] showed that microalgae-derived biodiesel is a potentially important replacement for petroleum fuels using mathematical modeling and engineering calculations. Finding microalgae species with the advantage of fast growth rate and high lipid content is the primary key to providing a solution to this problem [4]. Furthermore, screening and nurturing microalgae with high productivity, high lipid content and strong stress resistance are very important for its industrialization [5].

Pennate diatoms are one of the major groups of microalgae, which hold great promise as source of valuable long
chain polyunsaturated fatty acids (LC PUFAs) or highly unsaturated fatty acids (HUFAs), the main species of which are eicosapentaenoic acid (EPA), arachidonic acid (ARA), and docosapentaenoic acid (DHA) [6]. *Nitzschia* is a common pennate marine diatom and one of the most difficult genera for species identification. One reason for these difficulties is that no comprehensive monograph of the genus has been published during the 20th century [7]. Some experimental work has been carried out to study the effects of environmental conditions on the valve morphology and growth of *Nitzschia* species [8, 9]. Furthermore, researches on how to optimize growth and improve lipid content of *Nitzschia* sp. to enhance lipid productivity are still lacking [4].

Nitrogen limitation and light intensity are well known to trigger high amount of lipid accumulation. The main target of this study was to compare the effects of environmental conditions on the valve morphology and growth of *Nitzschia* species [8, 9]. Furthermore, researches on how to optimize growth and improve lipid content of *Nitzschia* sp. to enhance lipid productivity are still lacking [4].

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**Materials and methods**

**Microalgae and production conditions**

*Nitzschia* sp. EgeMacc-049 was obtained from Ege University Microalgae Culture Collection, Izmir, Turkey. The culture was monoalgal and cultivated in F/2 medium [10]. A 2 L plexiglass internal loop air-lift PBR was equipped with an on-line controller (Biosis, Pikolab, Turkey), consisting of a combined temperature-dissolved oxygen probe and pH probe. The pH was maintained at 8.0 by the automatic addition of 1 N HCl. The temperature was kept constant at 20±0.5°C in the temperature-controlled incubator. Air was supplied to the culture by air pump continuously and air flow rate was adjusted to 2 L min⁻¹ (1.1 vvm) with flow meter (RST Electronic Ltd. Sti, LZM-6T, Turkey). Illumination was provided by LED downlight lamps (Cata 10 W CT-5254) from the top of the PBR with a 16:8 h light:dark photoperiod. Light intensity was measured by a quantum meter (Lambda L1-185) on the surface of the PBR.

**Analytical procedures**

The cell concentration was determined by counting triplicate samples in a Neubauer hemocytometer. Dry weight was determined by filtering a 5-mL culture sample through pre-weighed GF/C filter (Whatman, UK) and drying at 105°C for 2 h. Chlorophyll in the cells was extracted with 100% (v/v) methanol as reported by Imamoglu et al. [11]. Lipid was extracted from lyophilized diatom biomass according to the method described by Isleten-Hosoglu et al. [12]. Fatty acids were analyzed by gas chromatography equipped with a flame ionization detector (Agilent 6890 GC-FID, US) using Turkish standard methods: TS EN ISO 12966-2:2011 and TS EN ISO 15304.

The specific growth rate (μ) and doubling time (DT) of the cells were calculated as reported by Tebiani et al. [13]. The data were analyzed using one-way analysis of variance (ANOVA).

**Results and discussion**

**Cultivation of *Nitzschia* sp.**

Investigating the effects of environmental parameters on the growth of *Nitzschia* sp. is a primary barrier for reaching fast growth rate. As shown in Figure 1, an increasing trend was observed throughout the cultivation in F/2 medium, it can be seen that a peak value of 19±0.82×10⁴ cells/mL is reached on day 14 while the obtained minimum cell concentration was only 1.75±0.60×10⁴ cells/mL in N-free F/2 medium under the light intensity of 56 μE m⁻² s⁻¹. Additionally, the cell concentration decreased by 47.7% in
N-free F/2 medium compared with F/2 medium under the light intensity of 11 μE m⁻² s⁻¹. This is the main outcome of the presence of nitrogen which leads to more cells grown efficiently.

As shown in Figure 2, there were significant differences on the chlorophyll-a contents beginning of the day 4, the maximum chlorophyll-a content of 3.56 ± 0.18 mg/L was found in F/2 medium under the light intensity of 56 μE m⁻² s⁻¹ for Nitzschia sp. On the other hand, chlorophyll-a contents were close to each other in both N-free F/2 media between the days of 1 and 8. It is also important to underline that the decrease in chlorophyll-a content leads to lower photosynthesis efficiency or vice versa, and thereby the inhibition of microalgal growth occurred. Considering these results, the increase in chlorophyll content went parallel to the increase in dry weight (Table 1).

The maximum specific growth rate of 0.26 day⁻¹, which corresponded to the doubling time of 2.68 days, was obtained found in F/2 medium under the light intensity of 56 μE m⁻² s⁻¹ for Nitzschia sp. (Table 1). As reported Jiang et al. [14], in the summer condition, the specific growth rates of Nitzschia sp. increased from 0.14 ± 0.04 to 0.25 ± 0.04 day⁻¹ in GP medium, while the specific growth rate in the winter varied from 0.06 ± 0.001 day⁻¹ to 0.12 ± 0.02 day⁻¹, but significantly lower than those in summer and spring/fall conditions. Smayda [15] reported that a combination of temperature, salinity, and light played an important role in the cell division of diatoms. Moreover, the estimation of the optimal growth rate in different environmental conditions is very important for mass culture of benthic diatoms [16].

**Lipid content and fatty acid profile of Nitzschia sp.**

Over the past few decades, thousands of algae and cyanobacterial species have been screened for high lipid production, and numerous oleaginous species have been isolated and characterized [17]. Quantity, quality and productivity of lipid are obviously of primary relevance. They depend not only on the strains, but also on culture conditions; for example, it is well known that nitrate starvation can trigger lipid accumulation, especially triacylglycerols (TAGs) suitable for biodiesel production [3, 18]. The proposed optimal ratio of fatty acids for biodiesel is: 5 : 4 : 1 of C16:1 : C18:1 : C14:0 [14, 19]. Currently, the commercialization of algae-derived biodiesel is still in its infancy stage [17].

In this study, the total lipid contents ranged from 10% to 32% of dry biomass weight of Nitzschia sp. for all growth conditions. Maximum lipid productivity of 5.76 mg/L/day, which corresponded to the maximum biomass production of 0.236 g/L, was obtained in F/2 medium under the light intensity of 56 μE m⁻² s⁻¹ for Nitzschia sp. Lipid content (32.004%) in F/2 medium was increased by about 1.2 times as compared to the lipid content (27.389%) in N-free F/2 medium under the light intensity of 56 μE m⁻² s⁻¹ (Table 1).

Weldy and Huesemann [20] argued that increasing the biomass yield was the most effective way to improve the lipid productivity of green algae Dunaliella salina [4]. It is worthy to note that these data are obtained from algal species under specific conditions and vary greatly when algal cells are exposed to different environmental or nutritional conditions such as temperature, pH, light intensity, or nitrogen concentration [21, 22].

**Table 1: Results of obtaining kinetic parameters of Nitzschia sp. production in air-lift photobioreactor.**

|                     | Chlorophyll-a (mg/L) | Dry weight (g/L) | Biomass productivity (g/L/day) | Specific growth rate (μL, day⁻¹) | Doubling time (DT, day) | Lipid content (% w/w) | Lipid productivity (mg/L/day) |
|---------------------|----------------------|------------------|-------------------------------|---------------------------------|-------------------------|-----------------------|-----------------------------|
| F/2 medium, 56 μE m⁻² s⁻¹ | 3.558 ± 0.18         | 0.236 ± 0.02     | 0.018                         | 0.258                           | 2.682                   | 32.004                | 5.76                        |
| N-free F/2 medium, 56 μE m⁻² s⁻¹ | 0.825 ± 0.06         | 0.158 ± 0.01     | 0.012                         | 0.149                           | 4.654                   | 27.389                | 3.29                        |
| F/2 medium, 11 μE m⁻² s⁻¹ | 2.544 ± 0.13         | 0.191 ± 0.02     | 0.015                         | 0.174                           | 3.978                   | 26.959                | 4.04                        |
| N-free F/2 medium, 11μE m⁻² s⁻¹ | 0.473 ± 0.05         | 0.123 ± 0.03     | 0.010                         | 0.102                           | 6.781                   | 10.789                | 1.08                        |
Different microalgae species react to different stresses by producing different fatty acids or by altering their composition of fatty acids [23]. As seen in Table 2, the most abundant saturated and monounsaturated fatty acids were pentadecanoic acid (C15:0) and palmitoleic acid (C16:1) which constituted 17%–42% and 15%–48% of total fatty acids for all growth conditions, respectively. Increased level of C16:0 and decreased level of C15:0 were observed in response to nitrogen deficiency. It was recorded that palmitoleic acid was always present at higher concentrations than palmitic acid. This result is consistent with other study that found by Jiang et al. [14].

PUFAs such as eicosapentaenoic acid (EPA; C20:5n-3) and docasahexaenoic acid (DHA; C22:6n-3) are essential for invertebrates (e.g. shrimp, oysters) and are valuable nutraceuticals [14, 24]. EPA has potential as an antibacterial agent and has been recommended for topical application on human infections [25]. It is also antibacterial for aquaculture pathogens [6, 26]. In this study, PUFAs were essentially absent for Nitzschia sp. except for eicosapentaenoic acid (C20:5n3), representing EPA, which constituted 2.5%–7.5% of total fatty acids for all growth conditions.

### Table 2: Fatty acid profile of Nitzschia sp.

| Fatty acid methyl esters (FAMEs) | Symbol | F/2 medium, 56 μE m⁻² s⁻¹ | N-free F/2 medium, 56 μE m⁻² s⁻¹ | F/2 medium, 11 μE m⁻² s⁻¹ | N-free F/2 medium, 11 μE m⁻² s⁻¹ |
|---------------------------------|--------|-----------------------------|---------------------------------|-----------------------------|---------------------------------|
| Lauric acid methyl ester        | C12:0  | ND                          | ND                              | 0.908                       | 0.042                           |
| Myristic acid methyl ester      | C14:0  | ND                          | ND                              | 25.440                      | 0.170                           |
| Myristoleic acid methyl ester   | C14:1  | ND                          | ND                              | 8.716                       | 0.548                           |
| Pentadecanoic acid methyl ester | C15:0  | 20.718                      | 17.732                          | 41.673                      | 20.102                          |
| cis-10-Pentadecenoic acid methyl ester | C15:1 | 0.432                      | 0.496                          | 0.231                       | 0.312                           |
| Palmitic acid methyl ester      | C16:0  | 17.207                      | 25.440                          | 8.716                       | 29.093                          |
| Palmitoleic acid methyl ester   | C16:1  | 48.985                      | 44.776                          | 15.338                      | 35.880                          |
| Heptadecanoic acid methyl ester | C17:0  | ND                          | ND                              | 2.026                       | 0.295                           |
| Stearic acid methyl ester       | C18:0  | 2.275                       | 1.048                          | 5.774                       | 2.736                           |
| Oleic acid methyl ester         | C18:1n9c | 2.669                    | 0.791                          | 10.379                      | 3.442                           |
| Linoleic acid methyl ester      | C18:2n6c | 1.138                    | 1.772                          | 1.496                       | 1.383                           |
| Trans-Linolenic acid methyl ester | C18:2n6l | 1.564                  | 2.778                          | 0.984                       | 1.402                           |
| Arachidic acid methyl ester     | C20:0  | 0.070                       | ND                              | ND                          | ND                              |
| cis-11,14-Eicosadienoic acid methyl ester | C20:2 | 0.053                  | 0.041                          | ND                          | 0.062                           |
| cis-8,11,14-Eicosatrienoic acid methyl ester | C20:3n6 | 0.083          | 0.247                          | ND                          | 0.259                           |
| cis-5,8,11,14,17-Eicosapentaenoic acid methyl ester | C20:5n3 | 3.454                  | 3.652                          | 7.578                       | 2.487                           |
| Behenic acid methyl ester       | C22:0  | ND                          | ND                              | ND                          | 0.173                           |
| cis-13,16-Docosadienoic acid methyl ester | C22:2 | ND                          | ND                              | 0.579                       | 0.142                           |
| cis-4,7,10,13,16,19-Docosahexaenoic acid methyl ester | C22:6n3 | 0.754                  | 0.425                          | 2.199                       | 0.473                           |
| Tricosanoic acid methyl ester   | C23:0  | 0.340                       | 0.657                          | 0.712                       | 1.019                           |
| Lignoceric acid methyl ester    | C24:0  | 0.259                       | 0.146                          | 0.859                       | 0.357                           |

ND, Not detected.

**Conclusion**

The present study demonstrates the fatty acid profile and the growth of Nitzschia sp. under specific conditions. The maximum specific growth rate of 0.26 day⁻¹, which corresponded to the doubling time of 2.68 day, was obtained in F/2 medium under the light intensity of 56 μE m⁻² s⁻¹ for Nitzschia sp. The correlation between biomass amount and lipid content is significant. Maximum lipid productivity of 5.76 mg/L/day, which corresponded to the maximum biomass production of 0.236 g/L, was obtained in F/2 medium under the light intensity of 56 μE m⁻² s⁻¹ for Nitzschia sp. The noteworthy finding was that the lipid content increased with increasing the growth rate of Nitzschia sp. under phototrophic conditions. For Nitzschia sp., the most abundant saturated and monounsaturated fatty acids were pentadecanoic acid (C15:0) and palmitoleic acid (C16:1) which constituted 17%–42% and 15%–48% of total fatty acids for all growth conditions, respectively.

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