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# Effect of Nitrogen Source on Biomass and Lipid Production of a Marine Microalga, *Nannochloropsis oceanica* IMET1

**Yongxue Chi[1*], Feng Chen[2], Yasuyuki Takiguchi[1]**

[1]Department of Life and Environmental Sciences, Graduate School of Engineering, Chiba Institute of Technology, Chiba, Japan
[2]Institute of Marine and Environmental Technology, University of Maryland Center for Environmental Science, Baltimore, MD, USA
Email: [*]yongxue101@hotmail.com

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## Abstract

**The effects of the nitrogen sources sodium nitrate ($NaNO_3$) and urea ($CH_4N_2O$) on growth, lipid production, and fatty acid composition of *Nannochloropsis oceanica* IMET1 were investigated. Nitrogen source affected cell density, dry cell weight, and lipid production. Cells grown in the nitrate medium increased dry cell weight and lipid weight in comparison with cells grown in the urea medium. The composition of fatty acids varied with nitrogen sources. IMET1cultured in the nitrate medium mainly contained C18:2 (14.9%) and C16:0 (6.3%) fatty acids, while IMET1 in the urea medium mainly contained C22:0 (33.1%), C18:3 (8.6%), and C16:0 (6.8%). This study demonstrates that nitrogen source can strongly influence lipid production and composition of *N. oceanica* IMET1.**

## Keywords

***Nannochloropsis oceanica*, Nitrogen Source, Lipid Production, Growth Performance, Fatty Acid Composition**

## 1. Introduction

Microalgae are known as a next-generation energy source because of its high biomass productivity, high lipid productivity, and carbon neutrality. Among oil-producing microalgae, several species in the genus *Nannochlo-*

[*]Corresponding author.

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*ropsis* are excellent candidates for producing biofuel because of their high biomass and lipid productivity and wide environmental tolerance capacity [1]-[5].

*Nannochloropsis oceanica* MET1 is an algal strain maintained at the Institute of Marine and Environmental Technology (IMET). This strain (IMET1) produces 69% lipid (dry weight) in a 23-day batch culture [6]. It can be cultivated under a wide range of environmental conditions [1]-[8]. Recent studies have focused on increasing lipid yield by altering the cultivation conditions, including nitrogen concentration, salinity, light intensity, and temperature. The concentration of nitrogen in the culture can affect the lipid yield and composition of IMET1 [1]. Although IMET1 is considered to be a promising alga for biodiesel production, little is known about the effect of different nitrogen sources for biomass and lipid production [9]-[14]. An understanding of the effect of nitrogen sources on growth and the lipid content will help improve lipid productivity and aid large-scale commercial producers in selecting a suitable fertilizer.

In this study, we investigated the effect of two different nitrogen sources, sodium nitrate ($NaNO_3$) and urea ($CH_4N_2O$), on the growth, lipid yield, and fatty acid composition of *N. oceanica* IMET1.

## 2. Materials and Methods

### 2.1. Microalgae and Culture Conditions

*N. oceanica* IMET1 was cultured in BG11 medium at pH 7.4. Cultures were incubated at 30˚C in Erlenmeyer flasks illuminated continuously at 100 μmol $m^{-2}\cdot s^{-1}$ with white fluorescent lamps. $CO_2$ (2% v/v) composed by blending pure $CO_2$ and air using a gas mixture device with two gas flow meters was supplied. Two nitrogen sources, sodium nitrate ($NaNO_3$) and urea ($CH_4N_2O$), were tested. For investigating the effect of sodium nitrate, BG11 medium was used. For investigating the effect of urea, urea replaced the sodium nitrate in BG11 medium. The initial cell density was adjusted to 0.4 by absorbance at 600 nm ($OD_{600}$). Cultures were monitored over a period of 12 days.

### 2.2. Growth

Cell concentrations were determined by hemocytometer cell count. Dry cell weight was measured using the following procedure: A 50-mL sample was taken from the Erlenmeyer flask and placed in a centrifuge tube. The cells were separated by a centrifugal separator at 10,000 rpm for 15 min. The supernatant was removed and the precipitate was washed with deionized water and centrifuged at 10,000 rpm for 15 min. This process was repeated twice. The cells were frozen at −20˚C and lyophilized using vacuum freeze-drying equipment for 24 h. Dry cell weight was measured using an electric balance.

### 2.3. Lipid Production

The Blight–Dyer method [15] was used for the extraction of lipid products from the dried cells. The remained layer was mixed with pure hexane and neutral lipids were extracted. The hexane extraction process was repeated three times. The cell extracts were combined in a recovery flask and evaporated under vacuum. The weight of the remaining lipid was expressed as a proportion of the dry cell weight.

The lipid products were separated by TLC to extract only TAG. The TLC spot of TAG was scraped off the plate with a spatula and placed in a glass tube. To methylate fatty acid products, a fatty acid methylation kit (product number 06482-04) produced by Nacalai Tesque Inc. was used. Methylated fatty acids were analyzed using a gas chromatograph with flame ionization detector (GC-14B, Shimadzu Co.). UD-wax was used as the separation column. Analysis conditions were as follows: the oven temperature was initially set at 100˚C, rising to 240˚C at 15˚C/min, and being held for 10 min. The split ratio was 1:50. A 1-μL sample was injected. Lipid yield was expressed as the peak area ratio of each fatty acid.

## 3. Results and Discussion

### 3.1. Growth

**Figure 1** shows the growth performance of IMET1 incubated with nitrate and urea media, respectively. IMET1 grew similarly in nitrate or urea medium from day 4 to day 10. Interestingly, IMET1 in the urea medium reached higher cell density than IMET1 in the nitrate medium from day 10 to day 12.

The growth based on dry weight between the urea and nitrate media are compared in **Figure 2**. Dry cell weight also increased with culture time in the presence of both nitrogen sources. Although dry cell weight was almost the same irrespective of the nitrogen source until the 6$^{th}$ day, the dry cell weight of IMET1 in the nitrate medium was greater than that in the urea medium after day 10.

Higher cell count but lower dry weight for IMET1 grown in the urea medium is consistent with the finding based on the effect of nitrogen sources on *N. salina* strain 1776 reported by Campos *et al*. [8]. They also reported that urea led to more cells than nitrate, and nitrate-grown cells had larger diameters than urea-grown cells. Li *et al.* reported the effect of nitrogen sources on *Neochloris oleoabundans* UTEX 1185. They found that the dry cell weight with nitrate was greater than that with urea. Moreover, Goksan [11] also reported that the dry cell weight of *Haematococcus pluvialis* cultivated with nitrate was greater than that with urea.

Thus, it appears that microalgae grown in the nitrate medium tend to have a larger size or higher dry weight compared to those in the urea medium.

## 3.2. Lipid Products

The lipid content of IMET1 obtained after 12 days of incubation with nitrate or urea is shown in **Figure 3**. The lipid content of IMET1 with nitrate was about two times higher than that with urea. Higher lipid content in nitrate-grown algae than urea-grown algae has been reported by Campos *et al*. [8]. The chromatograms of fatty acid components of IMET1 grown under different nitrogen conditions are shown in **Figure 4**. When nitrate was

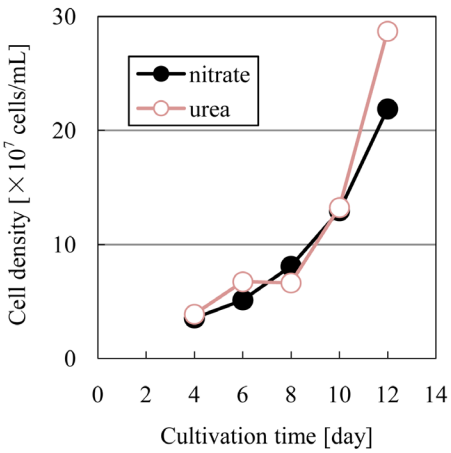


**Figure 1.** The growth of IMET1 measured by cell density under two different nitrogen sources.

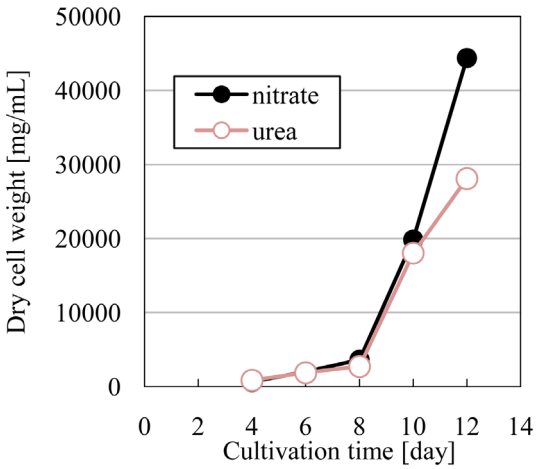


**Figure 2.** The growth of IMET1 measured by dry weight under two different N sources.

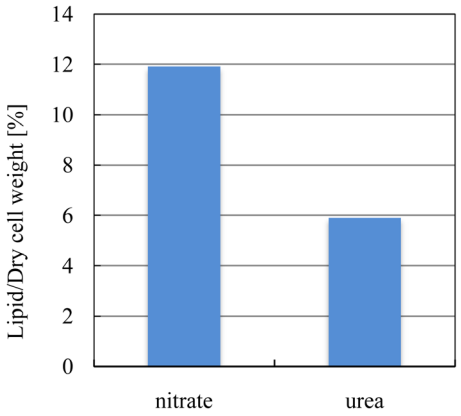


**Figure 3.** Lipid content of IMET1 after 12 days of incubation with different nitrogen sources.

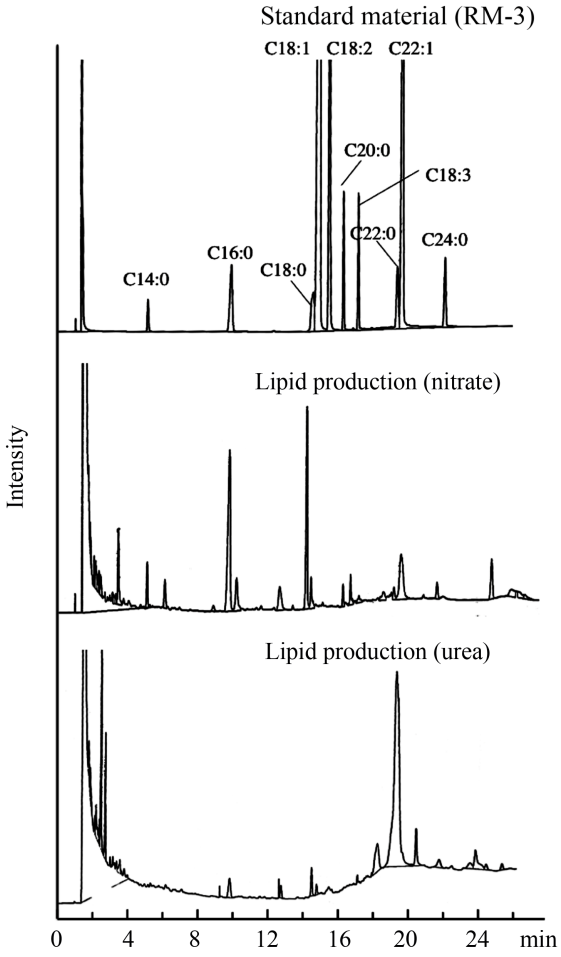


**Figure 4.** Chromatogram of fatty acid composition of IMET grown under different N sources at day 12.

used, C14:0, C16:0, C18:0, C18:1, C18:2, C20:0, C22:0, C22:1, and C24:0 were detected. When urea was used, C14:0, C16:0, C18:0, C18:1, C18:2, C18:3, and C22:0 were detected. Other small peaks were detected using both nitrogen sources. From the area of the main peak, the fatty acid component ratio was calculated (**Figure 5**). The relative abundance of main fatty acid products are different between IMET1 grown under nitrate and urea

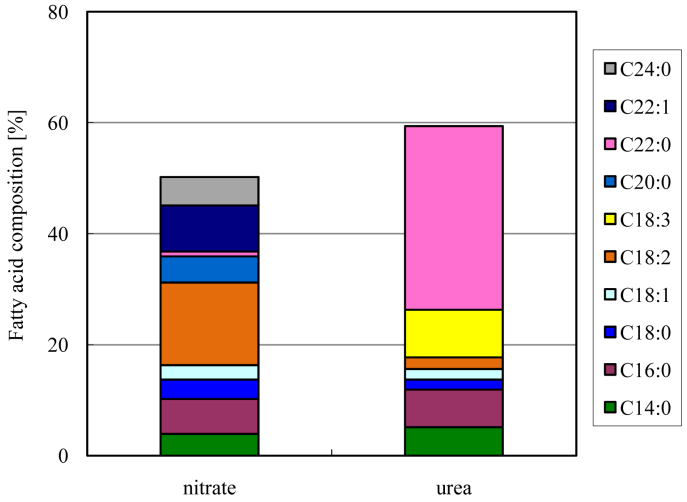


**Figure 5.** Fatty acid composition of lipid from *N. oceanica* IMET1 cultured with different N sources.

conditions. The main fatty acid composition of nitrate-grown nitrate comprised 6.3%, 14.9%, and 1% of C16:0, C18:2, and C22:0. In contrast, main fatty acid composition of urea-grown IMET1 comprised 6.8%, 8.6%, and 33.1% of C16:0, C18:3, and C22:0. In addition, a clear difference can be seen in C18:3 and C18:2 for cells grown in different nitrogen sources. Thus, it is obvious that different nitrogen sources can affect the composition of fatty acid in microalgae. However, in a previous study based on *N. salina*, Campos *et al.* reported almost the same lipid FAME fraction irrespective of nitrogen source [8]. The different results between our study and their observation could be due to the difference in fatty acid analysis. Only C14 - C20 fatty acid components were analyzed in their study, whereas we analyzed the C14 - C24 components in this study (**Figure 5**). Xiao *et al.* reported metabolic profiles of *N. oceanica* IMET1 cultured in a modified f/2 medium in 2013 [6]. The main fatty acids were C16:0 and C16:1, which differed from our results. They also analyzed C14 - C20 fatty acids, but found no > C22 fatty acids. The base medium is f/2 medium in their study, while BG11 is the base medium in our study. The difference in medium composition could also result in the variation in fatty acid composition.

## 4. Conclusion

Our study evaluates the impact of different nitrogen sources (nitrate and urea) on the growth, lipid production and composition of *N. oceanica* IMET1. We find that *N. oceanica* IMET1 yields much higher lipid content when cultivated with nitrate than with urea. Nitrate-grown cells mainly produce C16:0 (6.3%) and C18:2 (14.9%) and urea-grown cells mainly produce C16:0 (6.8%), C18:3 (8.6%), and C22:0 (33.1%) fatty acids. IMET1 grown with urea produces much higher C22:0 fatty acid in comparison with IMET1 grown with nitrate. Nitrate-grown cells have higher dry weight than urea-grown cells. We demonstrate that replacing nitrate with urea will have a negative effect on dry cell weight and lipid yield. However, urea based algal cultures have potential to produce more C22:0 fatty acid.

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