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# Effect of Different Nitrogen Sources on the Growth and Lipid Accumulation of *Chlorella variabilis*

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**Abstract**

Nitrogen is one of the major elements required for growth and other physiological activities of microalgae. Microalgae can use different forms of nitrogen such as nitrate, nitrite, ammonium, and urea. Although the type of nitrogen used by microalgae depends on the species, microalgae usually prefer ammonia as a nitrogen source and the usual order of preference is ammonium, urea, nitrate, and nitrite. However, types of nitrogen sources and their concentrations affect the growth of microalgae cultures and their biochemical structures. It has been reported in many studies that microalgae accumulate more lipids under nitrogen starvation growth conditions. Therefore,growth and lipid accumulation behavior of microalgae *Chlorella variabilisin* growth medium (BG11) containing different types of nitrogen source [sodium nitrate ($NaNO_3$), ammonium chloride ($NH_4Cl$) and urea($CH_4N_2O$)] was investigated in this work. Maximum cell concentration (1.59 g/L) and growth rate (0.054/h) were obtained in $NH_4Cl$ containing photobioreactor, whereas highest lipid content (16.4%) and productivity (4.21 mg/L.day) was obtained in $CH_4N_2O$ containing photobioreactor. Compared to the other nitrogen sources, $NaNO_3$ did not significantly improve the growth rate and the lipid productivity. According to the results, microalgae can be considered as a raw material for biodiesel production applications in the future.

**Keywords:** biodiesel, *Chlorella variabilis*, microalgae oil, nitrogen stress

## INTRODUCTION

Algae has been usedfor the production of different bioproducts for many years; proteins, vitamins, fatty acids, , carbohydrates, minerals and pigments, hydrocarbons, polysaccharides, antibiotics and other metabolites. Microalgae are photosynthetic organisms that are able to grow using carbon dioxide and light, and can be produced in photobioreactors which allow continuous production throughout the year. They can alter their metabolism in response toadverse environmental conditions. *Chlorella* and *Neochloris* species belonging to the class Chlorophyceae have been found to accumulate up to 60% lipidsper dry cell weightwhen cultured in nutrient deficient environment [1]. Nitrogen source type and concentration are known to be effective on the growth and biochemical compositionof microalgae. [2,3]. It has been documented that the nitrogen limitationenrich fatty acids in the cells[4,5]. The most commonnitrogen sources that are used in microalgae cultures are nitrate nitrogen, ammonium nitrogen and urea nitrogen [2,6]. Xu et al.have investigated the effects of different nitrogen sources [nitrogen nitrate ($NO_3$ - N), ammonium nitrogen ($NH_4^+$ N), urea nitrogen ($(NH_2)_2$ CO - N)] on growth and fatty acid composition of *Ellipsidion* sp. While no significant difference in growth rate was observed in the medium prepared with $NO_3$ -N and $NH_4$ -N, it was stated that the growth rate in the medium prepared with urea-N was slower. They found that fatty acids were more abundant in the nutrient media prepared with $NO_3$ -N and $NH_4$ -N.

In this study, the effects of different nitrogen sources on *Chlorella variabilis* microalgae growth and lipid content are investigated. To the best of our knowledge, there is no research published on the utilization of different nitrogen sources by *Chlorella variabilis*. Documenting the lipid accumulation response of the microalgae to the nutrient composition may improve its usage as a possible raw material for biodiesel production processes.

## MATERIALS AND METHODS

### Microalgae strain and nutrient medium

*Chlorella variabilis*was used in this study. The strain was donated by the Molecular Biology and Genetics Department of Istanbul Medeniyet University.Modified Blue-Green (BG-11) nutrient medium was used with the following chemical composition (g/L); $KH_2PO_4$, 0.04; $MgSO_4.7H_2O$, 0.075; $CaCl_2.2H_2O$, 0.036; $H_3BO_3$ 0.0029; $Na_2CO_3$, 0.02; Fe(III)citrate, 0.006; citric acid 0.006. One of the following compounds was added to the medium as the nitrogen source; $NaNO_3$ (Sodium nitrate), $NH_4Cl$ (Ammonium chloride), $CH_4N_2O$ (urea). The amounts added were 1.5 g/L for each compound.Chemicals were analytical grade and were purchased from Merck. The prepared nutrient medium was sterilized thermally before inoculation. 3% inoculation was made to the photobioreactors from fresh microalgae culture.

### Experimental Setup and Operating Conditions

Microalgae were cultured in 500 ml glass erlenmeyer-flasks (photobioreactors).LED lamps (2700K) were used to provide continuous illumination to the photobioreactors throughout the experiments. 3.80 klux light intensity was achieved on the surface of the photobioreactors. The temperature was kept constant at 25 ± 2° C. The aeration of the samples was provided by an air pump at a rate of 300 L/h. The air from the air pump was sterilized by filtration (0.45 µm) before being fed into the photobioreactors.

### Analyses

Growth of microalgae was monitored spectrophotometrically during the runs. For the analysis of optical density , 3 mL sampling was made from the photobioreactors. Jenway 6800 UV-VIS spectrophotometer was used for analysis, samples were placed in quartz cuvettes and the measurements were made at 600 nm wavelength.After the runs were completed, in order to determine the dry weight of the microalgae biomass, thephotobioreactor output was centrifuged at 4000 rpm for 30 minutesand then dried at 60 °C for 24

hours.

pH measurements were made using a Mettler Toledo Easy S-20K pH meter.

Lipid analysis was performed according to the method of Bligh and Dyer (1959)[7]: 0.2 grams of homogenized microalgae samples were kept at 105 ºC for 2 hours in the oven. After cooling, a mixture of 120 mL methanol/chloroform (1/2) was added onto the samples. These samples were stirred for 3 hours at 200 rpm by a mechanical stirrer, then filtered through a filter paper. Samples were kept in dark overnight. The next day the upper layer consisting of the methanol-water mixture was separated. Chloroform was removed from the media by means of a rotary evaporator. The residual lipid was cooled to the room temperature and weighed to determine the lipid amount of the sample [7].

**Calculations**

Maximum specific growth rate ($\mu_{max}$) was calculated according to the equation:

$$\mu_{max} = (\ln X_2 - \ln X_1) / (t_2 - t_1)$$

where $X_2$ and $X_1$, are biomass concentrations during the logarithmic phase of growth at $t_2$ and $t_1$

Cell doubling time ($t_d$) was calculated according to the equation:

$$t_d = \ln(2) / \mu_{max}$$

## RESULT AND DISCUSSION

The time-dependent growth and pH profile of *Chlorella variabilis*under different nitrogen sources are shown in Figure 1 and Figure 2, respectively.

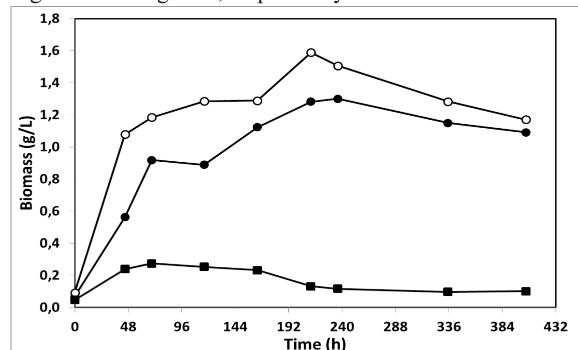
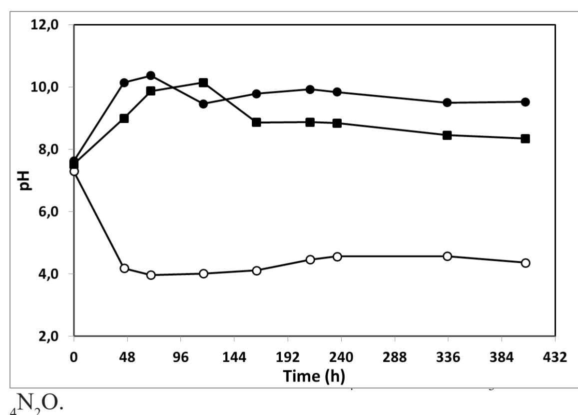



**Figure 1.** Effects of different nitrogen sources on the growth of *Chlorella variabilis*.(○): $NH_4Cl$,(●): $NaNO_3$,(■): $CH_4N_2O$.




$_4N_2O$.

Figure 1 shows that themicroalgae in the medium prepared with $CH_4N_2O$ showed a slow growth which reached stationary phase at 45th hour. In the nutrient media prepared with $NaNO_3$ and $NH_4Cl$, a rapid growth was observed up to the 69th hour, then slowed but continued with fluctuations until 212th hour, after when the death phase started.

According to Figure 2, initial pH values at the start of the runs were measured to be in the range of 7.3 -7.6.After the inoculation, the pH of the flaskcontaining$NH_4Cl$decreased down to 4 due to the slight acidic effect of $NH_4Cl$, then remained stable between 4.0-4.6 during the rest of the run. The pH of the flasks containing $NaNO_3$ and $CH_4N_2O$media increased up to 69th hour and reached to more than 10, then remained stable in the range of 8.3-9.9 until the end of the runs.

The effects of different nitrogen sources on *Chlorella variabilis* growth rate and lipid content are given in Table 1.

**Table 1.** The effects of different nitrogen sources on *Chlorella variabilis* growth rate and lipid content

| Type of N source | Maximum cell concentration (g/L) | $\mu_{max}$ ($h^{-1}$) | Lipid content (%) | Doubling time (h) | Lipid productivity (mg/Ld) |
|---|---|---|---|---|---|
| $NaNO_3$ | 1.30 | 0.038 | 15.2 | 18 | 4.15 |
| $NH_4Cl$ | 1.59 | 0.054 | 13.9 | 12 | 1.90 |
| $CH_4N_2O$ | 0.27 | 0.018 | 16.4 | 38 | 4.21 |

The maximum cell concentration (1.59 g/L) and the specific growth rate (0.054/h) were reached in the nutrient medium prepared with $NH_4Cl$. However, the lipid content, doubling time and lipid productivity of this run was lower than the others. The cell lipid contentsand lipid productivites obtained in $NaNO_3$ and $CH_4N_2O$containing photobioreactors were comparable. Doubling times of the cells were determined to be 12,18 and 38 hours, for $NH_4Cl$, $NaNO_3$ and $CH_4N_2O$containing photobioreactors, respectively.

It needs more energy of microlgae in assimilation of nitrate-N than that of ammonium-N, because nitrate-N must be transformed into ammonium-N before being utilized [4]. This explains our findings ofhigher growth rate and lower doubling time in the ammonium containing medium.

Sharma et al [8] conducted a comparable study in which the effect of different nitrogen sources (sodium nitrate, potassium nitrate and urea) were analyzed on growth and lipid accumulation on *Chlorella sp*. They found that the effect of nitrogen source on the specific growth rate and doubling time was insignificant, but lipid yield was better in sodium nitrate and potassium nitrate media compared to urea media.

In another study, the influence of different nitrogen source (potassium nitrate, sodiumnitrate, urea, calcium nitrate, ammonium nitrate and ammonium chloride) of varying concentrations on biomassproduction of green algae *Scenedesmus* was investigated. It was found that among the various nitrogen sources, nitrate was the favorable source for biomass growth of Scenedesmus. Among the ammonical forms, urea resulted in almost equal biomass as nitrates, making it an economical substitute for nitrogen source in large scaleculturing of algae being commercially available [9].

Soni et al [10] also investigated the effect of various nitrogen sources (ammonium sulphate, urea and potassium nitrite) on microalgal growth and lipid content in two different microalgae strains; *Chlorella pyrenoidosa* NCIM 2738 and ANK-1. They reported that the most effective nitrogen source for *Chlorella pyrenoidosa* was urea and potassium nitrate while for ANK-1 the suitable nitrogen source was urea for growth.*Chlorella pyrenoidosa* showed high lipid content in nitrogen source ammonium sulphate and urea whereas ANK-1 showed high lipid in potassium nitrate and urea.

Qiang Lin et al. examined the effects of nitrogen source ($(NH_4)_2CO_3$, urea, $NaNO_3$, urea and $NaNO_3$ mixture)and concentration on the biomass and oil accumulation and pro-

ductivity of *Scenedesmusrubescens* and found that the microalgae fed with the mixture of urea-N and $NaNO_3$-N had the highest biomass productivity [11].

## CONCLUSION

For the first time in literature this study expresses the effect of different nitrogen sources on growth and lipid accumulation of microalgae *Chlorella variabilis*.

$NH_4Cl$ was the best nitrogen source to obtain the maximum cell concentration (1.59 g/L) and growth rate (0.054/h), whereas highest lipid content (16.4%) and productivity (4.21mg/L.day) was obtained in $CH_4N_2O$ containing photobioreactor.

The findings show that the microalgae can be considered as a raw material for biodiesel production applications in the future.

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