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Research Paper

Effect of Nitrogen Source on Growth and Protein and Lipid Amounts of a Freshwater Microalga *Scenedesmus acutus*

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Abstract: *Scenedesmus acutus* (*S. acutus*) is a green freshwater microalga. This study investigated the impact of nitrogen sources on growth, protein and lipid amounts of *S. acutus*. The reaction of *S. acutus* to different N sources was tested in liquid nutrients by adding KNO_3, $CaNO_3$, $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$, instead of nitrogen sources, separately in a standard medium. KNO_3 and $CaNO_3$ resulted in the highest growth. The amount of protein was highest in KNO_3 and NH_4NO_3 cultures, while the amount of lipid was highest in KNO_3 and $NaNO_3$ cultures.

Key words: Nitrogen sources, *Scenedesmus acutus*.

Azot Kaynaklarının *Scenedesmus acutus*' un Gelişim, Protein ve Lipit Miktarına Etkisi

Öz: Bu çalışma, yeşil mikroalg *Scenedesmus acutus*'un gelişim, protein ve lipit miktarı üzerinde azot kaynağının etkilerini incelemeyi amaçlamıştır. Bu amaçla Scenedesmus acutus' un farklı azot kaynaklarına tepkileri standart besiyeri ortamındaki azot kaynakları yerine sadece KNO_3, $CaNO_3$, $NaNO_3$, NH_4NO_3 ve $(NH_4)_2SO_4$, ayrı ayrı ilave edilerek sıvı besi ortamlarında test edildi. Test edilen beş farklı azot kaynağı arasında KNO_3 ve $CaNO_3$ en yüksek gelişimi sağladı. Protein miktarı azot kaynağı olarak KNO_3 ve NH_4NO_3 kullanılan kültürlerde maksimum olurken lipit miktarı KNO_3 ve $NaNO_3$ kullanılan kültürlerde en yüksek miktarda tespit edildi.

Anahtar kelimeler: Azot kaynağı, *Scenedesmus acutus*.

1. Introduction

Microalgae have long been used for different purposes. However, natural, renewable, and eco-friendly products have become more prevalent in recent years. Microalgae are once again the center of attention because they are a source of valuable compounds [1-3]. When we think about overcoming protein deficit, the first source that comes to mind is algae. Microalgae have an important place in human and animal nutrition as they contain proteins, carbohydrates, amino acids, lipids, vitamins, and pigments [4-6]. Microalgal biomass cultivation is affected by physicochemical factors, such as nutrients, temperature, light source, pH, and salinity. However, production costs are the greatest problem in the cultivation of microalgae. Nutrients and light should be used efficiently in microalgae cultures to produce large quantities of low-cost biomass. Nitrogen, in particular, is a critical nutrient for growth. It is a macro element essential for the synthesis of nucleic acids, amino acids, proteins, and pigments, and thus, cellular growth. The cell growth rate and biochemical composition of microalgae depend on nitrogen concentration in a culture medium. Research shows that nitrogen starvation in a culture medium slows down the cell growth rate of microalgae and reduces protein synthesis by increasing lipid or carbohydrate content [7]. Many microalgae species use inorganic nitrogen sources (nitrate, nitrite, and ammonium) and synthesize cellular organic nitrogen compounds. Some microalgae (*Dunaliella tertiolecta* and *Botryococcus braunii*) prefer nitrate [8-9], while some *Chlorella* species prefer ammonium for growth [10-11]. Nitrogen source affects the biochemical content of microalgae. For example, *Dunaliella salina* has twice as much protein in nitrate media as in ammonium media [12], while *Chlorella sorokiniana* has twice as much lipid in ammonium media as in nitrate and urea media [13]. Different algae species need different nitrogen sources for growth. Not only growth but also biochemical content depend on the nitrogen source and amount. Therefore, we need to compare nitrogen sources and choose the best one for each type of microalgae. We need to identify suitable culture media to increase the efficiency of target products (lipid, protein, carbohydrate, etc.) and reduce culture costs.

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This study investigated the impact of nitrogen sources on growth and protein and lipid amounts of *Scenedesmus acutus* (*S. acutus*).

2. Material and Method

2.1. Medium and Culture Condition

S. acutus was sampled from the *Keban* Dam Lake, Turkey, and isolated at the algal biotechnology laboratory of Fırat University. The samples were cultured in a Jaworski's medium with 80 mg $NaNO_3$, 20 mg $Ca(NO_3)_2.4H_2O$, 36 mg $Na_2HPO_4.12H_2O$, 12.4 mg KH_2PO_4, 50 mg $MgSO_4.7H_2O$, 2.25 mg EDTAFeNa, 15.9 mg $NaHCO_3$, 2.25 mg $EDTANa_2$, 2480 µg H_3BO_3,1390 µg $MnCl_2.4H_2O$, 1000 µg $(NH_4)_6Mo_7.4H_2O$, 40 µg biotin, 40 µg cyanocobalamin (B12), and 40 µg thiamin (B1). The culture medium was sterilized for 15 minutes at 121 °C and an atmospheric pressure of 1. The culture medium, called "standard medium," was used as control cultures. Liquid nutrient media with equal final concentrations were generated by adding only KNO_3, $CaNO_3$, $NaNO_3$, NH_4NO_3, or $(NH_4)_2SO_4$, instead of nitrogen sources, separately in the standard medium to determine the reaction of *S. acutus* to different N sources.

The samples were inoculated into 250-mL flasks with a 100-mL standard medium. The Erlenmeyer flasks were left to grow at a light intensity of 55 µmol photon $m^{-2}sec^{-1}$ at 23±1 °C in a conditioning chamber. The cultures were sampled (10 mL) after they reached a certain density. The standard medium was used for control cultures. Modified media were used for experimental cultures. The modified media were media with $CaNO_3$, $NaNO_3$, NH_4NO_3, and $(NH_4)_2SO_4$ added into the standard medium with all nitrogen sources removed. The samples were inoculated into the modified media. The control and experimental cultures were allowed to grow for ten days in a climate cabinet at 23 ±1 °C under a 16h light/8h dark regime and 55 µmol photon $m^{-2}sec^{-1}$ light intensity. The Erlenmeyer flasks were shaken three times a day.

2.2. Growth, Lipid and Protein Content

Growth was calculated at the optical density (OD) of 680 nm using a visible density spectrophotometer. Measurements were performed three times.

Lipid content was determined gravimetrically using a solution of chloroform and methanol mixture [14]. A mixture of 40 mL methanol and 80 mL chloroform was added onto a 0.2 g sample, and 20 mL of 0.4% $CaCl_2$ was added to the mixture, which was then was filtered through filter paper and left in the dark overnight. The following day, a fraction of methanol and water was separated using a separatory funnel, and the chloroform was evaporated in a 60 °C water bath. The remaining part was kept in an oven at 90 °C for one hour to allow the chloroform to evaporate completely and then was weighed.

Total protein content was measured using the Lowry method [15]. 0.1-mL DOC solution was added in a 1-mL sample, which was then kept at room temperature for ten minutes. Afterward, 0.1-mL TCA was added onto the sample, which was then centrifuged at 7500 rpm for ten minutes. Following the removal of the supernatant, 1-mL Lowry solution was added to the precipitate, which was then kept at room temperature for 20 minutes. Later on, 1-mL folin reagent was added to the sample and kept for 30 minutes. Lastly, absorbance was plotted at 750 nm to generate a standard curve, which was used to evaluate the results.

3. Results and Discussion

Nitrogen is essential for synthesizing amino acids, proteins, nucleic acids, coenzymes, and chlorophylls, which are vital to all living things. Nitrogen deficiency significantly affects the growth of microalgae as well as all living things [6]. Nitrogen source is important for protein and lipid accumulation during microalgal growth. This study looked into the impact of different nitrogen sources on growth and protein and lipid amounts. Standard media were used as control cultures, and modified standard media were used as test cultures to determine which nitrogen source was effective in the growth of *S. acutus*. Figure 1 shows the trend of change in OD during cultivation. OD_{680} was 0.040 in the standard and modified liquid cultures on the day of inoculation. From the first day after inoculation, the increase in cell density in both standard and modified cultures led to an increase in OD, which reached a maximum on day six. The control, KNO_3, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, and $CaNO_3$ cultures had

an OD of 0.123, 0.358, 0.228, 0.315, 0.284, and 0.344, respectively. The optical density decreased after day six (Fig.1). Weak and short-term microalgal growth due to lack or insufficiency of nitrogen is observed in many algae [16]. Nitrate-N is a more common nitrogen source than ammonium nitrogen for microalgae [17]. However, some microalgae (e.g., *Isochrysis galbana*) can use ammonium-N as a nitrogen source and grow rapidly [18-19]. Some microalgae grow faster in ammonium media than in nitrate media. We found that the cells in all cultures continued to grow until day six but that the greatest growth took place in KNO_3 and $CaNO_3$ media, indicating that *S. acutus* prefers NO_3-N for growth.

Proteins are large multi-atom organic molecules composed of carbon, hydrogen, oxygen, and nitrogen, as well as sulfur and phosphorus. Research shows that microalgal proteins are promising alternative sources of protein [4-5]. We collected samples from liquid media for ten days to determine the effect of nitrogen source on the protein amount of *S. acutus*. Figure 2 shows the changes in the protein amount of *S. acutus*. On the day of inoculation, all cultures had a protein content of 24.35 µg/ml, which increased until day six. The control, KNO_3, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, and $CaNO_3$ cultures had a protein content of 154.78, 275.65, 225.55, 260,40, 200.45, and 242.05 µg/ml, respectively, on day six, after which the protein content decreased.

We monitored the variation in the amount of lipid of *S. acutus* based on nitrogen sources for ten days. Figure 3 shows on the day of inoculation, all cultures had a lipid content of 9.20%, which increased until day six after inoculation. On day six, the control, KNO_3, $NaNO_3$, NH_4NO_3, $(NH_4)_2SO_4$, and $CaNO_3$ cultures had protein contents of 58.35%, 78.64%, 68.15%, 45.65%, 40.35%, and 52.35% µg/ml, respectively.

Different strategies for improving microalgal growth and biochemical composition have become popular in recent years. Some of those strategies focus on increasing growth rates and protein, lipid, and pigment contents by altering environmental conditions [2, 20-25]. The primary objective of those strategies is to select the right nitrogen source and provide efficient and cost-effective microalgae cultivation for high-value products. Many microalgae can utilize different forms of nitrogen by following the pathways leading to the assimilation of nitrogen into amino acids [26]. For example, NH_4NO_3 cultures increase the growth rate and lipid content of *Scendesmus abundans* and *Chlorella ellipsoidea* [27], while nitrate cultures are better for the growth of *Tetraselmis* cells, as they result in twice as much biomass as ammonium cultures [28].

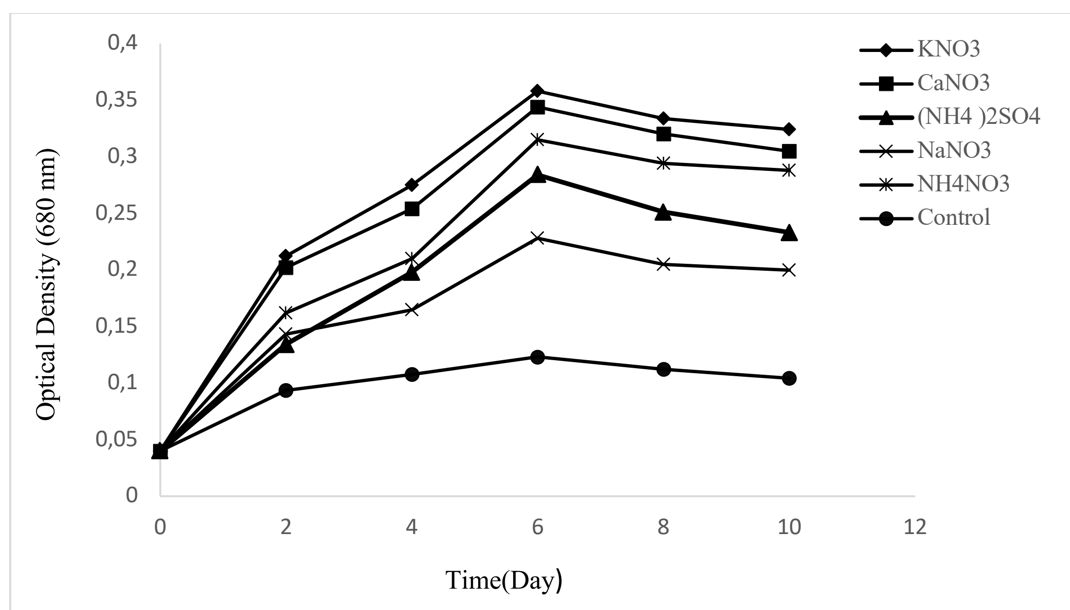

Figure 1. Effects of different nitrogen sources on growth of *Scenedesmus acutus.*

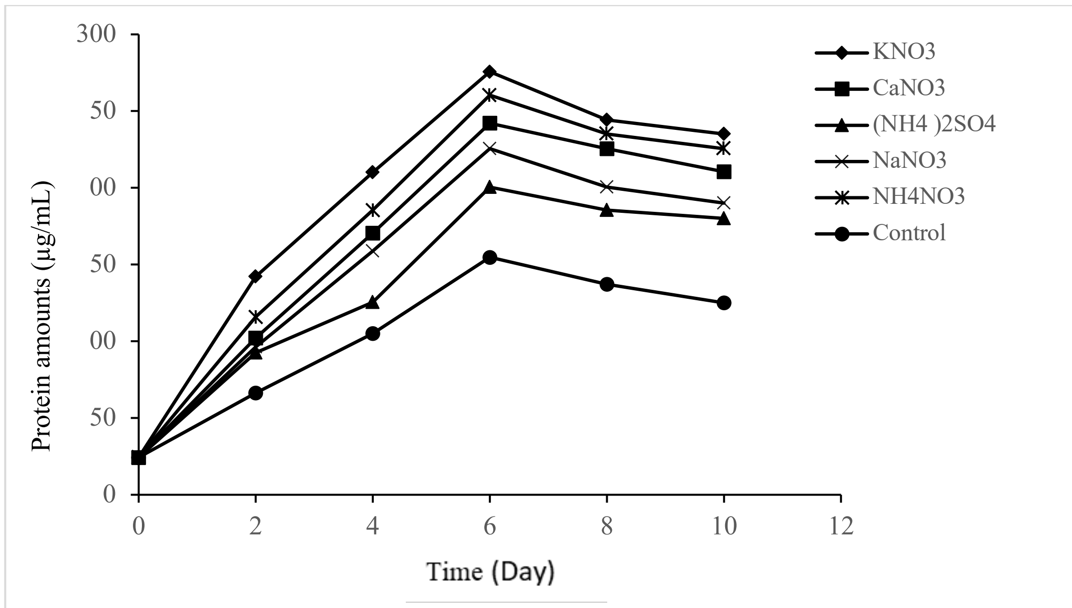

Figure 2. Effects of different nitrogen sources on protein amounts of *Scenedesmus acutus.*

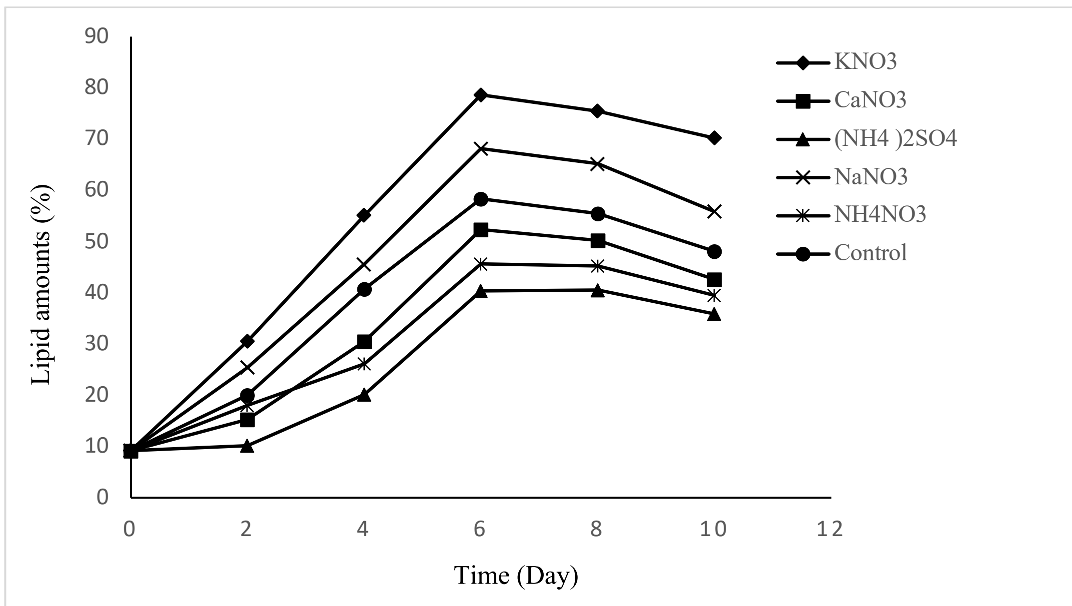

Figure 3. Effects of different nitrogen sources on lipid amounts of *Scenedesmus acutus.*

4.Conclusion

The results show that microalgae prefer different sources of nitrogen. The highest growth was observed in KNO_3 and $CaNO_3$ cultures. The protein content was highest in KNO_3 and NH_4NO_3 cultures, while the lipid content was the highest in KNO_3 and $NaNO_3$ cultures. If we are interested in increasing biomass in *S. acutus*, we should use KNO_3 or $CaNO_3$ cultures as nitrogen sources. We should use KNO_3 or NH_4NO_3 cultures to obtain high amounts of protein, and we should use KNO_3 or $NaNO_3$ cultures to obtain high amounts of lipid. However, more research is warranted to optimize the culture parameters necessary for high cultivation rates of *S. acutus*. We think that this study will pave the way for further research.

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