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Menba Kastamonu Üniversitesi Su Ürünleri Fakültesi Dergisi 2023; 9(1): 7-11 Araştırma Makalesi/Research Article

Effect of Selenomethionine and Concentration on Growth and *Chlorophyll-a* of *Scenedesmus quadricauda*

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Abstract

Selenium is an essential element for all living organisms. Since its deficiency can cause health Selenium is an essential element that plays an important role in the growth and development of all living organisms. However, the correct dose must be used for all living things. Otherwise, high selenium concentrations can cause toxic effects. In this study, the effect of adding selenomethionine (SeMet), an organic form of selenium at different concentrations of 50, 75, 100, and 250 mg/L, to the culture medium of the freshwater green microalgae *Scenedesmus quadricauda*, on microalgae growth and chlorophyll-a content was investigated. The results obtained after the study; It has been observed that the presence of high seleniummethionine in microalgae culture may cause toxic effects, but when 50mg/L is used as an effective dose, it positively supports the cell count as 64±4.9 x106 cells/mL and the chlorophyll-a pigment content of 0.593±0.1µg/L.

Selenometiyonin ve Konsantrasyonun *Scenedesmus quadricauda*'nın Büyüme ve *Klorofil-a* Üzerine Etkisi

Makale Bilgisi

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Anahtar Kelimeler:
- Selenometiyonin
- Selenyum
- *Scenedesmus*
- *Klorofil-a*

Öz

Selenyum, tüm canlı organizmaların büyüme ve gelişiminde önemli rol oynayan esansiyel bir elementtir. Ancak tüm canlılar için doğru dozun kullanımı gerekmektedir. Aksi takdirde yüksek selenyum konsantrasyonları toksik etkiye sebep olabilmektedir. Bu çalışmada, tatlı su yeşil mikroalgi *Scenedesmus quadricauda*'nın kültür ortamına 50, 75, 100 ve 250 mg/L olmak üzere farklı konsantrasyonlarda selenyumun organik formu olan selenometiyonin(SeMet) eklenmesinin mikroalg büyümesi ve klorofil-a içeriği üzerindeki etkisi araştırılmıştır. Yapılan çalışmanın ardından elde edilen sonuçlar; mikroalg kültüründe yüksek selenyummetiyonin varlığının toksik etkiye neden olabileceği ancak efektif doz olarak 50mg/L kullanıldığında hücre sayısını 64±4,9 x106 hücre/mL ve klorofil-a pigment içeriğini 0,593±0,1µg/L olmak üzere pozitif yönde desteklediği görülmüştür.

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INTRODUCTION

Selenium (Se) is an essential trace element that functions as the 21st amino acid selenocysteine (Sec) in a defined set of proteins, which is very important in animals and plants, especially the human body (Qian et al., 2019). Selenium affects organisms in a dose-dependent manner, being an important element that is beneficial at low concentrations and toxic at high concentrations. Organically, it is found in the form of selenocysteine in animal foods, while it is found in the form of Selenomethionine (SeMet) in plant foods (Kangalgil and Yardimci, 2017). In aquatic ecosystems, microalgae have begun to be recognized as vector organisms for Se transfer. It has been investigated that microalgae can accumulate selenium in their metabolism by exposure to a selenium-containing environment. However, what kind of changes in the morphological and physiological properties of microalgae produced in the presence of selenium have not been fully revealed yet. Green algae are accepted as target organisms in Se bioaccumulation studies. Therefore, the obtained biomass, product amount, and enriched pigment content of freshwater microalgae grown in a selenium-enriched nutrient medium have the potential to be used as food, cosmetics, fish feeding in aquaculture, inclusion in the food chain in feed areas or as biofertilizer to enrich the soil.

In this study, the effect of adding different concentrations of Selenomethionine (SeMet), the organic form of selenium, to the culture medium on the growth and chlorophyll-a content of *Scenedesmus quadricauda* was evaluated. Investigation of the

effect of selenium addition on microalgae pigments and in terms of obtaining natural pigment contents from selenium-enriched microalgae biomass will be evaluated in line with the results to be obtained.

MATERIAL AND METHODS

The Scenedesmus quadricauda were obtained from Ege University Fisheries Faculty Aquaculture Department, Plankton Culture Laboratory (Figure 1).

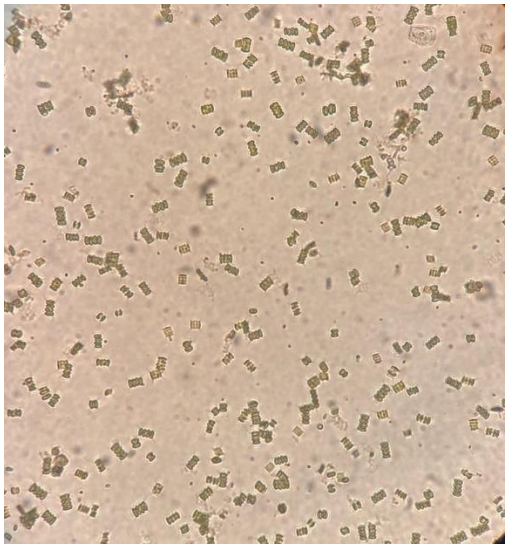

Figure 1. Light microscope image of *Scenedemus quadricauda.*

Microalgae Culture Condition

The preferred nutrient medium for the culture of *Scenedesmus quadricauda* was The Bold Basal Medium (BBM). For the culture, the flasks were illuminated with LED (Philips TLM 40W/54RS) lighting for 24 hours, and the ambient conditions were kept constant at 24°C with the air conditioning system. In addition, an aeration system was added to prevent biofilm formation, but no CO2 was added to the flasks. Different concentrations of SeMet were added to the found culture medium and cultured in a 1 L flask at concentrations of 50 mg/L, 75 mg/L, 100 mg/L, and 250 mg/L.

Number of Cells

The daily cell count of Scenedesmus quadricauda was followed. As the culture entered the stationary phase at the end of the 11th day, the trial set was terminated. Cell numbers were determined using a Neubauer hematocytometer with a light microscope (Nikon 250, Japan). The specific growth rates (μ) were calculated using the formula below.

$$\mu = \frac{\ln x_2 - \ln x_1}{t_2 - t_1}$$

X2 and X1 represent the culture densities at t2 and t1 times.

Chlorophyll-a Analysis

Chlorophyll-a analysis for *Scenedesmus quadricauda* was performed according to the spectrophotometric method. After taking 5 mg of dried sample and treated with 5 ml of methanol (Merck 100%, Germany). The cells were homogenized with an Ika (Ultra Turrax T25) brand homogenizer for 5 minutes and then ultrasonically (Sonorex) at 70°C for 10 minutes. After the extract obtained is separated by centrifugation (Elektromag M 615 P) at 3500 rpm. The samples were read at 666 nm wavelengths using the spectrophotometer (Boe co, S-20 VS, England) and the chlorphyll -a values were calculated with the below formula. Analyzes were performed in triplicate.

Chlorophyll-a(mg/g) = 13.9 x A_{666} (Sanchez et al., 2005)

(A_{666}: absorbance reading at 666 nm)

Statistical analysis

All cultivation experiments and pigment analysis were performed in triplicate and all data are expressed as the mean with standard deviation. Results were analyzed by one-way ANOVA with significance level at $P \leq 0.05$ and Tukey's multiple comparison test was performed by using SPSS Statistics 23 (IBM, US).

RESULTS AND DISCUSSION

Cultural Densities

The initial culture density of *Scenedesmus quadricauda* in the study was 4x10^{6}cells/mL (Figure 2). The highest cell count has achieved at 64±4.9 x10^{6}cells/mL on day 8 when used 50mg/L SeMet concentration in the culture medium. 75mg SeMet concentration reached higher levels than the control group ($p<0.05$). When it was used 100 mg/L and 250 mg/L SeMet concentrations, there were negative affected on growth *S. quadricauda*. As a result of, SeMet to use in the medium more than 100 mg cause a significant growth difference of *S. quadricauda* ($p>0.05$). For this reason, trial sets were terminated before the SeMet concentration caused a toxic effect.

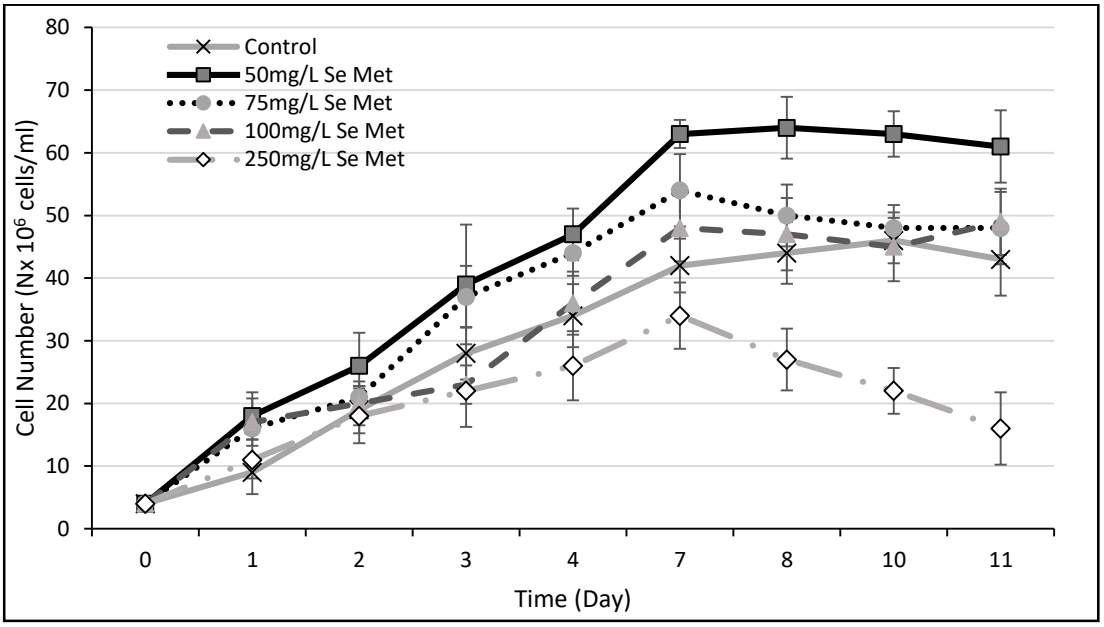

Figure 2. Cell density of *Scenedesmus quadricauda* at 11 days of production by addition of SeMet.

Chlorophyll-a Amounts

In the pigment analysis, chlorophyll values were measured every 3 days and the average results were taken. In all cultures, the highest chlorophyll value was observed in 0.593±0.1µg/L, and 50mg/L SeMet addition (Figure 3). The highest chlorophyll value for the control group was measured as 0.436±0.09µg/L. Therefore, it is seen that the addition of a maximum of 100 mg/L not a higher concentration of SeMet did cause a difference in the amount of chlorophyll per cell compared to the control group ($p<0.05$).

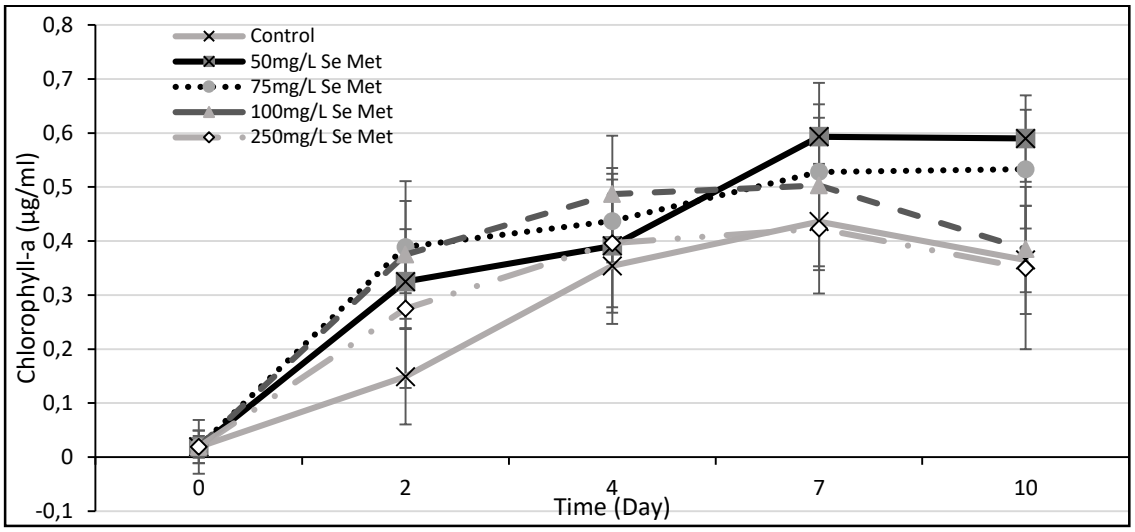

Figure 3. Chlorophyll-a values determined from the production of *Scenedesmus quadricauda* with the addition of SeMet.

Information on the presence and possible effects of selenium in microalgae culture media is limited. However, the effects of inorganic forms of selenium on growth rate and photosynthetic pigment (such as chlorophyll-a, and β-carotene) production in various microalgae species vary depending on the dose of selenium used. First, while the dose plays an active role, it is also an important factor in whether the form used is organic or inorganic. In the study, the effect of organic se form on

growth dynamics and chlorophyll-a amount was investigated since it is known that the toxic effect of organic se form is less (Demircan,2022). The results obtained showed that the presence of SeMet in *Scenedesmus quadricauda* culture medium was effective at 50mg/L to 75mg/L depending on the dose of use.

Pigments are one of the important metabolites for microalgae. Culture conditions that encourage pigment levels of microalgae, which have many uses in the biotechnological field thanks to their various pigment contents, have been studied for a long time. In line with the results obtained from the literature studies, the effects of selenium on various pigment substances during the culture of microalgae species are discussed. For example, While the effect of *Dunaliella salina* on the presence of beta-carotene in the 21-day period followed by the production of selenium was limited, the results obtained did not find a significant effect of the presence of selenium on pigment substances (Constantinescu-Aruxandei et al., 2019). During the example, the promotion of selenium has been observed. However, in previous studies, it has also been encountered that the inorganic form of Selenium has no effect or has a toxic effect. However, in another study by Sun et al., (2014) a steady increase in the amount of chlorophyll-a was observed in the cultivation of Chlorella vulgaris, and the inorganic form of selenium was used at a concentration of 75mg/L. Similarly, *Spirulina platensis* was used in the study conducted by Chen et al. 2008 and the addition of selenium during its production showed a stimulating effect on the chlorophyll content. It has been reported that the chlorophyll content reaches its maximum value (29.3 mg / g) on the 3rd day with the addition of 40 mg / L Se at the effective dose. In another study prepared by Pronina et al., 2001, it was reported that sodium selenite supplementation increased from 1.04 to 1.10 compared to the control group with the inclusion of 50mg/L. Therefore, in this study, findings parallel to the references compared for pigment sources were obtained and the effect of selenium addition on the chlorophyll-a content of *Scenedesmus quadricauda* was found to be positive. In addition, as the most recent example, by Demircan, (2022) in the study in which SeMet was added to *Scenedesmus dimorphus* culture medium when the effect of growth conditions and chlorophyll-a content was examined, approximately 1.5 times positive effect was reported in the flask containing 50mg/L SeMet compared to the control group.

When the experiment was evaluated, it was observed that the SeMet form positively promoted growth and chlorophyll-a content when used at an effective dose for the freshwater green microalgae *Scenedesmus quadricauda*.

CONCLUSION

According to our study results, 50, 75 and 100mg/L SeMet concentrations added to the *S. quadricauda* culture medium stimulated cell growth, while 250mg/L caused a toxic effect and reduced the cell number to a lower level than the control group. For this reason, it has been observed that selenium supplementation can be effective at different values for each species, but levels below 100mg/L have a positive effect. Thus, it was concluded that the potential of using selenium methionine form in the culture medium is positive in increasing the microalgae biomass and enriching the pigment contents.

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