The influence of the organo-mineral complex on the growth dynamics of the Scenedesmus quadricauda culture

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Abstract. The paper presents data on the biotechnology of growing accumulative cultures of algae Scenedesmus quadricauda when adding organo-mineral complex drag to the culture growth medium. It is shown that the trace elements that make up the drug (trade name "Helavit"), at the studied concentrations, act in various ways stimulating and inhibiting the growth of algological culture. Having a different effect in the concentration range from 0.1 to 1 mg / L. The maximum positive effect is achieved with a drug concentration of 0.3 mg / l (8986 ± 120 103*cells/ ml), which can be recommended for mass cultivation of algae. A possible cause of inhibition of culture growth, at a concentration of 0.1 mg / l (6960 ± 115 103*cells/ ml), are the inhibitory effects of copper and iodine ions.

1.Introduction
Currently, algae are widely used as an object of biotechnology. They are used for genetic engineering, the creation of biofuels [1] and laboratory studies [2], as an additive in fish feed [3], and also as an object for bioremediation of contaminated water [3, 4]. In such cases, specialized algal growth media are used for the cultivation of algae [5], which include nutrients and trace elements necessary for the productive development of the culture. In the mass cultivation of green algae biomass, simple mineral fertilizers and animal waste are used [6]. Such cultivation media are not balanced in micromineral composition and do not provide maximum culture growth. For this reason, the question of finding a method of enriching the environment during the mass cultivation of algae is very important. A number of studies have shown that most often the limiting element is the ions of iron, zinc and magnesium [7]. To eliminate the deficiency of macro and trace elements, water-soluble metal salts are most often used. Their disadvantage is that they quickly precipitate in the aquatic environment and become inaccessible to cultivation objects.

In this paper, we consider a method of introducing micronutrients and macronutrients in the form of chelate compounds, which are stored for a long time in solutions and consumed by algae during the entire cultivation time, thereby ensuring optimal crop growth [8]. In the experiments, a complex of mineral elements was used (Helavit), which includes the following components: iron, manganese, copper, zinc, cobalt, selenium, iodine.

The experiment was conducted on pure cultures of Scenedesmus quadricauda microalgae, which are widely used for laboratory studies to study photosynthesis, aquatic toxicology [9] and genetic engineering, due to the simplicity of cultivation and high biomass yield.

Purpose of the study: establish whether the growth of the algae culture depends on the concentration of the solution of chelated trace elements in the laboratory.
2. Materials and methods

The experience was carried out on the basis of the Russian-Scandinavian center for research and innovation in the field of aquaculture of Moscow State Technical University, Razumovsky K. G. (PKU), with double repetition, within 12 days. The initial culture of Scenedesmus quadricauda, obtained from the collection of the Faculty of Biology of Moscow State University, was grown on Uspensky-1 medium in accordance with the methodology [10]. The composition of the medium was (g / l): KNO3 - 0.250; KH2PO4·3H2O - 0.025; K2CO3 - 0.345; MgSO4 · 3H2O - 0.025; Ca(NO3)2 · 4H2O - 0.144; trace element solutions - 1 cm3 from a mixture of H3BO3 - 2.86; MnCl2 · 4H2O - 1.81; ZnSO4 · 7H2O — 0.222 g / L; MoO3 - 17.64; NH4VO3 - 22.96 mg / L. The initial algae culture was cultivated at a temperature of 20 ° ± 2 °C with constant illumination (30 μE / m²s).

The algae culture was poured into 100 ml containers. Then, in each container, except for the control, an organic-mineral preparation was added (composition mg / l: Fe - 13; Mn - 2.6; Cu - 1.3; Zn - 7.3; Co - 0.26; Se - 0.13; I - 0.4), according to the following concentrations: 0.1 mg/L, 0.3 mg/L, 0.5 mg/L, 1 mg/L. Cultivation was carried out at a temperature of 22 ± 2 °C, with constant illumination (30 μE / m²s).

Cell concentration was measured on the first, third, eighth and twelfth day of the experiment, using a Goryaev camera (Hemocytometer), based on the generally accepted method [11]. One of the evaluation criteria was the morphology of algal cells studied by microscopy (Olympus BX43P). The results were processed by static methods.

3. Results and discussion

According to the results of the study, it was revealed that from the first days of the experiment, the growth of the culture, with the addition of a mineral preparation, was higher than in the control. The maximum effect (Table 1), both in the number of cells and in the percentage of growth, was on the third day at a concentration of 0.5 (360.5%) and 0.3 mg / L (214%). A culture with a concentration of a mineral supplement of 1 mg / L (288.5%) showed less growth on the third day, which may be caused by a toxic effect [12].

On the eighth day of the experiment, in all concentrations, the number of cells per ml was above 40 thousand units. The concentration of 0.5 mg / L (7980 ± 123 10³* cells / ml) had the greatest number of cells with an increase of 221.13%. The maximum percentage growth was observed at a concentration of 0.1 mg / l (329.16%).

| Concentration mg / L | 1 | 3 | 8 | 12 |
|----------------------|---|---|---|----|
|                      | 10³ cells / ml | % | 10³ cells / ml | % | 10³ cells / ml | % | 10³ cells / ml | % |
| Control              | 1000±50         | 0 | 1536±45         | 53.6 | 4305±73         | 180.27 | 7290±143         | 108.89 |
| 0.1                  | 1000±50         | 0 | 1440±64         | 44 | 4740±88         | 329.16 | 7446±131         | 157.08 |
| 0.3                  | 1000±50         | 0 | 3140±90         | 214 | 5055±91         | 60.99 | 6140±124         | 12.14 |
| 0.5                  | 1000±50         | 0 | 3605±110        | 360.5 | 7980±123        | 221.13 | 8986±120         | 11.26 |
| 1                    | 1000±50         | 0 | 2885±79         | 2880.5 | 6450±114        | 223.57 | 6960±115         | 10.79 |

The minimum number of cells per ml was in the control, the minimum percentage of growth was recorded in the concentration of mineral additives 0.3 mg / l (60.99%).

When measured on day 12, the minimum percentage growth was noted in the medium with a concentration of 1 mg / l (10.79%), the maximum in the control (108.89%). The maximum number of cells for the entire observation period reached a concentration of 0.5 mg / l (8986 ± 120 10³*cells / ml). The difference between the minimum (0.1 mg / L) and maximum (0.5 mg / L) number of cells was 46.35%.
At a concentration of the mineral supplement of 0.1 mg / L, the pattern of cell growth repeated that in the control. Significant differences in the dynamics of growth of culture were not identified. From which we can conclude that such a low concentration of trace elements is insufficient to stimulate the growth of algae culture.

A smaller increase at a drug concentration of 1 mg / l may be due to the toxic effects of iodine and copper ions, the amount of which per 100 ml was respectively: 0.1 and 1.3, which are critical for the development of culture and inhibit the division processes [12].

From the graph it is seen (Figure 1A) that the presence of a mineral additive in the substrate changes the dynamics of the growth rate, shortening the period of reaching maximum productivity.

![Figure 1. Change in the concentration of algae in the experiment: A- percentage increase algae concentration; B- cell concentration; black triangle- 0.5 mg/; white triangle- 0.1 mg/l; white square-0.3 mg/l; black square- control; black cross- 1 mg/l.](image)

In the conditions of the accumulative culture, this can lead to accelerated aging, which requires a more frequent replacement of the nutrient substrate. This feature of growth when adding a mineral preparation under conditions of mass cultivation will not be weakened by how much a constant change of solutions and extraction of biomass takes place. The graph (Figure 1A) shows that the most effective concentration of the drug is 0.5 mg / L. At this concentration, in contrast to 0.3 and 1 mg / l, the growth effect is maintained throughout the entire experiment (Figures 2,3).

![Figure 2. The cenodia of S. quadricauda is in control for different start (A) and end (B) experiences.](image)

There were no morphological changes in the control over the entire study period (Figure 2A, B). Most of the cenodia consisted of four cells with pronounced caudal outgrowths. In cells, along with chloroplasts, nuclear structures were well distinguished.
In media supplemented with mineral additives on day 12 of the experiment, no significant morphological differences with control were found (Figure 3). However, at a concentration of 1 mg / L bowl, colonies of two cells were found (Figure 3 A). Also, algae in all concentrations could distinguish chloroplasts.

4. Conclusions

Based on the above, it can be concluded that the “Helavit” mineral complex showed the entire spectrum of effects on the algae Scenedesmus quadricauda from inhibition at the maximum concentration (1 mg / l) due to the toxic effect, to growth stimulation (0.5 mg / l).

The maximum culture growth was achieved at a concentration of 0.5 mg / L on the third day of the experiment and amounted to 350.5% of the control.

Morphological changes in cells at all concentrations except 1 mg / L were minimal. At a concentration of the mineral supplement of 1 mg / L, the colonies consisted predominantly of two cells.

In the conditions of accumulative culture, the addition of a mineral additive leads to a sharp increase in the first days of the experiment. Subsequently, the growth dynamics decreases as a result of depletion of stocks of nutrients.

If our assumptions about the mechanism of stimulation are reliable, then a similar effect will be obtained by adding a mineral additive to other algae of various systematic positions.

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