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$[CaL_3][Co(NCS)_4]$ - potential agent for enhancing the productivity of *Chlorella Vulgaris* Beijer. microalga

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Abstract. The advancing of modern biotechnologies requires the development of new procedures for stimulating the biological activity of some cultures that produce substances of vital importance. Coordination compounds present an effective solution for achieving this objective. The inclusion of some biometals in the composition of the coordination compounds, as well as some organic ligands with electron-donating atoms, creates premises for the assembly of molecules that can influence biological systems. The productivity of the microalgae *Chlorella vulgaris* Beijer., cultivated on modified nutrient media and supplemented with a coordination compound containing calcium, was evaluated. It was found that increased concentrations of this compound have an inhibitory action on *Chlorella* strain. A stimulatory effect was registered when the concentrations of 10 mg/L, 5 mg/L and 1 mg/L of the tested coordination compound were administered, the biomass obtained was higher by 10.8%, 7.6% and 5.4% in relation to the control group.

Keywords: *Chlorella vulgaris*, Ca(II), biomass, biostimulator, cultivation.

$[CaL_3][Co(NCS)_4]$ - potențial agent pentru sporirea productivităţii microalgei *Chlorella Vulgaris* Beijer

Rezumat. Dezvoltarea biotehnologiilor moderne solicită elaborarea noilor procedee de stimulare a activității biologice a unor culturi producătoare de substanțe cu importanță vitală. Compușii coordinativi prezintă o soluție eficientă pentru realizarea acestui obiectiv. Includerea în componența compușilor coordinativi a unor biometale, precum și a unor liganzi organici cu atomi donori de electroni crează premise pentru asamblarea unor molecule ce pot influența sistemele biologice. A fost evaluată productivitatea microalgei *Chlorella vulgaris* Beijer., cultivată pe medii nutritive modificate și suplimentate cu un compus coordinativ cu conţinut de calciu. S-a constatat că concentraţiile sporite ale acestui compus au acţiune inhibitoare asupra tulpinii *Chlorella*. Efect stimulator s-a înregistrat la administrarea concentraţiilor de 10 mg/L, 5 mg/L și 1 mg/L a compusului coordinativ testat, unde biomasa obţinută a fost mai mare cu 10,8%, 7,6% și 5,4% în raport cu martorul.

Cuvinte-cheie: *Chlorella vulgaris*, Ca(II), biomasă, biostimulator, cultivare.

1. Introduction

The growing needs of society in substances of natural origin that possess properties to influence biological systems require new solutions for increasing the productivity of certain cultures. Algae are a rich natural source of bioactive compounds, which have different applications in agriculture, pharmaceutical, food, cosmetic and perfumery industries, etc. Microalgae and cyanobacteria biomass extracts, as well as the filtrates resulting after their cultivation, can be used as biostimulators and antimicrobial substances in cultivation of some crops. These substances can have a positive impact on some organisms with an important role in substituting synthetic products with toxic action on the environment and human health [6; 8]. A number of factors, including the chemical one, can influence the synthesis of biologically active substances. A class of chemical compounds with a special potential are the coordination complexes which, due to the diversity of their composition and structural architecture, show various useful properties, including stimulation of biochemical processes in some species of algae [2; 3]. Combining in one compound one or more bioactive metal ions, as well as organic molecules containing various functional groups, creates favorable prospects for expressing the synergistic effect of metal complexes on the physiological systems of microorganisms.

Chlorella vulgaris Beijer. is a single-celled autotrophic protist with a coccoidal structure. The cells are spherical or ellipsoidal, solitary of 2.2-7.5 μ in diameter (or aggregated), with thin and smooth cellulosic walls, which do not gel. In some senescent cells (larger in size – 10.0-13.3 μ) the vacuoles can be observed [5]. They have a parietal cupular chromatophore, with a single pyrenoid (sometimes missing) surrounded by 2-4 starch granules. *Chlorella* reproduces mainly asexually via 2-8 or, rarely, 16 spores, which are formed in autosporangia (mother cells). Autosporangia have a spherical-ellipsoidal shape with a diameter of 7-8 μ. It is a α-mesosaprobe species commonly distributed in all types of freshwater pools. It can be found on all types of soil. *Chlorella vulgaris* is an autotroph, but under conditions of excess organic substances dissolved in the water tank, it switches to heterotrophic mode of nutrition. This species has a high tolerance to environmental conditions, increased adaptability; therefore, it can easily be cultivated in controlled laboratory conditions, serving as test object in various physiological, biochemical, biophysical, genetic researches, etc. [1].

The purpose of this work is evaluating the action of the coordination complex containing calcium and cobalt metal cations $[CaL_3][Co(NCS)_4]$ (L_3 – dimethylpyridine-2,6-dicarboxylate) on the accumulation of the microalga *Chlorella vulgaris* Beijer. biomass.

2. Materials and Methods

Heterobimetallic complex $[CaL_3][Co(NCS)_4]$ was obtained in the reaction of 2,6-pyridinedicarbonyl dichloride with calcium(II) thiocyanate tetrahydrate and cobalt(II) thiocyanate trihydrate in methanol, according to the method described in the literature [10, 11]. The synthesis of the coordination complex was carried out in the Laboratory of Coordination Chemistry of the Institute of Chemistry of the MSU.

The biological investigations were carried out in the "Ecological Biotechnologies" scientific laboratory of "Ion Creanga" State Pedagogical University. The *Chlorella vulgaris* Beijer. microalga strain supplied by the Institute of Microbiology and Biotechnology (deposited in the National Collection of Nonpathogenic Microorganisms) served as the object of study. Cultivation was carried out in 100 mL Erlenmeyer flasks closed with cotton plugs with periodic slow stirring. Borsch liquid medium with the following chemical composition was used as a nutrient substrate (g/L): NH_4NO_3 - 0.1; KH_2PO_4 - 0.04; $FeSO_4 \cdot 7H_2O$ - 0.00001; $MgSO_4 \cdot 7H_2O$ - 0.04; $CaCl_2$ - 0.02; solution of microelements - 1.8 mL (g/L: H_3BO_3 - 2.86; $MnCl_2 \cdot 4H_2O$ - 1.82; $ZnSO_4 \cdot 7H_2O$ - 0.222; MoO_3 - 0.01764; NH_4VO_3 - 0.02296) [9].

In the case of the experimental series, the liquid culture medium was supplemented with $[CaL_3][Co(NCS)_4]$ coordination complex in concentrations of 50 mg/L, 10 mg/L, 5 mg/L and 1 mg/L.

The amount of inoculum was 0.625 g/L of fresh biomass [4]. The Erlenmeyer flasks inoculated with *Chlorella* chlorophyte were placed on special racks under artificial light of about 4000 lx and a temperature of 27°C.

After 8 days of action of the coordinating compound, the *Chlorella vulgaris* strain was subjected to study. The statistical processing of the data obtained was carried out using the "STATISTICA 7" software, and the standard error of the mean was also determined. The productivity of *Chlorella vulgaris* chlorophyte was determined according to the current methodology [6; 7].

3. Results and Discussions

For the successful cultivation of microalgae under controlled conditions, providing them with the chemical elements necessary for mineral nutrition is of great importance. For the synthesis of proteins, carbohydrates, lipids and other cellular components, most of them require such macroelements as N, P, K, Mg, S, Ca, etc., as well as the microelements Fe, Mn, B, Sr, Cu, Zn, Ba, Ti, Mo et al. Under favourable nutritional conditions microalgae cultures accumulate impressive amounts of biomass in a short time [6].

The investigations carried out demonstrated that the calcium coordination compound content added to the Borsch liquid nutrient medium has a different stimulatory/inhibitory action on *Chlorella vulgaris* chlorophyte. The result depends primarily on the concentration of the chemical complex used, as well as on the light intensity, temperature and other abiotic environmental factors that accompany the cultivation process. Thus, after 8 days of *Chlorella vulgaris* Beijer strain cultivation on the Borsch culture medium supplemented with the calcium coordination compound, a greater amount of biomass was detected in the experimental series with the calcium compound concentration ranging from 1 mg/L to 10 mg/L. The optimal concentration of the calcium coordination complex with a stimulating effect on the productivity of *Chlorella vulgaris* strain proved to be 10 mg/L, where the fresh biomass obtained was of about 7.38 mg/L, being 10.8% higher than the accumulated biomass in the control series (Tab. 1).

The accumulated biomass of the microalgae tested in this experimental series after 8 days of cultivation was about 12-fold higher compared to the one initially administered in Erlenmeyer flasks. The microalgae productivity decreased together with concentration of the complex. In the case of series supplemented with the calcium compound in a concentration of 5 mg/L, the productivity of the chlorophyte was about 7.17 g/L, while at the concentration of 1 mg/L it accumulated about 7.02 mg/L, exceeding the control values by 7.6% and 5.4%, respectively. The complex administered in concentrations lower than 1 mg/L had an insignificant stimulatory effect and the fresh biomass accumulated by the *Chlorella* strain was approximately at the respective level in the control series.

Table 1. Fresh biomass accumulated by *Chlorella vulgaris* when treated with the $[CaL_3][Co(NCS)_4]$ compound

Nr.	Series		Starting fresh biomass, g/1000 mL	Fresh biomass after 8 days of cultivation, g/1000 mL		Δ
				x±mx	σ	
1.	Control		0.625	6.66±0.18	0.36	-
2.	$[CaL_3][Co(NCS)_4]$	50 mg/L	0.625	6.04±0.41	0.72	-9.3
3.		10 mg/L	0.625	7.38±0.08	0.17	10.8
4.		5 mg/L	0.625	7.17±0.18	0.36	7.6
5.		1 mg/L	0.625	7.02±0.10	0.20	5.4

In the experimental series with the calcium complex supplemented to the Borsch culture medium in a concentration of 50 mg/L, a lower productivity of the *Chlorella* culture was

recorded, the biomass accumulated being by about 6.04 g/L, or 9.3% lower than the values in the control group. Thus, increased concentrations of the complex inhibit the growth and development of the *Chlorella vulgaris* strain.

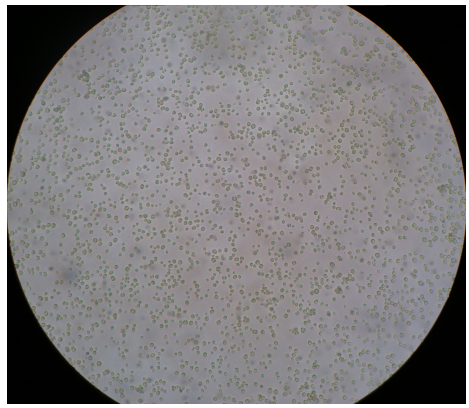

Figure 1. *C. vulgaris* strain in series with complex 1 in concentration of 10 mg/L (400 x).

In the first days of cultivation, the *Chlorella vulgaris* strain was in the phase of latency and growth acceleration. Under the microscope, numerous small cells with dimensions of 2.5-3.0 μ (autospores), distributed among larger and senescent cells of 5.5-7.5 μ, were observed. On the 8th day of cultivation, the biomass accumulated in the series administrated with 50 mg/L of the Ca(II) had a different colour compared to the control – a green-yellowish hue, while the series treated with coordination compound concentrations of 10 mg/L, 5 mg/L and 1 mg/L biomass had a green colour (Fig. 1; 2).

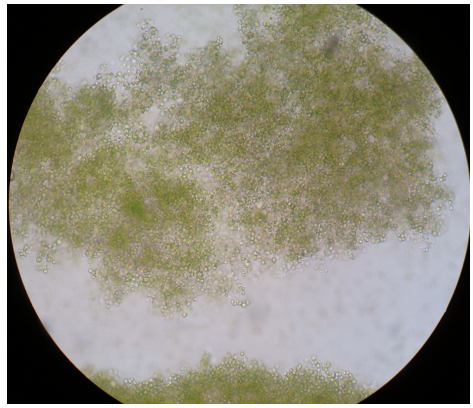

Figure 2. *C. vulgaris* strain in series with complex 1 in concentration of 50 mg/L (400 x).

In case of the samples treated with the Ca(II) complex in concentrations of 50 mg/L, the cells viewed in the microscope had a more rounded shape, were deformed, had a diameter of 4.0-8.0 μ and were included in large agglomerations (aggregates).

The results obtained confirm the importance of the calcium coordination complex content added to the Borsch liquid nutrient medium for the growth of the *Chlorella vulgaris* Beijer. chlorophyte, as well as the concentration of the administered active substance, which stimulates its development.

4. Conclusions

(1) The results obtained demonstrated that the calcium coordination compound supplemented to the Borsch liquid nutrient medium has a different stimulating action on the *Chlorella vulgaris* culture. Its effect depends primarily on the concentration of the administered compound, as well as on the abiotic factors (temperature, lighting, etc.) accompanying the cultivation.

(2) Increased concentrations of 50 mg/L proved to be inhibitory on the *Chlorella* culture. The most significant data were obtained in the series with the Ca(II) complex concentration of 10 mg/L, where the investigated strain accumulated up to 7.38 g/L of the biomass, as well as the concentration of 5 mg/L, and where a higher productivity of 10.8% and 7.6% was recorded compared to the control series.

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