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PIGMENT CONTENT OF *Chlorella vulgaris* Beij. UNDER INFLUENCE OF SODIUM SELENITE AND METALS IONS

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The aim of the research was to determine the conditions of *Chlorella vulgaris* Beij. algosubstantion enriched with selenium and bioactive metals obtaining in aquaculture. The content of seaweed pigments under the action of sodium selenite in concentrations of (calculated using Se^{4+}): 0.5, 5.0, 10.0 and 20.0 mg/dm^3 within 1, 3 and 7 days and under simultaneous action of selenite (10.0 mg Se^{4+}/dm^3) and Zn^{2+}, Mn^{2+}, Co^{2+}, Cu^{2+}, Fe^{3+} in concentrations of 5.00, 0.25, 0.002, 0.008 and 0.05 mg/dm^3, respectively, within 7 days of cultivation was investigated. The content of pigments was determined spectrophotometrically; the cell wall was separated in percoll gradient and investigated microscopically. The pigments content in *Ch. vulgaris* increases 1.5–2.5 times in comparison with the retention sample under the influence of 10.0 mg of Se^{4+}/dm^3 with and without of metal ions; chlorophylls *a*/*b* ratio increases, that is accompanied by formation of the secondary cell wall in cells, and is the sign of successful chlorella adaptation for these factors. The cultivation of chlorella enriched with selenium and bioactive metals is possible within 7 days under the influence of selenite (10 mg of Se^{4+}/dm^3) and mentioned concentration of appropriate metal ions.

Key words: *Chlorella vulgaris* Beij., sodium selenite, metal ions, pigments.

Selenium as an essential trace element in algae is involved in many metabolic processes, including the regulation of photosynthesis [1]. However, selenium compounds in high concentrations have a toxic effect, inhibit growth, disrupt metabolism and can cause seaweed death [2]. In addition, the effect of selenium on algae depends on morphological and functional features of their individual species, compounds concentration and selenium degree of oxidation in them, as well as on physical and chemical characteristics of water environment [3]. The availability of selenium compounds for microalgae substantially depends also on the content of metal ions in the culture medium [4]. Previously we found that some metal ions, especially biogenic ones, can cause a violation of physiological and biochemical processes in algae and regulate their metabolism within adaptive responses [5, 6]. The results of these studies show that certain amounts of selenium compounds have the ability to reduce the toxic effect of some metals. Selenites are considered also as a source of this trace element and they are able to interact with metal ions and promote their accumulation by cells [4, 7]. High bioaccumulation of inorganic salts and their biocomplexes with algae cell macromolecules formation *in vitro* may be used to produce dietary supplements that contain essential minerals, including selenium and ions of biogenic metals [8–10].

Algae stability as response to environmental factors changing is provided primarily by photosynthetic activity adaptation. Since the synthesis of lipids in chlorella takes place mainly in chloroplasts [11], the changes of photosynthetic apparatus are also markers for evaluating the overall functional status and effectiveness of algae adaptive reactions formation in response to the impact of cultivation conditions.

Photosynthetic apparatus of cells is primarily changed under the influence of stressors on algae [12]. Adaptive role of photosynthetic pigments — chlorophylls *a*, *b*, carotenoids, and pheophytins — is of particular interest for the study [13]. In addition, the changes that cause extreme states in cells induce the formation of secondary cell wall, which is a structure that is observed in many plant cells, located between the first cell wall and plasma membrane [12]. We have previously

found the formation of such wall, called the phenomenon of "double concentric membranes" formation [14] under the action of zinc ions if it reaches critical accumulation levels. At that from pool of metabolites necessary compounds for toxins binding withdraw that also needs biosynthetic processes strengthening [15]. So the study of changes occurring in *Ch. vulgaris* photosynthetic apparatus is an important stage for the comprehensive assessment of sodium selenite and metal ions impact on the algae.

The aim of the study was to establish the changes in pigment composition and morphology of the cell wall in *Ch. vulgaris* by action of sodium selenite separately and by its joint action with metal ions, and also to determine the optimal concentration of these substances for algosubstantion enriched with selenium and bioactive metal ions obtaining.

Materials and Methods

The study was performed with the micropopulations of algologically pure culture *Ch. vulgaris* Beij. CCAP-211/11в, which is grown in batch culture in Fitzgerald nutrient media in the modification of Zehnder and Gorham № 11 at a temperature of 22–25 °C and under 2 500 lux lighting for 16/8 hours [16]. In the experiment sodium selenite aqueous solution in concentrations of (calculated using Se^{4+}) 0.5; 5.0; 10.0 and 20.0 mg/dm^3 was added to the algae culture taken in the logarithmic growth phase. Biomass of living cells was collected at the 1st, the 3rd and the 7th day of experiment.

Studying the combined effect of sodium selenite and metal ions, we introduced selenite (calculated using Se^{4+}) — 10 mg/dm^3, and metal salts (calculated using metal ions): Zn^{2+} ($ZnSO_4 \cdot 7H_2O$) — 5 mg/dm^3, Mn^{2+} ($MnSO_4$) — 0,25 mg/dm^3, Cu^{2+}($CuSO_4 \cdot 5H_2O$) — 0,002 mg/dm^3, Fe^{3+} ($Fe_2(SO_4)_3$) — 0,008 mg/dm^3, Co^{2+} ($CoSO_4$) — 0,05 mg/dm^3. The culture which was grown in a medium without selenite and metal salts served as a control. Sampling analysis was performed on the 7th day of cultivation.

The content of chlorophylls *a* and *b* and carotenoids was determined spectrophotometrically, after their 90 % acetone extraction, at wavelengths corresponding to the absorption maximum of: 430 nm, 480 nm, 630 nm, 645 nm, 663 nm, 750 nm [16]. Pheopigments determination was performed by measuring the difference in optical density of pigment extract at 665 nm before acidification of the sample with 0.1 N hydrochloric acid and 5 min later [17].

The cell wall was isolated from algae homogenates in 40 mM Tris-HCl (pH 7.6) by the technique of Findley and Evans [18] as described previously [14]. Morphological changes in cells were fixed with the microscope MBI-15 followed by integrated digital analysis with the complex SSTU-camera Manual Vision SSD-color-WOYV00020 after their painting with chlorine-zinc-iodine reagent [19].

Results and Discussion

The maximum increase of chlorophyll a as compared to the retention sample (more than 3 times) was detected under the influence of sodium selenite in concentrations of 0.5 mg/dm^3 and 20.0 mg/dm^3 at the 7th day of action (Table 1).

Under the action of selenite at the concentration of 0.5 mg/dm^3 at the 1st and the 3rd day of exposition the content of chlorophyll *a* increased by 36.21% and 68.48% as related to control, respectively, and at the concentration of 20 mg/dm^3 — more than 2.6 times, starting from the 1st day of the experiment. Under selenite action at the concentration of 10.0 mg/dm^3 the chlorophyll *a* content at the 3rd and the 7th day increased respectively by 48.71% and 81.68% towards control. Under the action of selenite at the concentration of 5.0mg/dm^3 the reduction of this pigment content against control by 16.77% at the 1st day of exposition and by 27.37% — at the 3rd day of the action was marked. On the 7th day the amount of chlorophyll *a* was slightly higher than in retention sample.

As concerns the content of chlorophyll *b*, then under the action of sodium selenite in all investigated concentrations, its increase relating to the control was found; as in the case of chlorophyll *a* the maximal magnification was observed under the action of 0.5 and 20.0 mg of Se^{4+}/dm^3. Thus, at the 1st day of the experiment under the actions of selenite in the concentration of 0.5 mg/dm^3 the pigment content increased by 79.22% compared to control. At the 3rd and the 7th day the chlorophyll *b* content exceeds its content in retention sample more than twice. Under selenite at the concentration of 20.0 mg/dm^3 action the content of this pigment increased by 69.67 % when compared to control. At the 3rd day the content of chlorophyll *b* exceeded the benchmark 2 times. At the 7th day the amount of pigment decreased, but was higher than the value in the control by 69.58%.

Carotenoids content under the action of sodium selenite in all investigated concentrations increased (Table 2).

Table 1. **Pigments content in *Ch. vulgaris* Beij. cells under the influence of sodium selenite**

Se^{4+} concentration, mg/dm^3	Duration of chlorella cultivation with sodium selenite, days	Pigments content			
		Chlorophyll *a*, μg/dm^3	Chlorophyll *b*, μg/dm^3	Chlorophylls ratio, *a*/*b*	Pigment index: the sum of carotenoids / chlorophyll *a*
Control		160.14 ± 9.02	81.70 ± 4.98	1.96	0.22
0.5	1	218.15 ± 12.17*	146.42 ± 8.78**	1.49	0.33
	3	269.80 ± 14.69**	190.80 ± 10.45**	1.41	0.27
	7	494.20 ± 30.17**	231.80 ± 15.70**	2.13	0.20
5.0	1	133.28 ± 5.71*	106.12 ± 6.52*	1.26	0.38
	3	116.31 ± 9.21*	102.92 ± 7.43*	1.13	0.62
	7	168.71 ± 8.25	131.03 ± 9.41*	1.29	0.32
10.0	1	208.26 ± 22.16	105.79 ± 12.81	1.97	0.42
	3	309.70 ± 21.68*	137.05 ± 10.87	2.26	0.34
	7	378.37 ± 26.83**	149.52 ± 9.82*	2.53	0.27
20.0	1	431.14 ± 24.21**	138.60 ± 7.19**	3.11	0.11
	3	435.80 ± 24.62**	199.55 ± 11.05**	2.18	0.14
	7	501.31 ± 30.35**	238.50±13.92**	2.10	0.13

Note. Hereinafter: $M \pm m$, $n = 5$; differences of the indicators in comparison with the control are significant: * — $P < 0.05$; ** — $P < 0.01$.

Table 2. **Carotenoids and pheophytins content in *Ch. vulgaris* Beij. cells under the influence of sodium selenite**

Selenite concentration, mg Se^{4+}/dm^3	Duration of chlorella cultivation with sodium selenite, days	Pigments content	
		Carotenoids, mcSPU/dm^3	Pheophytins, μg/dm^3
Control		35.05 ± 1.91	154.90 ± 9.32
0.5	1	71.30 ± 3.98**	217.62 ± 11.97*
	3	73.05 ± 4.11**	273.04 ± 14.77**
	7	97.02 ± 5.70**	478.67 ± 26.86**
5.0	1	50.01 ± 2.31*	112.20 ± 10.46*
	3	72.00 ± 3.67*	107.04 ± 2.99*
	7	54.00 ± 4.16*	97.45 ± 0.77*
10.0	1	87.52 ± 7.30	211.46 ± 26.53
	3	104.20 ± 9.12	215.19 ± 3.93*
	7	103.09 ± 7.21	166.77 ± 17.78*
20.0	1	46.30 ± 2.9*	413.33 ± 23.36**
	3	62.02 ± 3.52**	320.45 ± 18.05**
	7	64.45 ± 3.40**	340.40 ± 18.50**

The maximal increase of these pigments content was observed under the action of selenite at the concentration of 0.5 mg/dm^3 over the entire exposure and at the concentration of 5.0 mg/dm^3 at the 3rd day of the experiment — more than 2 times against control. Under the action of selenite at the concentration of 10.0 mg/dm^3 at the 3rd and the 7th day of the experiment, the carotenoids content exceeded the benchmarks by 19.06% and 17.79%, respectively. Under the action of selenite at the concentration of 20.0 mg Se^{4+}/dm^3 the carotenoids content

increased by 32.10% and 76.35% at the 1st and the 3rd day, respectively. At the 7th day the content of these pigments was higher than benchmarks by 83.88%.

Regarding pheophytins, the dynamics of their content was similar to the changes of chlorophyll *a* content, namely, on the 7th day under selenite action at the concentration of 0.5 mg/dm^3, and throughout the duration of the experiment at the concentration of 20.0 mg Se^{4+}/dm^3, the increase of their number more than twice toward control was noted (Table 2). At the concentration of 5.0 and 10.0 mg/dm^3 the reduction of pheophytins content relative to control at the 7th day by 37.09% and 21.13%, respectively, was observed.

It should be noted that despite the increase in the total content of photosynthetic pigments under the influence of selenite at the concentration of 0.5 mg/dm^3 the ratio of chlorophyll *a*/*b* decreased as related to the control at the 1st day by 23.99%, at the 3rd — by 27.86%. As for the pigment index, then under the action of selenite at this concentration, due to the increased carotenoid biosynthesis versus chlorophyll *a*, its value increased: at the 1st day by 49.33%, at the 3rd — by 23.71% toward the control. At the 7th day of exposition the characteristic values of the ratio between pigments were close to the values in the control.

Under the action of 5.0 mg of Se^{4+}/dm^3 the ratio between the two forms of chlorophyll changed: at the 1st day the characteristic value decreased by 35.92% compared to control, at the 3rd — by 42.34%, at the 7th — by 34, 31%. Pigment index still rose: at the 1st day by 71.44%, at the 3rd — 2.8 times and at the 7th — by 46.24%. Perhaps this is due to the conversion of the "young" molecules of chlorophyll *a* into the chlorophyll *b*, which takes place in the dark phase of photosynthesis [20].

Selenite adding at the concentrations of 10.0 mg Se^{4+}/dm^3 as well as at the concentration of 0.5 mg/dm^3 helped increase the total amount of photosynthetic pigments. However, the ratio of chlorophylls a/*b* was higher than the benchmarks during the entire period of the experiment: at the 3rd day by14.80%, at the 7th — by 28.55%, that is due to the prevalence of chlorophyll *a* formation. Pigment index as a result of lower intensity of carotenoids formation compared to green pigments reduced: at the 3rd day — by 19.27%, at the 7th day — by 35.17%. The same tendency was observed for the action of 20.0 mg of Se^{4+}/dm^3 — a significant increase in the content of all pigments and increase of chlorophylls *a*/*b* ratio: at the 1st day — by 58.71%, at the 3rd — by 11.14%, and at the 7th — by 7.24% as compared to the control. Thus, as under the action of 0.5 mg of Se^{4+}/dm^3 the characteristic value of this ratio at the end of the exposure was approaching the characteristic values in the control. Pigment index reduced by half at the 1st day, whereas at the 3rd day it exceeded benchmarks by 34.98% and at the 7th — by 41.26%, respectively.

Chlorophyll *a*/*b* ratio can characterize the potential photochemical and biosynthetic activity of algae. Thus under stress action a decrease in chlorophyll *a* content, as less stable in comparison with chlorophyll *b*, takes place, and accordingly the relationship between these two forms of pigment decreases. When this happens, pigment index increases due to enhanced formation of carotenoids that perform a supporting and protective function in the course of photosynthesis. These changes were observed under the influence of 5.0 mg of Se^{4+}/dm^3. Chlorophyll *a* content increase, and hence the increase of chlorophyll a/*b* ratio when compared with the control is a sign of successful formation of chlorella physiological adaptation that appeared most clearly at 10.0 mg of Se^{4+}/dm^3 within 7 days of action [9, 21]. Just these conditions have been simulated in subsequent investigations.

Under the combined action of sodium selenite and metal ions in all variants of the experiment the content of green pigments in chlorella was growing (Table 3).

Thus, under the Se^{4+}+Co^{2+} action the amount of chlorophyll *a* increased by 33.33% compared to the control and was close to the values registered for selenite. Under the action of Se^{4+}+Mn^{2+} these characteristic values increased by 77.32% and 30.58%, respectively, under the Se^{4+}+Zn^{2+} action — by 96.91% and 45,01%, Se^{4+}+Fe^{3+} — by 80.83% and 33.16%, Se^{4+} and Cu^{2+} — 2.1 and 1.5 times, respectively.

The content of chlorophyll *b* increased by 94.77% compared with the control for Se^{4+} and Co^{2+} action compared to the effect of selenite separately (by 30.96%), in the event of Se^{4+} and Mn^{2+} these indicators increased 3.1 and 2.1 times, respectively, Se^{4+} and Zn^{2+} — 3.8 and 2.6 times, Se^{4+} and Fe^{3+} — 4.0 and 2.7, Se^{4+} and Cu^{2+} — 4.0 and 2.6 times, respectively (Table 3).

The content of carotenoids in chlorella cells under the action of Se^{4+} ions and metal ions also increased compared with the control (Table 4).

In this case the amount of carotenoids under simultaneous action of selenite and

Table 3. **Pigments content in *Ch. vulgaris* Beij. cells under simultaneous influence of sodium selenite and metal ions within 7 days of cultivation**

Variant of experiment	Chlorophyll *a*, μg/dm^3	Chlorophyll *b*, μg/dm^3	Chlorophylls ratio *a*/*b*	Pigment index: the sum of carotenoids/ chlorophyll *a*
Control	142.22 ± 19.03	60.59 ± 5.20	2.35	0.33
10 mg Se^{4+}/dm^3	193.13 ± 12.18	90.11 ± 9.70	2.14	0.33
Se^{4+}+Co^{2+}	189.62 ± 10.01	118.01 ± 3.37*	1.61	0.37
Se^{4+}+Mn^{2+}	252.18 ± 8.11*	185.55 ± 16.25*	1.36	0.32
Se^{4+}+Cu^{2+}	295.65 ± 9.90*	239.48 ± 9.15*	1.23	0.25
Se^{4+}+Zn^{2+}	280.05 ± 10.60*	229.79 ± 2.54*	1.22	0.27
Se^{4+}+Fe^{3+}	257.17 ± 20.30*	243.75 ± 5.71*	1.06	0.28

Table 4. **Carotenoids and pheophytins content in *Ch. vulgaris* Beij. cells under the simultaneous influence of sodium selenite and metal ions within 7 days of cultivation**

Variant of experiment	Carotenoids, мсSPU/dm^3	Pheophytins, μg/dm^3
Control	46.41 ± 6.50	211.46 ± 26.53
10 mg Se^{4+}/dm^3	64.03 ± 4.04	166.77 ± 6.57*
Se^{4+}+Co^{2+}	70.25 ± 4.02*	88.71 ± 8.16
Se^{4+}+Mn^{2+}	81.08 ± 5.28*	240.36 ± 5.26*
Se^{4+}+Cu^{2+}	74.65 ± 6.86	211.61 ± 20.02
Se^{4+}+Zn^{2+}	74.61 ± 9.23	137.11 ± 6.11
Se^{4+}+Fe^{3+}	71.00 ± 6.89	159.13 ± 2.29

metals slightly differed from that under the action of selenite separately. Under the action of Se^{4+} and Co^{2+} the content of these pigments increased by 51.37% compared with the control and by 9.7% compared with the effect of selenite separately. Carotenoids amount under the action of Se^{4+} and Mn^{2+} increased by 74.7% as compared to control and by 26.63% for selenite action, Se^{4+} and Cu^{2+} — by 60.85% and 16.59%, respectively, Se^{4+} and Zn^{2+} — by 60.76% and 16 52%, Se^{4+} and Fe^{3+} — by 52.98% and 10.89%, respectively.

Regarding pheophytins, under the selenite action their number decreased by 21.13% compared with the control. The same tendency has kept in the event of Se^{4+} and Zn^{2+} action (by 48.09% in comparison with the control) and Se^{4+} and Fe^{3+} (by 54.89%). Under the action of Se^{4+} and Cu^{2+} the pheophytins content was close to the benchmarks, and Se^{4+} and Mn^{2+} — by 13.67 % more than characteristic values in the control (Table 4).

Thus, under the combined influence of sodium selenite and metal ions when compared with the effect of selenite alone the content of chlorophylls *a* and *b* increased considerably. The content of carotenoids increased significantly relative to the control, but compared with the effect of selenite the changes of their amount in algae cells were minor. An interesting fact is that against the background of the increase in the amount of pigments under the influence of sodium selenite, the pigment index and the ratio of chlorophylls *a*/*b* has not been changed essentially, indicating that there is no negative impact of selenite on chlorella photosynthetic system. However, addition of metal salts into the medium with sodium selenite for chlorella cultivation caused chlorophylls ratio reduction by half. Pigment index was mostly lower compared with the control and only under the action of Se^{4+} and Co^{2+} has increased by 12.27%. This may be due to the increasing of chlorophyll *a* amount as a result of its degradation and also chloroplasts membrane lipids oxidation preventing by the action of selenite providing antioxidant effect, which increases in the presence of metal ions that are included in active centers of enzymes [22].

Chlorophyll *a* is the part of reaction centers and peripheral complexes of the photosystems

I and II, and chlorophyll *b* is the component of light-collecting complex of the photosystem II. Therefore, changing the ratio of chlorophylls *a*/*b* may indicate a shift of stoichiometric balance between the reaction center complexes of both photosystems and light-collecting complex of the photosystem II. It is believed that the main causes decreased the activity of the photosystem II under excess of metal ions in cells is the change of reaction center proteins structure and replacing of metal atoms in the reaction center (Mn^{2+}, Ca^{2+}, etc.) by some metal ions. Usually the target of metal ions action is a primary electron donor of the photosystem II reaction center — P-680, which is a reducing agent of pheophytin. Perhaps, metal ions, damaging P-680, cause the reduction of pheophytin in algae cells (Table 4). Pheophytins are the first electron carriers in the photosystem II. Since it is known that the amount of these pigments is directly proportional to the number of the reaction centers of the photosystem II, such changes of their content show the reduction of the functional activity of photosynthetic reaction centers [23]. Possible cause of photosynthetic electron transport inhibition by metal ions is changes in chloroplast ultrastructure, including thylakoids damage [24].

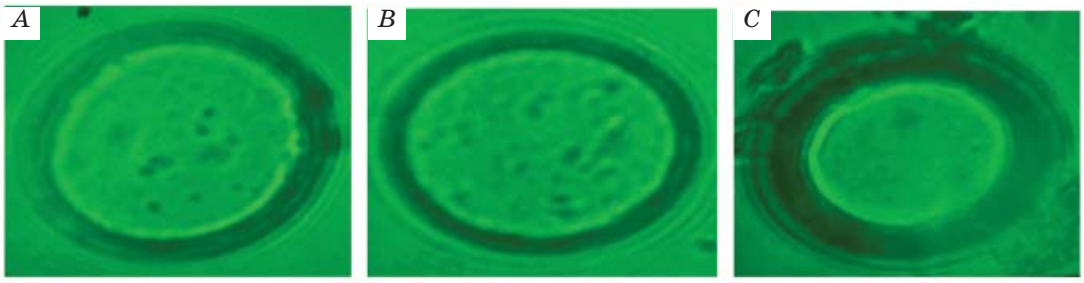
Changes in chlorophyll content may depend on the amount of carotenoids. The last, due to their antioxidant properties, are involved in the photosynthetic membranes protecting against photooxidation and in peroxide radicals neutralization, preventing lipid membranes of chloroplasts and chlorophyll degradation [22] and, consequently, increases the amount of green pigments in cells.

Adding of sodium selenite in chlorella cultivation medium was accompanied by increased content of photosynthetic pigments in the algae cells in almost all experiments. Perhaps this is due to the need of chloroplasts, which partially lost photosynthetic efficiency, updating. The latter could occur as a result of SeO_3^{2-} ions binding by lipids and chlorophyll-protein complexes [1]. It has been established that selenium is present in all lipid fractions, and the maximal amount of selenium-containing lipids has been found in carotenoids [25, 26]. Carotenoids content increase plays an important role in antioxidant activity of photosynthetic membranes that protects chlorophyll [27] and, therefore, increases its amount in cells. These changes in the functioning of *Ch. vulgaris* photosynthetic apparatus affect the whole complex of metabolic transformations.

The pigment index increase is explained by chlorophyll *a* initially destruction under unfavorable conditions, whereas carotenoids are more stable. The lasts are important protectors for green pigment and no-enzyme antioxidant components of cells. In addition, in the biotransformation of these pigments the biologically active metabolites are formed which are involved in the regulation of adaptation to the environment, including the impact of sodium selenite and metal ions. Formation of algae adaptations to stress factors is observed under the action of selenite in the concentration of 10.0 mg Se^{4+}/dm^3 and under its simultaneous action with Zn^{2+}.

Under the action of sodium selenite at a concentration of 10.0 mg Se^{4+}/dm^3 at the 7^{th} day the thickening of the membrane in chlorella cells is revealed (Figure). Dimensions of nuclear-cytoplasmatic volume of cells are changed by 3.55%, and the thickness of the outer part of the shell — by 12.23% compared to the control (Table 5). Previously it has been shown that zinc ions at a concentration of 5.0 mg/dm^3 cause the reduction of chlorella cells linear dimensions twice and the formation of the second ring of cell wall [14].

Under the simultaneous action of selenite and zinc ions at the 7^{th} day the nuclear-plasmatic volume reduction is also observed (by 4.42%) and the thickening of concentric cell wall system (by 29.14%). However, as mentioned above, the changes in photosynthetic



Photomicrographs of *Ch. vulgaris* cells:
A — control; *B* — under the action of Se^{4+} at a concentration of 10 mg/dm^3;
C — under simultaneous action of Se^{4+}+Zn^{2+}. ×9 000

Table 5. **The main morphometric parameters of *Ch. vulgaris* cells under the influence of sodium selenite at a concentration of 10 mg Se^{4+}/dm^3**

Conditions of algae cultivation	The diameter of the cells, μm	The distance from the center of the cell to the inner ring of the cell wall, μм	The distance from the center of the cell to the outer ring of the cell wall, μм	The thickness of the cell wall, μм
Control	3.948 ± 0.355	1.518 ± 0.156	1.976 ± 0.177	0.458 ± 0.124
10 mg Se^{4+}/dm^3	3.808 ± 0.491	1.39 ± 0.205	1.904 ± 0.245	0.514 ± 0.095
$Se^{4+}+Zn^{2+}$	3.564 ± 0.481	1.172 ± 0.165	1.782 ± 0.24	0.61 ± 0.095

apparatus pointed to the successful formation of chlorella cells adaptation to these factors.

Therefore, according to research the dynamics of photosynthetic pigments content, changes in their ratio as well as morphological parameters indicate chlorella adaptation in response to the impact of metal ions at sodium selenite adding in the culture medium. In this regard, at selenite concentration of 10.0 mg of Se^{4+}/dm^3 and mentioned metal ions concentration, selenium and biogenic metals enriched chlorella cultivation for 7 days can be successful. This, in turn, is essential for appropriate plants obtaining for the production of biotechnological products.

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ПІГМЕНТНИЙ СКЛАД *Chlorella vulgaris* BEIJ. ЗА ДІЇ СЕЛЕНІТУ НАТРІЮ ТА ІОНІВ МЕТАЛІВ

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Метою роботи було встановлення умов отримання в аквакультурі *Chlorella vulgaris* Beij. альгосубстанції, збагаченої селеном та біоактивними металами. Досліджували вміст пігментів водоростей за дії селеніту натрію у концентраціях з розрахунку на Se^{4+}: 0,5; 5,0; 10,0 і 20,0 мг/дм^3 протягом 1, 3 та 7 діб і за одночасної дії селеніту 10,0 мг Se^{4+}/дм^3 та іонів Zn^{2+}, Mn^{2+}, Co^{2+},Cu^{2+}, Fe^{3+} у концентраціях 5,00; 0,25; 0,002; 0,008 та 0,05 мг/дм^3 відповідно упродовж 7 діб культивування. Вміст пігментів визначали спектрофотометрично, клітинну стінку виділяли центрифугуванням у градієнті перколу і досліджували під мікроскопом. За дії 10,0 мг Se^{4+}/дм^3 окремо та одночасно з іонами зазначених металів вміст пігментів у хлорели збільшується порівняно з контролем в 1,5–2,5 раза, зростає співвідношення хлорофілів *a*/*b*, що супроводжується утворенням у клітинах вторинної клітинної стінки і є ознакою успішної адаптації хлорели до цих чинників. За концентрації селеніту 10,0 мг Se^{4+}/дм^3 та зазначених концентрацій іонів відповідних металів протягом 7 діб можливе культивування хлорели, збагаченої селеном та біоактивними металами.

Ключові слова: *Chlorella vulgaris* Beij., селеніт натрію, іони металів, пігменти.

ПИГМЕНТНЫЙ СОСТАВ *Chlorella vulgaris* BEIJ. ПРИ ДЕЙСТВИИ СЕЛЕНИТА НАТРИЯ И ИОНОВ МЕТАЛЛОВ

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Целью работы было установление условий получения в аквакультуре *Chlorella vulgaris* Beij. альгосубстанции, обогащенной селеном и биоактивными металлами. Исследовали содержание пигментов водорослей при действии селенита натрия в концентрациях из расчета на Se^{4+}: 0,5; 5,0; 10,0 и 20,0 мг/дм^3 в течение 1, 3 и 7 сут и при одновременном воздействии селенита 10,0 мг Se^{4+}/дм^3 и ионов Zn^{2+}, Mn^{2+}, Co^{2+},Cu^{2+}, Fe^{3+} в концентрациях 5,00; 0,25; 0,002; 0,008 и 0,05 мг/дм^3 соответственно в течение 7 сут культивирования. Содержание пигментов определяли спектрофотометрически, клеточную стенку выделяли центрифугированием в градиенте перкола и исследовали под микроскопом. При действии 10,0 мг Se^{4+}/дм^3 отдельно и одновременно с ионами указанных металлов содержание пигментов у хлореллы увеличивается по сравнению с контролем в 1,5–2,5 раза, возрастает соотношение хлорофиллов *a*/*b*, что сопровождается образованием в клетках вторичной клеточной стенки и является признаком успешной адаптации хлореллы к этим факторам. При концентрации селенита 10,0 мг Se^{4+}/дм^3 и указанных концентраций ионов соответствующих металлов в течение 7 сут возможно культивирование хлореллы, обогащенной селеном и биоактивными металлами.

Ключевые слова: *Chlorella vulgaris* Beij., селенит натрия, ионы металлов, пигменты.