INFLUENCE OF $Fe_2Se_3O_9 \cdot 6H_2O$ ON SOME BIOLOGICALLY ACTIVE SUBSTANCES IN *Spirulina platensis* BIOMASS UPON ITS CULTIVATION

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Abstract. The article presents the results of study of the effect of the chemical compound $Fe_2Se_3O_9 \cdot 6H_2O$ on the growth of cyanobacterium *Spirulina platensis*, evaluation of its biochemical parameters (proteins, phycobiliproteins, lipids and carbohydrates), as well as the level of iron/selenium accumulation into its biomass. The optimal concentration (30 mg/l) of $Fe_2Se_3O_9 \cdot 6H_2O$ was determined to induce biomass production of selenium and iron enriched *Spirulina platensis*.

Keywords: *Spirulina platensis,* $Fe_2Se_3O_9 \cdot 6H_2O$, selenium, iron acetate, biochemical composition.

INTRODUCTION

In recent decades, microalgae are increasingly drawing attention as a potential source of bioactive substances: protein, vitamins, essential amino acids, β-carotene, etc., as well as for their further use as valuable food additives and drugs [4, 8, 27, 34]. Their metabolic plasticity allows to provide additional opportunities for biomass quality control using directed changes in terms of growth. One of the most promising objects among them is cyanobacterium *Spirulina platensis* [31]. The wide range of applicability of spirulina include two main directions: the use of biomass itself and spirulina biomass utilization as a raw material for obtaining valuable substances.

The first direction includes various ways of using spirulina biomass as a food supplement in the diet of humans and animals, in biomedical procedures of therapeutic and preventive nature. A special place is occupied by the use of spirulina biomass as a source of trace elements (iodine, selenium, zinc, chromium, etc.) that are known to be essential for human life [5, 11, 22]. Spirulina biomass is used in various spheres of human activity: medicine, cosmetics, sports, animal husbandry, beekeeping, fish farming, poultry farming, veterinary medicine, etc. [17, 21, 25, 33].

The second direction is no less important – obtainment of substances such as amino acids, proteins, various carbohydrates, lipids, pigments, vitamins, etc from microalgae biomass. In spirulina, natural dyes (pigments), namely, phycobiliproteins (phycocyanin) come out on top. The content of such pigments in spirulina is sufficiently high (about 10%), which always makes possible the use of biomass as a raw material for the production of pigments [35].

Efficiency in the development of these directions is determined by the optimization of the controlled cultivation of cyanobacterial cells, thus, ensuring their potentially high production properties. The scientifically based possibility of adjusting the chemical composition of nutrient media is of great importance, allowing to control the biosynthesis processes in cyanobacterial cells, shifting them towards the accumulation of protein, carbohydrates, lipids, or activating the formation of certain vitamins, as well as regulating the accumulation of a particular trace element [12]. Thus, it is possible to improve the biomass quality by directed synthesis, changing the composition of culture medium by adding compounds that are not usually found in it. Of particular interest is the inclusion of selenium in the composition of spirulina biomass, since selenium is an essential trace element required for the normal development of a living organism [18, 23, 24]. Incorporated from plants into the food chain of humans or animals, selenium plays an important role in some metabolic processes and its deficiency has adverse effects. Selenium activates the process of tissue respiration, regulates redox reactions, influences immune responses, protein metabolism, especially the metabolism of sulfur-containing amino acids [28]. Its therapeutic possibilities are also widely known: it has antiviral, antimicrobial, antitumor effects, etc. [15, 16]

The numerous investigations from the last two decades on the effect of selenium on cyanobacterium *Spirulina platensis* mention only three selenium containing compounds: Na_2SeO_3, Na_2SeO_4 и H_2SeO_3 [13, 19, 26, 38, 39], which serve to improve its biochemical composition: the protein content increases, particularly, the content of phycobiliproteins. The rise in β-carotene and polysaccharide levels could also be accounted by adding selenium upon spirulina cultivation.

Of remarkable interest, the use of iron selenite $Fe_2Se_3O_9 \cdot 6H_2O$ during cultivation will contribute to the accumulation of such an important element as iron, along with selenium. The formation of haemoglobin and the enrichment of red blood cells with oxygen are the most important functions of iron in the body. This element increases the activity of many enzymes, it is involved in maintaining the normal state of the immune system. Iron deficiency has a negative effect on mental development. Iron also supports the formation and maturation of T-lymphocytes [1, 14].

The aim of the work was to study the effect of $Fe_2Se_3O_9 \cdot 6H_2O$ on the growth and evaluation of biochemical composition of cyanobacterium *Spirulina platensis*, as well as the accumulation of selenium and iron in biomass.

MATERIAL AND METHODS

The object of study. Cyanobacterium *Spirulina platensis* strain CNMN-CB-11 [32], stored in the National Collection of Non-Pathogenic Microorganisms.

The process and conditions of cultivation. The modified Zarrouk's medium with a certain ratio of macro- and microelements for normal development and growth of spirulina culture was used [29]. In addition, $Fe_2Se_3O_9 \cdot 6H_2O$ was introduced into the nutrient medium in three concentrations: 10, 20 and 30 mg/l on the first day of cultivation. These three concentrations were chosen due to the fact that a significant amount of selenium accumulates and biochemical composition does not change significantly.

Cultivation was carried out in Erlenmeyer flasks with a volume of culture liquid of 100 ml for 144 hours at a temperature of 28±2°C, illumination of 40.5 μM $m^{-2}s^{-1}$, pH of 9.5-10.0, that is, the necessary conditions for the biosynthesis of all intracellular components of *S. platensis*.

During cultivation, the following process parameters have been observed: inoculum – 0.4-0.45 g/l ADB (absolutely dry biomass); temperature of 28-32 °C, pH-optimum of medium 8-10, illumination (during growth of culture at light) of ~ 37-55 μM photons/m^2/s. The culture was stirred daily for 2 hours on a universal WU-4 laboratory shaker with the oscillation frequency of 2500 hz. The duration of the cultivation was 7 days.

Methods for the determination of the biochemical content of spirulina biomass.

Spirulina productivity: The spirulina productivity in the experimental variants and control was determined photometrically with the recalculation of the cell mass to absolute dry biomass (ADB). Productivity was expressed in g/l absolute dry biomass (ADB) and in % relative to control, in order to compare research results [29, 30].

The protein content in biomass was determined spectrophotometrically by the Lowry method using Folin-Ciocalteu reagent [20].

The carbohydrate content was determined based on the dehydration of hexoses in the presence of concentrated sulfuric acid, followed by their condensation with the anthrone reagent [37].

Lipids were determined spectrophotometrically using the phospho-vanillin reagent in the chloroformic extract of spirulina [10].

Phycobiliproteins were determined in hydric extract obtained from spirulina biomass by the method proposed [2].

Determination of selenium content in spirulina biomass was performed using AAnalyst 800 high performance atomic absorption spectrophotometer (PerkinElmer Inc., USA), according to GOST R 51309-99 „Drinking water. The measurements were carried out in Geolab Testing Laboratory of Institute of Chemistry of the Academy of Sciences of Moldova. Determination of elements content by atomic spectrometry methods" , which was based on the vaporization of solution to be analysed that contains selenium in air flame with acetylene and the measurement of flame absorbance (selenium-containing vapors) at wavelengths 196-207.5 nm. The results were compared with the reference solution with a known concentration of selenium according to the calibration curve. Sensitivity: 100.0 mcg/l. Optimal concentration: 0.5 L.

The iron content in spirulina biomass was determined according to the method described by Filippovich [9].

The determination of selenium and iron was preceded by biomass mineralization using H_2O_2 and concentrated HNO_3. Biomass was mineralized for 2-3 hours on a sand bath until a colourless solution was obtained.

All experiments were performed in triplicate. The experimental results were subjected to formal statistical analysis with the application of descriptive statistics tools (calculation of arithmetic means, standard deviation, coefficient of variation and fiducial limits), inferential statistics (tests of statistical significance and validity). Calculation of statistical indicators has been conducted using the possibilities of MS Excel.

RESULTS

The results obtained in the experimental variants with spirulina cultivation in the presence of iron (III) selenite are shown in Figure 1. Thus, iron selenite exerts a biological effect characterized as a moderate stimulator on the growth of spirulina culture. In the concentration range from 10 mg/l to 30 mg/l, spirulina productivity showed values within the limits of the control sample (spirulina grown in the absence of iron selenite)) - 3-6%, or about 10-13% over the productivity level of the control. Regarding the process of selenium accumulation, it presented more active dynamics of this element accumulation in biomass. In view of preserving a good level of spirulina productivity, the selenium content increased with the concentration of iron selenite in the medium and reached maximum level at the concentration of 30 mg/l, which enhanced the content of selenium in biomass by 280 mg%. These results suggest that this compound can be used on future large-scale production of selenium-enriched spirulina. As for the accumulation of iron in spirulina biomass, it followed the same trend as selenium. The higher the concentration of $Fe_2Se_3O_9 \cdot 6H_2O$ in the medium, the more iron was accumulated in the biomass. The maximum accumulation of iron (320 mg%) was observed at a concentration of 30 mg/l.

Investigations were also conducted on the biochemical composition of spirulina biomass with the addition of $Fe_2Se_3O_9 \cdot 6H_2O$. In general, iron selenite had a positive effect on the content of the major bioactive substances in the biomass of cyanobacterium

S.platensis. First of all, the protein content was investigated. Thus, in view of obtained results (Fig. 2), it can be seen that the compound $Fe_2Se_3O_9 \cdot 6H_2O$ manifested a positive effect on protein synthesis in spirulina biomass. Iron selenite in concentrations of 10-30 mg/l generally promoted protein synthesis (4-12.8% more protein, compared to control, $P<0.05$). The highest protein content of 70.33% (12.8% more than control, $P<0.05$) was observed when adding $Fe_2Se_3O_9 \cdot 6H_2O$ with the concentration of 30 mg/l.

According to the obtained data (Fig. 3), $Fe_2Se_3O_9 \cdot 6H_2O$ had no significant effect on the content of phycobiliproteins in biomass. The level of phycobiliprotein accumulation was practically the same as in the control sample. Only with the addition of iron selenite in a concentration of 30 mg/l, the phycobiliprotein content increased slightly (by 3.6%, $P<0.05$) in comparison with the control sample.

The study of the effect of $Fe_2Se_3O_9 \cdot 6H_2O$ showed (Fig.4) that it contributed to an increase in lipid accumulation in spirulina biomass within the entire range of studied concentrations (from 10 to 30 mg/l). The addition of iron selenite in all these concentrations significantly stimulated the lipid content: 15-67% more compared to the control sample. The maximum lipid content of 7.38% ADB (67% more compared to the control, $P<0.05$) was observed with the introduction of iron selenite into nutrient medium at a concentration of 30 mg/l.

Analyzing the obtained results (Fig.5), one can observe that $Fe_2Se_3O_9 \cdot 6H_2O$ in the studied concentration had a positive effect on the synthesis of carbohydrates. Their content increased by 14-26.4% compared to the control, depending on the concentration of iron selenite in the medium. The minimum input concentration of 10 mg/l contributed to a maximum increase of carbohydrate content by 14.28% ADB (22% higher than in the control, $P<0.05$) in spirulina biomass, and with the further increase in concentration to 30mg/l, the carbohydrate content decreased, but still remained higher than in the control sample.

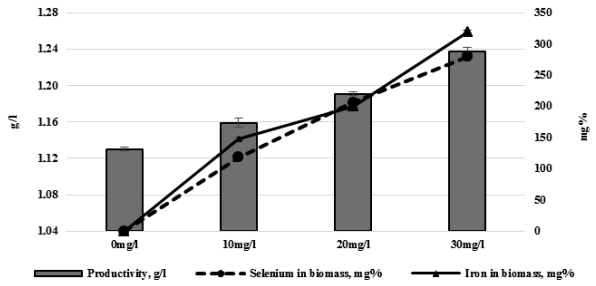

Figure 1. Productivity of *S. platensis* and the level of selenium/iron accumulation in biomass to its cultivation in the presence of iron selenite – $Fe_2Se_3O_9 \cdot 6H_2O$

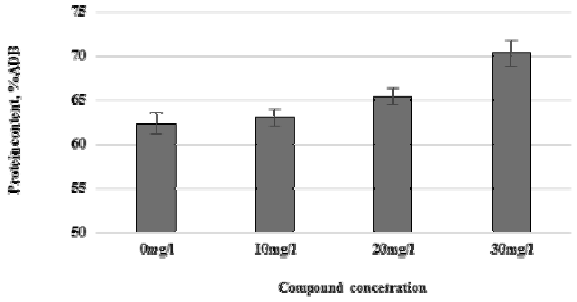

Figure 2. Protein content in *S. platensis* biomass grown in the presence of iron selenite $Fe_2Se_3O_9 \cdot 6H_2O$

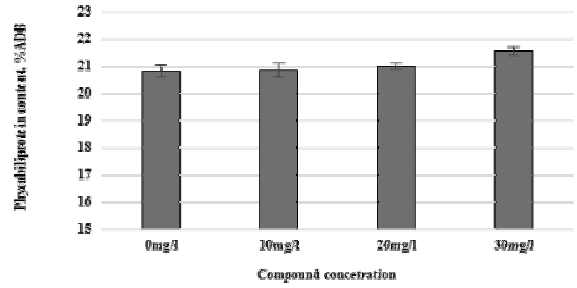

Figure 3. Phycobiliprotein content in *S. platensis* biomass grown in the presence of iron selenite $Fe_2Se_3O_9 \cdot 6H_2O$

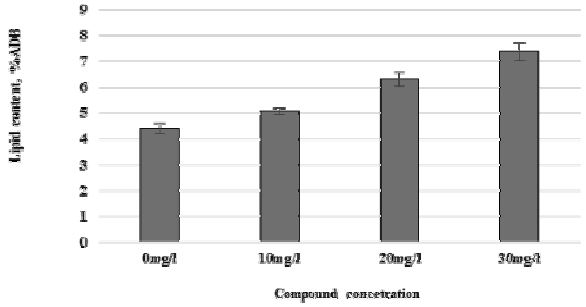

Figure 4. Lipid content in *S. platensis* biomass grown in the presence of iron selenite $Fe_2Se_3O_9 \cdot 6H_2O$

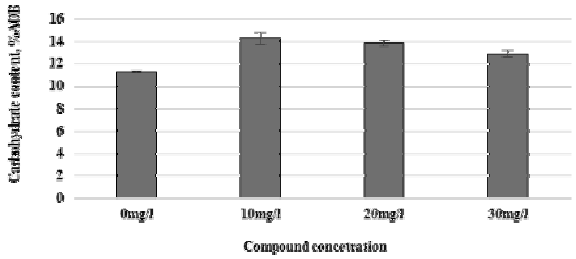

Figure 5. Carbohydrate content in *S. platensis* biomass grown in the presence of iron selenite $Fe_2Se_3O_9 \cdot 6H_2O$

DISCUSSIONS

Studies geared toward elucidating the bioaccumulation mechanisms of metals in algal and cyanobacterial biomass indicate the involvement in the accumulation process of the membrane and cell wall, as well as the formation of metal bonds with cytoplasmic ligands, phytochelatins and metalloproteins and other intracellular molecules. The functional groups involved, such as -OH (hydroxyl); -PO_3O_2 (phosphoric); -NH_2 (amine); -COOH (carboxylic); -SH (sulfhydric) not only have each a

specific dissociation constant, but also belong to different classes of organic substances – proteins, glycolipids, peptidoglycans, polysaccharides, etc. Cyanobacteria and microalgae, and, in particular, *Spirulina platensis* offer through biomass these bioactive components, considered as donors of active functional groups, which are involved in the processes of bioaccumulation and biotransformation of inorganic forms of metals into organic ones [7, 19].

According to some authors, the forms of selenium accumulated in spirulina biomass are: organic selenium (up to 80-85% of accumulated selenium) and two inorganic forms – (1) selenium (IV) (up to about 13%) and (2) selenium (VI) (about 1%), showing that in the process of spirulina growth most of the inorganic forms of selenium are converted into organic ones [3, 39].

The accumulation of metal ions depends on external concentration of metal ions in the solution until their concentration leads to toxic effects and which leads to decreased performance of bioaccumulation [36].

The compound studied in the present work is composed of selenium and iron. There is no data on the simultaneous effect of selenium and iron on the growth of cyanobacterium *Spirulina platensis*, there is only information on the effect of a particular compound separately. Some investigations on the influence of iron (III) heterotrinuclear coordination compounds demonstrate a positive effect on the growth dynamics of cyanobacteria, and some of them ([Fe_3O-Gly] and [Fe_3O-Ala]), in certain concentrations, contribute to an increase in the amount of biomass of up to 50% as compared to control. The author also notes that with an increase in the concentration of iron (III) heterotrinuclear coordination compounds, the level of iron accumulation in the biomass of cyanobacterium *Spirulina platensis* increased. [40]. As for selenium, a group of Chinese researchers [19] showed that sodium selenite in concentrations not exceeding 400 mg/l stimulates the growth of spirulina, and this effect is most pronounced in the concentration range of 5-40 mg/l

The same dynamic was observed in this study: the chemical compound $Fe_2Se_3O_9{\cdot}6H_2O$ with an increase in concentration contributes to an increase in the productivity of spirulina (at a concentration of 30 mg/l an increase of 13% compared with the control was recorded). The same trend is observed in the accumulation of selenium and iron in the biomass of cyanobacteria: with an increase in the compound concentration, the level of accumulation of these trace elements in biomass was also increased (Fig. 1).

In our investigation iron selenite had a positive effect on the content of protein in the biomass of cyanobacterium *S. platensis* (Fig. 2). $Fe_2Se_3O_9{\cdot}6H_2O$ in concentrations of 10-30 mg/l generally promoted protein synthesis (4-12.8% more protein, compared to control). The highest protein content − 70.33%, (12.8% more than control) was observed when adding $Fe_2Se_3O_9{\cdot}6H_2O$ with the concentration of 30 mg/l. Accoding to Ciumac et al [6] some Fe(III) coordination compounds have a stimulating effect on protein synthesis, depending on the concentration and nature of the substance. Yuhui Q. and Shutian Sh. also observed a positive effect of sodium selenite on protein accumulation at concentrations of 10 and 50 mg/l, while the protein content increased by 5.13% and 6.97%, respectively [38].

According to the obtained data (Fig. 3), $Fe_2Se_3O_9{\cdot}6H_2O$ had no significant effect on the content of phycobiliproteins in biomass. The levels of phycobiliproteins were practically the same as in the control sample. Only with the addition of iron selenite in a concentration of 30 mg/l, the phycobiliprotein content increased slightly (by 3.6%) in comparison with the control sample. Huang et al. [16] reported an increase in the content of phycocyanin and alophycocyanin by 12% and 15%, respectively, as compared with the control at spirulina cultivation in the presence of Na_2SeO_3 in concentration of 10 mg/l. As for the iron compounds, they have a positive effect on the synthesis of phycobiliproteins. For example, some Fe(III) coordination compounds promote an increase in phycobiliprotein content from 4 to 45% depending on the concentration and nature of the substance [40].

The study of the effect of $Fe_2Se_3O_9{\cdot}6H_2O$ showed (Fig.4) that it contributed to an increase in lipid accumulation in spirulina biomass within the entire range of studied concentrations (from 10 to 30 mg/l). The addition of iron selenite in all these concentrations significantly stimulated the lipid content: 15-67% more as compared to the control sample. The maximum lipid content of 7.38% (with 67% more compared to the control) was observed with the introduction of iron selenite into the nutrient medium at a concentration of 30 mg/l. Pronina et al. [26] also reported that Na_2SeO_3 in concentrations of 10-100 mg/l contributes to a significant increase in the content of lipids in the biomass of *S. platensis*. At a concentration of 100 mg/l of sodium selenite, the author reports a two fold increase in the lipid content.

The obtained results (Fig.5) demonstrate that $Fe_2Se_3O_9{\cdot}6H_2O$ in the studied concentrations had a positive effect on the synthesis of carbohydrates. Their content increased by 14-26.4% as compared to the control, depending on the concentration of iron selenite in the medium. The minimum input concentration of 10 mg/l contributed to a maximum increase of carbohydrate content by 14.28% ADB (22% higher than in the control) in spirulina biomass, and with the further increase in concentration to 30mg/l, the carbohydrate content decreased, but still remained higher than in the control sample. In their work, Pronina et al. also note that with an increase in the input concentration of Na_2SeO_3, the carbohydrate content was increased [26]. Iron coordinative compounds can also lead to an increased synthesis of carbohydrates in the biomass of spirulina [40].

According to the obtained data, the chemical compound $Fe_2Se_3O_9{\cdot}6H_2O$ in all investigated

concentrations manifested a positive effect on the productivity of cyanobacterium *S. platensis* and on the enrichment of biomass with selenium and iron. The best variant was observed when adding this compound at a concentration of 30 mg/l. In this case, productivity was 10% higher than in control sample, and the accumulation of selenium and iron was 280 mg% and 318 mg%, respectively. At the same time, an increase in protein content by 12.8% compared to the control was observed. The content of phycobiliproteins stayed at the control level. In case of lipid content, their maximum accumulation was observed (67% higher than in the control) at a concentration of 30 mg/l. The level of carbohydrates at all concentrations was higher compared to the control.

Thus, this compound containing both selenium and iron does not negatively affect the cyanobacterium *Spirulina platensis*, but rather leads to an increase in the content of some biologically active substances and allows to obtain biomass enriched with selenium and iron. In conclusion, the chemical compound $Fe_2Se_3O_9 \cdot 6H_2O$ can be used to obtain *Spirulina platensis* biomass enriched with selenium and iron. This biomass can be used as a source of bioactive preparations with antimicrobial, antiviral, antitumor and, also antianemic properties.

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