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Application of Blue-green Alga Spirulina for removing Caesium ions from polluted water

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A B S T R A C T

The presented work concerns studying a possibility of using blue-green alga (cyanobacteria) Spirulina (*Spirulina platensis)* for removal of Caesium ions form polluted waters. For this aim, tolerance of alga to Cs^+ ions, in particular, influence of different concentrations of heavy metal on ultrastructure of Spirulina cells, as well as on biomass production by Spirulina and chlorophyll accumulation in alga cells have been studied. According to obtained data, the highest concentration of Cs^+ (100 ppm) blocked biomass formation only by 25%, and the content of chlorophyll decreased by 20%. This concentration of heavy metal caused only an increase of hydrocarbons-containing space between lamella in Spirulina cells, which can be a result of accumulation of Cs^+ ions in alga cells. Thus, 100 ppm concentration of Cs^+ ions has been chosen for testing possibility of using Spirulina to clean artificially polluted water in the model large-scale experiment. The obtained data have shown that Spirulina has capability to assimilate up to 90% of Cs^+ ions from polluted water during 15 days. These results indicate that Spirulina is potentially useful for cleaning waters polluted with nonradioactive Cs^+ ions as well as with $^{137}Cs^+$ ions.

Keywords: Phytoremediation, *Spirulina platensis*, Water pollution, Heavy metals, Caesium ions, Radioactive and Stable Cesium.

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1. Introduction

Nowadays, water pollution by different chemicals especially by hazardous heavy metals and radionuclides is one of the most important ecological problem. Among them ^{137}Cs is very dangerous. Nonradioactive Caesium ions that occur in nature and are released into environment through mining and milling of ores, are only mildly toxic [1]. Both radioactive and stable cesium, when they occur in human or animal body, act the same way [2].

Presented work devotes to elaboration of method for cleaning water contaminated with Cs^+ ions by using blue-green alga Spirulina (*Spirulina platensis)*. Spirulina (*Spirulina platensis*) should have prospects for phytoremediation of waters polluted by different toxic compounds. Spirulina has unique chemical content and biological properties. This blue-green alga is characterized by reproduction and fast biomass formation in extreme conditions. Spirulina cells have rich content of proteins and peptides, and these compounds should be chelating ions of heavy metals. During the aging, the vacuoles of Spirulina cells inflate with air bubbles and as a result, Spirulina colonies float onto the water surface. As a result, Spirulina biomass will be easily (mechanically) separated from cleared water body after remediation. These factors have attracted our interest in Spirulina as to potential phytoremediator agent. It is known the information about using of algae for cleaning the environment polluted with such toxicants as heavy metals [3-

8], radioactive elements [9], fluoride ions [10] and others. There is only limited data concerning using Spirulina as a phytoremediator agent in case of water pollution with Cs^+ ions, but for this aim, Spirulina seems to have prospects, because there is a lot of information about heavy metals uptake processes by Spirulina [3-5].

In presented work, potential of Spirulina to uptake of Cs^+ ions from water solutions has been evaluated. For this aim, tolerance of alga to Cs^+ ions, in particular, the influence of different concentrations of heavy metal on ultrastructure of Spirulina cells, as well as on biomass formation and chlorophyll accumulation by Spirulina have been studied. According to the obtained data, the phytoremediation technology for cleaning of water polluted with Caesium based on application of Spirulina have been elaborated and tested via model experiments.

2. Materials and methods

2.1 Materials

The biomass of Spirulina platensis obtained via cultivation in standard Zarrouk's medium (pH – 8.7; content in g/L: $NaHCO_3$ – 16.8, K_2HPO_4 - 0.5, $NaNO_3$ – 2.5, K_2SO_4 – 1.0, NaCl – 1.0, $MgSO_4 \cdot 7H_2O$ – 0.2, $CaCl_2 \cdot 2H_2O$ – 0.04, $FeSO_4 \cdot 7H_2O$ – 0.01, EDTA – 0.08; and microelements kit A5 – 1 mL) were used in the experiments. Incubation was carried out with permanent air barbotage (rate of air flow 2 L/min), at temperature 25°C, and under following illumination conditions: a photoperiod of lighting 16L/8D (16 hours of light: 8 hours of dark), a total photosynthetic photon flux density (PPFD) of ≈15 $\mu mol \cdot m^{-2} \cdot s^{-1}$.

2.2 Methods

For measurement of fresh biomass productivity and chlorophyll formation by Spirulina, the following method was elaborated: the incubation medium was centrifuged at 1000 g during 20 min and obtained pellet was weighted. The obtained fresh biomass was treated by acetone and the content of chlorophyll was determined spectrophotometrically at 652 nm according to standard method [11].

The biomass of Spirulina in incubation medium have been measured spectrophotometrically at 750 nm [12].

The content of Cs^+ ions in the samples was determined by the method of atomic absorption (flame emission) analysis [13]. Conditions for analysis are the following: Instrument – PerkinElmer HGA900 Graphite Furnace; wavelength – 852.1 nm; slit – 0.2/0.4 nm; flame – air-acetylene; stock standard solution – CESIUM 1000 mg/L; light source – EDL; interfence – ionization controlled by addition of 0.1% KCl.

To study the influence of Cs^+ ions on cell ultrastructure, biomass of Spirulina was fixed in 2.5% glutaraldehyde and then in 1% OsO_4. After dehydration in ethanol of increasing concentrations, the samples were embedded in Epon–Araldite resin (1.5: 1.0) and poured into gelatin capsules [14]. Thin serial sections (50–60 nm) were made using an LKB III ultra-microtome, stained with 2% uranyl acetate, and analyzed by electron microscope Tesla BS 500 (Czech Republic) (specifics, transmission; resolution, 0.8 nm).

2.3 Experimental design

For estimation of influence of different concentrations of Cs^+ on growing parameters of Spirulina, alga was cultivated in standard Zarrouk's media (volume 50 mL) containing different concentrations of Cs^+ (1, 10 or 100 ppm). The initial biomass of Spirulina was 3-4 g/L. The incubation was carried out in flasks during 72 h (120 h for ultrastructural investigations), at temperature 25°C, without air barbotage, a photoperiod of lighting 16L/8D, PPFD ≈15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. After incubation the biomass productivity by Spirulina and chlorophyll content in cells by above mentioned methods were determined. The ultrastructure analysis was carried out in same samples.

The model large-scale experiment was carried out in following conditions: Spirulina was preliminary growing in Zarrouk's medium (volume 20 L) and after 7 days 20 L of Cs^+ containing solution was added. The volume of incubation medium in the beggining of incubation of Spirulina with Cs^+ was 40 L. At that moment, the biomass of Spirulina was 3.5 g/L and Cs^+ concentration was 100 ppm. The incubation was carried out in a glass container with sizes 60 x 21 x 40 (in cm, length x width x height), with permanent air barbotage (rate of air flow 2 L/min), at temperature 25°C, under following illumination conditions: a 24-h lighting (24L/0D), PPFD ≈15 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In the control variant instead of Cs^+ solution 20 L of water was added. The content of Cs^+ ions and biomass of Spirulina were determined after 5, 10 and 15 days from addition of heavy metal ions.

3. Results and Discussion

3.1. The influence of Cs^+ on Spirulina cells

The influence of different concentrations of Caesium ions (1, 10 and 100 ppm) on biomass and chlorophyll accumulation by Spirulina has been studied. The obtained results are given in Fig. 1.

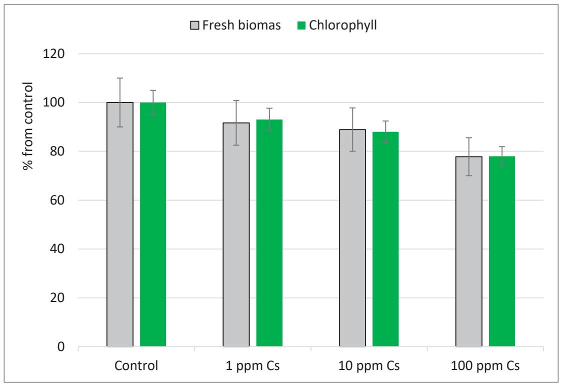

Fig. 1. *The influence of different concentrations of ceasium ions on biomass formation and chlorophyll accumulation by Spirulina. Incubation conditions are described in part 2.3.*

According to the obtained results, low concentrations of heavy metal revealed insignificant effect on Spirulina vital processes - reduction of biomass formation (by 5-6%) and chlorophyll content (by 8-9%). The high concentration of Cs^+ (100 ppm) blocked biomass production by 25%, and the content of chlorophyll decreased by 20%. According to the obtained results, Spirulina revealed tolerance to all tested concentrations of Cs^+ ions.

3.2. Study of penetration and localization of Cs^+ in Spirulina

On the next stage, the influence of Cs^+ ions on ultrastructure of Spirulina cells using electron microscopic method has been studied (Fig. 2 A-D).

As Fig. 2 shows, the influence of the highest concentration of Cs^+ ions (100 ppm) caused only an increase of hydrocarbons-containing space between lamella in Spirulina cells, which can be a result of accumulation of heavy metal ions. Thus, 100 ppm concentration of Cs^+ ions has been chosen for the model experiments.

3.3. The results of model experiment

The model experiments for elaboration new approach of phytoremediation technology for cleaning water polluted by Cs^+ ions have been carried out. For this aim, Spirulina cultivated in water artificially polluted with 100 ppm Cs^+ ions. The obtained data are given in Fig. 3.

As it seen from Fig. 3, Spirulina has capability to assimilate up to 90% of heavy metal ions from polluted water during 15 days. At the same time biomass of alga decreases by 25%. These results indicate that Spirulina is potentially usable for cleaning of waters polluted with nonradioactive Cs^+ ions as well as with $^{137}Cs^+$ ions.

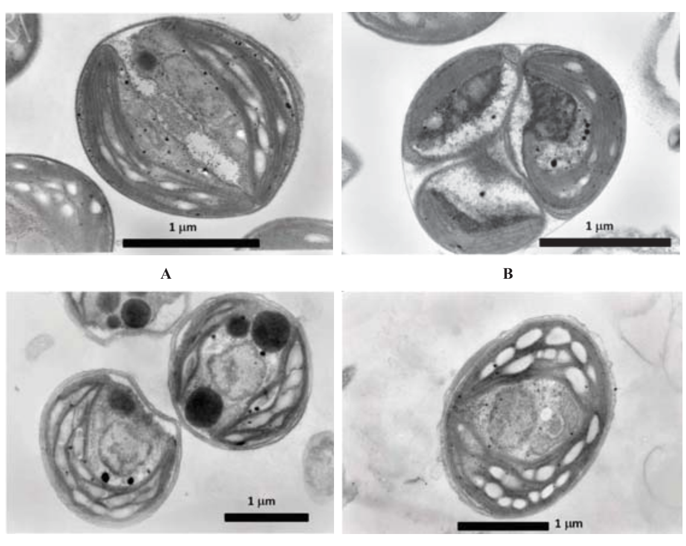

Fig. 2. *Electron micrographs of cells of Spirulina, cultivated on Zarrouk's medium without Cs^+ ions (A and B), and with 100 pm of Cs^+ ions. Incubation conditions are described in part 2.3.*

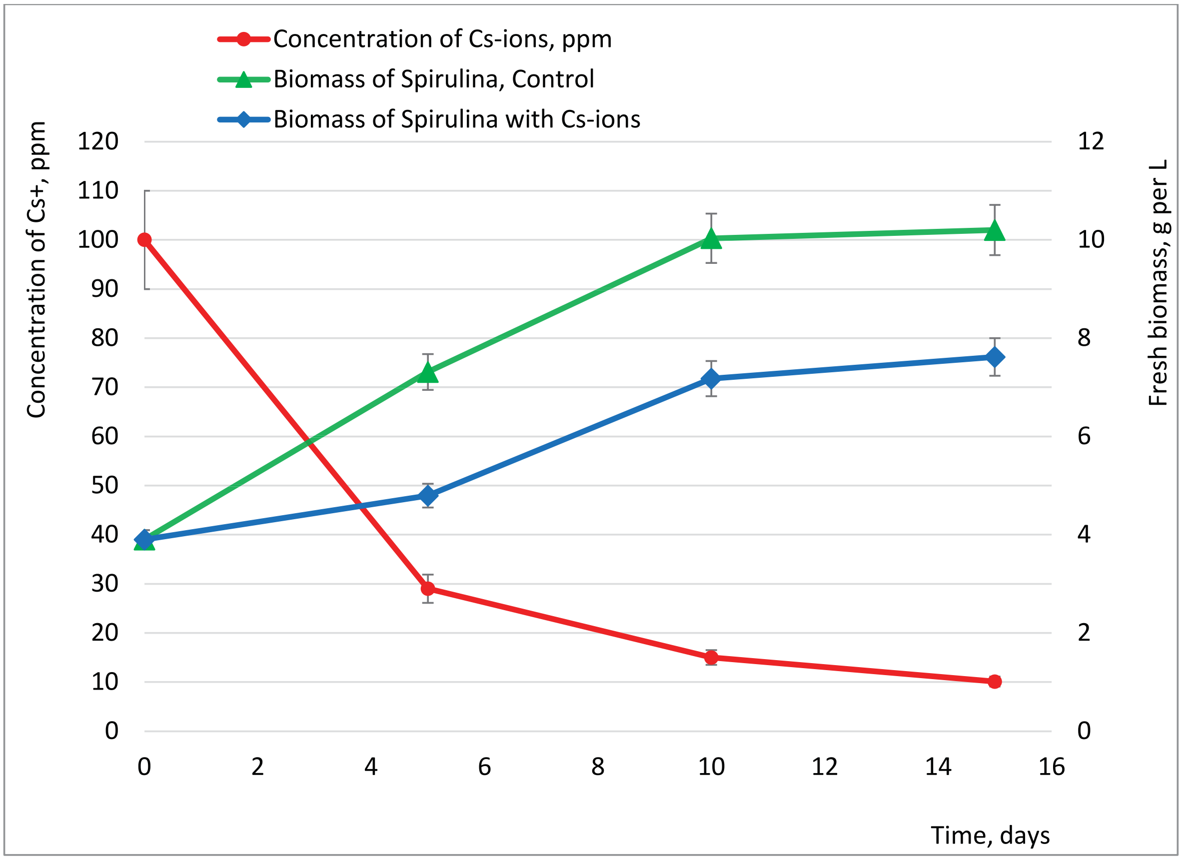

Fig. 3. *The results of model experiment for cleaning Cs^{+} containing water by using Spirulina. The conditions of experiment are described in part 2.3.*

5. Conclusions

Thus, the work carried out has shown that Spirulina cells have an ability to effectively remove Cs^{+} ions from artificially polluted water. These results can become a basis for development of a new phytoremediation technology for cleaning waters polluted with radioactive Caesium ions ($^{137}Cs^{+}$) based on application of Spirulina.

6. Acknowledgment

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