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Biotechnology

Biotechnology in Georgia for Various Applications

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ABSTRACT. The results of collaborative work carried out in the field of biotechnology at the Frank Laboratory of Neutron Physics (FLNP) of the Joint Institute for Nuclear Research (JINR) (Dubna, Russia) jointly with scientists from Georgia are presented. Using instrumental neutron activation analysis (NAA), significant results were obtained in the following directions – medical biotechnology, environmental biotechnology and industrial biotechnology. In the biomedical experiments a blue-green alga *Spirulina platensis* biomass has been used as a matrix for the development of pharmaceutical substances containing such vitally important trace elements as selenium, chromium and iodine. The feasibility of target-oriented introduction of these elements into *Spirulina platensis* biocomplexes retaining its protein composition and natural beneficial properties has been proved. The adsorption of such toxic metal as mercury by *Spirulina platensis* biomass in dynamics of growth has been studied also. NAA has been successfully applied to investigate the biotechnology of toxic Cr(VI) transformation into less toxic Cr(III) complexes by Cr(VI)-reducer bacteria isolated from polluted basalts in Georgia. This method was used to track accumulation of chromium in the bacterial cells. To monitor and identify Cr(III) complexes in these bacteria, electron spin resonance (ESR) spectrometry was employed. For the first time, the elemental composition of Cr(VI)-reducer bacteria has been studied, using epithermal NAA.The natural organic mass of vegetal origin – peat – was applied as a source of microorganisms to study the bacterial leaching of some metals from lean ores, rocks and industrial wastes. *© 2008 Bull. Georg. Natl. Acad. Sci.*

Key words: *Spirulina platensis, neutron activation analysis, microelements, pharmaceuticals.*

Introduction

At present investigations in the field of biotechnology by using various microorganisms is one of actual trends in life sciences and industry. These are obvious reasons why since the 1999 collaboration of the Institute of Physics of Georgia and FLNP JINR (Dubna) was extensively developed. Neutron activation analysis (NAA) using epithermal neutrons was used as a well-proven analytical technique for determination of elemental composition of biological objects. Due to activation with resonance neutrons, the technique makes it possible to minimize matrix effects of biological samples and at the same time to determine concentrations of over 30 major, minor and trace elements.

Biochemical investigations for substantiation of the experimental technique were carried out at the Institute of Physics (Tbilisi, Georgia). Analytical research was conducted at the IBR-2 pulsed fast reactor of the Frank Laboratory of Neutron Physics JINR (Dubna, Russia).

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Experimental techniques and analytical information treatment procedure are described in [1, 2].

Quality control of analytical measurements was carried out using certified standards for biological samples—Lichen (IAEA, Lichen 366), Bottom Sediments (IAEA SDM-2T) and Danish Moss (DK-1).

Biotechnology of blue-green algae *Spirulina platensis* (*S. Platensis*) [3–10], *Arthrobacter oxydans* [11–13] and other microorganisms [14] was substantiated by using ENAA.

I(a). *Spirulina platensis* for pharmaceuticals

Object of study. The advisability of using blue-green algae *S. platensis* in pharmaceutical industry was fairly well proved during the last decade. Owing to easy assimilability (85–95%), high protein content (60–70%) and other biologically active substances, *S. platensis* is recognized today by the experts of the World Health Organization as one of the best health-improving and therapeutic preparations [15, 16]. Spirulina, being an effective immunostimulant and having antiviral and anticarcinogenic properties, is often administered prior to, or simultaneously with medications intended to clean an organism from harmful substances and supply it with a number of useful biologically active compounds. Spirulina is a living microorganism and in the process of cell cultivation it is capable of assimilating certain amounts of some microelements from a nutrient medium and of incorporating them into the composition of its biological macromolecules. Analytical control of this process allows to establish a unique dependence between the element concentration in the nutrient medium and its content in the obtained *S. platensis* biomass. This dependence serves as a basis for substantiation of biotechnology for production of substances for pharmaceutical preparations with required doses of a given essential element. It is very important that concentrations of compounds added to the nutrient medium as loading have no influence on the conditions in which spirulina cells grow normally and retain their beneficial natural properties.

First, the multi-element composition of *S. platensis* biomass was studied by the NAA technique and the concentrations of certain elements were compared with the corresponding permissible level values [3]. The results of the investigations showed that the concentrations of such toxic elements as As, Hg, Cd, Pb, *etc*. did not exceed those permissible for the human organism, according to the data at the website: http://www.spirulina.com/SPBNutrition.html.

Biotechnology of target-oriented incorporation of certain elements into *S. platensis* biomass composition in the process of cultivation was developed using as an example such vitally important elements as Se, Cr and I.

As a rule, in metabolism and exchange processes, microelements in pharmaceuticals are best assimilated by the organism in a biologically accessible form, *i.e.* when they are included in a biological macromolecule. Hence the desirability of using biologically active biomass as a substance base, i. e. biomass of *S. platensis* which is capable of assimilating required elements in prescribed quantities.

Experimental

The strain IPPAS B-256 of *S. platensis* from the algological collection of the Timiryazev Institute of Plant Physiology of RAS was used in the experiments. The cultivation of *S. platensis* cells was carried out in the Zaroukh standard water-salt nutrient medium at pH 8.5–11, at a temperature of +32–34 °C, continuous stirring and illumination with 5000 lx sodium lamp.

In each experiment, after cultivating for 5 days, the harvest of *S. platensis* biomass was separated from the nutrient medium by filtering, rinsing and centrifugation. The resulting substance was lyophilically dried.

Samples for NAA were prepared in the form of small pellets using a special titanium mould.

Selenium. Selenic acid H_2SeO_3 of various concentrations in the range of 0.016–2770 μg/l was used as a loading of the nutrient medium to obtain selenium-containing *S. platensis* biomass. Starting from Se concentration of 100 μg/l, an intensive growth of its accumulation by cells with a possible maximum in the range of 1100–1200 μg/l is observed. For pharmaceutical purposes, the range 100–1000 μg/l seems to be the most advantageous: at high degree of Se assimilation the significant steepness of the curve enables a fairly precise determination of its doses in the substance obtained.

Visual microscopic observation of the state of culture, determination of total protein content in the biomass as well as investigation of its electrophoregrams revealed natural properties of the obtained selenium-containing biomass [9, 10]. Thus, by including Se in the composition of its biological macromolecules, *S. platensis* preserves its beneficial properties and compares favourably with other similar preparations, which are either a mechanical mixture of Se compounds with spirulina powder [22] or are obtained at such high Se concentrations in a medium that cells grow against the background of struggle for survival and no normal quality of biomass can be retained [23].

Iodine. Iodine-containing *S. platensis* biomass was cultivated in the nutrient medium with loading of potas-

sium iodide KI in the concentration range of 10^{-8}–10^{-4} g/l. The curve of iodine concentration in biomass *versus* its concentration in the nutrient medium is presented in [4].

Chromium. Chromium-containing *S. platensis* biomass with vitally essential form Cr(III) was cultivated at the loading of the nutrient medium with chromium acetate $Cr(CH_3COOH)_3$ in concentrations from 0.5 to 15 mg/l. The accumulation of toxic Cr(VI) form by spirulina at loading of the nutrient medium with potassium bichromate $K_2Cr_2O_7$ in a similar range of concentrations was also investigated [7,10]. As can be seen from the curves obtained, spirulina assimilates mainly Cr(III), while the degree of binding of Cr(VI) is approximately three times lower. As opposed to other microorganisms such as, for example, *Arthrobacter îxydans, S. platensis* prefers a non-toxic form of chromium to the toxic one, even if both forms are present in the solution.

Due to the fact that some microorganisms in the process of metabolism can interact with a number of elements and change their valence, it was necessary to verify whether under loading of the nutrient medium with Cr(III) compounds a toxic form Cr(VI) arises in the possible chain of its valence change Cr(III) → Cr(V) → Cr(VI) or not. For this purpose, the technique of colorimetric determination of Cr(VI) was applied [25]. The investigations showed that Cr(VI) was absent in all cases of *S. platensis* cultivation with Cr(III) loading.

The presence of intermediate form Cr(VI) was checked by the electron paramagnetic resonance (EPR) technique with a sensitivity of the order of 5×10^{-10}g of Cr(V). The obtained results show the absence of a resonance signal, which is typical of Cr(V), in all samples under investigation.

At loading concentrations within 5–12 mg/l, the curve of Cr(III) accumulation in *S. platensis* biomass does not reach its saturation level [10]. This makes it possible to obtain optimal chromium doses in the resulting biomass and to recommend 30–100 μg/l for food supplement and 200–250 μg/l for therapeutic and prevention purposes.

The microscopic control of the cytological state of the culture as well as of protein content of the obtained biomass demonstrated in all cases that the normal state of *S. platensis* and, consequently, its natural beneficial properties are retained.

The performed investigations demonstrated that the cultivation of *S. platensis* cells under selected conditions allows target-oriented introduction of the required elements (Se, Cr, I, *etc*.) into the composition of biological macromolecules with preservation of their protein composition and natural properties of biomass. With the application of NAA the curves of concentrations of necessary elements in the resulting *S. platensis* substance *versus* concentrations of these elements in the nutrient medium were obtained. The feasibility of accurate determination of therapeutic and preventative doses of each element according to these curves was demonstrated.

I(b). *Spirulina platensis* as a sorbent of Hg

Mercury and its compounds are widely used in various branches of industry, agriculture and medicine, finding their way into the environment in one way or another. As for toxicity, mercury holds the first position among the other heavy metals and, according to the accepted classification, it belongs to the first group of toxic substances. Thus, the necessity of studying the peculiarities of Hg interaction with living systems is quite obvious. A blue-green microalgae *S. platensis* is considered as a living system. The processes of accumulation and adsorption of mercury by the live *S. platensis* biomass as a function of Hg concentration in the nutrient medium was studied.

Non-destructive epithermal neutron activation analysis (ENAA), which represents the most efficient and sensitive analytical technique, was used for Hg determination [8].

Cultivation of *S. platensis* biomass was carried out in a standard Zaroukh medium. Mercury glycinate was used for nutrient loading. In the first series of experiments to study Hg accumulation by *S. platensis* cells, the concentrations of nutrient medium loading by mercury constituted 100, 50, 5, 1, 0.1 mg/l.

Cultivation of *S. platensis* cells was conducted for 6 days. In the second short-term series of experiments to study Hg adsorption by *S. platensis* cells, the mercury concentration of nutrient medium loading was 500 mg/l.

The dynamics of the adsorption processes, usually taking place during 1–2 h, were studied during 1 h. The mercury content in the samples was determined by ENAA at the pulsed fast reactor IBR-2 (FLNP, JINR, Dubna).

The results of experiments to study Hg accumulation from a nutrient medium by *S. platensis* biomass during cell cultivation over 6 days at various Hg concentrations are presented in [8]. In all cases the exponential character of the decrease of Hg content can be observed. Such a character of dependence seems to be clear: as the number of *S. platensis* cells grows exponentially, the number of sites for Hg(II) ion binding surpasses considerably the number of Hg(II) ions in the nutrient medium. This results in the blocking of toxic Hg

ions and their removal from the nutrient medium. Such a mechanism may serve as one of the important ways for the biosphere to "self-purify" itself of heavy metals with the help of microorganisms.

The maximum Hg content adsorbed by the *S. platensis* biomass is reached within 50 min and then a diminution of concentration is observed. Theoretical calculations of the adsorption isotherm on the basis of the experimental data were performed in accordance with the Freundlich model, which takes into account both physical adsorption and chemisorption [25, 26].

The result obtained can be regarded as a confirmation of the predominance of biosorptive processes at the initial stage of the *S. platensis* cell cultivation. If we take into account that Hg content in the control samples is 0.007 ppm [3], then it turns out that in 1–2 days of its growth the *S. platensis* biomass accumulates mercury about 500 times more effectively at Hg concentration 100 mg/l in the nutrient medium.

As for absorption, for 50 min *Spirulina* absorbs mercury about 300 times more effectively at Hg concentrations 500 mg/l. Here, it should be also noted that the *S. platensis* biomass consisting of long trichoms can be easily separated by filtration, which makes the technological process of the waste water cleaning considerably cheaper and simpler.

II. Biotechnology of Cr(VI) transformation into Cr(III)

Contamination with chromium is widespread throughout the environment because of its use in dyes, pigments, refractory material, leather tanning, and electroplating. As an environmental contaminant, chromium is found mostly in its oxidized, hexavalent form. Cr(VI) is a toxic, soluble species that moves fairly rapidly in the subsurface and that can readily enter a cell, whereas the reduced form, trivalent chromium Cr(III), is relatively insoluble, and thus not bioavailable and nontoxic. Some indigenous microorganisms, especially those residing at heavy metal contaminated sites, have developed abilities to co-exist with toxic metals. Among these microorganisms are common soil bacteria that can grow not only in the presence of Cr(VI), but they can also reduce it to Cr(III). Bacterial reduction of Cr(VI) into less toxic Cr(III) is one of most promising strategies for the bioremediation of contaminated environments. In our experiments the mechanisms of Cr(VI) transformation into Cr(III) by Cr(VI)-reducer bacteria belonging to *Arthrobacter* genera have been studied [11–13]. The tested bacteria were isolated from polluted basalts of Georgia. Besides, one of these strains (*Arthrobacter oxydans)* was isolated from Columbia basalt-rocks (USA). *Arthrobacter* species is of interest because of its high potential for the reduction and immobilization of chromium in aerobic environments [25]. *Arthrobacter* species are the member of the high mol % G+C actinomycete-coryneform bacteria [26]. The life cycle of *Arthrobacter* is characterized by its cells changing from rods to cocci (almost spherical form), *i.e.*, in the exponential phase of growth the bacterial cells are rods that change in size and shape. In the course of exponential growth, the rods get shorter and are eventually transformed into coccoid forms characteristic of a stationary phase structure [27].

Experimental

Sample preparation. The bacteria were grown in the following nutrient medium: 10 g of glucose, 10 g of peptone, 1 g of yeast extract, 2 g of caseic acid hydrolysate, 6 g of NaCl and 1 liter of distilled water. Bacterial cells were grown in 250 ml Erlenmayer flasks as a suspension. The medium was inoculated with 0.1 ml of overnight broth and incubated at 21°C, being shaken continuously. After being cultivated for 5 days, the cells were harvested by centrifugation (10,000 rpm, 15 min, 4 °C), rinsed twice in a 20 mM phosphate buffer. This wet biomass was then placed in an adsorption-condensation lyophilizer and dried following the procedure reported elsewhere [13]. The dry native biomass was finally pelletized into 5-mm pills using a special titanium press form. The elemental composition of the bacterial biomass was determined by ENAA.

For Cr(VI) reducing tests, Cr(VI) [as K_2CrO_4] was added to the nutrient medium at an early stationary phase of growth to provide the chromium concentration within a range of 50–1000 mg/L Cr(VI). After being cultivated for 5 days the cells were harvested by centrifugation (10,000 rpm, 15 min, 4 °C), rinsed twice in a 20 mM phosphate buffer and subjected to both NAA and ESR spectrometry. NAA was used to track accumulation of chromium in the bacterial cells. To monitor and identify Cr(III) complexes in these bacteria**,** electron spin resonance (ESR) spectrometry was employed.

Electron Spin Resonance (ESR) measurements of Cr(III) complexes were carried out in the Andronikashvili Institute of Physics, Tbilisi, at the RE 1306 radiospectrometer with computer-based digital systems for data acquisition and processing. Cr(III) ESR signals were measured at liquid nitrogen temperature (T=77 K) by the method described in detail in [28].

In the first set of experiments the elemental composition of *A. oxydans* isolated from the Columbia basalt-rocks (USA) was studied [12]. The chemical composi-

tion of this bacterium was compared to those from Georgia basalts [11].

The concentrations of 12–19 elements were determined in each bacterium simultaneously. The concentration range was over 8 orders of magnitude, from major to ultra-trace elements. Some similarity in the elemental composition of bacteria was observed.

In all bacteria, potassium and sodium were the dominant elements. The concentrations of both Na and K were in the range of 10^4 mg/g.

The content of Mg was about an order lower than that for Na and K and about an order higher than that for Fe and Al. In the tested bacteria the concentrations of the other elements were much less.

The relatively high contents of Fe detected in bacteria (140–340 mg/g of dry weight) indicate bacterial adaptation to the environmental conditions typical of basalts.

Elemental analysis of these bacteria also revealed that basalt-inhabiting bacteria are distinguished by relative contents of essential metals such as Na, K, Mg, Fe, Mn, Zn, Co. As, Sb, Rb, Br were found in all bacteria. In some isolates we also detected Ba and Sr and ultra-trace amounts of Au, Ag, Cs, Sm. All of these elements have to be considered as toxins. The basalt-inhabiting bacteria were found to have an obligated requirement for Co. Co was detected in all isolates. In bacterium having the maximal rate of Cr(III) formation, the concentration of Co was found to be larger. Cr(VI)-reducing ability of bacteria was tested by ESR method, showing that different isolates have different ability to reduce Cr(VI).

In the second set of experiments the dose-dependent formation of Cr(III) complexes and uptake of chromium by *Arthrobacter oxydans* – a Gram-positive bacterium from contaminated Columbian basalt rocks (USA) – were studied along with the testing under aerobic conditions of two bacterial strains of *Arthrobacter* genera isolated from polluted basalts from the Republic of Georgia [13].

The behavior of relative intensity of Cr(III) ESR signal (with a *g*-factor of 2.02 and line width of 650 Gauss, corresponding to Cr(III) hydroxide), which is in direct proportion to the concentration of Cr(III), has the same character. Besides, according to estimations for Cr(III), in bacterial cells the concentration of Cr(III) is the same order of magnitude than that of total chromium, demonstrating that the main part of accumulated Cr(VI) was transformed into Cr(III).

To quantify Cr uptake by bacteria the Langmuir-Freundlich (LF) model (solid lines) was successfully applied:

$$q = \frac{q_{\max}(bc)^n}{1+(bc)^n}.$$

Here c is concentration of metal ions, q_{max} represents the maximum metal accumulation, b is an affinity parameter of the isotherm reflecting the high affinity of the biosorbent for the sorbate, and n is an empirical parameter that varies with the degree of heterogeneity.

As follows from the Table, all tested bacteria are heterogeneous (n<1) with a similar ability of chromium accumulation. In the case of *Arthrobacter globiformis* the maximum Cr accumulation is reached at concentrations higher than 1000 mg/L of Cr(VI). In comparison with other bacterial strains, *Arthrobacter sp.* shows the highest affinity for Cr(VI). Thus, by combined implication of INAA and ESR spectrometry the behaviour of chromium in basalt-inhabiting bacteria of *Arthrobacter genera* exposed to high concentrations of Cr(VI) was studied. It was shown that the tested bacteria of *Arthrobacter genera* could efficiently detoxify high concentrations of Cr(VI). NAA measurements revealed that under aerobic conditions accumulation of chromium in bacteria is dose-dependent and its character changes significantly at higher concentrations of Cr(VI). The chromium accumulation process fits well with the Langmuir-Freundlich model.

By ESR method it was established that the main part of accumulated chromium consists of Cr(III) complexes (in *general*, Cr(III) hydroxide).

Comparative analysis of dose-dependent formation of Cr(III) complexes and uptake of chromium revealed that Cr(VI) transformation mechanism is rather similar in *Arthrobacter oxydans,* and *Arthrobacter sp.*, and is different in *Arthrobacter globiformis.*

III. Bacterial leaching of metals

The variety of microbiological species provides numerous biotechnology applications for the leaching of various metals. However, intensively developing methods of bacterial leaching for ores deal mostly with the extraction of a limited number of metals to be found in substrates at more or less increased concentrations. An attempt to study the technological process of bacterial leaching of a wide range of rare and scattered elements contained at low concentrations in lean ores, rocks and industrial wastes in Georgia was undertaken by instrumental epithermal neutron activation analysis at the IBR-2 reactor, FLNP JINR, Dubna.

Peat, natural organic mass of vegetal origin, was used as the source of microorganisms. Abundance of peat bogs in West Georgia and thus the opportunity of

Table

The R^2 and fitting parameters for the LF fit to the accumulation curves of tested bacteria [13]

Bacteria	q_{max} [mg/g]	b [L/mg]	n	R^2
A. oxydans	12.2 ± 0.41	0.004 ± 0.00018	0.32 ± 0.05	0.99
A. globiformis	15.3± 1.88	0.003± 0.0005	0.56 ± 0.11	0.99
Arthrobacter sp.	17.8± 1.05	0.006±0.0007	0.42 ± 0.09	0.98

obtaining a cheap source of microorganisms with their inherent optimal bacterial content predetermined our choice of peat as the natural source of microorganisms. This stimulates both the simplicity of replicating the technological process and the economic profitability of the proposed method [14].

Experiments were conducted on bacterial leaching of metals from various types of rocks, ores and production waste.

The peat suspension with the energetic material for microorganisms, prepared and pre-stored under anaerobic conditions for 15-20 days, is filtered and poured into a vessel with initial material and thoroughly mixed ground up to <0.1-mm-size. After 20-day leaching the solid and liquid phases are separated and the analyses of the chemical composition of the solution and the remaining unleached mass are performed.

The study of the bacterial composition of the microbial-community applied in our experiments indicates that a great number of microbes take part in metal leaching. Fifteen different groups of microorganisms, both in solid and liquid nutrient media, have been investigated (Fig. 11). The study of the behaviour of different elements during bacterial leaching was conducted on the basis of neutron-activation analysis of 30 elements of initial as well as leached metals.

Each solution version for a certain field, *i.e.* solutions of the same-type samples, distinctly retains the inherent standard content of these or other microorganisms. For example, in ores and wastes of manganese-ore deposits extraction of manganese is mainly effected by heterotrophic microorganisms existing at the expense of oxidation and consumption of organic matter and specific stimulants for manganese reduction. In the leached solutions of copper-ore concentrate and copper-pyrite and complex-ore tailings such microorganisms dominate as saprophytic fungi, butyric-acid bacteria and sulphur- and iron-reducing bacteria. The conducted research demonstrated that at bacterial leaching there started both subtraction of certain metals and a considerable enrichment of the remaining leached mass with some elements, so that now this leached mass features concentration of metals many times. The results of NAA showed that bacterial leaching may be used for extraction of such elements as Au, Se, Rh, In, Cd, Ir, Ru, Hf, Ta, Zr, as well as radioactive elements Sr, U, Th.

The results of the research into the ability of the investigated types of rocks, ores and waste to get leached enable inference concerning the perspective of applying bacterial leaching to certain rock varieties, depending on the object in view (purification of waste, extraction of scarce metals, enrichment with a certain element, *etc*.). These results present the basis for deciding on the promising trend of the further work.

The obtained data indicate the perspectiveness of further investigations aiming at practical application of the technique for enriching rocks and wastes.

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ბიოტექნოლოგია

ზოგიერთი გამოყენებითი ბიოტექნოლოგიის განვითარება საქართველოში

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(წარმოდგენილია აკადემიკოს მ. ზაალიშვილის მიერ)

ნაშრომში წარმოდგენილია ბირთვულ კვლევათა გაერთიანებული ინსტიტუტის ფრანკის სახელობის ნეიტრონული ფიზიკის ლაბორატორიის (დუბნა, რუსეთის ფედერაცია) და ქართველ მეცნიერთა ერთობლივი გამოკვლევების შედეგები ბიოტექნოლოგიის დარგში. ინსტრუმენტული ნეიტრონულ-აქტივაციური ანალიზის მეთოდის გამოყენებით ჩატარდა ექსპერიმენტები სამი მიმართულებით – სამედიცინო ბიოტექნოლოგია, გარემოს დაცვის ბიოტექნოლოგია და სამრეწველო ბიოტექნოლოგია. ბიოსამედიცინო ექსპერიმენტებში ლურჯ-მწვანე წყალმცენარის ***Spirulina platensis*** ბიომასა გამოყენებულ იქნა როგორც მატრიცა ადამიანის ორგანიზმისთვის მნიშვნელოვანი კვალური ელემენტების (სელენი, ქრომი, იოდი) შემცველი ფარმაცევტული ბიოკომპლექსების შესაქმნელად. ექსპერიმენტულად დასაბუთებულ იქნა, რომ შესაძლებელია დასახელებული ელემენტების ***Spirulina platensis*** ბიომასაში მიზანმიმართული შეყვანა ისე, რომ წყალმცენარე არ დაკარგავს თავის სასარგებლო თვისებებსა და ცილოვან შემადგენლობას.

ასევე შესწავლილ იქნა ***Spirulina platensis*** უნარი მოახდინოს ისეთი ტოქსიკური მეტალის ადსორბცია, როგორიცაა ვერცხლისწყალი.

ნეიტრონულ-აქტივაციური ანალიზის მეთოდი ასევე გამოყენებულ იქნა ბაზალტებში მობინადრე ქრომის აღმდგენი ბაქტერიების მიერ ტოქსიკური ექვსვალენტიანი ქრომის Cr(VI) ნაკლებად ტოქსიკურ სამვალენტიან ქრომად Cr(VI) ტრანსფორმაციის პროცესების შესასწავლად. ამავე დროს ეპითერმული ნეიტრონულ-აქტივაციური ანალიზის მეთოდის მეშვეობით პირველად იქნა დადგენილი ქრომის აღმდგენი ბაქტერიების ელემენტური შემადგენლობა.

მცენარეული წარმოშობის ბუნებრივი ორგანული მასა (ტორფი) გამოყენებულ იქნა როგორც მიკროორგანიზმების წყარო გაღარიბებული მადნებიდან, ქანებიდან და სამრეწველო ნარჩენებიდან ზოგიერთი მეტალის ბაქტერიული ამოტუტვისთვის. ამ პროცესების შესასწავლად ასევე ეფექტური აღმოჩნდა ნეიტრონულ-აქტივაციური ანალიზის მეთოდი.

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