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# Bioaccumulation and biosorption of some selected metals by bacteria *Pseudomonas putida* from single- and multi-component systems

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**ABSTRACT**

The effectiveness of lanthanum, chromium, uranium, and vanadium ion bioaccumulation and time-dependent biosorption from single- and multi-component systems by bacteria *Pseudomonas putida* was studied. The process of metal uptake was traced using reactor neutron activation analysis. In the experiment on accumulation, the efficiency of metal uptake was observed as follows: La > U > Cr > V (single-metal system) and U > Cr > La > V (multi-metal system). In the 2-h biosorption experiments, the highest rate of metal biosorption was noticed for chromium and uranium in single-component systems, and for uranium and vanadium in multi-metal system. Pseudo-first-order Lagergren model was applied when simulating the kinetic experiment results. Fourier transform infrared spectroscopy was used to identify the functional groups responsible for metal binding. The results of the present work have shown that *Pseudomonas putida* biomass can be efficiently implemented for industrial effluents treatment.

*Keywords:* Biosorption; Bioaccumulation; Chromium; Lanthanum; Vanadium; Uranium; Neutron activation analysis; FTIR spectroscopy; *Pseudomonas putida*

## 1. Introduction

Metals constitute a group of chemicals whose presence in aquatic ecosystems pose a risk to human health, as well as the well-being of many other living organisms [1]. Along with metals, radionuclides, especially long-lived isotopes of uranium, plutonium, and other actinides, present a serious problem for the environment. The most important anthropogenic sources of such metals and radionuclides are associated with mining, burning of fossil fuels, nuclear weapons and nuclear fuel production, manufacturing of fertilizers, metal plating and alloying, and wood preservation among others [2–5]. Industrial wastewaters contain different types of inorganic and organic pollutants in either soluble or insoluble forms [6].

Over the past decades, due to wide aquatic environmental pollution, great attention has been focused on different environmentally friendly methods of pollutant recovery or removal from wastewaters [7].

Coagulation–precipitation, ion exchange, complexation, reverse osmosis, chemical oxidation or reduction, and

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sorption are the techniques commonly applied for metal removal. The main disadvantages of these methods are their high energy consumption, high operational cost, generation of large amount of sludge and in some cases secondary pollution, low efficiency especially at metal concentrations 1–100 mg/L [1,7,8].

Application of biological objects for the removal of metal, defined as biosorption and bioaccumulation, has been recommended as a simple, economic, efficient, and environmentally friendly technique [6]. Biosorption is a metabolically passive physicochemical process of pollutant removal, while bioaccumulation is a two-step process, which includes biosorption and transport of pollutant inside cells [9]. Thus, in bioaccumulation, more binding sites are available for metal binding, and lower residual concentrations can be reached. Non-living biomass, naturally abundant biomass types, and/or industrial biomass waste can be used as biosorbents. Such types of biomass do not require maintenance and nutrition, and do not produce toxic effects on microorganisms and their sorption capacity [6,9,10]. Bioaccumulation, however, can occur only by living cells and thereby complicates the process [6,9].

Biosorption and bioaccumulation of various metal ions by different types of microorganisms: fungi, bacteria, microalgae, have been reported in the literature [11–15]. A number of investigations on radioactive waste treatment through bioprecipitation, bioreduction, and organic carbon oxidation processes are known [16–18]. The main focus of published works has demonstrated metal, and radionuclides, mainly in cationic form, biosorption, and bioaccumulation from single-metal systems. At the same time, many toxic compounds in the environment are present as anionic species. Most of the industrial effluents represent complex solutions; thus, it is important to evaluate the efficiency of metal uptake from multi-metal aqueous solutions, containing metal ions both in cationic and anion forms.

The purpose of the present study was to study the effectiveness of lanthanum, chromium, uranium (cationic forms), and vanadium (anionic form) ions bioaccumulation and time-dependent biosorption from single- and multi-component batch systems by bacteria *Pseudomonas putida*, ubiquitous bacteria frequently present in water and soil, and widely exploited for environment bioremediation [19–22], and to choose which process is more suitable for industrial application. For the first time reactor multi-element neutron activation analysis (NAA) has been applied to study the elemental content of the biomass after the accumulation and sorption processes by bacteria *Pseudomonas putida*.

## 2. Materials and methods

### 2.1. Materials

#### 2.1.1. Reagents

For the analysis, Merck reagents: uranyl nitrate salt, sodium vanadate, lanthanum nitrate, and chromic potassium sulphate of analytical grade were used.

#### 2.1.2. Biomass

Gram-negative bacterial pure culture *Pseudomonas putida* (*P. putida*) strain Ghk1 was isolated from deepwater horizons (185 m depth) of Krasnoyarsk region [23]. Cultivation of bacteria for sorption static experiments was carried out in aerobic conditions in a bioreactor with a 30-L volume in the Adkins growth medium [24] with the following composition g/L: $NH_4Cl$ – 1.0, $K_2HPO_4$ – 1.5, $KH_2PO_4$ – 0.75, $CaCO_3$ – 0.2, NaCl – 0.8, $MgSO_4x7H_2O$ – 0.1, KCl – 0.1, $NaNO_3$ – 1.0, yeast extract – 0.5, and glucose 2 at temperature 22°C–25°C, pH 7.0–7.5 and at constant stirring. The cultivation of the *P. putida* cells was conducted for 7 d. After cultivation, biomass was separated from the growth medium by centrifugation at 7,000 g and dried at 50°C (for biosorption experiment).

#### 2.1.3. Single-component system

In the present study two types of experiments were performed. The first experiment looked at metal ion accumulation, where metal salts in concentrations of 100 mg/L by metal were added to the bacterial biomass at the beginning of exponential growth phase (after the first day of cultivation) in 100 mL of Adkins growth medium [24] with 1 g/L sodium acetate and 2 g/L glucose (as a carbon and energy source). The biomass was grown for 7 d until it reached the stationary phase (the optical density [OD] stopped increasing) at the temperature 25°C and pH 7.0–7.5. The initial amount of bacterial inoculum in experiments was 1 mg/10 mL or $10^6$ cell/mL. During the first experiment biomass concentration was determined spectrophotometrically (OD650) at 650 nm. As a control, the *P. putida* biomass in the same growth medium (without any metal additions) was used.

The second experimented focused on determining the biosorption of metal by bacterial biomass. 100 mg of *P. putida* biomass was added to solution with metal concentrations 100 mg/L in teflon vessels (on a rotary shaker set at 100 rpm, temperature 25°C, and initial pH 7.0–7.3, without its further change). The dynamics of the adsorption processes was studied during 2 h. The samples were obtained after 5, 15, 30, 60 and 120 min. At the end of all experiments, the obtained biomass was filtered and dried to constant weight. All experiments were performed in duplicate. The *P. putida* biomass without any metal additions was used as a control.

#### 2.1.4. Multi-component system

Solutions containing four selected element concentrations were prepared with the same salts mentioned above in concentrations 100 mg/L by metal. The scheme of experiments was the same as for the single-component experiment.

### 2.2. Methods

#### 2.2.1. UV–Vis spectrometry

To determine total biomass concentration during bioaccumulation experiment, every day OD of obtained suspension was measured on the Varian Cary 4000 spectrophotometer at the wavelength 650 nm. The amount of biomass was calculated according to the calibration curve OD\biomass concentration in 1 L of media. The biomass concentration was determined by the dry weight method, which includes drying a sample to constant weight in a conventional oven.

*2.2.2. Neutron activation analysis (NAA)*

NAA is one of the most important nuclear techniques for samples analysis, as it allows simultaneous measuring of more than 30 elements in biological samples. Unlike other analytical techniques, NAA does not require any chemical sample pretreatment (digestion or dissolution). The main NAA advantages include independence of matrix effects, high sensitivity, and selectivity. Application of NAA is limited by its high cost, formation of hazardous wastes, and availability of nuclear reactor. To determine the elemental composition of *P. putida* biomass, NAA at the pulsed fast reactor IBR-2 (FLNP JINR, Dubna) was applied. The description of the irradiation channels and the pneumatic transport system REGATA of the IBR-2 are given in [25]. To determine vanadium content, the samples were irradiated for 3 min and measured for 15 min. In the case of chromium, uranium, and lanthanum, the samples were irradiated for 4 d under a resonance neutron fluency rate of ~$3.6 \times 10^{11}$ n $cm^{-2}$ $s^{-1}$, repacked in clean containers and measured using HP germanium detector twice (after 4–5 d and 20–23 d of decay). The lanthanum content in the samples was determined by a γ-line with the energy of 1,596.5 keV of isotope $^{140}$La, vanadium by a γ-line with the energy of 1,434.1 keV of isotope $^{52}$V, chromium by a γ-line with the energy of 320.1 keV of isotope $^{51}$Cr, and uranium by a γ-line with the energy of 228 keV of isotope $^{239}$Np.

The quality of the experiment was assured through the use of the certified reference material IAEA-433 (trace elements and methylmercury in marine sediments) and NIST Standard reference materials (trace elements in coal fly ash [1633c] and oysters tissue [1566b]), which were irradiated in the same conditions as the samples. The NAA data processing and determination of element concentrations were performed using the software developed in FLNP JINR [26].

*2.2.3. Fourier transform infrared spectroscopy*

Fourier transform infrared (FTIR) spectroscopy was used to confirm the presence of the functional groups in the samples of *Pseudomonas putida* and to observe the chemical modification after heavy metal adsorption. Infrared spectra were recorded in the 4,000−550 $cm^{-1}$ region using a Thermo Nicolet Nexus 4700 FT-IR spectrometer.

## 3. Results and discussion

*3.1. Bioaccumulation*

Microorganisms play a significant role in bioremediation of heavy metal contaminated wastewater. Bioaccumulation is a complex two-stage process, which includes pollutants bound to the surface of cell wall (biosorption) and their intracellular accumulation.

The biomass concentration values after 7 d of growth in metal-containing medium are presented in Table 1.

According to the influence of the elements on the *P. putida* growth, they can be placed in the following line: mix > C = Cr > La = U > V. Increase of biomass concentration for Cr, La, U, and mixture of metals indicates active bioaccumulation process behaviour. The concentration of biomass at the addition of metal mixture showed the highest values, which can be explained by metal chelation with cell components and intracellular/extracellular precipitation that lead to the reduction of their toxic effect and stimulation of the growth of bacteria. The lowest rate of biomass growth was observed at vanadium loading. At addition of vanadium salt to bacterial inoculum after 1 d of cultivation, the biomass yellowing was observed. $V^{5+}$ is the most toxic form of vanadium. It was suggested that at the presence of vanadate ions in the cultivation medium pseudomonads cells were exposed to osmotic shock, which leads to their damage. In Valko et al. study [27] it has been reported that the toxicity of vanadate ions is related to their capacity to induce the formation of intracellular reactive oxygen, which impairs the mitochondrial function. Low vanadium ions accumulation can be also connected with their ability to inhibit several enzymes, due to their similarity with phosphate ions [28].

Fig. 1 presents the results of metal accumulation by biomass for single- and multi-component systems. The results obtained by NAA showed the high efficiency of *P. putida* for lanthanum and uranium uptake from single-component systems. The amount of lanthanum and uranium in the biomass after 14 d of cultivation increased from 0.6 to 30,600 μg/g and from 1.2 to 22,400 μg/g, respectively.

Despite its widespread application in industry and agriculture, knowledge about lanthanum level of toxicity and possible recovery is limited. Soil bacteria *Myxococcus xanthus* was able to accumulate 0.6 mmol La/g of wet biomass and/or 0.99 mmol/g of dry biomass. Authors suggested that the main

Table 1
Values on biomass grow under metal loading

| Metal | Initial biomass concentration, mg/L | Biomass concentration on the 7th day, mg/L |
|---|---|---|
| Cr | 20 | 200 |
| V | 20 | 120 |
| La | 20 | 160 |
| U | 20 | 160 |
| Mix | 20 | 240 |
| Control | 20 | 200 |

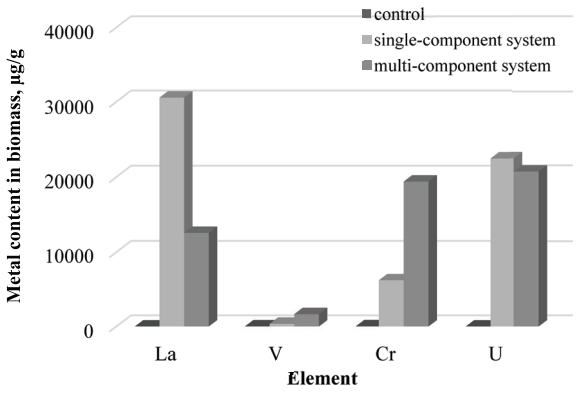


Fig. 1. Bioaccumulation of La, V, Cr, and U by *P. putida* (*T* 25°C; pH 7–7.5; $C_i$ 100 mg/L).

role in lanthanum binding by *Myxococcus xanthus* belongs to phosphate groups and the phosphoryl residues of phospholipids, lipopolysaccharides, nucleic acids, polyphosphates, etc. [29]. On the basis of the FTIR data obtained in the present study and the data presented in works [4,30], it can be concluded that phosphate, carboxyl, hydroxyl, and amine groups play important role in the lanthanum binding by bacteria *Pseudomonas putida.*

Radionuclides like uranium that have no biological function and are considered severe environmental and public health hazards due to their toxicity and long half-lives. Ion exchange may be one of the mechanisms of uranium uptake by *P. putida*. During the process of uranium accumulation by *P. putida*, the sodium content in biomass decreased from 65,700 mg/g to 21,500 μg/g and for potassium from 16,700 to 4,600 μg/g. Uranium accumulated by the cells of *Bacillus sphaericus* JG-A12 was located at the cell surface, and its uptake was associated with its binding to phosphonate groups and phospholipids from cell membrane [31]. In case of *Pseudomonas* sp. it was shown that uranium sequestration is a result of combined ion-exchange–complexation–microprecipitation mechanism [32]. The ability of *Pseudomonas* sp. EPS-5028 to uptake uranium was examined at different uranium concentration in solution. The maximum amount of uranium accumulation by cells was 55,000 μg/g [33].

The content of chromium in biomass in comparison with native biomass increased from 32 to 6,090 μg/g. The sequestration of the trivalent chromium ions occurs in a passive way, opposite to the hexavalent ions, and their toxicity can be reduced by their chelating with cell ligands with the formation of the coordination compounds [34].

In single-metal systems, the lowest rate of accumulation was observed for vanadium. Its content increased from 17.4 to 355 μg/g. Vanadium ions resemble phosphate ions and consequently can inhibit many phosphate-metabolizing enzymes [35].

In complex systems binding of metal ions on biomaterials depends on different parameters such as: electronegativity, ionic radius, potential, redox potential of these metals, and preference for the ligand binding sites [8]. La, U, and Cr were presented in the studied systems in cationic form. FTIR data have shown that abovementioned ions bind to the same sites on the wall and a competition effect could result between the metals for binding site. In this case, the biosorptive capacity for metals in complex solution can be lower than in single-metal solutions [36]. In the present study lanthanum content in biomass decreased from 30,500 μg/g (single-component system) to 12,500 μg/g (multi-component system); at the same time, the content of chromium increased 3-fold. It can be concluded that competition between chromium and lanthanum for binding sited takes place. The decrease of lanthanum content in multi-component system can be also explained by the phenomenon of negative cooperation [37]. Vanadium, present in anionic form, content increased 4.5-fold in comparison with the single-component systems. It can be suggested that vanadium ions uptake was improved in comparison with single-metal solution due to pairing of vanadate ions charge with charges of cationic forms present in the solution, thus decreasing the repulsion between the negative vanadate ions adsorbed on the surface. The improvement of Cr(VI) ions adsorbed onto lignin in the presence of zinc ions in the solution was shown in Albadarin et al. work [38].

*3.2. Biosorption*

Biosorption is a first stage of bioaccumulation process. The aim of the second type of experiment was to determine the equilibrium time for metal biosorption. The influence of pH, temperature, sorbent dosage, and initial metal concentrations will be discussed for each metal in forthcoming papers. It can be seen from Figs. 2 and 3 that the rate of sorption of all studied metals was very fast in the first 5–15 min of sorbent–sorbate interaction, after reaching equilibrium.

The Lagergren pseudo-first-order model was applied to determine the time required to reach equilibrium.

The following equation was used for calculations:

$$\log(q_e - q) = \log q_e - \frac{K_a}{2.303} t,$$

where $q$ and $q_e$ are the adsorbed amounts (mg/g) at time ($t$) (min) and at equilibrium time, respectively; $K_a$ (min$^{-1}$) is the rate constant of the first-order biosorption [39].

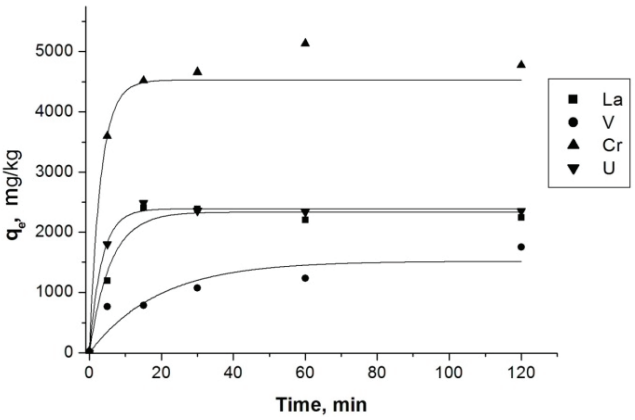


Fig. 2. Biosorption of La, V, Cr, and U by *P. putida* biomass (data for single-component systems), (*T* 25°C; pH 7–7.3; $C_i$ 100 mg/L; 0.1 g of sorbent).

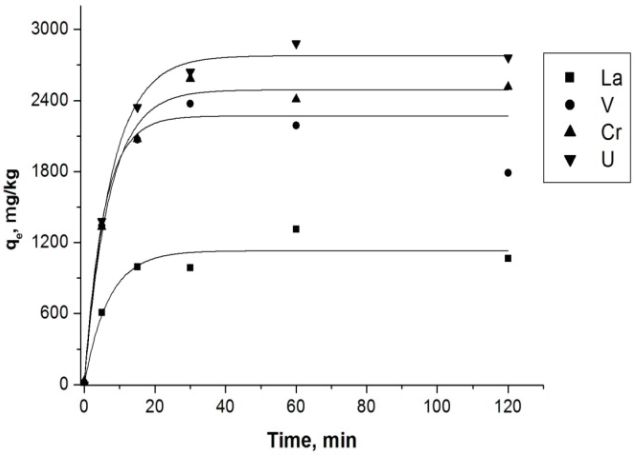


Fig. 3. Biosorption of La, V, Cr, and U by *P. putida* biomass (data for multi-component systems), (*T* 25°C; pH 7–7.3; $C_i$ 100 mg/L; 0.1 g of sorbent).

Table 2
The value of $q_e$ and $K_a$ and the correlation coefficients for the Lagergren first-order model ($T$ 25°C; pH 7–7.3; $C_i$ 100 mg/L; 0.1 g of sorbent)

| | Single-metal system | | | | Multi-metal system | | | |
|---|---|---|---|---|---|---|---|---|
| | La | V | Cr | U | La | V | Cr | U |
| $q_e$, mg/g | 2,330 ± 96 | 1,514 ± 100 | 4,529 ± 251 | 2,385 ± 35 | 1,128 ± 68 | 2,268 ± 219 | 2,491 ± 61 | 2,776 ± 44 |
| $K_a$, min$^{-1}$ | 0.17 ± 0.03 | 0.05 ± 0.001 | 0.32 ± 0.01 | 0.29 ± 0.03 | 0.15 ± 0.04 | 0.18 ± 0.09 | 0.14 ± 0.02 | 0.13 ± 0.01 |
| $R^2$ | 0.97 | 0.92 | 0.94 | 0.99 | 0.95 | 0.9 | 0.99 | 0.99 |

The value of $q_e$ and $K_a$ and the correlation coefficients for the Lagergren first-order model are listed in Table 2. It was observed that Lagergren pseudo-first-order model fit well the experimental data. The results obtained for pseudo-second-order model (nor presented) were inferior to pseudo-first-order model.

In the case of single-metal solutions (Fig. 2), the highest rate of metal uptake was observed for chromium – 5.1 mg/g (in control biomass 30 μg/g). For lanthanum and uranium, the maximum levels of metal in the pseudomonas biomass reached the values 2.4 and 3.5 mg/g, respectively. In case of vanadium, during the mentioned period, bacterial biomass sorbed 1.8 mg/g of vanadium (content in control 17.4 μg/g).

In the multi-component system (Fig. 3), lanthanum and chromium content in biomass decreased approximately twice, and the increase of vanadium content (up to 2.9 mg/g) was observed. As was mentioned above, lanthanum and vanadium binding belong to phosphate groups. Additionally, the slight increase of uranium concentration in biomass by 430 μg/g in comparison with single-metal system was observed.

In biosorption process metal sorption occurs through binding of metal ions to functional groups on the microbial cell wall. It is a very quick process. Bioaccumulation is a long two-step process, which include: biosorption (first step) and intracellular metal uptake (second step). Thus, the amount of metal accumulated during the bioaccumulation process is considerably larger than during biosorption process. In the present study, the amount of metals accumulated during the bioaccumulation process was ~20 times higher than in the biosorption experiments.

To understand better the nature of functional groups responsible for the metal uptake, FTIR analysis was performed (Fig. 4). The FTIR spectrum of V-loaded biomass showed no changes in the spectrum relative to control spectrum. The changes in the FTIR spectra of La-, Cr- and U-loaded biomass were mainly associated with the fluctuation of amino acid residues of proteins and carbohydrates in the range 1,200–900 cm$^{-1}$, where CO, CC, COC, PO, and COH groups' adsorption bands appears.

The maximum of intensive combined absorption band in the spectrum of the control sample is accounted for by the absorption band 1,026 cm$^{-1}$. In La- and Cr-loaded biomass the shift of this adsorption bands by 6 cm$^{-1}$ and its significant broadening in the low-frequency region are observed. FTIR studies revealed involvement of C=C, C=O, and O–H functional groups in binding of chromium by *Streptomyces* sp. (MB2) [40]. For U-loaded biomass the shift was more pronounced by 35 cm$^{-1}$, and a new band 924 cm$^{-1}$ related to uranyl ion is also present.

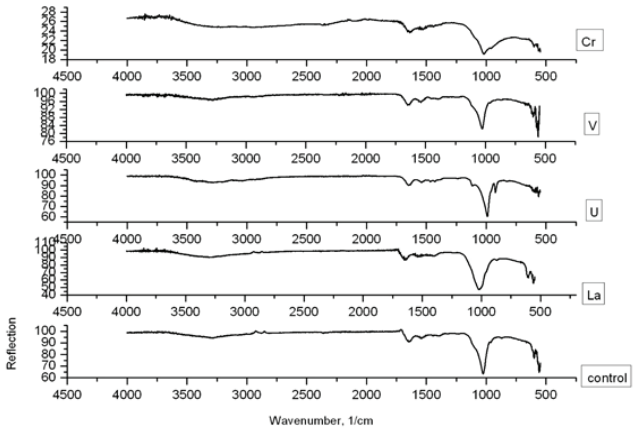


Fig. 4. FTIR spectra of *P. putida* biomass.

## 4. Conclusion

Two processes of metal uptake by *P. putida* biomass: bioaccumulation and biosorption were studied. The amount of metals accumulated during the bioaccumulation process was ~20 times higher than in the biosorption experiments. In bioaccumulation process, the metal uptake occurred in the order: La > U > Cr > V (single-component system) and U > Cr > La > V (multi-metal system). In biosorption experiments, *P. putida* removal efficiency followed the order: Cr > U > La > V for single-metal systems and U > Cr > V > La in multi-metal system. FTIR spectra showed that the main role in studied metal binding belongs amino acid residues of proteins and carbohydrides. *P. putida* biomass was found to be suitable for metal removal from monometal solutions and complex industrial effluents.

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