# INFLUENCE OF SODIUM ON ASSIMILATION PROCESS OF Cr(VI) AND Cu BY *ARTHROBACTER* GLOBIFORMIS 151B CHROMIUM-RESISTANT BACTERIUM

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The process of assimilation of Cr(VI) and Cu by chromium-resistant bacteria (*Arthrobacter globiformis* 151B) and the influence of high-concentration Na ions on this process have been studied. The bacteria are known for their property to assimilate the hexavalent chromium ions from the environment intensively, to convert them into trivalent form and to accumulate it in the cell. Thanks to these properties, it is possible to use them for detoxification of the environment, polluted by highly toxic Cr(VI). The strain of bacteria under investigation was isolated from basalt samples taken from the places highly contaminated by Cr(VI) in Kazreti. The solutions of the studied elements (Cr and Cu) and Na were introduced simultaneously into the nutrient medium. We studied the influence of different concentrations of Na ions during a different period of time of bacteria cultivation (17h, 24 h, 48 h, 96 h and 144 hours) on the process of assimilation of Cr and Cu by bacteria. The concentration of Na in the nutrient medium was 2, 3.5, 6.5 and 9.5 g $mL^{-1}$. For determination of the content of metals (Cr, Cu, and Na) in the cell, after the cultivation of bacteria, the precipitation of cells by centrifuge and the preparation of the obtained bacterial pellet for the analysis were carried out. The content of metals was measured by atom-absorption spectrometry.

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## Introduction

Metals at the excess concentrations have toxic and carcinogenic properties. It is very important to develop the technologies by means of which it is possible to remove the toxic metals from the environment. Among the most prospective methods of remediation of the polluted environment are the biological technologies based on the use of different microorganisms.[1,2]

The pollution of the environment by the materials containing Cr(VI) is an urgent problem for many countries.[3] In Georgia, the places which are most contaminated by heavy metals, are Kazreti and Zestaphoni,[4] where the concentration of Cr in the soil and water reaches several hundreds of mg $L^{-1}$ (the permissible concentration of Cr(VI) in surface waters is less than 0.05 mg $L^{-1}$.[3]

Chromium can be extremely toxic or non-toxic depending on its concentration and valence state.[5] In nature usually, it is met in trivalent and hexavalent forms, which have different transport properties. Cr(VI)- compounds are well water-soluble and toxic, while Cr(III)-compounds are less water-soluble and relatively harmless. The genotoxic and carcinogenic action of Cr(VI)-contained material is caused by their ability to penetrate rapidly into the cell, as well as by activation of this ability as a result of the intercellular reduction process.[6]

Detoxification of Cr(VI) that appeared in the environment can be made by its conversion into trivalent chromium, mainly, in the form of $Cr(OH)_3$ or makes a complex with surrounding ligands.[7] Various bacterial species (e.g., *Escherichia*, *Pseudomonas*, *Shewanella*, *Desulfovibrio*, *Bacillus sp.*)[8] are able to reduce Cr(VI). The recent researches proved that most of the studied bacterial strains are not metal resistant/tolerant. They lose their viability in co-existence of high concentration of heavy metals. Thus, it is reasonable to isolate the bacteria under investigation directly from the soil, mineral strata and water contaminated by metals.[9–15] At present, the testing of technologies based on endogenic microorganisms is carried out intensively,[16–18] providing that recently the application of biotechnologies is of high priority in the process of environment reduction in many countries.[19] The efficiency of biotransformation depends on the mechanism of bacteria–metal interaction, thus, for bacteria of any specific species, it is necessary to study this mechanism preliminarily in detail.

A significant part of experiments was concentrated on Gram-negative bacteria – a number of experiments concerning Gram-positive bacteria are comparably less. Not much is the number of experiments concerning the interaction of Gram-positive bacteria with heavy metals. According to new data,[11] Gram-positive bacteria appeared to be tolerant to higher doses of Cr(VI), as compared to gram-negative bacteria. Few are the information about the reaction

of bacteria to high doses of chromium. The mechanism of origination of chemically active intermediate (Cr(V)/Cr(IV)) products in the process of reducing Cr(VI) by bacteria, practically, is not studied. In this context, the pioneering research was carried out by Georgian investigators (N. Tsibakhashvili et al.).[20]

The vital natural medium of bacteria, which we are interested in contains, alongside with the elements under investigation (Cr and Cu), the (macro) elements that are widely spread in nature (Na, K, Si, …). These elements have an influence on the growth–evolution of bacteria, including the process of assimilation of elements (Cr and Cu) by bacteria and the biochemical process proceeding in bacteria. It is interesting to study the influence of macroelements on the process of assimilation and distribution of Cr(VI) and other elements in bacteria. The experimental material obtained as a result of the proposed and the similar investigation makes it possible to draw a certain conclusion about the biochemical processes taking place in bacteria and about the mechanisms, by which the assimilation of metals and the conversion of their compounds are made.

## Experimentals

For the object of investigation, we chose the bacteria of *Arthrobcter globiformis* 151B. As is known,[21] the bacteria of Arthrobacter family are aerobic gram-positive bacteria living in the soil. They belong to Arthrobacteria class, type – Actinomycetales. According to the existing data,[22,23] they have a high potential of remediation of the chromium-contaminated environment. The selected bacteria were removed from basalt rocks**,** taken from ecologically the most contaminated regions of Georgia (Kazreti, Zestaphoni).[24] From the chosen basalts 157 endolithic bacteria resistant to Cr(VI) were singled out, among which 33 appeared to have the ability to remediated high concentrations of Cr(VI) (about 1000 mg $L^{-2}$). The objects of this investigation are bacterial strains isolated from Kazreti basalts.

For studying the influence of Na on the process of assimilation of Cr(VI), Cu and other elements by *Arthrobcter globiformis* 151B, we cultivated bacteria in 500 mL Erlenmaier flasks in 100 mL TSB broth. We additionally introduced Na solution in the form of NaCl into some samples (flasks), thus, the concentration of Na in the nutrient medium was 2, 3.5, 6.5 and 9.5 g $mL^{-1}$. In addition, the Cr(VI) solution was added in the same samples with concentrations of 40 μg $mL^{-1}$. The nutrient medium contained the studied elements in the following concentrations: Cr – 7 and Cu – 0.06 μg $mL^{-1}$.

The cultivation of bacteria proceeded during 17 and 24 h, and 2, 4 and 6 days. After cultivation we carried out the precipitation by centrifuge (3000 rpm, 10 min, 0 °C), we poured out supernatants and the remained bacterial pellet washed in sterile distilled water. We dried the obtained biomasses by low-temperature lyophilizer and weighted them (the whole masses). From the total quantity of bacterial pellet, we took the amount necessary for analyses, weighted it (~30 mg) and put it into test tubes. In order to convert the samples into a liquid state, we added the concentrated nitric acid (1 mL) into the test tubes, heated it and after complete ashing dissolved it. The analysis of the obtained samples on the content of metals was made by an atom-absorption spectrometer (Analyst 800, acetylene-air flame).

## Results and discussion

We studied the process of assimilation of Cr(VI) and Cu by bacteria and the influence of Na ions of this process. The process of Na absorption by the bacteria *Arthrobacter globiformis* 151B was also studied. The results of measurement are given in Figures 1 – 4.

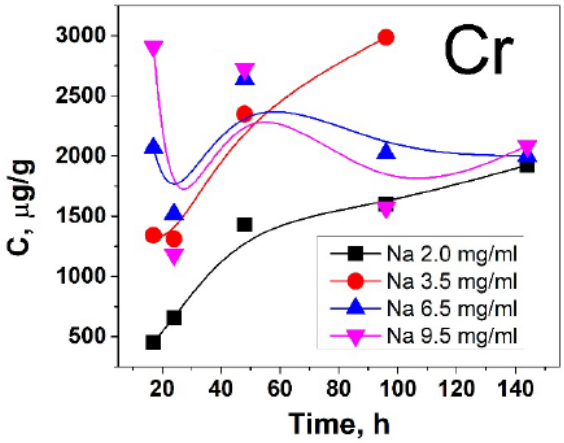


**Figure 1.** Dependence of Cr concentration (*C*, μg $g^{-1}$) in bacteria on time of growth–evolution of bacteria *t*(*h*). Concentration of Na in nutrient medium was 2.0, 3.5, 6.5, and 9.5 mg $mL^{-1}$.

As it is seen from the obtained results (Figure 1), Cr(VI) (40 μg $mL^{-1}$) added into the nutrient medium causes an abrupt increase of the content of Cr in bacteria during 4 days of their growth–evolution. In the nutrient medium with 7 and 40 μg $mL^{-1}$ contents of Cr, on the 6th day of cultivation, an equalization of Cr content takes place. In the samples taken after 17 h cultivation, together with the increase of the concentration of added Na, the content of Cr is increasing as well. In the medium containing Na, after 24-hour cultivation the content of Cr in bacteria decreases and makes about one and the same value

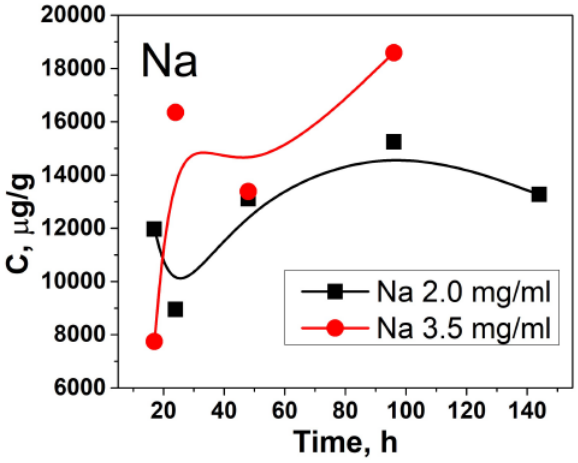


Figure 2. Dependence of Na concentration in bacteria (C, μg $g^{-1}$) on time of growth–evolution of bacteria *t* (*h*). The concentration of Na in the nutrient medium was 2 and 3.5 mg $mL^{-1}$.

The decrease of Cr content coincides with the increase of bacteria biomass (see below). In bacteria grown in 2 days, the rise of Cr is observed, coinciding with the decrease of bacterial biomass.

As shown in Figure 2, the Na content in bacteria drops sharply at 24 h like Cr, when the Na content in the food medium is 2 mg $mL^{-1}$. When the Na content in the food medium is 3.5 mg $mL^{-1}$, the Na content increases dramatically at 24 h.

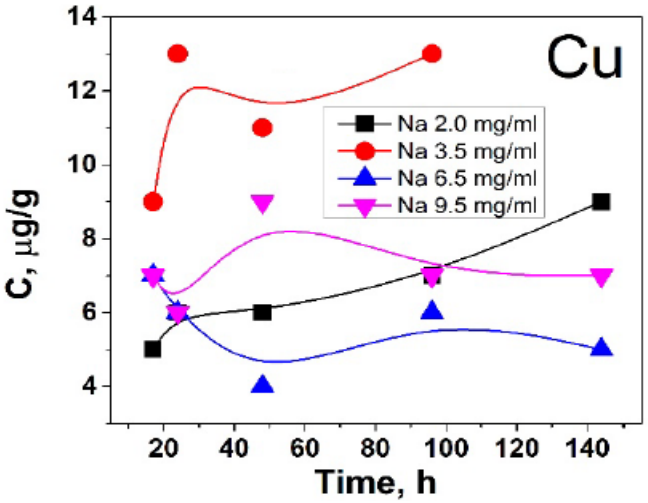


**Figure 3**. Dependence of Cu concentration (*C*) in bacteria on time of growth–evolution of bacteria *t*(*h*).

After 17 h of cultivation, the Cu content in bacterial cells increases, when Na is added to the nutrient medium. When the cultivation time is 24 hours, in the bacterial cells the Cu content is reduced, when in the nutrient medium the Na content is 6.5 mg/ml and 9.5 mg /ml. (Figure 3).

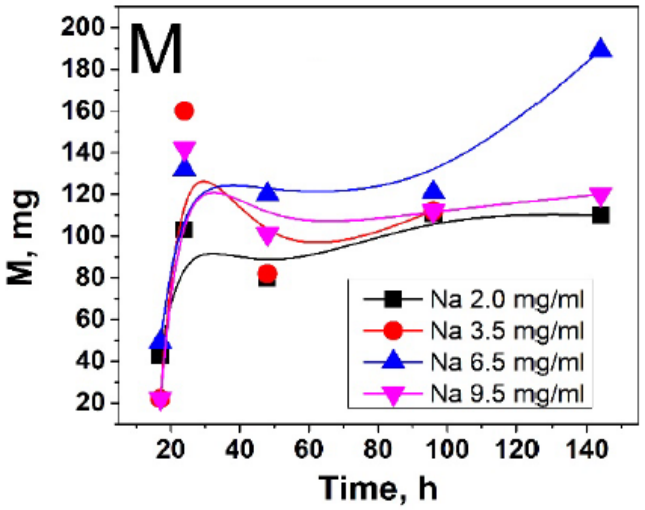


**Figure 4.** Dependence of bacteria masses *M* (mg) on time of growth–the evolution of bacteria *T*(*h*).

As it is seen from the results obtained (Figure 4), bacteria is evaluating rapidly during 17–24 h. Na added into nutrient medium favors the growth-development of bacteria.

Therefore, Na, added to the nutrient medium, contributes to the growth and development of bacteria during this period. After 48 h cultivation, in case of existence of different concentrations of Na in the nutrient medium, the bacterial biomass is decreased. For a further period (4 and 6 days) a gradual increase of the bacterial biomass is observed. It can be said that Na added into the nutrient medium slightly favors the growth of bacteria during the whole period of its growth–evolution.

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