Effect of Na(I) on Bioavailability for Cr(VI) and Cr(III) Arthrobacter species

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Abstract
The biosorption of Cr(VI) and Cr(III) of Arthrobacter species (Arthrobacter globiformis and Arthrobacter oxidas) was studied application dialysis and atomic absorption analysis at various Na(I) concentrations. It was shown significant difference between the binding constants as for Cr(VI) and Cr(III) Arthrobacter oxidas, as well as Cr(VI) and Cr(III) Arthrobacter globiformis at various Na(I) concentrations.

It was shown, that bioavailability increases in both cases with decreases Na(I) concentration.

Key words: Arthrobacter oxidas, Arthrobacter globiformis, metal ions

Introduction
Among the different heavy metals, chromium is one of the most toxic pollutants. This metal is introduced into natural waters through various industrial activities [1]. The two typical oxidative states of chromium in the environment are hexavalent, Cr(VI), and trivalent, Cr(III). Biotransformation of highly toxic and mutagenic hexavalent chromium [2] to relatively nontoxic trivalent Cr(III) form by chromate reducing bacteria offers an economical as well as ecofriendly option for chromium bioremediation. These two oxidation states have widely contrasting toxicity and transport characteristics: hexavalent chromium is more toxic, with high water solubility and mobility, while trivalent chromium is less soluble in water, less mobile and less harmful [3]. Due to the repulsive electrostatic interactions, Cr(VI) anion species are generally poorly adsorbed by the negatively charged soil particles and can move freely in the aqueous environments. In contrast, Cr(III) species normally carry positive electric charges and therefore can be easily adsorbed on the negatively charged soil particles [4].

Four chromate reducing bacterial strains, namely, Arthrobacter sp. SUK, Arthrobacter sp. SUK 1205, Pseudomonas putida SKPD 1202, and Corynebacterium paurometabolum SKPD 1204 were previously isolated and reported from chromite mine overburden and mine seepage samples and found to reduce chromate during growth under aerobic conditions [5]. The process of chromate reduction is adversely affected by the presence of additional metal ions possibly due to metal toxicity and inhibition of the Cr(VI) reduction process [6].

Chromate reduction by viable cells of Arthrobacter sp. SUK 1201 [7] was in general negatively affected when the reduction medium was supplemented with different heavy metals such as Ni(II), Zn(II), Mn(II), and Co(II) at equimolecular concentration. As compared to control, presence of Ni(II), Zn(II), Mn(II), and Co(II) showed nearly 66%, 74%, 60%, and 64% reduction,
respectively [7]. However, Cr(VI) reducing capability of the isolate was enhanced when Cu(II) was present in the medium along with Cr(VI). Such stimulatory effect of Cu(II) on Cr(VI) reduction activity has also been reported for Cr(VI)-reduction by Arthrobacter sp. SUK 1205 [8]. Many methods have been exploited to remove Cr (VI) in the environment, including empowering bacteria as bioremediation agent.

Effect of Na(I) on absorption Cr(VI) and Cr(III) of Arthrobacter species was studied in this paper using dialysis and atomic absorption analysis.

**Materials and Methods**

The other reagents were used: NaCl, CrCl₃, K₂CrO₄ (Analytical grade). Arthrobacter bacteria were cultivated in the nutrient medium. Arthrobacter species cells were centrifuged at 12000 rpm for 10 min and washed three times with phosphate buffer (pH 7.0). The centrifuged cells were dried without the supernatant solution until constant weight. After solidification (dehydrated) of cells (dry weight) solutions for dialysis were prepared by dissolving in phosphate buffer. This buffer was used in all experiments. A known quantity of dried bacterium suspension was contacted with solution containing a known concentration of metal ion. For biosorption isotherm studies, the dry cell weight was kept constant (1 mg/ml), while the initial chromium concentration in each sample was varied in the interval (10⁻³ -10⁻⁶ M). All experiments were carried out at ambient temperature. Metal was separated from the biomass with the membrane, which thickness was 30µm Visking (serva) and analyzed by an atomic absorption spectrophotometer „Analyst-900” (Perkin Elmer) λ_Cr=357.9 nm wavelength. Dialysis carried out during 72 h. Concentration of Na(I) 2mM, 20 mM, 50mM. The isotherm data were characterized by the Freundlich [9] equation, which by us in analogue cases were discussed in work [10].

**Results and Discussions**

Biosorption of Cr ion in anion and cation forms for two kinds of Arthrobacter (Arthrobacter globiformis 151B and Arthrobacter oxidas 61B) at room temperature at various Na(I) concentrations were studied. Freundlich parameters evaluated from the isotherms with the correlation coefficients are given in table 1. As seen from table 1, the change in sodium concentration strongly affects as Cr (III) _Arthrobacter_ species and also Cr(VI)_Arthrobacter_ species complexes. In particular, as the concentration of sodium increases, the binding constant decreases in all cases. Comparative biosorption characteristics for Cr(III) Arthrobacter species shown (table 1), that more decrease in bioavailability has been observed experimentally for Cr(III)-Arthrobacter oxidas as compared with Arthrobacter globiformis. This change is more pronounced for Cr (VI) _Arthrobacter globiformis 151B_ compared to Cr (VI) _Arthrobacter oxidas 61B_. (Decreases from 3.8 x10⁻⁴ to 2.51 x10⁻⁴ in the case of Cr (VI) _Arthrobacter oxidas 61B_, and for Cr (VI) -Arthrobacter globiformis 151B 2.09 x10⁻⁴ to 0.95 x10⁻⁴). Our results indicated that Cr(VI) and Cr(III) sorption at various Na(I) concentrations is depended of species of bacterial Arthrobacter. Differences between Arthrobacter species in metal ion binding may be due to the properties of the metal sorbates and the properties of bacterium (functional groups, structure and surface area, depending on the species). Functional groups within the wall provide the amino, carboxylic, sulfydryl, phosphate, and thiol groups that can bind metals [11]. It was shown, that the carboxyl groups were the main binding site in the cell wall.
Table 1. Biosorption parameters for Cr(III) and Cr(VI) _Arthrobacter_ species at various Na\(^+\) concentration

|                      | [Na\(^+\)] , mM | Biosorption constant , Kx10\(^{-4}\) | Absorption capacity, n | Correlation coefficient R\(^2\) |
|----------------------|-----------------|-------------------------------------|------------------------|-------------------------------|
| **Cr(III)-Arthrobacter oxidas 61B** |                |                                     |                        |                               |
| 50                   | 2.1             | 2.17                                | 0.98                   |                               |
| 20                   | 4.1             | 4.34                                | 0.92                   |                               |
| 2                    | 8.7             | 1.56                                | 0.97                   |                               |
| without Na\(^+\) [10] | 26.0            | 1.37                                | 0.98                   |                               |
| **Cr(III)-Arthrobacter globiformis 151B** |                |                                     |                        |                               |
| 50                   | 3.2             | 1.59                                | 0.98                   |                               |
| 20                   | 4.6             | 1.75                                | 0.94                   |                               |
| 2                    | 8.0             | 1.69                                | 0.96                   |                               |
| without Na\(^+\) [10] | 20.2            | 0.98                                | 1.23                   | 0.98                          |
| **Cr(VI)-Arthrobacter oxidas 61B** |                |                                     |                        |                               |
| 50                   | 2.51            | 1.92                                | 0.98                   |                               |
| 20                   | 3.23            | 1.58                                | 0.99                   |                               |
| 2                    | 3.8             | 1.03                                | 0.93                   |                               |
| without Na\(^+\) [10] | 4.6             | 1.25                                | 0.98                   |                               |
| **Cr(VI)-Arthrobacter globiformis 151B** |                |                                     |                        |                               |
| 50                   | 0.95            | 1.05                                | 0.97                   |                               |
| 20                   | 1.59            | 1.2                                 | 0.95                   |                               |
| 2                    | 2.09            | 1.49                                | 0.99                   |                               |
| without Na\(^+\) [10] | 3.4             | 1.35                                | 0.96                   |                               |

of gram positive bacteria [12]. Therefore, it can be concluded that sodium ions "screen" chlorine ions to contact the active centers of the bacterium, in particular carboxyl groups. Gram-positive bacteria have also a greater sorptive capacity due to their thicker layer of peptidoglycan which
contains numerous sorptive sites [13]. In our case n values which reflects the intensity of sorption presents the same trend for Cr(III) _ and Cr(VI) _ Arthrobacter globiformis 151B (from 1.69 to 1.59, from 1.49 to 1.05 respectively with increase Na(I) concentration) but, as seen from table 1 for Cr(III) _ and Cr(VI) _ Arthrobacter oxidas received sorption intensity indicator are different.

The effect of pH on Cr(VI) reduction and removal from aqueous solution was studied in the range of 1-4. Arthrobacter viscosus biomass was used for Cr(VI) biosorption [14]. The best removal efficiency and uptake were reached at pH 4 [14]. Arthrobacter sp. SUK 1201, a potent isolate reported from chromite mine overburden of Orissa, India, has been evaluated for Cr(VI) reduction with immobilized whole cells. Optimum pH for Cr(VI) reduction was 7.0, and the process was inhibited by metal ions such as Ni(II), Co(II), Cd(II), Zn(II), and Mn(II) but not by Cu(II) and Fe(III) [7].

Binding constants for Cr(III) _ Arthrobacter species in all cases are more than for Cr(VI) _ Arthrobacter species. Similar results were obtained without Na (I) [10], but the difference was more significant than in the presence of Na (I). In particular, without Na (I), the biosorption constant for Cr (III) _ Arthrobacter oxidas and Cr (III) _ Arthrobacter globiformis is approximately 6 times greater than for Cr (VI) _ Arthrobacter oxidas and Cr (VI) _ Arthrobacter globiformis. At large concentrations of Na (I) ions (50 mmol) and without it, the difference between the binding constants for Cr (III) _ Arthrobacter oxidas is 12 times greater and for Cr (III) _ Arthrobacter globiformis 6-fold greater. In a same case for Cr (VI) _ Arthrobacter oxydas the difference is only 2-fold greater and for Cr (VI) _ Arthrobacter globiformis 4-fold greater.

Thus, effect of Na(I) ions are more significant in the case Cr(III) _ Arthrobacter species ,than for Cr(VI) _ Arthrobacter species. All this is natural considering that Cr(III) positively charged ions as Na(I) and both have the same binding active centers in contrast to negatively charged Cr(VI) ions.

References

1. U. K. Garg, M. P. Kaur, V. K. Garg and D. Sud, Removal of hexavalent chromium from aqueous solution by agricultural waste biomass, Journal of Hazardous Materials, 2007, 140, 60–68. doi: 10.1016/j.jhazmat.2006.06.056.

2. D. Bagchi, S. J. Stohs, B. W. Downs, M. Bagchi, and H. G. Preuss, Cytotoxicity and oxidative mechanisms of different forms of chromium, Toxicology, 2002,180(1), 5–22. doi: 10.1016/s0300-483x(02)00378-5

3. D. Mohan and C. U. Pittman Jr., Activated Carbons and Low-Cost Adsorbents for Remediation of Tri- and Hexavalent Chromium from Water: A Review, Journal of Hazardous Materials B, 2006,137, 762-811. doi: 10.1016/j.jhazmat.2006.06.060

4. B. Silva, H. Figueiredo, C. Quintelas, I. C. Neves and T. Tavares, Zeolites as supports for the biorecovery of hexavalent and trivalent chromium, Microporous and Mesoporous Materials, 2008,116,555-560. doi:10.1016/j.micromeso.2008.05.015
5. S. Dey and A. K. Paul, Hexavalent chromium reduction by aerobic heterotrophic bacteria indigenous to chromite mine overburden, Brazilian Journal of Microbiology, 2013, 44(1), 307–315. doi: 10.1590/S1517-83822013000100045

6. J. Mclean and T. J. Beveridge, Chromate reduction by a Pseudomonad isolated from a site contaminated with chromated copper arsenate, Applied and Environmental Microbiology, 2001, 67(3), 1076–1084. doi: 10.1128/AEM.67.3.1076-1084.2001

7. Satarupa Dey & A. K. Paul, Reduction of Hexavalent Chromium by Immobilized Viable Cells of Arthrobacter sp. SUK 1201, Bioremediation Journal, 2014, 18(1), 1-11, doi: 10.1080/10889868.2013.834866

8. S. Dey and A. K. Paul, Optimization of chromate reduction by whole cells of Arthrobacter sp. SUK 1205 isolated from metalliferous chromite mine environment, Geomaterials, 2012, 2(4), 73–81. doi: 10.4236/gm.2012.24012

9. H. Freundlich, Adsorption in solutions, Phys. Chem., 1906, 57, 384-410.

10. E. Gelagutashvili, E. Ginturi, D. Pataria, M. Gurielidze, Biosorption of Cr (VI) and Cr(III) of Arthrobacter species, arXiv:1106.2918 Chem-ph, 2011.

11. Y. P. Ting, F. Lawson, I. G. Prince, Uptake cadmium and zinc by the Chlorella vudguris. part II. Multi-ion Situation, Biotechnology and Bioengineering, 1991, 37, 445-455. doi: 10.1002/bit.260370506

12. M. G. Gadd, C. White, Microbial treatment of metal pollution—a working biotechnology, Trends in Biotechnology, 1993, 11, 353-359. doi: 10.1016/0167-7799(93)90158-6

13. E. D. Van Hullebusch, M. H. Zandvoort, P. N. L. Lens, Metal immobilization by biofilms. Mechanisms and analytical tools, Rev. Environ. Sci. Bio/Technol. 2003, 2, 9-33. doi: 10.1023/B:RESB.0000022995.48330.55

14. B. Silva, H. Figueiredo, I. C. Neves, and T. Tavares, The role of pH on Cr(VI) Reduction and Removal by Arthrobacter Viscosus, International Journal of Chemical and Biological Engineering, 2009, 2, 2.