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Biosorption of hexavalent chromium by *Arthrobacter viscosus*

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Abstract

Arthrobacter viscosus biomass was used for Cr(VI) biosorption. The effect of biomass concentration on Cr(VI) reduction and removal from aqueous solution was studied in the range of 1.2 to 5.3 g/L. The removal of Cr(VI) and total chromium increased linearly with the increase of biomass concentration. The best removal efficiencies of Cr(VI) and total chromium were reached for the highest biomass concentration, 72.2 % and 44.0 %, respectively. The increase in biomass concentration did not produce significant changes in the uptake values. The maximum uptake value, 8.2 $mg_{Cr}/g_{biomass}$, was obtained for a biomass concentration of 2.3 g/L.

1 Introduction

Heavy metals derived from the release of industrial wastewater present an ongoing and serious hazard to human health and to the environment, because these elements are toxic, not biodegradable and can accumulate in food chain and living tissues (Gavrilescu, 2004; Deng et al., 2006).

Chromium is a common and very toxic pollutant introduced into natural waters from a variety of industrial wastewaters. The major sources of contamination are electroplating, metal finishing industries and tanneries (Agarwal et al., 2006). Among the several oxidation states of chromium, the main forms present in the environment are trivalent and hexavalent, Cr(III) and Cr(VI), respectively. These two oxidation states have widely contrasting toxicity and transport characteristics. Hexavalent chromium poses a greater risk due to its carcinogenic properties to living organisms, while Cr(III) is generally only toxic to plants at very high concentrations and is less toxic or non-toxic to animals (Dakiky et al., 2002; Anderson, 1997). Depending on the solution pH values, Cr(VI) species may be in the form of dichromate ($Cr_2O_7^{2-}$), hydrochromate ($HCrO_4^-$), or chromate (CrO_4^{2-}) and Cr(III) species may take the form of hydrated trivalent chromium, $Cr(H_2O)_6^{3+}$, and chromium hydroxide complexes, $Cr(OH)(H_2O)_5^{2+}$ or $Cr(OH)_2(H_2O)_4^+$. 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 (Silva et al., 2008 a; Deng et al., 2004).

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The conventional methods used for heavy metals removal are generally expensive and potentially hazardous due to the generation of harmful by-products. Common methods include chemical precipitation, chemical oxidation or reduction, ion exchange, membrane filtration and carbon adsorption (Bailey et al., 1999). These technologies have significant disadvantages, such as incomplete metal removal and generation of huge volumes of toxic sludge, and are often inefficient and/or expensive mainly when applied to diluted solutions up to 100 mg/L (Sağ et al., 1995; Cossich et al., 2004).

As an alternative to traditional physicochemical treatments, a number of biological assays using microbial biomass have been studied and developed to treat chromium-contaminated waste streams and natural waters. Certain types of microbial biomass can retain relatively high quantities of metals by means of passive processes known as biosorption, which is dependent on the affinity between the metallic species and the binding sites on the cell wall (Raras, 1995). Biosorption is a metabolism-independent process and thus can be performed by both living and dead microorganisms. This adsorption is based on mechanisms such as complexation, ion exchange, coordination, adsorption, chelation and microprecipitation (Hu et al., 1996). The accumulation of metals by biological materials is a promising process that can reduce capital costs by 20%, operational costs by 36% and total treatment costs by 28%, compared with conventional systems (Loukidou et al., 2004).

The use of bacteria for biosorption is a fast growing field in metal remediation because of their ubiquity, ability to grow under controlled conditions and small size (Mohan et al., 2006). Many studies have demonstrated that various types of biomaterials such as bacteria are capable of transforming hexavalent chromium, Cr(VI), into the much less toxic and less mobile trivalent form, Cr(III). When Cr(VI) comes in contact with biomaterials, especially in an acidic solution, the Cr(VI) can easily or spontaneously be reduced to the Cr(III), because Cr(VI) has high *redox* potential value (above +1.3 V at standard conditions) (Park et al., 2004, 2005, 2007, 2008). *Arthrobacter* species are of particular interest because of its high potential for bioremediation (Asatiani et al., 2004). The bacteria used in this work, *Arthrobacter viscosus*, is a good exopolysaccharide producer, an aspect which would permit prediction of good metal ion entrapment (Figueiredo et al., 2008; Silva et al., 2008 b).

The purpose of the present work was to assess the potential of *A. viscosus* for the biosorption of hexavalent chromium and to study the effect of biomass concentration on its removal performance.

2 Experimental

2.1 Materials and reagents

Arthrobacter viscosus was obtained from the Spanish Type Culture Collection of the University of Valencia. Aqueous potassium dichromate solution was prepared by diluting $K_2Cr_2O_7$ (Panreac) in deionised water.

All glassware used for experimental purposes was washed in 10% nitric acid to remove any possible interference by other metals.

2.2 Preparation of the biomass

A medium with 10 g/L of glucose, 5 g/L of peptone, 3 g/L of malt extract and 3 g/L of yeast extract was used for the microorganism growth. The medium was sterilized at 121 ºC for 20 min, cooled to room temperature, inoculated with bacteria and kept at 28 ºC for 24 h with moderate stirring in an incubator. The cells were then harvested by centrifugation at 7000 rpm for 15 min and re-suspended in residual culture medium to obtain suspensions with different biomass concentrations to be used in the biosorption assays.

2.3 Biosorption assays

Batch experiments were conducted in 250 mL Erlenmeyer flasks using 15 mL of each suspension of *A. viscosus* and 150 mL of a potassium dichromate solution (100 mg_{Cr}/L). Biomass concentrations of 1.2, 2.3, 4.3 and 5.3 g/L were used and determined by dry weight measurement. The initial pH value of the dichromate solution was adjusted to 3, using a 4 M H_2SO_4 solution. The Erlenmeyer flasks were kept at 28 ºC, with moderate stirring. Samples of 1 mL were taken, centrifuged and analyzed for chromium determination.

2.4 Chromium analysis

Hexavalent chromium was analyzed by measuring absorbance at 540 nm of the purple complex of Cr(VI) with 1,5-diphenylcarbazide, in acidic solution (Eaton et al., 1995). For total Cr determination, the Cr(III) was first oxidized to Cr(VI) at high temperature by the addition of an excess of potassium permanganate previous to the reaction with 1,5-diphenylcarbazide. The Cr(III) concentration was calculated by the difference between the total Cr and Cr(VI) concentration.

3 Results

Cr(VI) removal and its reduction to Cr(III) was observed in the presence of *A. viscosus* cells. In Fig. 1 is shown the time-dependent concentration of Cr(VI) and the initial removal rates, for various biomass concentrations.

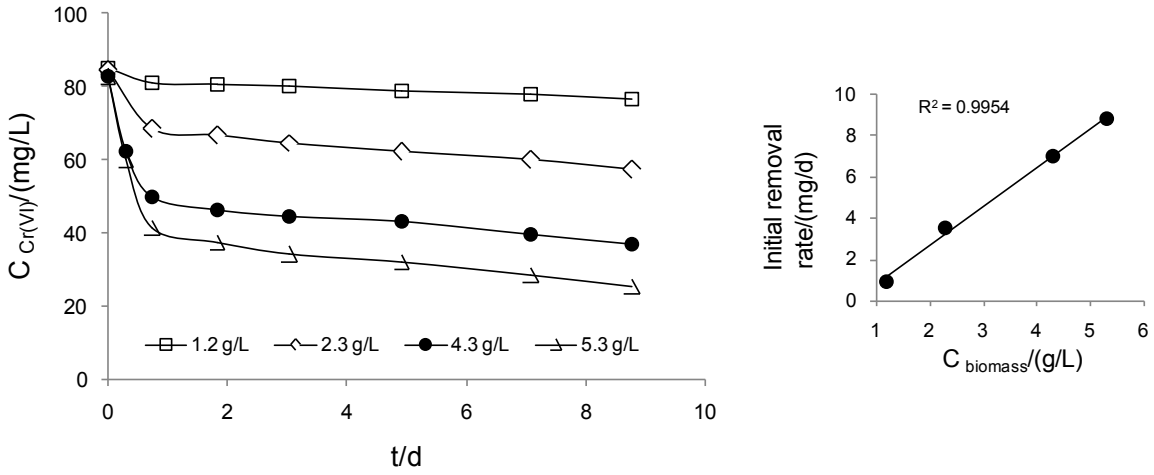

Fig. 1 Concentration of Cr(VI) as a function of contact time and initial removal rates, for biomass concentrations of 1.2, 2.3, 4.3 and 5.3 g/L.

It can be observed that the removal of Cr(VI) is enhanced with an increase in biomass concentration, as it was expected. The initial removal rates were plotted against biomass concentration, as presented in Fig. 1. The plot showed a good linearity, indicating that the reaction was pseudo-first-order with respect to biomass concentration. Total removal of Cr(VI) was not achieved, for the biomass concentrations in study. As the solution pH was not maintained at highly acidic values, the reduction of Cr(VI) to Cr(III) was restricted due to the high pH values of the solutions after 9 days of contact time, which varied from 4.6 to 5.1. Therefore, there was a deficit of protons in solution to allow the complete reduction of Cr(VI).

The concentration values of Cr(VI), Cr(III) and total chromium at the end of the contact time, are presented in Figure 2. It can be seen that the final chromium concentrations in solution showed a linear pattern with respect to biomass concentration, in the range of 1.2 to 5.3 g/L. Cr(VI) concentration decreased lienarly with the increase of biomass concentration, while Cr(III) concentration in solution increased proportionally with the increment of biomass. The lowest total chromium concentration was obtained for the highest biomass concentration, remaining in solution 25.4 mg/L of Cr(VI) and 24.6 mg/L of Cr(III).

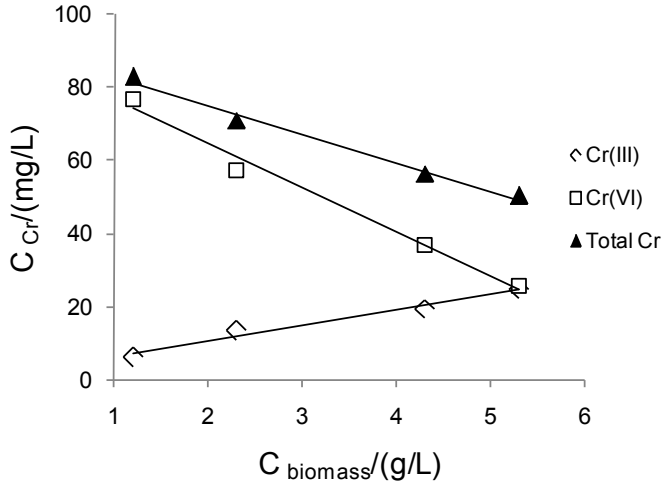

Fig. 2 Chromium concentrations in solution after 9 days of contact time, for different biomass concentrations.

In Fig. 3 are shown the removal efficiencies and the uptake of total Cr attained for the different biomass concentrations. As the biomass concentration increased, the removal efficiency of Cr(VI) and total Cr increased linearly, as discussed above. The best removal efficiencies of Cr(VI) and total chromium were achieved for a biomass concentration of 5.3 g/L, 72.2 % and 44.0 %, respectively. It can be observed in Fig. 3b) that the uptake values did not change significantly with the increase of biomass concentration. The highest uptake value, 8.2 $mg_{Cr}/g_{biomass}$, was obtained for a biomass concentration of 2.3 g/L.

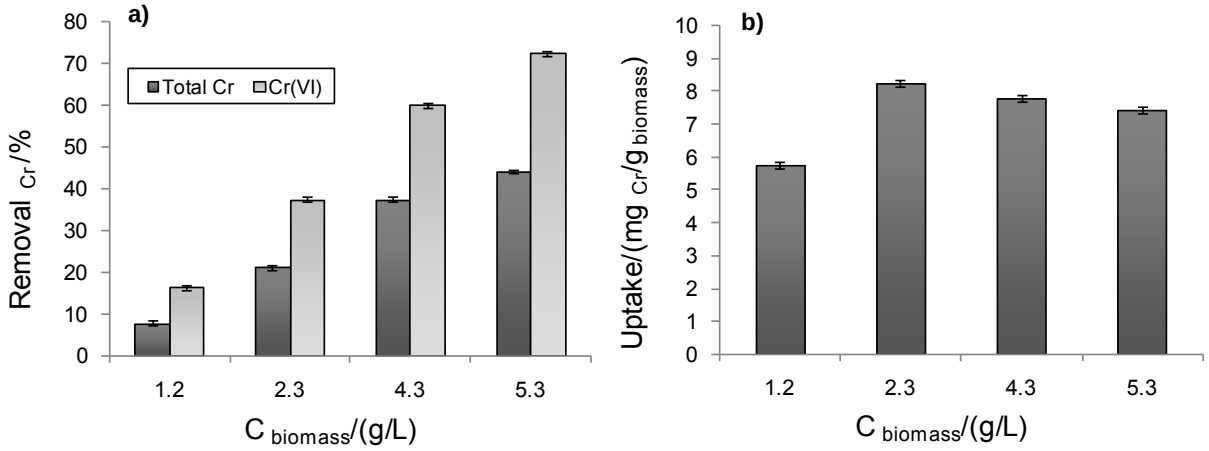

Fig. 3 Removal efficiencies of total Cr and Cr(VI) **(a)** and uptake of total chromium in terms of initial bacteria mass **(b)** after 9 days of contact time, for the different biomass concentrations tested.

4 Conclusions

This work demonstrate the ability of *Arthrobacter viscosus* biomass for the detoxification of Cr(VI) from contaminated wastewaters and highlights the efficacy of using biological agents as an alternative to conventional treatments.

The complete reduction of Cr(VI) was not attained for the biomass concentrations in study, due to the lack of protons in solution. For promoting the Cr(VI) reduction, it is necessary to work with highly acidic pH values. The removal efficiencies of Cr(VI) and total chromium increased linearly with the increase of biomass concentration, in the range 1.2 to 5.3 g/L.

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