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The role of pH on Cr(VI) Reduction and Removal by *Arthrobacter Viscosus*

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Abstract*—Arthrobacter viscosus* biomass was used for Cr(VI) biosorption. The effect of pH on Cr(VI) reduction and removal from aqueous solution was studied in the range of 1-4. The Cr(VI) removal involves both *redox* reaction and adsorption of metal ions on biomass surface. The removal rate of Cr(VI) was enhanced by very acid conditions, while higher solution pH values favored the removal of total chromium. The best removal efficiency and uptake were reached at pH 4, 72.5 % and 12.6 $mg_{Cr}/g_{biomass}$, respectively.

***Keywords*—**Biosorption, chromium, pH, reduction.

I. Introduction

HEAVY metals released into the environment have been increasing continuously as a result of several industrial activities. This causes a significant hazard to human health and to the environment, because of their toxicity, accumulation in the food chain, and persistence in nature [1].

Among the different heavy metals, chromium is one of the most toxic pollutants. This metal is introduced into natural waters through various industrial activities, such as steel production, electro-plating, leather tanning, textile industries, wood preservation, anodizing of aluminum, water-cooling and chromate preparation [2].

The two typical oxidative states of chromium in the environment are hexavalent, Cr(VI), and trivalent, Cr(III). 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 [3–5]. 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 [6-7].

The conventional methods for heavy metal removal from industrial effluents are precipitation, coagulation, ion exchange, cementation, electro-dialysis, electro-winning, electro-coagulation and reverse osmosis [8]. However, these processes have significant disadvantages such as incomplete metal removal, high reagent or energy requirements, generation of toxic sludge or other waste products and are generally very expensive when the contaminant concentrations are in the range 10–100 mg/L [9]. Due to these limitations, cost effective technologies or sorbents for treatment of metals contaminated waste streams are needed.

Biosorption of heavy metals by microbial cells has been studied extensively as an alternative technology for the treatment of wastewaters. It 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 [10]. Biosorption is generally defined as the accumulation of metals by biological materials without active uptake and can be considered as a collective term for a number of passive accumulation processes which may include ion exchange, coordination, complexation, chelation, adsorption and microprecipitation [11]. The applicability of bacteria as biosorbents has some advantages due to their small size, their ubiquity, their ability to grow under controlled conditions and their resilience to a wide range of environmental situations [12]. Numerous studies have demonstrated a reduction of toxic Cr(VI) to non-toxic Cr(III) by various types of biomaterials, such as bacteria. *Arthrobacter* species are of particular interest because of its high potential for bioremediation. Bacteria can detoxify chromium wastewater, by either reduction or accumulation inside the cells and/or adsorption of the ion on their surface [13]. The bacteria used in this work, *Arthrobacter viscosus*, is a good exopolysaccharide producer, an aspect which would permit prediction of good metal ion entrapment [14,15].

Although many studies on Cr(VI) biosorption claim that this anion is removed from aqueous systems by adsorption, recent reports reveal that the biosorption mechanism of Cr(VI) by biomaterials is not “anionic adsorption” but “adsorption-coupled reduction”. 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) [16-19]. These studies reveal that the

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removal of hexavalent and total chromium is strongly pH dependent, being this parameter the most important in the biosorption process. Other authors refer the importance of the solution pH on the binding behaviour of the chromium species due to the protonation/deprotonation of the cell wall functional groups hence making the surface positively or negatively charged [20-22].

The aim of the present work is to study the effect of solution pH in the reduction of hexavalent chromium and in its removal from solution, by living cells of *A. viscosus.*

II. Experimental

A. 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 deionized water.

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

B. Preparation of the Biomass

A medium with 10 g L^{-1} of glucose, 5 g L^{-1} of peptone, 3 g L^{-1} of malt extract and 3 g L^{-1} 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 a smaller volume of residual culture medium to obtain a concentrated suspension and provide a biomass concentration of 5 g/L in the biosorption assays.

C. Biosorption Experiments

Batch experiments were conducted in 250 mL Erlenmeyer flasks using 15 mL of *A. viscosus* suspension and 150 mL of a potassium dichromate solution (100 mg_{Cr}/L), with final bacterium concentration of 5 g/L. To study the effect of pH on Cr(VI) removal, pH values of 1, 2, 3 and 4 were used. The solution pH was regularly maintained at the desired value using H_2SO_4 or NaOH solutions. The Erlenmeyer flasks were kept at 28 ºC, with moderate stirring. Samples of 1 mL were taken, centrifuged and analyzed for chromium determination.

D. Analysis of Chromium Ions

Hexavalent chromium was analyzed by measuring absorbance at 540 nm of the purple complex of Cr(VI) with 1,5-diphenylcarbazide, in acidic solution [23]. 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.

III. Results

In Figs. 1 and 2 are shown the time-dependent concentration of Cr(VI) and total chromium, at various solution pH values in the range 1 to 4.

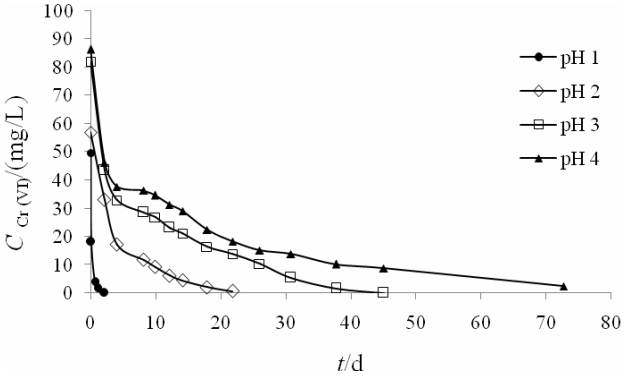

Fig. 1 Concentration of Cr(VI) as a function of contact time, for solution pH values of 1, 2, 3 and 4

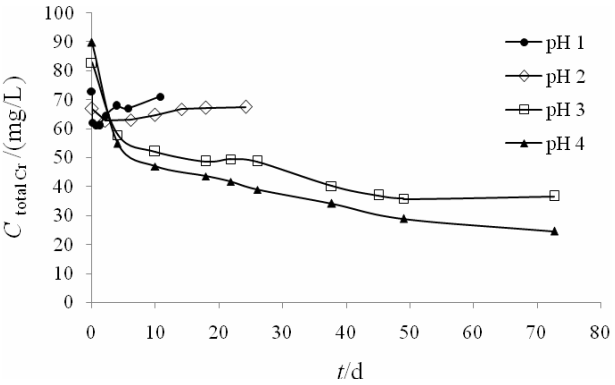

Fig. 2 Concentration of total chromium as a function of contact time, for solution pH values of 1, 2, 3 and 4

As it can be seen in Fig. 1, the removal rate of Cr(VI) was strongly pH dependent, increasing with a decrease in pH. According to the reduction reactions of Cr(VI) species, it is essential to supply numerous protons for promoting the rate of the reaction.

$$Cr_2O_7^{2-} + 14H^+ + 6e^- \leftrightarrow 2Cr^{3+} + 7H_2O \quad (1)$$

$$CrO_4^{2-} + 8H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \quad (2)$$

$$HCrO_4^- + 7H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \quad (3)$$

$$H_2CrO_4 + 6H^+ + 3e^- \leftrightarrow Cr^{3+} + 4H_2O \quad (4)$$

Hexavalent chromium was completely removal from solution for pH values of 1, 2 and 3. The contact time necessary for complete Cr(VI) removal was 52 hours, 22 days and 45 days for pH 1, pH 2 and pH 3, respectively. Total removal was not achieved at pH 4, for the contact time of 73 days, remaining in solution 2.5 mg/L of Cr(VI).

Although Cr (VI) reduction is favored by very acidic conditions, higher pH values enhance total Cr removal with

the increase in contact time as shown in Fig. 2. At the end of the assays, the lowest total chromium concentration remaining in solution was obtained at pH 4 (Table 1). These results are explained by the multiple phenomena involved in this system such as reduction of Cr(VI), adsorption/desorption of chromium ions and protonation/deprotonation of the cell wall functional groups depending on the solution pH. The increase of solution pH increases the negative charge on the cell surface due to the deprotonation of the metal binding sites hence attracting Cr(III) ions resulting from the reduction of Cr(VI). It should be noted that after a short period of contact time it can be observed a higher removal of total chromium for the lower pH values (pH 1 and pH 2) due to the strong protonation of functional groups, thus making the biomass more positively charged and hence creating an electrostatic attraction with Cr(VI) species. As the contact time increases it can be seen a releasing of chromium to the solution at these pH values. This is related to the electronic repulsion between the positively charged groups of the cell wall and the cationic Cr(III) species resultant from the reduction of hexavalent chromium on the bacterium surface.

TABLE I
FINAL Cr(VI) AND Cr(III)CONCENTRATIONS IN AQUEOUS SOLUTION

pH	$C_{Cr(VI)}$/(mg/L)	$C_{Cr(III)}$/(mg/L)
1	0.0	71.0
2	0.0	67.4
3	0.0	36.3
4	2.5	22.0

In Fig. 3 and Fig. 4 are shown the removal efficiencies and the uptake of total Cr. As the solution pH increased, the removal efficiency and uptake of total Cr increased, as discussed above. The best removal efficiency and uptake were achieved at pH 4, 72.5 % and 12.6 $mg_{Cr}/g_{biomass}$, after 73 days of contact time.

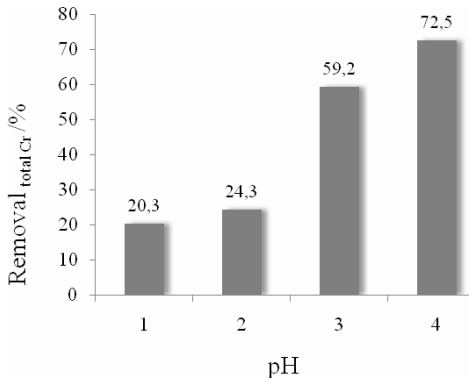

Fig. 3 Removal efficiencies of total chromium at the end of the contact time, for the different pH values tested

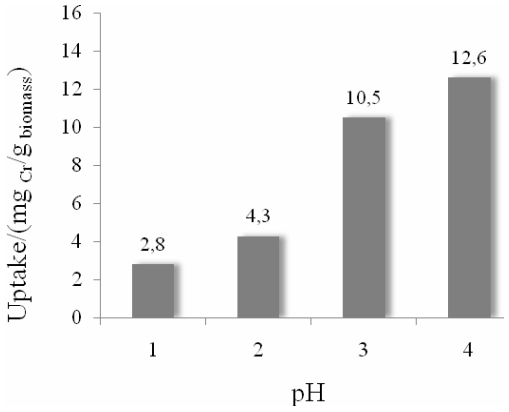

Fig. 4 Uptake of total chromium at the end of the contact time, in terms of initial bacteria mass, for the different pH values tested

IV. CONCLUSION

This work demonstrate that the use of *Arthrobacter viscosus* biomass can be an alternative process for the detoxification of Cr(VI) from contaminated wastewaters. Chromium biosorption was highly pH dependent, with lower pH values favouring Cr(VI) reduction and higher solution pH enhancing total chromium removal. The solution pH is one of the most important parameters in the practical use bacterial biomass in the Cr(VI) removal process. Wastewaters containing Cr(VI) at a concentration ranging from 10-100 mg/L are generally acidic thus making easier the control of this parameter in an industrial scale.

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