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A combined bacterial process for the reduction and immobilization of chromium

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Abstract

In this paper, we present the indirect Cr(VI) reduction by *Acidithiobacillus thiooxidans* using sulfur as energy source under aerobic conditions and the subsequent Cr(III) precipitation by *Desulfovibrio* sp. under anaerobic conditions. Cr(VI) reduction is promoted by intermediate-products such as sulfite and thiosulfate. Both processes have been operated sequentially under continuous flow conditions to decontaminate a 5 mg l^{-1} Cr(VI) solution.

Keywords: *Acidithiobacillus thiooxidans*; *Desulfovibrio* sp; Chromium decontamination

1. Introduction

Hexavalent chromium was classified as a primary contaminant because of its mobility in soil and groundwater and its reported harmful effects on organisms including humans. Wastewaters containing Cr(VI) are generated by many industrial processes as ore processing, electroplating, leather-tanning processes among others (Lawson, 1997). The reduction of toxic Cr(VI) leads to the formation of stable and non-toxic Cr(III); this reduction followed by precipitation or immobilization can be produced by chemical or biological action (Melhorn et al., 1994; Rajwade and Paknikar, 1997; Salunkhe et al., 1998).

The oxidation of sulfur by two species of chemoautotrophic *acidithiobacilli*, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* (Rawlings, 1997), generates a series of sulfur compounds with high reducing power (Steudel, 1989). Recently, it has been reported that these bacteria are capable of reducing Cr(VI) in aerobic conditions through similar mechanisms (Sisti et al., 1998) although this ability has not been proved in a continuous flow-regime nor by *Acidithiobacillus* cultures at pH values higher than 2.0.

Sulfate-reducing bacteria can be used to remove toxic metals in waters of moderate pH through the formation of insoluble metal sulfides (Newman et al., 1997). This group of anaerobic microorganisms uses sulfate as a terminal electron acceptor for respiration and organic compounds as carbon/energy sources (Voordouw, 1995). There are few reports on Cr(VI) reduction by *Desulfovibrio* bacteria (Lovley and Phillips, 1994; Fude et al., 1994) and on Cr(III) precipitation, although in this last case using an undefined culture of sulfate-reducing bacteria (White et al., 1998). However, there is no report on the use of a pure culture of *Desulfovibrio*, particularly *Desulfovibrio vulgaris*, for Cr(III) removal.

In this paper, we present the results of three experiments: (a) Cr(VI) reduction by *At. thiooxidans* cultures at different pH values, (b) Cr(III) immobilization in cultures of *D. vulgaris* or *Desulfovibrio* sp. and finally, (c) a combined process for decontamination consisting of Cr(VI) reduction by a *At. thiooxidans* culture followed by Cr(III) immobilization by a *Desulfovibrio* sp. culture.

2. Materials and methods

2.1. Chromium(VI) reduction by At. thiooxidans culture

Cultures were carried out in a reactor vessel containing 12 g of powdered sulfur and 0.6 l of iron-free 9 K medium (Silverman and Lundgren, 1959), inoculated at 10% v/v with a strain of *At. thiooxidans.* (DSM 11478) with the pH adjusted to 2.0, 4.0 or 6.0. pH was maintained with the automatic addition of 1.25 M KOH. Cultures were maintained at 30°C and stirred at 400 rpm. When free bacterial population reached 5×10^8 cells ml^{-1}, 100 ml of the culture were filtered through blue ribbon filter paper and

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then through a 0.45 μm filter. Then, 20 ml of a solution of $K_2Cr_2O_7$ containing 10 mg l^{-1} Cr(VI) at pH 2.0 were added and left in contact with the membrane (with the retained cells and sulfur particles of size < 3 μm) during 20 min to allow Cr(VI) reduction. Afterwards, the solution was forced to pass through the membrane using a vacuum pump. Membranes were washed with sulfuric acid (pH 2.0) and fixed with 10% glutaraldehide in phosphate buffer (pH 7.5) to allow microscopic/EDXA examination.

2.2. Chromium(III) immobilization by Desulfovibrio species

Sealed flasks under N_2 containing 250 ml of sterilized Postgate C medium (4.5 g l^{-1} Na_2SO_4, 1 g l^{-1} yeast extract and 6 g l^{-1} sodium lactate) without iron and with 5–25 mg l^{-1} Cr (III) (pH 7.5 ± 0.2), were inoculated at 10% v/v with cultures of *Desulfovibrio* sp. (ATCC 49975) or *Desulfovibrio vulgaris* (ATCC 29579) prepared in Postgate C medium. Flasks were kept at 30°C. Sterile controls were carried out under similar conditions. Samples were taken at different times, filtered through 0.22 μm membrane. The solid residues were washed with phosphate buffer (pH 7.5) and fixed with 10 % glutaraldehide to allow microscopic/EDXA examination. Chromium was analyzed in the supernatants.

2.3. Combined process (chromium(VI) reduction and chromium(III) immobilization)

At. thiooxidans culture was carried out in a reactor vessel containing 16 g of powdered sulfur and 0.8 l of iron-free 9 K medium (pH 5.1) at 30°C and magnetically stirred at 400 rpm. pH was maintained through the automatic addition of KOH 1.25 M. After 6 days, the medium was supplemented with Cr(VI) allowing an initial concentration of 5 mg l^{-1}. An influent medium containing Cr(VI) 5 mg l^{-1} was added with a peristaltic pump at a flow rate of 150 ml day^{-1}. The effluent was collected in a reservoir to allow colloidal sulfur sedimentation.

Desulfovibrio sp. culture was carried out in a similar reactor vessel containing 0.8 l of Postgate C medium without iron (pH 7.5 ± 0.2) at 30°C and magnetically stirred at 400 rpm. In order to obtain anaerobic conditions, sterilized N_2 was continually bubbled into the medium. pH was maintained with the automatic addition of H_2SO_4 0.1 M. After 6 days, the effluent medium from the *At. thiooxidans* reactor, supplemented with 4.5 g l^{-1} of lactate, was pumped into the *Desulfovibrio* sp. reactor at flow rate of 75 ml day^{-1}.

2.4. Analytical methods

Free (not attached) bacterial population was determined by using a Petroff–Hausser counting chamber in a microscope with a contrast phase attachment. Cr(VI) was determined by the diphenylcarbazide method (Urone, 1955) and total chromium concentration was determined using atomic absorption spectrophotometry. Sulfate concentration in cultures was determined by a turbidimetric method (Greenberg et al., 1985). Samples from *Desulfovibrio* cultures were digested with HNO_3 (30 min) before chromium determination. Solid residues were examined under a scanning electron microscope with an energy-dispersive X-ray (EDXA) probe.

3. Results and discussion

3.1. Chromium(VI) reduction

The capability of *Acidithiobacillus* cultures to reduce Cr(VI) was demonstrated in aerobic experiments (Sisti et al., 1998). Although sulfur-oxidation mechanism has not been completely explained yet, in *At. thiooxidans* cultures reduced glutathione (GSH) is a required intermediate for the oxidation of elemental sulfur (Steudel, 1989):

$$S_n + GSH \rightarrow GSS_nH. \quad (1)$$

The polysulfide formed is then successively oxidized to different compounds like sulfite, thiosulfate, other polythionates and finally sulfate. Sulfite and polythionates could be responsible for reductive reactions. It has been recently demonstrated that the capability of chromium reduction by *Acidithiobacillus* cultures could be related to reducing compounds associated with sulfur particles (with size less than 3 μm) and cells (Quintana et al., 2001). This agrees with a previous report (Steudel, 1989) indicating that colloidal sulfur in cultures of *Acidithiobacillus* would be present as long-chain polythionates forming micelles of globules of up to few μm. Because of this, in this paper the ability of chromium reduction by *At. thiooxidans* was evaluated using the sulfur particles and cells retained by the filtration membranes.

A SEM microphotograph of the membrane filter with the *At. thiooxidans* cells and sulfur particles is shown in Fig. 1A. Fig. 2 (outer graph) shows Cr(VI) reduction by the sulfur particles and *At. thiooxidans* cells retained on the membrane from cultures at different pH values. The inner graph shows free bacterial population and mmoles of added base to keep the pH constant in the cultures from which the samples were taken to evaluate the chromium reduction. No Cr(VI) reduction were detected in sterile controls. Although the *At. thiooxidans* growth showed a long lag phase at pH 6.0, samples from this culture reached higher chromium reduction values (close to 100% at the end of bacterial growth). At pH 2.0 and 4.0, cultures reached higher free bacterial populations but lower chromium reduction values. Thus, the higher the pH, the higher the amount of reducing compounds associated to the colloidal sulfur and cells. This could be explained because some of these reducing compounds are stabilized at high pH. In

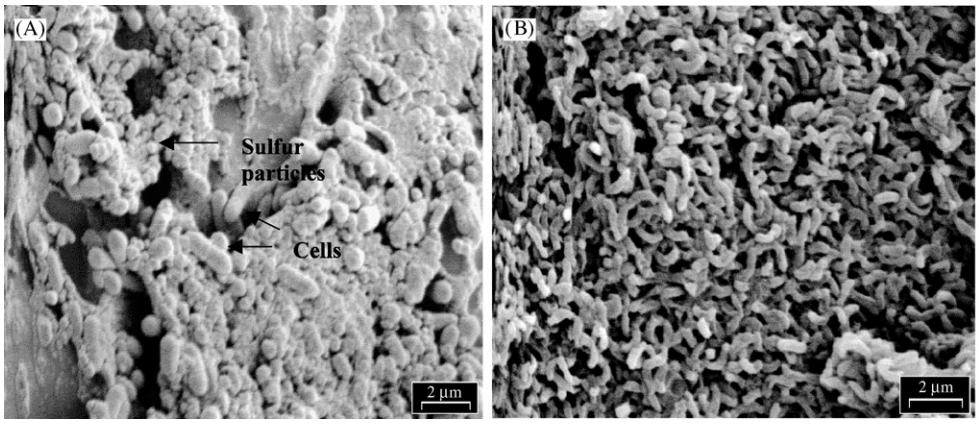

Fig. 1. (A) SEM Microphotograph of *Acidithiobacillus thiooxidans* cells and sulfur particles retained by the 0.45 µm membrane during the Cr(VI) reduction experiment. Bar represents 2 µm. (B) SEM micrograph of *Desulfovibrio* sp. after being exposed for 22 days to Cr(III) solution. Bar represents 2 µm.

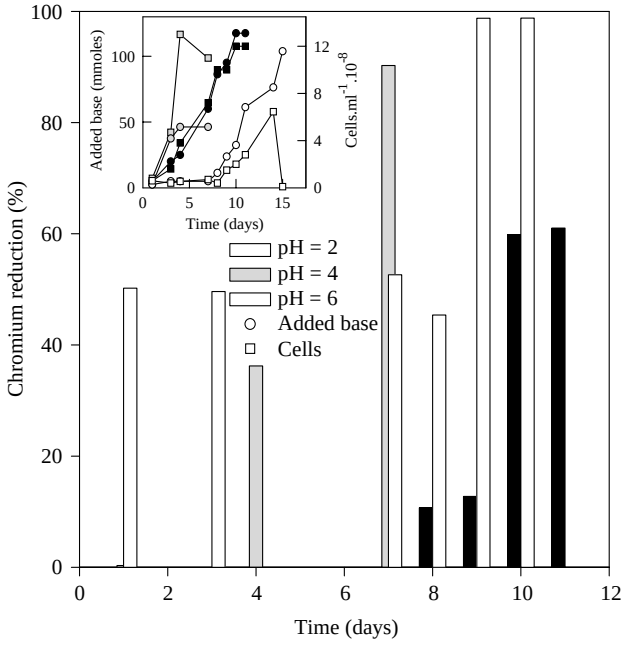

Fig. 2. Cr(VI) reduction by *Acidithiobacillus thiooxidans* at different pH values. Outer graph: Chromium reduction vs. time. Inner graph: mmoles of added base vs. culture time and number of cells ml^{-1} vs. time.

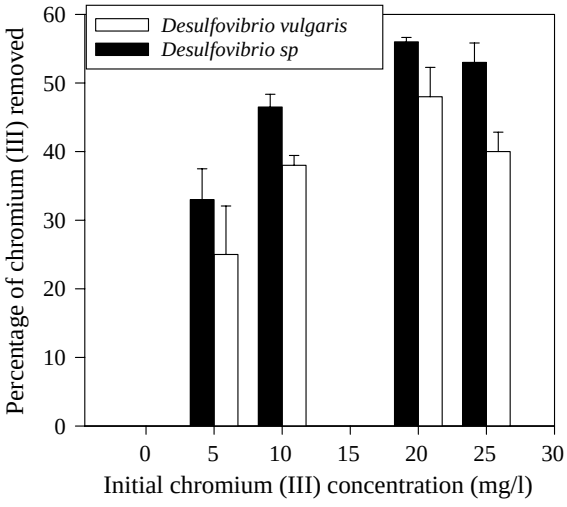

Fig. 3. Percentages of Cr(III) immobilization in *Desulfovibrio* sp and *Desulfovibrio vulgaris* cultures after 22 days, for different Cr(III) concentrations. Bars represent standard deviations for duplicates.

all cases, chromium reduction increased with the age of the culture. As a compromise between the capability of reducing Cr(VI) and the number of bacteria, we decided to use an intermediate pH value (about 5) in the combined experiment.

3.2. Chromium(III) immobilization

Fig. 1B shows a SEM microphotograph of a *Desulfovibrio* sp. culture in the modified Postgate C medium for 22 days and Fig. 3 illustrates the percentages of Cr(III) precipitation after 22 days in *Desulfovibrio* sp. and *Desulfovibrio vulgaris* cultures. The highest precipitation was about 60% using an initial Cr(III) concentration of 20 $mg\,l^{-1}$. According to these figures, the mixed population of the *Desulfovibrio* sp. culture was more efficient than the pure culture of *Desulfovibrio vulgaris* in removing Cr(III) from the solution for any initial concentration. Moreover, *Desulfovibrio* sp. was able to grow in the presence of a Cr(III) concentration as high as 25 $mg\,l^{-1}$. Thus, the former culture was selected for the combined process. EDXA analysis of the solid residues obtained in a culture of *Desulfovibrio* sp. (Fig. 4) showed the presence of chromium. The peaks corresponding to sulfur and phosphorus are higher in the presence than in the absence of chromium indicating that the precipitates could be chromium phosphate and chromium sulfide.

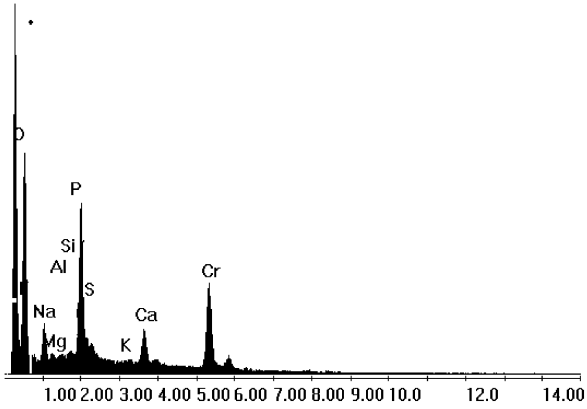

Fig. 4. EDXA spectrum of the precipitates obtained during the Cr(III) immobilization experiment showing peaks corresponding to phosphorus, sulfur and chromium.

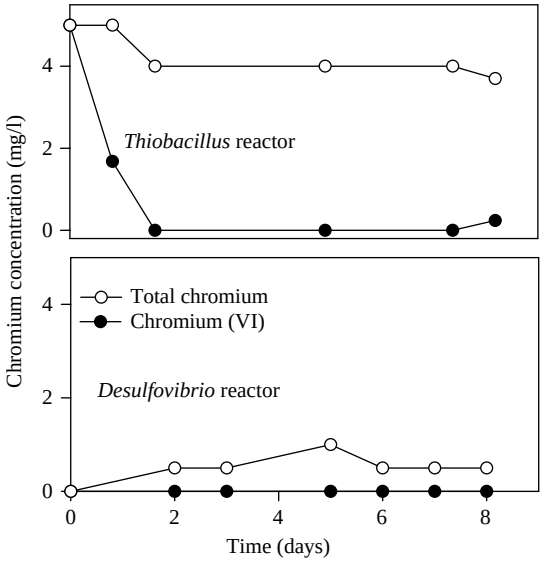

Fig. 5. Combined process for Cr(VI) reduction (by *Acidithiobacillus thiooxidans*) and Cr(III) immobilization (by *Desulfovibrio* sp.) showing Cr(VI) and total chromium concentrations in the effluents of both reactors when run in tandem.

3.3. Combined process

Levels of Cr(VI) and Cr(III) in both reactors are shown in Fig. 5. During the experiment, 6 mg of Cr(VI) passed through the *At. thiooxidans* culture. This amount was almost completely reduced to Cr(III), but the balance between Cr(VI) entering the reactor and Cr(III) in the effluent indicated that about 20% of total chromium was precipitated in the *At. thiooxidans* reactor. In the second reactor, only 0.3 mg of Cr(III) were not immobilized by *Desulfovibrio* sp. cells. As in flask experiments, EDXA analysis of the solid residues obtained showed the probable presence of chromium phosphate and chromium sulfide.

The combination of Cr(VI) reduction, using *At. thiooxidans* bacteria, and bioprecipitation of Cr(III), by *Desulfovibrio* sp. proved to be effective in removing chromium from a solution. Both processes operating sequentially under continuous flow conditions could be used for bioremediation of wastewaters containing hexavalent and trivalent chromium.

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