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Short communication

Chromate reduction by *Arthrobacter* CR47 in biofilm packed bed reactors

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Abstract

Bacterial strain Cr47 was isolated from a landfarming process soil sample. It was identified, by 16s rDNA sequencing, as *Arthrobacter* sp. The time course of the Cr(VI) reduction was monitored in batch operated packed bed biofilm reactors (12mL void volume) and in recirculating packed bed biofilm reactors (100 mL void volume) inoculated with bacterial strain Cr47. The reduction was evaluated with, 30 mg L^{-1} Cr(VI) laboratory solutions prepared with $K_2Cr_2O_7$ and enriched with glucose-minimal medium, and with 30 mg L^{-1} Cr(VI) industrial model solutions prepared with chrome plating waste waters enriched with sucrose-minimal medium. Under batch mode the reduction reaction by the biofilm seemed to fit well an exponential-decay model with a first order kinetic parameter of 0.071 mg(L h)$^{-1}$ Cr(VI). In the recirculating reactor, monitored after 4 weeks from inoculation and fed with laboratory solutions the removal rate was 0.79 mg(L h)$^{-1}$. In the reactor fed with the industrial model solutions the maximum Cr(VI) removal rate attained was 0.49 mg(L h)$^{-1}$. *Artrobacter* sp. packed bed biofilm reactors achieved Cr(VI) reduction rates comparable to other aerobic and anaerobic fixed film bioreactors previously reported.

Keywords: Chromate; Biofilm reactor; Biological treatment; Recirculating reactor; Reduction; Kinetics

1. Introduction

Cr(VI) (chromates) are a common residue of industrial processes such as, leather tanning, corrosion control, plating and pigment manufacture. In a study published by DAMA-IDEAM (Bogota's municipal environmental protection agency) on industrial effluents in Bogotá in the year 2002 was reported that the two kinds of economical activity that produced waste water discharges contaminated with Cr(VI) were the leather tanning and the metal plating industry. The percentage of those discharges that did not complied with the environmental regulation (0.5 mg L^{-1}) was 54.9% and 78.8% respectively. The average concentration of Cr(VI) in the discharges from the leather tanning industry was 13.23 mg L^{-1}, and 61.71 mg L^{-1} in the metal plating industry. This is causing a discharge of 450 kg day^{-1} of chromium to the city's river. There are reports of higher chrome urine levels in the general population (not directly involved in the tannery or chrome plaiting labor) that lives and works in those tannery and chrome plating districts. The estimated percentage of this population with urine chromium levels above the accepted 10 μg L^{-1} limit is 6.3%. Health problems that have been associated with labor in industries that use or produce chromates, has become in Bogota's case a public health issue. The great majority of those small tanneries and chrome plating shops will loose their financial viability if they had to implement traditional treatment methods, which require the use of large quantities of expensive chemical reagents.

Chromates are highly soluble in water which gives them a high mobility in aquatic environments. Chromates are strong oxidizing agents that can react with nucleic acids producing mutagenic and carcinogenic effects [1,2]. The most widely accepted hypothesis of the mechanisms of Cr(VI) toxicity involved entry into the cells by the sulfate transport channels and subsequent intracellular reduction to Cr(V) and reactive oxygen species. However According to recent data [3,4], extracellular reduction of Cr(VI) in animals may occur in the initial steps of Cr(VI) metabolism. Evidence is mounting that observed long lived Cr(V) complexes are stabilized by a combination of extra-

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cellular and intracellular species. In either case, typical aerobic, one electron reduction of Cr(VI), results in the formation of reactive oxygen species, however if they are formed extracelluarly damage to DNA can be reduced.

A great variety of bacteria, including strains from *Pseudomonas* sp., *Bacillus* sp. sulphate-reducing bacteria (SRB), and *Microbacterium* sp., have the ability of reducing Cr(VI) [5–9]. These bacteria reduce the highly toxic and soluble Cr(VI) to Cr(III) which spontaneously forms insoluble oxides (solubility <0.01 mg L^{-1}) and hydroxides at pH above 5, making the metal ion less bioavailable and less toxic [5,10]. The Cr(VI) reduction mechanism in the *Arthrobacter* sp. has been elucidated in recent studies with *Arthrobacter oxydans*. Its been reported that macromolecules at the cell wall of *A. oxydans* could act as ligands and electron donors to Cr(VI) and as stabilizers of Cr(V) intermediates. The reduction of Cr(VI) in *A. oxydans* begins with the formation of Cr(V)-diol complexes at the surface of bacteria [4].

Besides this ability to chemically transform Cr(VI), bacterial cells are excellent nucleation sites for the formation of Cr(III) complexes [6,12]. This is a desirable property for biological waste water treatments where biomass is usually separated, from the water, by sedimentation. Biofilms have been reported to be more resistant to Cr(VI) toxicity than suspended growth cells [11,13], concentration gradients, complex formation, ion entrapment and the stationary phase of the biofilm biomass are possible reasons for the superior resistance [11].

Conventional methods for removing chromates from industrial waste waters usually include chemical reduction, with sulfurous acid, followed by NaOH-induced precipitation [14]. This process has high energy costs and requires big quantities of chemical reagents. Other methods immobilize the chromate ions in a solid phase, without reducing it; these include ionic exchange, adsorption in activated carbon, among others. These methods may have low costs but desorption usually occurs, when the adsorbent solid is deposited in landfills, contaminating ground waters.

The use of biofilm packed bed reactors for the biological reduction of Cr(VI) has been previously reported by Chirwa and Wang [13]. *Bacillus* sp. was used in that work for the transformation of Cr(VI) into Cr(III). Dermou et al. [15] also reported the biological reduction of Cr(VI) in a pilot-scale tricking filter inoculated with an industrial sludge containing strains from the *Acinetobacter* sp. Other studies have reported the use of anaerobic bacteria and conditions in film fixed bioreactors to reduce Cr(VI) [16,17]. Among these micro-organisms, sulphate-reducing bacteria (SRB) are known to readily use hydrogen and indirectly reduce Cr(VI) by hydrogen sulphide or by using Cr(VI) as a terminal electron acceptor [16].

The aim of this study is to use the Colombian native Cr(VI)-reducing *Arthrobacter* Cr47 to form Cr(VI)-reducing biofilms in gravel packed bed reactors. The Cr(VI) reduction, in water contaminated with the average reported concentration of Cr(VI) for the discharges in Bogotá, was evaluated. The time course of the Cr(VI) reduction was monitored in batch operated packed bed biofilm reactors and in flow-through packed bed biofilm reactors with recirculation flask. To further evaluate the capacity, of the proposed system, to treat an industrial waste water contaminated with Cr(VI), tests were done with Cr(VI) industrial model solutions prepared with a chromic acid bath from a chrome plating industry. The Carbon source in the growth medium was changed from anhydrous glucose to commercial sucrose, a more available and cheaper sugar (in the Colombian market). For industrial applications the sugar cane molasses, a by-product of the sugar production, that contains mostly sucrose can be used as growth medium.

2. Materials and methods

2.1. Bacterial strain

The strain used in this study, for its potential as a chromate reducer, *Arthrobacter* Cr47, is an aerobic, non-spore-forming bacterium. The strain was isolated from a soil sample, contaminated with hydrocarbons and heavy metals, taken from a landfarming process in Cundinamarca, Colombia. It was selected from a group of 14 strains isolated, from the same soil sample, for its ability to grow in the presence of chromate and for its higher Cr(VI) reduction rate. It was identified using 16S rRNA analysis. The gene was amplified by PCR, first with external primers GM3F (5′ AGAGTTTGATCMTGGC 3′) and GM4R (5′AAGTCGTAACAAGGTA 3′) and then with internal primers 357F (5′ACTCCTACGGGAGGCAGCAG 3′) y 1087R (5′GGGTTAAGTCCCGCAACGAG 3′). The sequence obtained was compared with the available sequences in the internet web site Ribosomal Database (http://rdp.cme.msu.edu/index.jsp).

2.2. Media

Arthrobacter Cr47 was grown and maintained in Luria broth: 5 g L^{-1} of yeast extract, 10 g L^{-1} NaCl and 10 g L^{-1} triptone. For chromate reduction, a glucose-minimal medium was used. This was made in two parts, autoclaved separately, and mixed at room temperature prior to use. Part A consisted of 10 g L^{-1} of glucose, 2.67 g L^{-1} of NH_4Cl, 5.35 g L^{-1} of Na_2HPO_4. Part B (6 mL per L of A) consisted of, 0.1 g L^{-1} of $CaCl_2{\cdot}2H_2O$, 10 g L^{-1} of $MgSO_4{\cdot}7H_2O$, 0.07 g L^{-1} of $MnSO_4{\cdot}7H_2O$, 0.4 g L^{-1} of $FeSO_4{\cdot}7H_2O$. The sucrose-minimal medium contained the same salts but 30 g L^{-1} of sucrose, and it was not autoclaved, the sucrose and salts were dissolved together in tap water over a heater.

2.3. Cr(VI) analysis

Chromate reducing activity was measured as the decrease of Cr(VI) with time using the colorimetric reagent diphenylcarbazide [19]. The diphenilcarbazide solution was prepared by dissolving 250 mg of 1,5-diphenylcarbazide in 50 mL of acetone, the solution was stored in a brown bottle at 4 °C [20]. The reaction mixture was done in essay plastic tubes as follows, for the upper part of the concentration range (30–5 mg Cr(VI) L^{-1}): 4.72 mL of distilled water, 160 μL of sample, 20 μL of H_2SO_4 10% v/v, and 100 μL of the diphenylcarbazide solution, giving a total volume of 5 mL. For the concentration