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REDUCTION OF DICHROMATE BY *THIOBACILLUS FERROOXIDANS*

Sisti F.[1], Allegretti P.[2] and Donati E.[1]*
[1]Centro de Investigación y Desarrollo de Fermentaciones Industriales
[2]Laboratorio de Química Orgánica y Medio Ambiente
Facultad de Ciencias Exactas, 47 y 115 (1900) La Plata, Argentina

ABSTRACT
Chromium(VI) was reduced by *Thiobacillus ferrooxidans* grown with elemental sulphur as the sole energy source. Chromium(VI) reduction (as high as 2000 μM), was due to the presence of sulphite and thiosulphate, among others with high reducing power which was generated during the sulphur oxidation by the bacteria. Therefore, *Thiobacillus ferrooxidans* could be used to treat chromium(VI)-containing industrial effluents.

INTRODUCTION
Hexavalent chromium [mainly in the form of chromate (CrO_4^{2-}) and dichromate ($Cr_2O_7^{2-}$)] are used in a wide variety of commercial processes as well as chromite ore processing. As a result of these processes, significant amounts of both forms of chromium(VI) occurs in the environment. These compounds are toxic and mutagenic for most organisms including humans. Its reduction to chromium(III) represents a potentially detoxification process because this has less solubility and bioavaibality than chromium(VI). Usually, growing cells have been used for the direct reduction of chromium(VI) to chromium(III) (Lovely and Philips, 1994; Shen and Wang, 1995; Cifuentes *et al*, 1996; James, 1996; Llovera *et al*, 1993; Turick *et al*, 1996). Most of the micro-organisms used were heterotrophic and they needed organic sources which could be reduced by chromium(VI) fast. However, there were not adequate controls in some cases.

The chemiautotrophic bacteria *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are able to obtain energy from the catalysed oxidation of partially-oxidised sulphur compounds using O_2 as the last acceptor of electrons (Ehrlich, 1990; Tuovinen, 1990). Moreover, *T. ferrooxidans* also catalyse the iron(II) oxidation in an aerobic condition. The oxidation of such sulphur compounds, particularly that of elemental sulphur, generates a series of sulphur compounds (sulphite and thiosulphate, among others) with high reducing power. This activity has been used in cultures of *T. thiooxidans* to catalyse the reduction of manganese(IV) (Porro *et al*, 1990), iron(III) (Donati *et al*, 1995) and vanadium(V) (Briand *et al*, 1996).

In this study, we used the reduced power of *T. ferrooxidans* cultures (without organic sources) for the direct reduction of dichromate which is the most important form of chromium(VI) at the pH of those cultures.

MATERIALS AND METHODS
Micro-organisms. A *T. ferrooxidans* strain (from Santa Rosa de Arequipa, Perú) was used. The strain was routinely sub-cultured in iron-free 9 K medium (Silverman and Lundgren, 1959) with powdered sulphur (10 g/l) as energy source.
Chromium toxicity. To estimate the toxicity of chromium(III) and chromium(VI), *T. ferrooxidans* was grown in flasks containing 0, 50, 100, 200 and 400 μM of chromium(III) or

chromium(VI) with 10 g/l of powdered sulphur as the sole energy source. The flasks were inoculated with a *T. ferrooxidans* culture to allow a bacterial population of $4x10^7$ bact/ml. The experiments were carried out at 180 rpm and 30 °C.

Reduction of dichromate. The assays were carried out in agitated flasks at 180 rpm and 30 °C. 100 ml of a iron-free 9 K medium with an initial bacterial population of $5x10^8$ bact/ml were added in all flasks. The initial pH was 1.70. Analytical-grade potassium dichromate was added to allow concentrations of chromium(VI) between 200 and 2000 μM (200, 500, 1000, 1500 and 2000 μM) in the flasks. Additionally, 1 gram of analytical grade powdered sulphur was added as the sole energy source. The experiments were carried out by duplicate. The disappearance of chromium(VI) in the solution was used as a measure of chromium(VI) reduction to chromium(III) because species of chromium(VI) and chromium(III) are fully soluble at the ph of the cultures.

Control system. Sterile controls were carried out in similar conditions for each dichromate concentration. A inoculated system with chromium(VI) (1000 μM) as dichromate was boiled during 20 minutes before adding dichromate to estimate the dichromate reduction due to the organic matter of bacteria.

Analytical determinations: The amount of residual dichromate was estimated spectrophotometrically at 350 nm. Previously, the samples were centrifuged for 30 minutes at 4500 rpm (to eliminate cells and elemental sulphur) and 0.5 ml of 0.05 M H_2SO_4 were added. Bacterial populations were determined by using a Petroff-Hausser counting chamber in a microscope with a contrast phase attachment. The sulphuric acid formed by the microbial oxidation of sulphur was analysed by tritation with a sodium hydroxide solution.

RESULTS AND DISCUSSION

Acid production in cultures of *T. ferrooxidans* was not affected by the addition of chromium(III) (figure 1), indicating that chromium(III) (concentrations between 50 and 400 μM) was not toxic for the bacteria. The results in acid production and bacterial populations (data not shown) in similar cultures with chromium(VI) instead of chromium(III) demonstrate growth inhibition (a long lag phase). Chromium(VI) reductions were less than 20 % of the initial concentrations in the inoculated systems. There was no significant chromium(VI) reduction in the control systems.

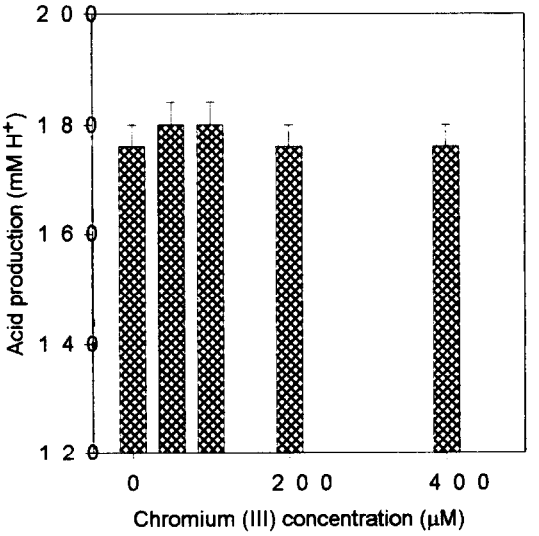

FIGURE 1: Influence of chromium(III) on the acid production by cultures of *T. ferrooxidans* on elemental sulphur. The error in acid production determination was ±4 mmoles/l.

Figure 2 shows the time profiles of chromium(VI) reduction by cultures of *Thiobacillus ferrooxidans* when the initial population was very high (the culture was in exponential phase growth when chromium(VI) was added).

As it can be seen, a minimal chromium(VI) reduction occurred in the control systems for 20 days. Thus, the abiotic chromium(VI) reduction with elemental sulphur as electron donor is very slow.

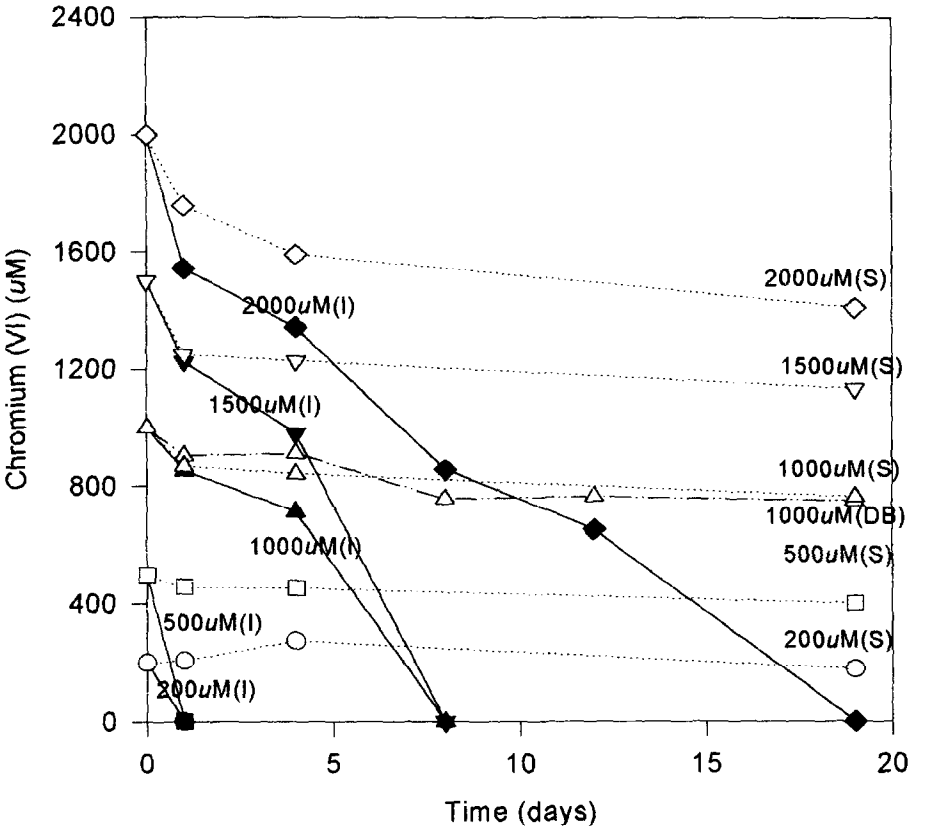

FIGURE 2: Chromium(VI) reduction in cultures of *Thiobacillus ferrooxidans*. Initial chromium(VI) concentrations: (○): 200 μM; (□): 500 μM; (Δ): 1000 μM; (▽): 1500 μM; (◊): 2000 μM. Black symbols represent inoculated cultures (I), grey symbols represent sterile controls (S) and white symbols represent dead bacteria (DB). All values are the means from two systems. The errors in chromium(VI) concentration were lower than 36 μM.

The chromium(VI) reduction in inoculated systems was very fast when chromium(VI) concentrations were lower than 500 μM. On the other side, the chromium(VI) reduction required 19 days when the initial chromium(VI) concentration was 2000 μM.

Additionally, there was little acid consumption while chromium(VI) was present. Later, there was an important acid production (data not shown) which is in agreement with the bacterial sulphur oxidation to sulphuric acid. Bacterial population (data not shown) only increased after the total chromium(VI) reduction. Moreover, there was an initial decrease of bacterial population in cultures with chromium(VI) concentrations higher than 1000 μM.

The chromium(VI) reduction was probably due to the action of the series of sulphur compounds (mainly sulphite) which were generated in the bacterial oxidation of elemental sulphur. The following mechanism can be proposed for the process:

$$S + O_2 + H_2O \xrightarrow{T.\ ferrooxidans} SO_3^{2-} + 2\,H^+$$

$$Cr_2O_7^{2-} + 8\,H^+ + 3\,SO_3^{2-} \rightarrow 2\,Cr^{3+} + 4\,H_2O + 3\,SO_4^{2-}$$

This proposal is consistent with the acid consumption while chromium(VI) was present (see above). Additionally, the indicated evolution of bacterial population showed an evident bacterial growth inhibition by chromium(VI) (specially for chromium(VI) concentrations higher than 1000 μM). In these cases, the bacterial sulphur oxidation was very slow. The very fast reduction in cultures with 200 and 500 μM of chromium(VI) was surely produced by the reducing sulphur compounds which were initially present in the cultures.

Since dichromate is a strong oxidising agent, its reduction could be a result of a chemical reaction with the organic matter. However (as it can be seen in figure 2), there was a little disappearance of chromium(VI) (similar to the control) in a culture with cells that had been previously boiled for 20 minutes. Thus, the chromium(VI) reduction was due to the indirect bacterial action.

Most of the previous reports show chromium(VI) reduction at concentrations not higher than 500 μM and the using micro-organisms which require a heterotrophic medium.. However, this paper shows chromium(VI) reduction by an autotrophic micro-organism at concentrations at least as high as 2000 μM. Moreover, the chromium(VI) reduction may be enhanced by using chromium(VI)-adapted cells that we are at present trying to obtain. Thus, this micro-organism may be used for the efficient remediation of chromium(VI)-contaminated effluents.

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