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Bacterial removal of chromium (VI) and (III) in a continuous system

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Abstract The capacity of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* to reduce different concentrations of hexavalent chromium in shake flask cultures has been investigated. *A. ferrooxidans* reduces 100% of chromium (VI) at concentrations of 1, 2.5 and 5 ppm, but in the presence of 10 ppm only 42.9% of chromium (VI) was reduced after 11 days of incubation. *A. thiooxidans* showed a lower capacity to reduce this ion and total reduction of chromium (VI) was only obtained for concentrations of 1 and 2.5 ppm, whereas 64.7% and 30.5% was reached for 5 and 10 ppm, respectively, after 11 days. A continuous flow mode system was subsequently investigated, in which *A. thiooxidans* was immobilized on elemental sulphur and the acidic medium obtained was employed to solubilize chromium (III) and to reduce chromium (VI) present in a real electroplating waste [30% of chromium (III) and 0.1% of chromium (VI)]. The system enabled the reduction of 92.7% of hexavalent chromium and represents a promising way to treat this type of waste in the industry.

Keywords *A. ferrooxidans* · *A. thiooxidans* · Chromium (VI) · Chromium (III) · Reduction · Solubilization

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Introduction

Due to industrial expansion, large quantities of industrial wastes are accumulating in many countries and cannot be disposed without prior special treatments. In particular, waste products from the mining and metal refining industries, sewage sludges and residues from power station and waste incineration plants can contain heavy metals at high concentrations. Usually, these heavy metals can be leachated from the soil to the surface water system (Chang et al. 1984; Dowdy et al. 1991) at concentrations higher than they are allowed (CEC 1986). For this reason, they cannot be disposed into wastewaters plant and must be submitted to special treatment in order to reduce metals contents. Therefore, the management of waste sludge produced from the industrial activity becomes the most important issue of environmental protection.

Among heavy metals presents in sludge, chromium is one of the most common. This metal

exists in two stable oxidation states, trivalent and hexavalent chromium. The trivalent state is less toxic and mobile, while hexavalent chromium is easily soluble and 100-fold more toxic than trivalent. So, the reduction of Cr (VI) to Cr (III) is an attractive and useful process for remediation of Cr (VI) pollution, and the technologies focusing on transformation of Cr (VI) to Cr (III) have accordingly received much more concerns. Many studies have reported that hexavalent chromium could be reduced into trivalent chromium by chemical methods (Erdem 2006; Guo et al. 2006; Souza et al. 2006; Panswad et al. 2001). Although these physical and chemical treatment techniques have been extensively applied in practice, they show some limitations such as low efficiency and high cost (Rulkens et al. 1995). Generally, biotechnology is a powerful and versatile alternative to chemical and physical methods for resolving many problems of environmental pollution because of low demand of energy and materials, and low generation of waste and emissions. Recently, biological chromium (VI) reduction has been developed with some advantages due to the lower costs and the significant smaller quantities of the produced sludge (Sisti et al. 1996; Ganguli and Tripathi 1999; Konovalova et al. 2003; Liu et al. 2006).

Industrial residues can be finally disposed and even utilized as fertilizer on agricultural lands (Davis 1987; Scheltinga 1987). However, if their metal content of metals as Zn, Al, Mn, Ni or Cu is high (Sreekrishan et al. 1996; Chen et al. 2004; Tichy et al. 1998), previous leaching to decrease the content below the guidelines set for each country should be done. Bioleaching process is largely documented in literature (Solisio et al. 2002; Porro et al. 1990; Krebs et al. 1997; Gadd 2000); it could be to the extraction of metals from various ore concentrates (Veglio et al. 1999; Ebner et al. 1978) but it has been recently developed to remove heavy metals from sludges, sediments and soils containing metals at high concentrations (Sreekrishan et al. 1996; Chen et al. 2004; Tichy et al. 1998). The microorganisms intensively used in bioleaching processes belong to the genus *Acidithiobacillus*. The oxidation and acid producing activity of sulphur-oxidizing bacteria are the primary mechanisms of solubilization of heavy metals in the bioleaching process. These sulphur-oxidizing bacteria can obtain energy from the oxidation of elemental sulphur or reduced inorganic sulphur (Tuovinen 1990), and cause bioacidification and solubilization of heavy metals. This process is a novel technology for treatment of heavy metals from sludges by application of the sulphur biocycle. After the solubilization of the metals, the leachates can be re-used into the industrial processes or even treated to metal recovery. The performance of bioremediation process is affected by various physical, chemical and biological parameters (Jensen et al. 1995). Among these affecting parameters, variation of the pH has been considered to be a key factor in determining the solubilization of heavy metals in the process (Chen et al. 2001). Moreover, the nature of the sludge, type of contact between cells and solids and operation mode would be important in the efficiency of the bioremediation process. Therefore, a through understanding of these parameters is useful to optimize the process in order to its application in a industrial purpose.

The aim of this study is to evaluate the capacity of two *Acidithiobacillus* species (*ferrooxidans* and *thiooxidans*) to reduce different concentrations of chromium (VI). After this study, a continuous flow system to treat an electroplating waste with high content of chromium (III) and chromium (VI) was investigated.

Materials and methods

Microorganisms and maintenance

Strains used in this study were *A. ferrooxidans* (DSM 11477) and *A. thiooxidans* (DSM 11478). *A. ferrooxidans* was routinely sub-cultured in an iron-free medium to remove the presence of this element as an energy source. Cells of both microorganisms were cultivated in iron-free 9 K medium (Silverman and Lundgren 1959), with powdered sulphur (10 g/l) as the energy source. Media were inoculated at 10% v/v with cultures in exponential growth phase. Each medium was adjusted to pH 1.8 for *A. ferrooxidans* and pH 2.5 for *A. thiooxidans*, cultures were incubated in a

shaker at 150 rpm and 30°C. Media were replaced with fresh medium when cultures reached a pH near to 1.

Chromium (VI) reduction in shake flasks

The experiments were carried out in 250 ml Erlenmeyer flasks supplemented with 90 ml of iron-free 9 K medium (pH 4.0), 1 g of powdered sulphur and different concentrations of potassium dichromate [between 1.0 and 10 ppm of chromium (VI)]. These assays were inoculated at 10% v/v with cultures of *A. ferrooxidans* or *A. thiooxidans* in exponential growth phase. Experiments were incubated in a rotary shaker at 150 rpm and 30°C.

A. thiooxidans immobilization

Immobilization of *A. thiooxidans* was carried out in a column reactor that was prepared as follows: 170 g of elemental sulphur (particle size: in the range 2–4 mm) and 150 ml iron-free 9 K medium (initial pH 2.5) inoculated at 10% v/v with an *A. thiooxidans* culture in exponential growth phase was added to a column (150 ml working volume). A flow of 9.9 l/h of air was continuously fed into the reactor. The system was maintained at 30°C and when the pH value was 1.0, all the medium was replaced by fresh iron-free 9 K medium adjusted to pH 5.0. This ''draw and fill'' procedure with fresh medium at pH 5.0 was repeated until a constant rate of sulphuric acid production was obtained. It was assumed that the biofilm had then formed. At that moment, the system started to work in continuous operation mode, with the influent (feed) and the effluent (acid medium) flows supplied by two peristaltic pumps.

Chromium (VI) reduction and chromium (III) solubilization in a continuous regime system

The chromium waste consisted of a fraction of clay-containing filter press from the electroplating industry. The press had been used to retain chromium during the electroplating process. A total of 157 g of this waste was added to a glass column. The chromium composition of the waste was as follows: 30% of chromium (III) and 0.1% of chromium (VI). The acidic medium obtained from the *A. thiooxidans* reactor was supplied to the column containing the chromium waste. The effluent was collected in a reservoir and this was designated as ''leachate''.

When the pH of *A. thiooxidans* was above 1.5, the addition of acid medium from the biological reactor to the waste column was interrupted and the system was operated in two ways:

Initially, both reactors were maintained in recirculation. However, due to adverse effects in the 'leachate', the operation mode was subsequently changed and the biological reactor was in recirculation and the waste column was drawn slowly to facilitate contact between the acid medium and the chromium waste. This column remained empty until a new charge was added after the pH in the biological reactor rose above 1.5 again. A diagram of the system is presented in Fig. 1.

Analytical methods

A CRISON (52-02) pH meter with an Ag electrode was used to follow the pH. The sulphuric acid obtained by bacterial oxidation of sulphur was analysed by titration with a 0.02 N sodium hydroxide solution. Free bacterial population was determined by counting in a Neubauer chamber in conjunction with an optical microscope (Olympus BH-2).

Hexavalent chromium was determined by the diphenylcarbazide method (Urone 1955): 0.25 ml of solution (0.5 g of diphenylcarbazide in 10 ml of acetone) was added to 5 ml of sample (diluted where necessary). 0.05 M H_2SO_4 was used to acidify the samples. After 10 min of incubation at room temperature, the absorbance at 540 nm was determined by spectroscopy (HP8453).

The total chromium concentrations of samples were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Iris Intrepid—Thermo-elemental Series 11393, Model 14425501) (Clesceri et al. 1998). Prior to analysis the samples were filtered to remove biomass and other solid particles, acidified (2N HNO_3) and stored at 4°C until the measurement was taken.

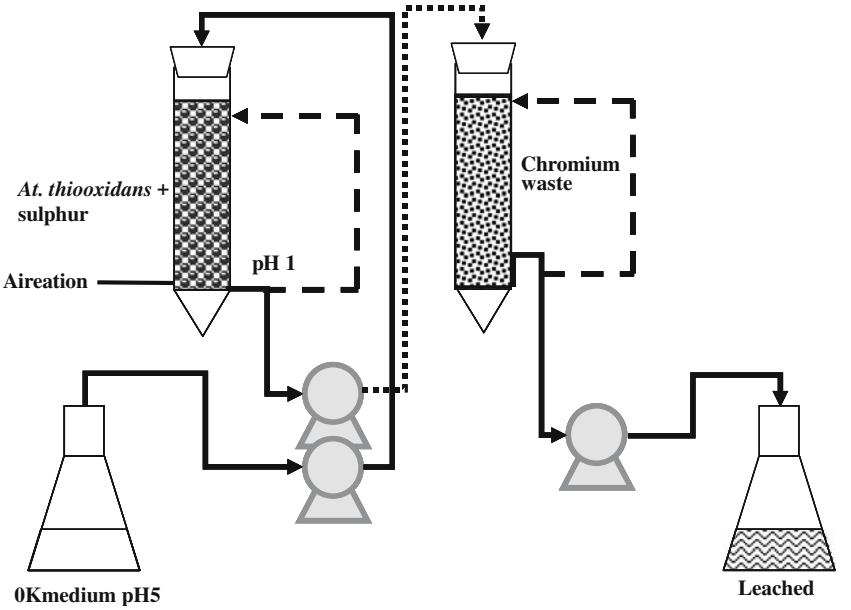

Fig. 1 Diagram of system for chromium (VI) reduction and chromium (III) solubilization in continuous operation mode (0 K medium is iron-free 9 K medium)

Results and discussion

Chromium (VI) reduction in shake flasks

A study of the chromium (VI) tolerance and reduction capacities of *A. ferrooxidans* and *A. thiooxidans* was carried out with 1, 2.5, 5 and 10 ppm of chromium (VI). The evolution of pH, bacterial population and chromium (VI) concentration in solution versus time at different initial chromium concentrations for *A. ferrooxidans* and *A. thiooxidans* are shown in Figs. 2 and 3, respectively.

The reduction percentage was estimated by the ratio between chromium (III) concentration [determined as the difference between total chromium and chromium (VI)] and the initial chromium (VI) concentration. The maximum chromium (VI) reduction percentage in each experiment is shown in Table 1 along with the number of days necessary to achieve these values.

As a result of sulphur oxidation, the *A. ferrooxidans* culture showed a large decrease in pH (since 1.8 to 0.8) in less than eleven days (Fig. 2) for chromium concentrations up to 5 ppm. This effect corresponds with a final bacterial population between 3.2 and 9.3×10^8, approximately, for cultures with those concentrations. A decrease in pH was not observed for cultures with higher concentrations until total chromium (VI) reduction was achieved; this behaviour was accompanied by sulphur oxidation and bacterial growth. This fact is consistent with the observation reported by Sisti et al. (1998), who detected significant bacterial growth just when chromium (VI) disappeared from the medium—indicating that bacterial sulphur oxidation, partially inhibited previously, re-started from that moment. The initial fall in the chromium (VI) concentration can be attributed to reducing compounds attached to colloidal sulphur present in the inoculum added to each experiment. In the presence of 10 ppm of chromium (VI), the behaviour was different due to significant changes in pH and bacterial population were not observed and only a reduction of around 3 ppm of chromium (VI) was produced in the first day of incubation. This small decrease is probably due to reducing compounds which were included into the inoculum. The bacterial inhibition led to a decrease in the sulphur oxidation and, subsequently and just 32.7% of the chromium (VI) was reduced after 11 days.

A. thiooxidans was more sensitive to the presence of chromium (VI) than *A. ferrooxidans* (Fig. 3). The lower ability of *A. thiooxidans* to reducechromium (VI), in comparison to *A. ferrooxidans*, was also observed by Sisti et al. (1998).

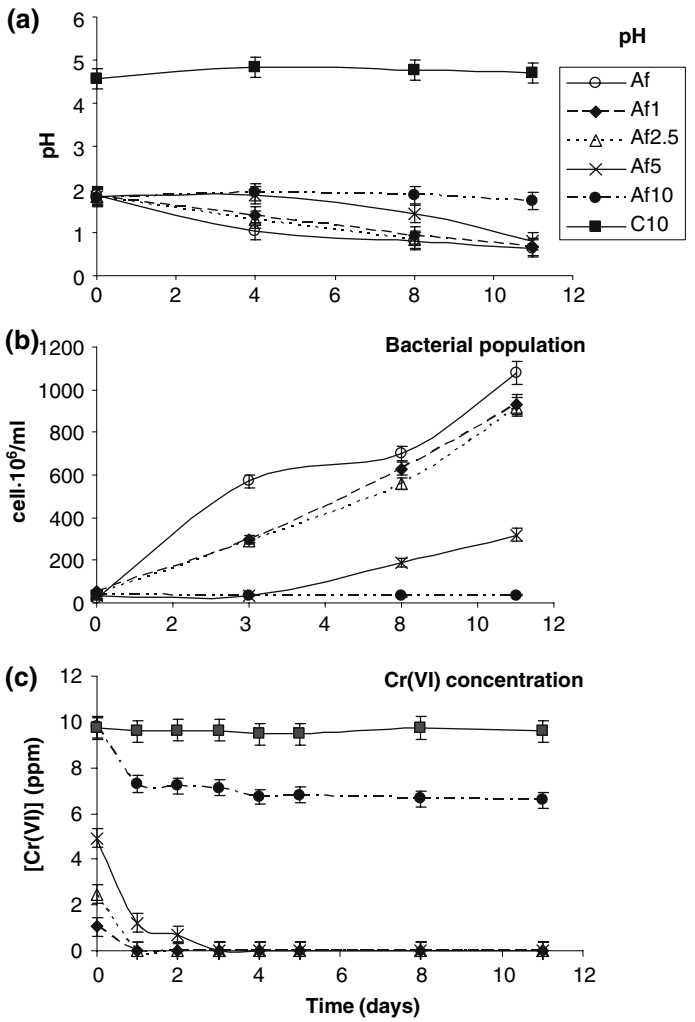

Fig. 2 Evolution of pH (**a**), bacterial population (**b**) and Cr (VI) concentration (**c**) for *A. ferrooxidans* in the presence of 1, 2.5, 5 and 10 ppm of Cr (VI) and for a control [10 ppm of Cr (VI)]

A pH decrease indicating a significant bacterial growth was observed in the presence of 1 and 2.5 ppm of chromium (VI); total reduction was reached in 24 h. Higher concentrations (5 and 10 ppm) of chromium (VI) inhibited the bacterial activity; that is why pH and bacterial population were maintained practically constant. Chromium reduction was mainly observed in the initial few days, that probably means the reducing compounds were present into the inoculum. After 11 days, only 64.7% and 16.5% of the added chromium (VI) were reduced for experiments with 5 and 10 ppm, respectively. The control assay, prepared with 10 ppm of chromium (VI) and maintained at a pH of about 4.7, showed a very low level of chromium (VI) reduction (1.5%) throughout the experiment, thus confirming that the chromium reduction was due to bacterial sulphur oxidation.

The level of chromium reduction reported in this work is much lower than that found by Allegretti et al. (2006), although in that case the experimental conditions were different as they involved the use of filtrates (fraction between 0.45 and 3 μm) of growth cultures that contained biomass, sulphur particles and associated sulphur compounds; thus, the concentration of reducing compounds was much higher than in the study reported here.

The low tolerance of the *Acidithiobacillus* strains studied led us to consider an indirect continuous system for chromium waste treatment. This approach involved the generation of an acidic reducing medium by sulphur-oxidizing

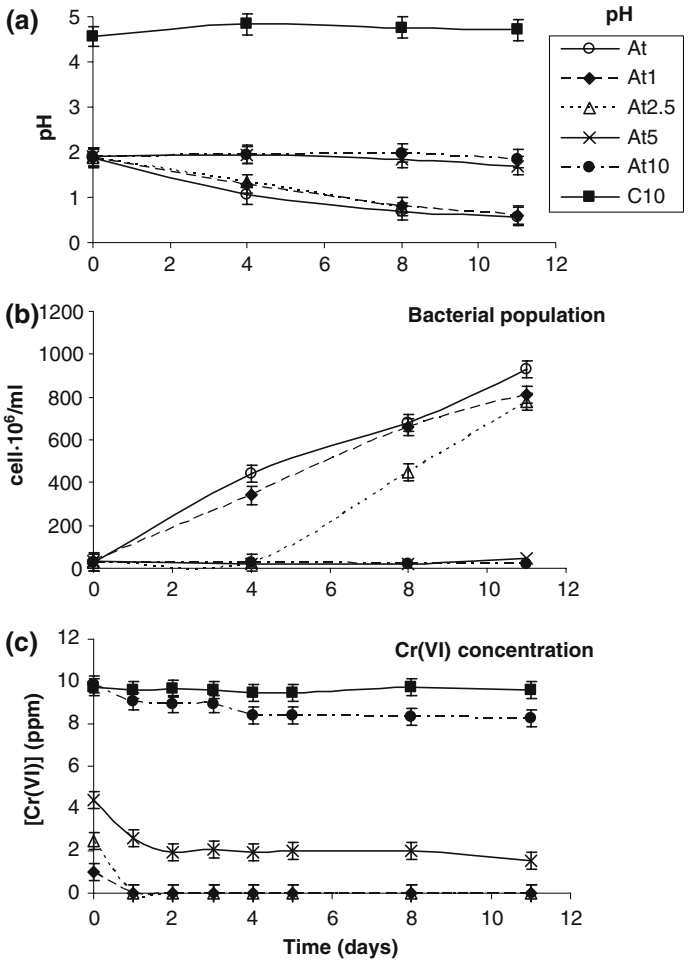

Fig. 3 Evolution of pH (**a**), bacterial population (**b**) and Cr (VI) concentration (c) for *A. thiooxidans* in the presence of 1, 2.5, 5 and 10 ppm of Cr (VI) and for a control [10 ppm of Cr (VI)]

bacteria and the treatment of chromium waste with the medium in an independent reactor.

Chromium (VI) reduction and chromium (III) solubilization in a continuous regime system

The aim of this experiment was to exploit the ability of sulphur-oxidizing bacteria to produce an acidic reducing medium to solubilize chromium (III) from insoluble compounds and to reduce chromium (VI) to chromium (III) in a continuous regime system.

Of the two strains of sulphur-oxidizing bacteria studied, *A. thiooxidans* cells were selected for this process due to their high capacity to oxidize elemental sulphur and because of their ability to survive at low pH. The immobilization of *A. thiooxidans* onto elemental sulphur particles was carried out for 250 h until the culture reached 3×10^8 cell/ml and pH 1; a ''draw and fill'' method was then carried out until the pH was near to 1. Immobilization cycles were repeated

Table 1 Mass reduced of chromium (VI), maximum chromium (VI) reduction percentage and the time necessary to reach these values for *A. ferrooxidans* and *A. thiooxidans* in the presence of 1, 2.5, 5 and 10 ppm of Cr (VI) and for a control [10 ppm of Cr (VI)]

	Mass reduced of Cr (VI) (ppm)	% Cr (VI) reduction	Time (days)
Af1	1.00	100	1
Af2.5	2.50	100	1
Af5	5.00	100	2
Af10	4.29	42.9	11
At1	1.00	100	1
At2.5	2.5	100	1
At5	3.24	64.7	11
At10	3.05	30.5	11
C10	0.15	1.5	11

until they were found to have similar durations; at this point it was supposed that the formation of the biofilm was complete.

When the immobilization process had finished, the acidic medium from the sulphur-oxidizing bacteria reactor was transferred to the ''waste column'' until the pH was above 1.5; at this moment the immobilization reactor was connected in recirculation.

The evolution of pH, chromium (VI) and chromium (III) concentrations in the daily leachate is shown in Fig. 4.

When a pH of 1.5 was attained in the acidic medium from the *A. thiooxidans* reactor, the waste column was operated in recirculation. During this first stage two effects were observed:

- pH fluctuation in the leachate. Initially, the acidic medium dissolved alkaline compounds from the waste but the acidic conditions were not suitable for the solubilization of chromium (III). In this period a significant change was observed when 1,030 ml of leachate had been collected. At this point, a marked decrease in pH and significant chromium (III) leaching were observed. This occurrence was isolated and could be due to acidification of a local waste zone due to preferential routes. With the exception of this single occurrence, the homogenization of waste was carried out every day in order to avoid this effect.
- The leachate contained hexavalent chromium. This finding could be due to either insufficient contact time between the acid medium and chromium waste or, alternatively, to low levels of reducing compounds from the *A. thiooxidans* reactor for the reduction of chromium (VI).

As a result of these adverse effects, in a second stage, the operation mode was changed after 1,900 ml of leachate had been obtained. During the recirculation period in the *A. thiooxidans* reactor (pH higher than 1.5), the effluent from the waste column did not operate in recirculation; it was drawn slowly and the waste was kept dry until a new charge was added. This change immediately produced a positive effect. At levels of between 1,900 and 3,300 ml of leachate, the pH decreased from 7 to less than 3 and the chromium (VI) concentration was so low that it could not be detected. At the same time, a progressive increase in the chromium (III) concentration was found in the leachate.

A stationary phase was reached when 3,300 ml of chromium liquor had been collected: the chromium (III) concentration was around 4,000 ppm and the pH was less than 2.5.

After 4 l of leachate, 12% of the total chromium (III) from the waste had been solubilized [5.68 g from 47.1 g of initial chromium (III)]. It is worth noting that 8% of the solubilized chromium was collected in the last 900 ml of leachate, i.e., when constant conditions had been reached. If the system were continuously operating in the stationary stage, approximately 10 l would be necessary for the solubilization of the remaining chromium contained in the system.

The quantity of chromium (VI) in the total leachate was 11.5 mg of chromium (VI), which indicates that 92.7% of chromium (VI) had been

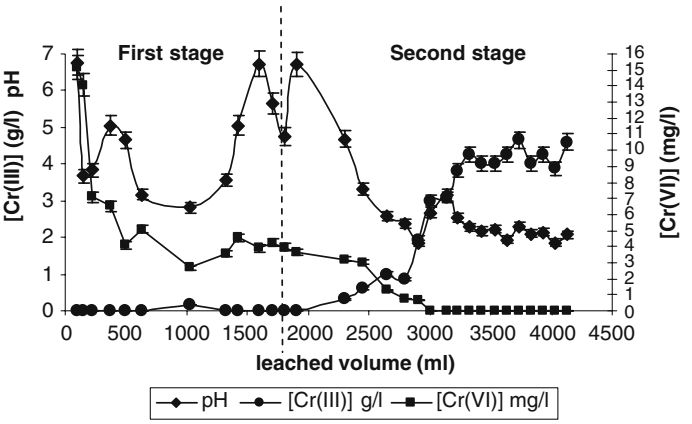

Fig. 4 Evolution of pH, Cr(III) and Cr(VI) concentrations for the leachate versus volume of acid medium passed through the ''waste column''

reduced to chromium (III) by the first 2,900 ml of acid medium from *A. thiooxidans*.

Conclusions

A. ferrooxidans and *A. thiooxidans* cells have the ability to reduce hexavalent chromium. *A. ferrooxidans* reduces 100% of chromium (VI) at concentrations of 1, 2.5 and 5 ppm, while in the presence of 10 ppm only 42.9% of chromium (VI) was reduced after 11 days of incubation. *A. thiooxidans* showed a lower capacity to reduce this ion, with total reduction of chromium (VI) only obtained for 1 and 2.5 ppm, while levels of 64.7% and 30.5% were reached for 5 and 10 ppm, respectively, after 11 days. The treatment of chromium waste with an acid medium from *A. thiooxidans* cultures in a continuous flow system allows the reduction of chromium (VI) and the solubilization of chromium (III) with high efficiency.

The results of our studies show the chance to use *A. thiooxidans* biofilms on elemental sulphur as a feasible technology (from economic and environmental points of view) for the removal of chromium from electroplating sludge or other solid residues to their final disposition. In addition, this technology could be useful to reduce chromium (VI), much toxic and much difficult to eliminate than chromium (III), eventually present into the residues. Finally, the leachates should be re-used into the industrial processes or treated abiotic or biotically to precipitate chromium (III) under controlled conditions in order to generate much less volume of solids (in comparison with the initial residues). Regard to the use of this process as a real technology, further researches will be conducted on the performance of the reactor and optimal conditions to the process to be applied in the industry.

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