

# Activation of bacteria in agglomerated ores by changing the composition of the leaching solution

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## Abstract

This work presents preliminary results of a study to determine the technical feasibility of heap bioleaching of copper sulfides in a mixed ore that had previously undergone a chemical leach to recover the copper from the copper oxides in the ore. The recovery of the copper sulfides is economically and environmentally important. The first step in this study was to determine the presence of bacteria in samples of both the agglomerated ores taken from the heaps and the samples from the irrigation solution used in the leaching process. No bacteria were detected in either sample, probably because of the very low pH, the high concentration of sulfate (200 g/l), and the presence of other ions in the irrigation solution. On the other hand, the cultivation of bacteria from recently crushed, fresh mixed ore (oxide-sulfide), and from cured, agglomerated ore, showed the presence of a small bacterial population, with ferrous iron-oxidizing activity. This indicates that the ore initially contained viable bacteria that were inhibited in the heap. Based on these results, a glass laboratory column containing 700 g of agglomerated ore from a partially, chemically leached heap was set up. The ore in the column was irrigated in a closed circuit with nutrient medium containing 2 g of ferrous sulfate per liter. Bacteria were detected, and the redox potential in the leaching solution began to increase on day 20 of the bioleaching experiment, indicating the beginning of the bioleaching stage of the sulfide mineral. The data indicate that the bacteria initially present on the ore can be recovered after acid curing, and also after prolonged contact with a solution containing a high concentration of sulfate. These results indicate that it is technically possible to induce a change from a chemical leaching process with high ionic strength to a bacterial leaching process, by dilution of the irrigation solution.

*Keywords:* Heap bioleaching; Sulfide minerals; Activation of bacteria; Agglomerated ore; Irrigation solution

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## 1. Introduction

The bioleaching of copper sulfide ores in heaps is a technology widely developed in Chile, with more than 85,000 tons of ore processed per day (Brierley, 1999). The process permits recovery of copper, zinc and other metals using the catalytic activity of several strains of ferrous iron and sulfur-oxidizing chemolithotrophic bacteria (Norris, 1990). Numerous studies

have demonstrated the presence of bacteria attached to the ores and/or present in the pregnant leaching solutions (PLS) in these operations. However, in some processes the bacteria are not active because of environmentally adverse conditions. A frequent problem in the operation of heap bioleaching, according to results obtained in our laboratory, is the high concentration of sulfate, and other ions in the raffinate used as irrigation solution on the heaps, after copper has been removed by solvent extraction. Additionally, organic compounds used in copper removal might

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be introduced into the raffinate solutions, generating adverse conditions to bacteria (Rusin et al., 1995).

In the case of mixed copper oxide/sulfide ores that have been chemically treated to leach the copper oxides from them, the irrigation solutions not only contain high ion concentrations, but also exhibit a very low pH. Some authors believe that bacteria that are active in sulfide bioleaching processes can grow in the presence of high concentrations of sulfate and hydrogen (Espejo and Romero, 1997). Pregnant leaching solutions (PLS) from chemically leached copper oxide minerals inhibit the development of bacterial populations because of the high concentration of sulfuric acid used in these processes. Under these conditions, bacteria are not able to catalyze the leaching of the remaining unleached sulfide ore from which copper oxide was leached. It is well recognized that, to leach sulfide minerals in heaps, it is better to use an acid–ferric sulfate solution, produced by ferrous- and sulfur-oxidizing bacteria (Fletcher and Weiss, 1985). From an environmental standpoint, bioleaching of sulfide minerals under uncontrolled conditions can result in acid mine-water generation and metal ion contamination of the soil over time. In this work, we studied the technical feasibility of bioleaching an old, exhausted mixed ore heap, from which copper oxide had been extracted, but that still contained copper sulfides.

## 2. Materials and methods

### 2.1. Mineral

We utilized a mixed ore from a copper mine in northern Chile, containing 1% copper oxide and 0.63% of copper sulfide. The ore was sampled during the following stages of treatment: untreated, immediately after crushing, after recent acid curing and agglomeration, and before, and after deposition in a heap for leaching.

### 2.2. Detection of ferrous iron- and sulfur-oxidizing bacteria

To determine the presence of ferrous iron-oxidizing bacteria, 10 g of each mineral sample were contacted in sterilized flasks with basal medium (0.4(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.4MgSO<sub>4</sub>·7H<sub>2</sub>O, and 0.056 KH<sub>2</sub>PO<sub>4</sub> g/l) of pH 1.8,

containing 2 g of ferrous sulfate per liter. A similar setup was used for sulfur-oxidizing bacteria. In this case, we employed basal medium at an initial pH of 3.5 to detect their presence by a pH decrease in the culture medium as a result of sulfur oxidation. The flasks were shaken (100 rpm) at 30 °C for 30 days, and periodically sampled to determine the concentration of ferrous and total iron, pH variations, and the number of ferrous iron- and sulfur-oxidizing bacteria in the irrigation solution. All experiments were carried out in duplicate.

### 2.3. Column bioleaching

To determine the feasibility of bioleaching of chemically leached ore, 700 g of agglomerated ore taken directly from the surface of a heap was placed in a glass column 30 cm in height and 8 cm in width. The column was irrigated in a closed circuit with basal medium at pH 1.8 containing 2 g of ferrous sulfate per liter. The irrigation solution was periodically sampled to determine the number of bacteria, the concentrations of ferrous and total iron, and the variation in pH and Eh.

### 2.4. Chemical and microbiological determinations

Raffinate and pregnant leach solutions (PLS) from the heap leaching process were analysed by atomic absorption to determine total Cu, Fe, As, and sulfate concentrations. Ferrous and total iron were determined by an *o*-phenanthroline colorimetric method (Muir and Anderson, 1977; Herrera et al., 1989). The number of bacteria in the irrigation solutions was determined microscopically by direct count with a Petroff–Hauser chamber. At the end of each culture experiment, the presence of ferrous iron- or sulfur-oxidizing bacteria in solution was confirmed using a Most Probable Number technique (Escobar and Godoy, 1999). To determine active bacteria in the agglomerated ore, an Electrochemical Respirometer Sapromat was used (Wiertz et al., 2001).

## 3. Results and discussion

### 3.1. Analysis of the heap solutions

Table 1 presents the measurements of some parameters determined initially in the raffinate, and in the

Table 1

Chemical composition of leaching solutions

Solution	pH	Eh, mV	Cu, g/l	As, g/l	Sulfate, g/l	Fe, g/l
PLS	0.74	350	0.795	0.14	214.4	20.7
Raffinate	1.14	339	0.331	0.055	209.2	22.9

PLS. The data show a low pH and a high sulfate concentration in the raffinate, indicating that bacteria were not able to develop at the high ionic strength existing in the solutions, and therefore on the agglomerated ore in the heaps. We have studied the effect of solutions with high sulfate concentrations on the bacteria, and we have determined that concentrations higher than 70–80 g per liter inhibited ferrous iron-oxidizing bacteria present in these process (data not published). Al, Zn and other cations frequently encountered in this type of solution could also have been present, but they were not determined. The absence of bacteria in the irrigation solutions was confirmed by a low Eh value, and by direct microscope counts of a suspension obtained after microfiltration of 500 mL of irrigation solution through a membrane filter of 0.1  $\mu\text{m}$ . Also, an MPN determination of iron- and sulfur-oxidizing bacteria resulted in detection of  $< 10^2$  bacteria/mL in both solutions. These results indicate that the bacterial development in the heap, necessary to begin bacterial oxidation of the sulfide minerals (Das et al., 1997), is not possible, with raffinate as inoculum, because the bacteria were either absent in it or their number was very low.

### 3.2. Bacterial activity in different stages of the leaching process

The development of bacteria at different stages of the leaching process is presented in Fig. 1. Ferrous iron oxidation was evidenced by an Eh increase in these enrichment solutions (Fig. 2). Variations in the number of bacteria in the different solutions can be attributed to variations in the pH due to acid consumption by the ore and by bacterial oxidation of ferrous iron, followed by a drop in pH by hydrolysis of ferric ion resulting in  $\text{Fe}(\text{OH})_3$  precipitation which can adsorb bacteria in solution.

The presence of ferrous iron-oxidizing bacteria in the enrichment solutions at the end of these experiments was demonstrated by the MPN technique

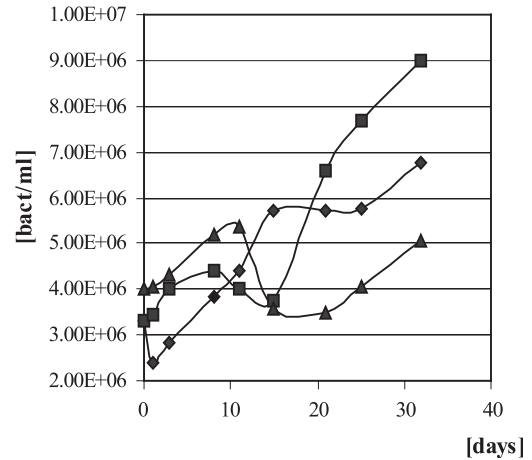


Fig. 1. Bacterial development in solutions.

(Table 2). This indicates that ferrous iron-oxidizing bacteria were present initially in the respective ore samples. It is important to consider that the number of bacteria shown in Table 2 represents only planktonic bacteria. A higher number of bacteria would be expected to adhere to the ore. On the other hand, the number of sulfur-oxidizing bacteria in the respective samples was less than  $1 \times 10^2$  bacteria/mL. An important conclusion derived from these results is that ferrous iron-oxidizing bacteria were present on the acid cured, agglomerated ore. It is probable that initially, the acid curing treatment killed most of them

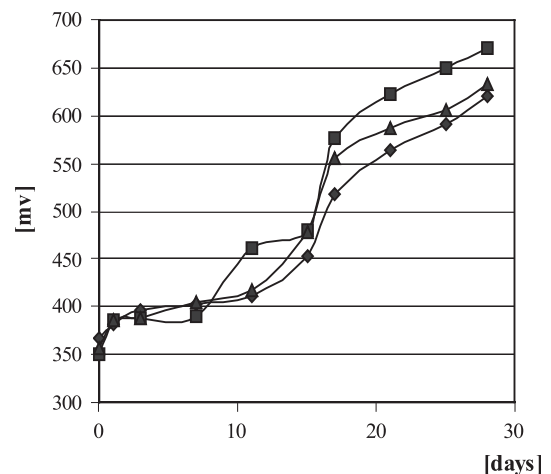


Fig. 2. Eh evolution in solutions. Mineral recently crushed (◆); acid cured agglom. (■); agglom. after deposition in the heap (▲).

Table 2

Number of Fe iron-oxidizing bacteria isolated from different stages of the leaching process

Samples	Crush (1)	Crush (2)	Aggl. (1)	Aggl. (2)	Heap (1)	Heap (2)
Fe oxid. Bac bact/ml	1.40e+02	2.80e+02	1.10e+06	1.20e+06	2.30e+05	2.80e+05

Crush: Mineral crushed. Aggl.: Acid cured, agglomerated ore. Heap: Agglomerated ore coming from the heap. Mean of two plates.

(Bruhn et al., 1999), however, it is also possible that this treatment promoted the formation of more soluble compounds that permitted a better development of the bacteria surviving the acid treatment. It has been considered that the colonization of the mineral in the heaps is produced by bacteria present in the irrigation solutions (Brierley, 1999; Bruhn et al., 1999). Here we have demonstrated that bacteria were not present in the solutions; besides, the composition of the solutions made them very toxic and thereby inhibited the bacterial growth. Hence, bacterial colonization of the ore in heaps would only be possible by starting with bacteria initially present in the ore.

### 3.3. Bioleaching of a copper oxide/sulfide ore

The presence of Fe(II)-oxidizing bacteria in ore samples from the different stages of the leaching process became evident only when the ore samples were contacted with fresh basal medium. These bacteria were not active in the heap process when the solution utilized had a low pH, and contained a high ion concentration, in particular sulfate. Starting with values of ions toxicity published by Das et al. (1997), it is possible to determine levels of toxicity of sulfate on *Thiobacillus ferrooxidans*. Toxicity can be observed starting with 70 g of sulfate per liter in solution; therefore, we consider that 200 g of sulfate per liter is toxic or constitutes a bacterial inhibitor. To study the activation of bacteria on the agglomerated ore, a glass column filled with agglomerated ore taken directly from a heap was set up, and irrigated in a closed circuit for 2 months with basal medium containing 2 g of Fe(II) sulfate per liter at pH 1.8. Initially, the ore consumed acid, resulting in pH increase. This pH increase was controlled by adding 0.5 N sulfuric acid. After 20 days, the pH was maintained at 2.6 or lower (Fig. 3), and the redox potential increased continuously (Fig. 4), while the Fe(II) ion concentration in the solution decreased (data not shown). Bacteria were detected in the irrigation solution only on day 40,

probably because they first colonized the ore and only then appeared in the irrigation solution. We counted  $3.5 \times 10^6$  bacteria/mL microscopically. It is possible that most of the bacteria were not yet cultivable because not all the bacteria counted microscopically can grow in a liquid medium; some of them are not cultivable in the utilized liquid medium, others could have died or were not viable, so that only  $6.4 \times 10^3$ /ferrous iron-oxidizing bacteria and  $2.3 \times 10^3$  sulfur-oxidizing bacteria per 100 mL were determined by respective MPN counts.

At the end of the experiment, a sample of the partially bioleached agglomerated ore from the column was used to confirm that active ferrous iron-oxidizing bacteria were attached to the agglomerated ore. A population of  $4.8 \times 10^6$  bacteria/g was estimated by measuring oxygen consumption by the attached bacteria using a Respirometer Sapromat (Wiertz et al., 2001). The bacterial population estimated on the ore was higher than that detected by Bruhn et al. (1999) on their mixed oxide/sulfide copper study. This can be explained in terms of the more suitable conditions resulting from dilution of the sulfate and other ions

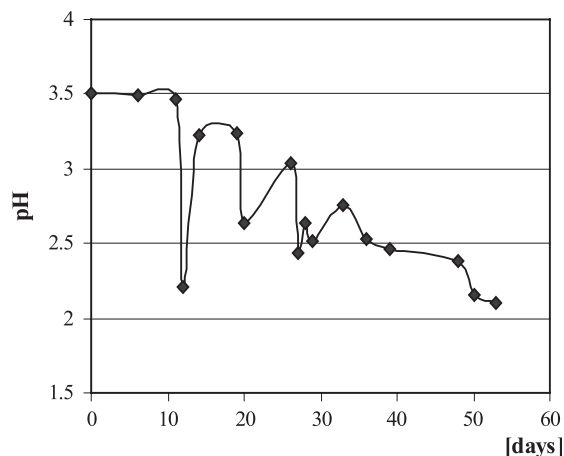


Fig. 3. Evolution of pH in the solution of column bioleaching.

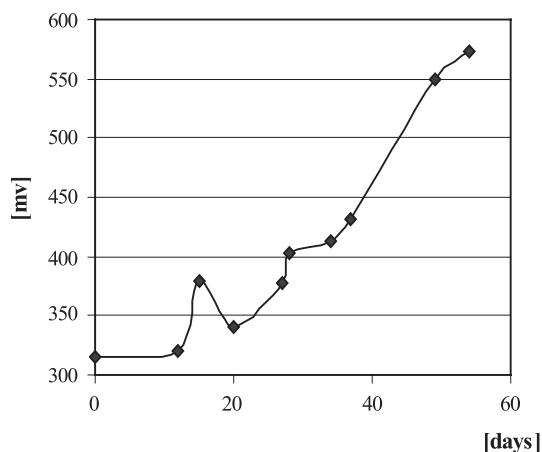


Fig. 4. Evolution of Eh in the solution of column bioleaching.

through mineral washing with basal medium. Initially, the basal medium used in this experiment contained 3 g of sulfate ion per liter. After 55 days, the sulfate ion concentration in the irrigation solution increased to 43.8 g/l, demonstrating the release of sulfate and other ions from the ore in the column by the washing of the agglomerated ore. The decrease of the sulfate ion concentration in the irrigation solution in this column permitted the bacterial growth and, therefore, the beginning of the bioleaching of the sulfide minerals present in the ore. This was confirmed by a chemical analysis of the solids taken from the column. At day 60, 72% of the copper oxide and 32% of the copper sulfide originally present in the ore had been dissolved.

This preliminary study, which was conceived as a contribution to the understanding of the operational limitations of the heap bioleaching processes, demonstrates that it is technically possible to bioleach a mixed oxide/sulfide copper oxide ore, starting with bacteria that are originally present in the acid cured, agglomerated ore. From the environmental point of view, this indicates that it is most important to maintain a strict control on the bioleaching of sulfide minerals present in an exhausted copper oxide ore heap because of the possibility of generating acid drainage containing ferric iron, copper and others ions, which may contaminate soils. The acid drainage results in this case from the presence of bacteria initially “inhibited”, but “potentially active” that can oxidize sulfide minerals when environmental conditions are favorable.

#### 4. Conclusion

The preliminary results presented here indicate that it is technically possible to change a chemical leaching process with high ion concentration to a bacterial leaching process. Colonization of the mineral selected for bioleaching can be initiated with the bacteria originally present on the acid cured, agglomerated ore. It is concluded that the acid curing treatment used to agglomerate the mineral does not kill the entire bacterial population, and that part of it maintains some ferrous iron-oxidizing capacity, which represents a valuable antecedent for a process of bioleaching. Besides, this bacterial population is enhanced by changing the irrigation solution, and therefore, by diluting the initial high ion concentrations.

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