THE ROLE OF *ACIDITHIOBACILLUS CALDUS* IN THE BIOLEACHING OF METAL SULFIDES

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***Abstract*— In the absence of iron, dissolution of zinc sulfide was enhanced by the action of *Acidithiobacillus caldus* at 40°C. The bioleaching mechanism was similar to that observed for mesophilic species of *Acidithiobacillus* at 30°C, although the final metal recovery was lower. When iron was added to the cultures, the solubilization of zinc and copper from the sulfides was higher than that in sterile controls. The activity of the cells was through two indirect mechanisms (acid and oxidant mechanisms, for zinc and copper sulfides respectively). *A. caldus* did not enhance the dissolution of nickel sulfide neither in the absence nor in the presence of iron.**

***Keywords*— Metal sulfides, *Acidithiobacillus caldus*, bioleaching, sulfur.**

I. INTRODUCTION

Acidithiobacillus ferrooxidans (*A. ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A. thiooxidans*) are considered the most important microorganisms in the bacterial dissolution of metal sulfides (bioleaching). These two species of *Acidithiobacillus* are gram-negative, aerobic and chemoautotrophic organisms. They develop at ambient temperatures (mesophilic bacteria) and are common in acid-polluted environments. Both bacteria are able to grow using energy obtained from reduced sulfur compounds. *A. ferrooxidans* is also capable of oxidizing iron(II) using oxygen as the last electron acceptor (Barrett *et al.*, 1993; Rawlings, 1997). Since the discovery of bacterial leaching, two different mechanisms have been proposed to explain bacterial attack by *A. ferrooxidans*: a direct one and an indirect one. The direct mechanism is based on catalytic sulfide oxidation, while the indirect one implies sulfide oxidation by ferric ions producing sulfur and ferrous ions. These products are oxidized by the microorganisms allowing the iron redox cycle to be repeated (Rawlings, 1997; Donati *et al.*, 1996).

Recently, two indirect leaching mechanisms have been proposed to explain degradation of sulfides. Both mechanisms combine characteristics of the former direct and indirect mechanisms. One is based on the oxidative attack of ferric iron on acid-insoluble metal sulfides involving thiosulfate as the main intermediate (Schippers *et al.*, 1996). The other mechanism is started by proton and/or ferric iron attack on acid-soluble metal sulfides with polysulfides and sulfur as intermediates (Schippers and Sand, 1999).

Lately, another sulfur-oxidizing bacterium was found in continuous flow biooxidation tanks operating at temperatures between 40 and 50°C. This bacterium named *Acidithiobacillus caldus* (*A. caldus*) is a moderately thermophilic unable to oxidize iron(II) and it is a close relative of the mesophilic *A. thiooxidans* (Rawlings, 1997; Hallberg *et al.*, 1996; Dopson and Lindstrom, 1999; Rawlings *et al.*, 1999). *A. caldus* is also an aerobic, gram-negative and chemoautotrophic organism that generates energy from the oxidation of reduced sulfur compounds.

The fact that under certain conditions *A. caldus* can dominate the sulfur-oxidizing bacterial populations in commercial bioleaching and biooxidation plants, suggests that its role in the bioleaching of sulfides is more important than that recognized up to this moment. Studies on the bioleaching of sulfide ores have used mixed populations of *A. caldus* and iron-oxidizing bacteria (*A. ferrooxidans* or *Leptospirillum. ferrooxidans*) but pure population of *A. caldus* has not been used yet (Dopson and Lindstrom, 1999). In this paper, we have studied the role of a pure culture of *A. caldus* in metal sulfide bioleaching at moderately high temperature in the presence and in the absence of iron.

II. METHODS

A. Bacteria

A. caldus (ATCC 51756) was grown in batch culture at 40°C in a medium (Dopson and Lindstrom, 1999) consisting in the basal salts (g/l)

$(NH_4)_2SO_4$ (3.0), $Na_2SO_4.10H_2O$ (3.2), KCl (0.1), KH_2PO_4 (0.05), $MgSO_4.7H_2O$ (0.5) including 1 % w/v elemental sulfur as energy source. The medium was adjusted to pH 2.5 with H_2SO_4. After removal of sulfur by filtration through blue ribbon filter paper (pore size 3 μm), cultures were centrifuged at 10000 g for 10 minutes and finally cells were suspended in the medium at pH 2.5. These suspensions were used as inocula in the leaching experiments. Bacterial population in these inocula was 1.0-2.7x10^8 cells/ml.

B. Experiments

Leaching experiments were carried out in 500-ml flasks with 140 ml of medium (see above) inoculated with 10 ml of the bacterial suspension. Medium was previously sterilized by filtration through a 0.22-μm pore-size filter. In some experiments, 1 g/l Fe(II) as ferrous sulfate instead of sulfur, was added to the medium. Different pure sulfides (CuS, NiS and ZnS) were used at pulp densities of 0.10, 0.25 and 0.50 % weight/volume (w/v) in experiments without iron and 0.20 % in experiments with iron. The particle size was <200 mesh. The initial pH was 2.5 and it was not controlled throughout the experiments. Sterile controls were prepared replacing inocula by the same volume of sterile medium. Flasks were incubated in an orbital shaker at 180 rpm and at 40°C. All experiments were carried out at least in duplicate.

C. Analytical methods

In periodic samples (previously filtered) the release of metal (zinc, copper and nickel) was followed by atomic absorption spectrophotometry. Sulfate concentration was monitored by a turbidimetric method (Vogel, 1978). Bacterial population in liquid phase was counted with a Petroff-Hausser chamber using a phase-contrasting microscope. Solid residues were analyzed using X-ray diffraction in order to detect compounds produced at the substrate surfaces during bioleaching.

III. RESULTS AND DISCUSSION

A. Experiments without iron

Figure 1 shows the results obtained when *A. caldus* was grown using sulfur as the sole energy source. The four parameters represented in this figure (proton and sulfate concentrations, pH and free bacterial population) show the evolution of the bacterial growth on elemental sulfur. In sterile controls there was not change in proton and sulfate concentrations (data not shown).

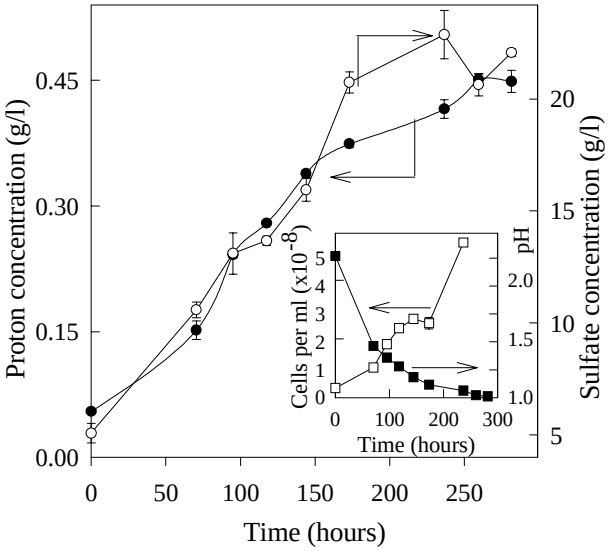

Figure 1. Bacterial growth on elemental sulfur. Proton and sulfate concentrations (outer graph). Free bacterial population and pH (inner graph).

The progress of proton concentration correlates with the corresponding evolution of sulfate concentration for the first 150 hours. During this period the ratio between proton and sulfate production was 2.8 which is higher than the stoichiometric ratio (mol H^+/mol SO_4^{2-}= 2) for sulfur oxidation according to the equation:

$$S + 3/2\ O_2 + H_2O \longrightarrow H_2SO_4 \qquad (1)$$

This low sulfate production indicates that the mechanism of sulfur oxidation is similar to that accepted for other species of *Acidithiobacillus*. According to this mechanism, the first step of sulfur oxidation should generate protons and intermediate compounds such as sulfite and thiosulfate. In the last step of the sulfur oxidation, the intermediate compounds should be oxidized to sulfate with no significant proton production. Our results suggest that intermediate compounds are accumulated in the culture during the first 150 hours.

At the end of the growth the ratio between proton and sulfate concentration tends to reach the theoretical value. This could mean that cells oxidize mainly the intermediate compounds when sulfur begins to be the limiting nutrient.

This capability of producing sulfuric acid through the oxidation of sulfur shows the two roles

of *A. caldus* during the bioleaching processes. One of them is the continuous acidification of the medium allowing the solubilization of metal compounds. The other role is the dissolution of the sulfur layer deposited on the sulfide during the bioleaching process, allowing further dissolution of the sulfides.

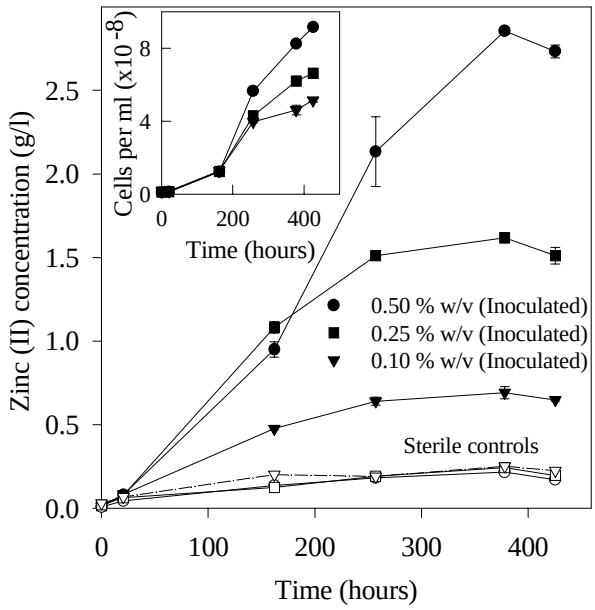

Figure 2. Bioleaching of zinc sulfide using *A. caldus*. Zinc concentration (outer graph) and free bacterial population (inner graph).

Figure 2 shows zinc concentration and free bacterial population versus time during the bioleaching of ZnS. Zinc extraction was higher in *A. caldus* culture than in the sterile control. It can be observed in the inner graph that the evolution of free bacterial populations has the same behavior than zinc extraction.

Figure 3 shows the percentages of metal recovery in the leaching of NiS and CuS after 15 days. As it can be seen, results of the chemical leaching experiments were similar to those for the bacterial leaching experiments.

No significant bacterial action was detected in our experiments using *A. caldus*, except in the case of ZnS. This suggests a strong correlation between solubility of the sulfide and the efficiency of bioleaching. Thus, there was a high metal extraction in the case of the ZnS, which is more soluble than the other sulfides. Our results could be explained by the mechanism reported for *A. thiooxidans* (Pistorio *et al.*, 1994). The first step of this mechanism is the chemical dissolution of the sulfide producing Zn^{2+} and H_2S (Eqn. 2) followed by the bacterial oxidation of these species (Eqn. 3).

The removal of H_2S allows the continuous dissolution of the sulfide due to the displacement of the equilibrium indicated by Eqn. 2. Sulfur formed in Eqn. 3 can be oxidized by cells according to Eqn. 1.

$$ZnS + 2\,H^{+} \longrightarrow Zn^{2+} + H_2S \quad (2)$$

$$H_2S + 1/2\,O_2 \longrightarrow S + H_2O \quad (3)$$

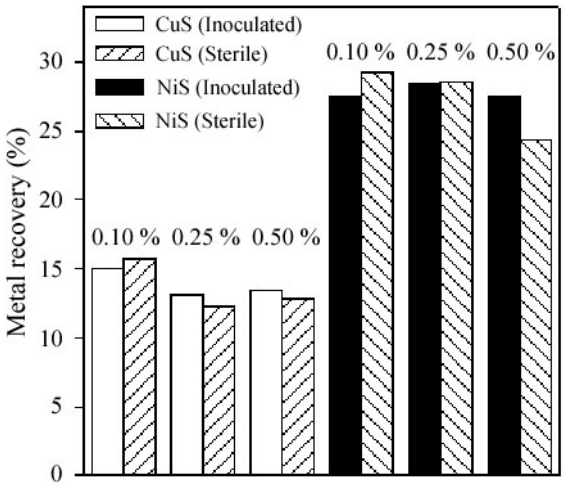

Figure 3. Percentages of metal recovery during the bioleaching of nickel and copper sulfides.

The behavior of pH (Figure 4) is in agreement with these processes: an initial slight increase of the pH (Eqn. 2) and a final decrease due to sulfur oxidation. In sterile controls the pH decrease was not observed and final pH was about 2.6 when the maximum yield of metal extraction (from 0.17 to 0.22 g/l according to the pulp density) was reached (Figure 2).

Yields of zinc solubilization were 0.65 g/l (96 %), 1.51 g/l (90 %) and 2.73 g/l (81 %) and the rates of solubilization were 0.181, 0.103 and 0.044 g/l.day for 0.50, 0.25 and 0.10 % w/v pulp densities respectively. Although ZnS bioleaching was effective, the yields of zinc recovery and rates of zinc solubilization were lower than those obtained in cultures of *A. ferrooxidans* or *A. thiooxidans* at 30°C where the rates of solubilization were 0.233 and 0.270 g/l.day respectively at 0.20 % w/v pulp density (Pistorio *et al.*, 1994). In *A. caldus* cultures, free bacterial populations reached higher values than those reported for other species of *Acidithiobacillus*. This suggests that *A. caldus* cells present less

affinity for sulfur (Eqn. 1) reducing the number of attached cells. Because of that, the two roles of the bacteria are partially repressed and consequently the efficiency of metal recovery decreases significantly.

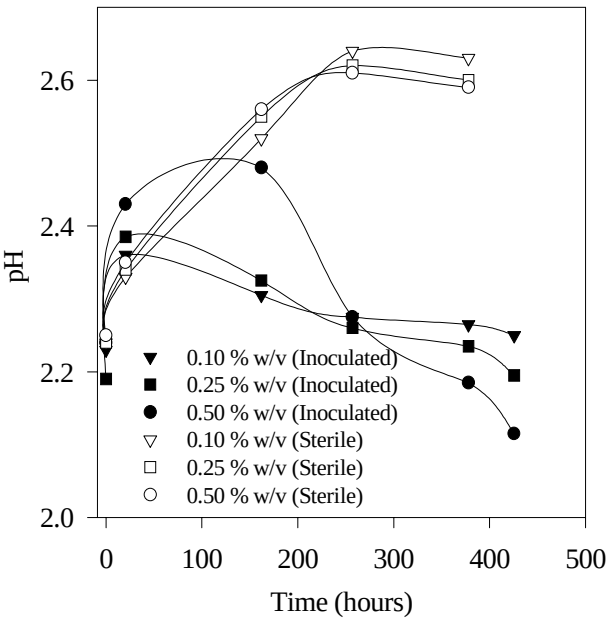

Figure 4. pH evolution during bioleaching of zinc sulfide using *A. caldus* cells.

According to Fig. 3, it can be seen that there was not enhancement of nickel or copper dissolution in the presence of *A. caldus* cells. However, there was a small growth of the free bacterial population especially for NiS at the lowest pulp density (data not shown). A significant increase of pH could have inhibited the bacterial activity and the consequent sulfide dissolution. Similar behavior was observed in the case of *A. thiooxidans* although *A. ferrooxidans* enhanced significantly the copper solubilization (Porro *et al*., 1997); these results suggest that the mechanisms involved in the oxidation of very insoluble sulfides (as CuS) and moderately insoluble sulfides (as ZnS) are different (Pogliani *et al.*, 1990).

B. Experiments with iron

In other series of experiments, the effect of iron on the metal recoveries was analyzed. Figure 5 (outer graph) shows the evolution of zinc and copper concentration during leaching experiments. The inner graph shows suspended bacterial population in the cultures. Inoculation improved metal extraction from both pure sulfides (ZnS and CuS) reaching 0.99 g/l (73.5 %) and 0.92 g/l (69.1 %) of metal concentration respectively after 40 days. The rate of released zinc (0.0336 g/l.day) from the sulfide was much lower than those obtained in the absence of iron.

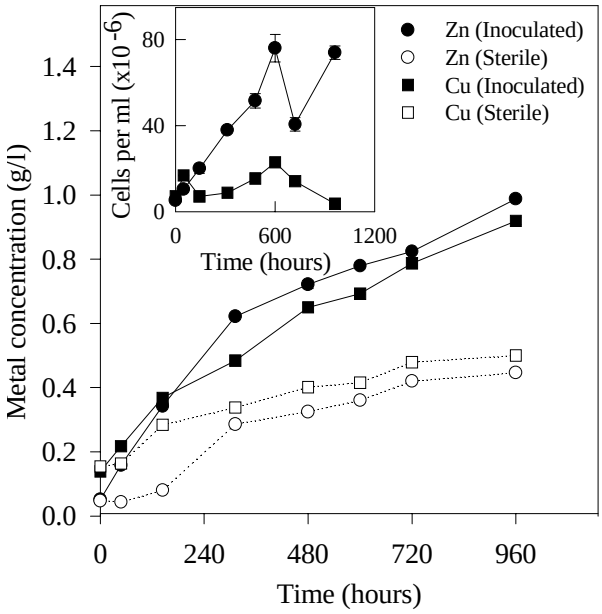

Figure 5. Zinc and copper concentration (outer graph) and free bacterial population (inner graph) during leaching experiments using *A. caldus* cells in the presence of iron.

In cultures and in sterile controls with zinc sulfide, a slight decrease of soluble iron was detected (data not shown). Soluble iron was mainly as iron (II). Thus, in agreement with experiments in the absence of iron, ZnS should have been solubilized by acid action. This process increased the pH (decreasing the solubilization rate) as it was found in sterile control where the final pH was 3.4. In cultures, there was not pH increase because *A. caldus* cells are able to oxidize H_2S to H_2SO_4 allowing further acid action (data not shown). The sulfate production (19 mM) during the bioleaching agrees with the amount of solubilized zinc (15 mM) indicating that ZnS was solubilized according to the mechanism proposed in the absence of iron (Eqns. 2, 3, 1).

In the case of CuS there was not a significant pH change neither in the absence nor in the presence of bacteria. Because of this, CuS (a very insoluble sulfide) is slightly solubilized by acid action. However, ferric iron was detected in these systems; uncompleted ferrous iron oxidation was due exclusively to the action of air either in cultures or in sterile controls. In this way, CuS was

oxidized by ferric iron producing sulfur and solubilizing copper (Eqn. 4).

$$CuS + 2\,Fe^{3+} \longrightarrow S + Cu^{2+} + 2\,Fe^{2+} \quad (4)$$

A sulfur layer was formed on the substrate protecting it from further chemical oxidation. Finally, *A. caldus* dissolved the sulfur layer deposited on the sulfide surface allowing a further oxidation of CuS by ferric iron. This mechanism was confirmed by the difference between copper solubilized in cultures and in sterile controls (4.9 mM) which is very close to the difference between iron (II) concentration in the same systems (5.6 mM) and to the sulfate production in the cultures (about 4.6 mM). Moreover, elemental sulfur was detected by X-ray diffraction analysis in the solid residues from the sterile controls (but not from the cultures). Similar mechanism was previously observed for *A. thiooxidans* culture (Curutchet *et al.*, 1995; Pogliani and Donati, 2000). Bacterial population in suspension was lower than that in cultures with zinc sulfide; this agrees with the amount of sulfate produced in both cases. In these systems, we found a great iron decrease in solution (higher than 50 % of the initial amount). In solid residues from inoculated and sterile systems, jarosite, troilite (FeS) and pyrrhotite (Fe_7S_8) were also detected.

Results of the chemical and bacterial leaching of NiS were similar. In both systems, the decrease in iron concentration was almost equivalent to the increase of metal concentration. Although the mechanism has not been elucidated yet, a replacement of the metal (nickel) in the solid phase by iron was confirmed by the formation of phases as $(Fe,Ni)_9S_8$ and pyrrhotite and troilite. Another sulfide (Ni_7S_6) has also been found in the solid residues. Finally, jarosite deposits were also detected in the solid residues (the iron (III) precipitation was as high as in cultures with CuS).

IV. CONCLUSIONS

Summarizing, this paper shows that in the absence of iron, *A. caldus* cells are only able to oxidize sulfur or to leach soluble sulfides as ZnS. In the presence of iron, the solubilization of zinc and copper from the sulfides was higher than that in sterile controls. During the leaching of ZnS, *A. caldus* enhanced zinc extraction through the oxidation of H_2S produced for the acid action on the sulfide; moreover, the sulfur biooxidation did not allow the consequent increase of pH. In the case of CuS, there was a low oxidation of iron(II) by air. Iron(III) oxidized the sulfide producing sulfur which was deposited on the sulfide. The role of the cells was to dissolve the sulfur layer allowing further dissolution of the sulfide. These mechanisms are similar to those observed in cultures of *A. thiooxidans* although the solubilization rates detected in these last cultures were higher. Finally, *A. caldus* was unable to enhance the solubilization of NiS neither in the presence nor in the absence of iron.

ACKNOWLEDGEMENTS

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