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 $1.0\text{-}2.7 \times 10^8$ 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