SULPHIDE BIOLEACHING BY *THIOBACILLUS CALDUS* AND *LEPTOSPIRILLUM FERROOXIDANS*

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ABSTRACT

A complex population of chemolithotrophic microorganisms is involved in bioleaching processes. Recently, using PCR-based identification techniques, it has been found that *Leptospirillum ferrooxidans* and *Thiobacillus caldus* dominated the bacterial population in commercial bioleaching and biooxidation plants.

Thiobacillus ferrooxidans, which was considered to be the most important microorganism in bacterial leaching, was present in low numbers in the same conditions. Thus, in media supplemented with iron, the combined action of those bacteria should be similar to the one reported for *T. ferrooxidans*. The objective of the present study is to investigate if a mixed culture forming by *L. ferrooxidans* and *T. caldus* is able to contribute to the dissolution of different metal sulphides in the presence of iron. Zinc sulphide, copper sulphide, nickel sulphide, molybdenum sulphide or cobalt sulphide were used as substrates in shake-flasks leaching experiments at 40 °C and inoculated with cell suspensions from pure cultures with similar bacterial populations of iron-grown *L. ferrooxidans* and sulphur-grown *T. caldus*.

Solid residues were analysed using X-ray diffraction in order to detect compounds produced at the substrate surfaces during bioleaching. Metal recoveries have been evaluated as a function of the amount of supplemented iron and compared with chemical leaching.

INTRODUCTION

For many years *Thiobacillus ferrooxidans* was considered to be the most important bacterium in commercial bioleaching and biooxidation plants operating at 40 °C or less (Barrett et al, 1993; Rawlings, 1997; Rawlings 1998). In more recent years, using the analysis of the 16S rDNA amplification products of total DNA isolated from different commercial applications, it has been established that under certain conditions *Leptospirillum ferrooxidans* and *Thiobacillus thiooxidans* dominated the bacterial population (Rawlings et al., 1999) and they can be at least as important as *T. ferrooxidans* for metal recovery (Pistorio et al., 1994; Curutchet et al., 1995; Donati et al., 1996; Porro et al., 1997; Pogliani and Donati, 2000).

The two species of *Thiobacillus* are gram-negative, mesophilic and chemoautotrophic organisms that generate their energy from the oxidation of reduced sulphur compounds. In addition, *T. ferrooxidans* is also capable of oxidising iron (II) using oxygen as the last electron acceptor. *L. ferrooxidans* is a mesophilic, vibrioid-shaped, iron-oxidising bacterium which presents higher acid tolerance than that of *T. ferrooxidans*. This bacterium normally grows at temperature as high as 40-45 °C (Helle and Onken, 1988; Sand et al., 1992; Breed and Hansford, 1999).

Recently, two novel mechanisms have been proposed to explain the role of these microorganisms in the bioleaching of different metal sulphides (Schippers et al., 1996; Schippers and Sand, 1999). Both mechanisms are basically associated to an indirect process through the iron (III) or protons action on the sulphide.

Another sulphur-oxidising bacterium was found in continuous flow biooxidation tanks operating at temperatures between 40 and 50 °C. This bacterium named *Thiobacillus caldus* is a moderately thermophilic (optimum temperature 45 °C) unable to oxidise iron (II), is a close relative of the mesophilic *T. thiooxidans* (Hallberg et al., 1996[a]; Hallberg et al., 1996[b]; Dopson and Lindström, 1999).

In this study, we have tested the role of a mixed culture of *T. caldus* and *L. ferrooxidans* in metal sulphide bioleaching at moderate high temperatures in the presence of iron.

EXPERIMENTAL

Thiobacillus caldus (ATCC 51756) was previously grown in batch culture at 40 °C in a *Tc* medium consisting of the basal salts (grams per liter) $(NH_4)_2SO_4$ (3.0), $Na_2SO_4.10H_2O$ (3.2), KCl (0.1), KH_2PO_4 (0.05) and $MgSO_4.7H_2O$ (0.5) with the pH adjusted to 2.5 with H_2SO_4 10 % wt/vol using elemental sulphur as the energy source.

Leptospirillum ferrooxidans (ATCC 29047) was previously propagated in batch culture at 30 °C in a similar medium (see above) with $FeSO_4.7H_2O$ as the energy source (9 g/l of iron (II)), pH adjusted to 1.8. In both cases, bacteria were harvested after removal of sulphur (*T. caldus*) or iron precipitates (*L. ferrooxidans*) by filtration through blue ribbon filter paper. Cultures were centrifugated at 10000 g for 10 minutes and finally cells were suspended in *Tc* medium without sulphur or iron at pH 2.5. These suspensions were used as inocula in the leaching experiments. Bacterial population in these inocula was 8.7-10x10^7 cells/ml.

Leaching experiments were carried out in 500-ml flasks with 140 ml of *Tc* medium described (see above) with 1 g/l ferrous iron (pH=2.5). Medium was previously sterilised by filtration (through a 0.22- µm pore-size bacterial filter). Flasks were inoculated with 5.0 ml of each inoculum and incubated in an orbital shaker at 180 rpm and at 40 °C. 0.30 g of different pure sulphides (ZnS, CuS, MoS_2, NiS and CoS) was added to each flask. The particle size was <200 mesh. Sterile controls were prepared, replacing inocula by the same volume of sterile medium. All experiments were carried out at least in duplicate.

The progress of the sulphide leaching was followed by monitoring the release of metal (zinc, copper, molybdenum, nickel, cobalt and iron), measured by atomic absorption spectrophotometry in periodic samples previously filtered. Soluble iron (II) was measured by *o*-phenanthroline method. Sulphate concentration was monitored by a turbidimetric method.

RESULTS AND DISCUSSION

Figure 1 (outer graph) shows the evolution of zinc and copper concentration during leaching experiments. The inner graph shows suspended bacterial population in the cultures.

As it can be seen in the figure, inoculation improved metal extraction from both pure sulphides (ZnS and CuS) reaching 73.5 and 69.1 % of metal extraction respectively after 40 days.

The rate of zinc released (33.6 mg/l.day) from the sulphide were much lower than those obtained in leaching experiments (in the absence of iron) using pure cultures of *T. caldus* at the same temperature (100 mg/l.day; unpublished results) or pure cultures of *T. ferrooxidans* (233 mg/l.day) or *T. thiooxidans* (270 mg/l.day) at 30° C (Pistorio et al., 1994)

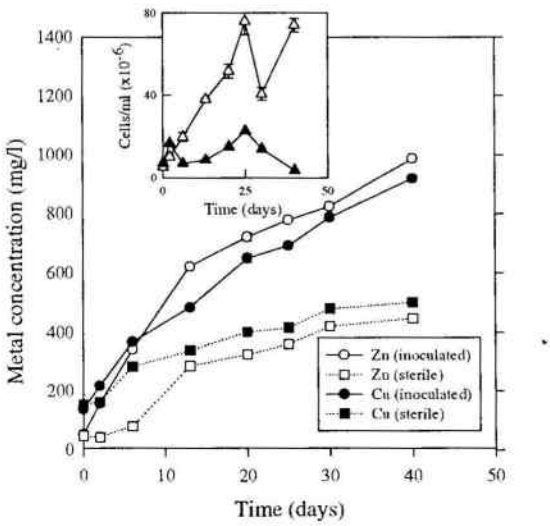

Figure 1: Zinc and copper concentration (outer graph) and free bacterial population (inner graph) during leaching experiments with a mixed culture.

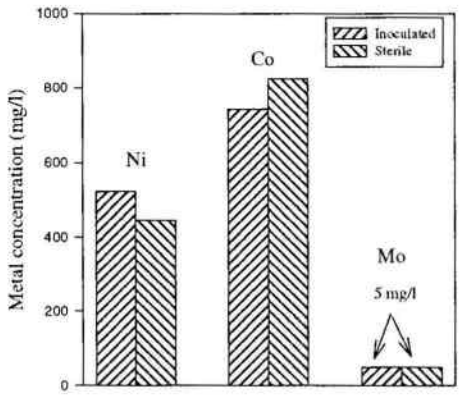

Figure 2: Metal concentrations in the leaching experiments with a mixed culture after 40 days.

The final metal concentrations from the other sulphides are shown in figure 2. As it can be seen, results of the chemical leaching experiments were similar to those for the bacterial leaching experiments. According to these results, the presence of these

microorganisms did not enhance the metal solubilisation except in the leaching of copper and zinc sulphides.

The possible inhibition of the two bacteria under the leaching conditions has also been investigated. *T. caldus* was grown in the same medium used in the leaching experiments but using sulphur (10 g/l) as the sole energy source. Figure 3 shows the results in this culture.

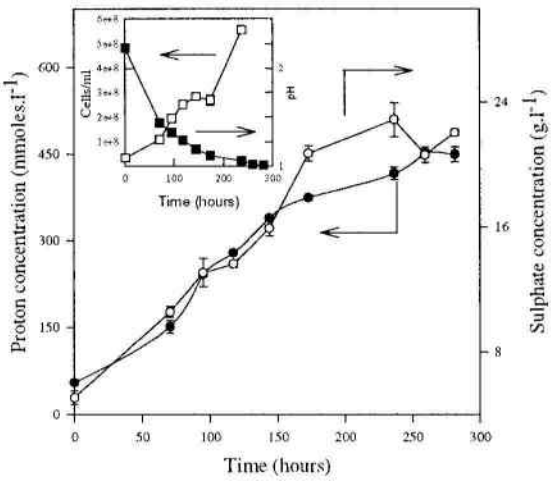

Figure 3: Growth of *T. caldus* on elemental sulphur at 40°C.

The rates of acidification and sulphate production indicate that the growth of this sulphur-oxidising bacterium was successful replacing each metal sulphide by sulphur. These cultures had a bacterial growth greater than in the leaching experiments although this agrees with the amount of disposable energy source.

On the other hand, ferrous iron oxidation was similar (and not completed) in chemical leaching and bacterial leaching. In order to explain this fact, cultures and sterile controls cultures of *L. ferrooxidans* were incubated on *Tc* medium using 1 g/l iron (II) as energy source or in 9 K medium (Donati et al., 1996). Ability to oxidise iron (II) at 40°C was compared with that at 30°C. Other conditions were the same as in the leaching experiments. Figure 4 shows ferrous iron oxidation in inoculated and sterile controls.

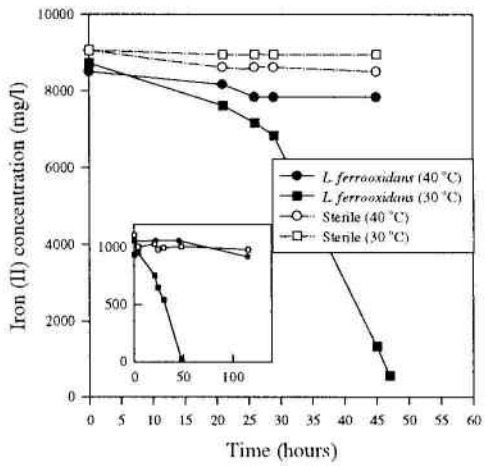

Figure 4: Comparison of ferrous iron oxidation by *L. ferrooxidans* in *Tc* medium (inner graph) or in 9K medium (outer graph) at 30°C and 40°C.

In contrast to our results, previous reports showed that ferrous iron oxidation by *L. ferrooxidans* increased with increasing temperature, other reports indicated that ferrous iron oxidation was not affected by temperature while a strain with optimum temperature lower than 30 °C has also been reported (Gómez et al., 1999). The reason why this strain of *L. ferrooxidans* presented no significant bacterial growth in *Tc* or 9 K medium at 40°C is not known. Since the strain of *L. ferrooxidans* was clearly inhibited by temperature and perhaps by metal ions, bacterial action in the leaching experiments should be adjudicated to *T. caldus* rather than to *L. ferrooxidans*. Thus, uncompleted ferrous iron oxidation was due exclusively to the action of air either in cultures or in sterile controls.

In cultures and in sterile controls with zinc sulphide, we detected a little decrease of soluble iron that was almost completely as iron (II). Thus, in agreement with previous experiments (unpublished results), zinc sulphide should have been solubilised by acid action. This process increased the pH (decreasing the solubilisation rate) as it can be seen in sterile control where the final pH was 3.4. In cultures, there was not pH decrease because *T. caldus* cells are able to oxidise H_2S to H_2SO_4 allowing further acid action. The sulphate production (about 19 mM) during the bioleaching agrees with the amount of solubilised zinc (about 15 mM) indicating that all zinc sulphide was solubilised according to these processes in inoculated flasks:

$$ZnS + 2\ H^+ \rightarrow H_2S + Zn^{2+} \quad [1]$$

$$H_2S + 2\ O_2 \rightarrow 2\ H^+ + SO_4^{2-} \quad [2]$$

In cultures and in sterile controls with copper sulphide there was not a significant pH change. This is

why copper sulphide is slightly solubilised by acid action although the presence of an oxidant agent significantly enhances the solubilisation. In these cultures (as in the sterile control), there was a low but significant ferric iron concentration indicating that copper sulphide was oxidised by ferric iron. This process formed a sulphur layer on the substrate and protected it from further chemical oxidation.

$$CuS + 2Fe^{3+} \rightarrow S + Cu^{2+} + 2Fe^{2+} \quad [3]$$

Finally, the sulphur layer deposited on the sulphide was dissolved by *T. caldus* action and copper sulphide was further oxidised by ferric iron. This mechanism was confirmed because the difference between copper solubilised in cultures and in sterile controls (4.9 mM) is very close to the difference between iron (II) concentration in the same systems (5.6 mM) and to the sulphate production in the cultures (about 4.6 mM). Moreover, in the solid residues from the sterile controls (but not from the cultures), elemental sulphur was detected by X-ray diffraction analysis. Bacterial population in suspension was lower than in cultures with zinc sulphide; this agrees with the amount of sulphate produced in both cases. In these leaching systems, we found a great iron decrease in the 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.

In bioleaching experiments with cobalt or nickel sulphide, according to the sulphate production and the free bacterial population, there was a small bacterial growth (higher in the case of nickel sulphide). In both culture and sterile control, the decrease of 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 or cobalt) in the solid phase by iron was confirmed by the formation of phases as Co_8FeS_8 or $(Fe,Ni)_9S_8$ and pyrrhotite and troilite. Other sulphides (Co_9S_8 or Ni_7S_6) have 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 copper sulphide).

Our results show that molybdenum was not extracted in any case confirmed previous results showing that sulphur-oxidising bacteria or iron-oxidising bacteria in the absence of iron could not leach molybdenite (Porro et al., 1997).

CONCLUSIONS

In similar way to *T. thiooxidans*, *T. caldus* was not able to enhance the solubilisation of cobalt, nickel and molybdenum sulphides probably due to these less soluble sulphides did not generate sulphur as the product of iron(III) action. On contrast, our results showed the ability of *T. caldus* cells to enhance the solubilisation of zinc and copper sulphides by mechanisms (acid and oxidant respectively) similar to those observed in *T. thiooxidans* cultures although the solubilisation rates detected in these last cultures were higher.

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