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E. Donati · C. Pogliani · J. L. Boiardi

Anaerobic leaching of covellite by *Thiobacillus ferrooxidans*

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Abstract *Thiobacillus ferrooxidans* was able to grow under anaerobic conditions on copper sulphide with ferric ion as the electron acceptor. The dissolution of covellite under these conditions (68% after 35 days) was higher than values observed aerobically in cultures with similar media composition and almost as high as under aerobic conditions without iron. From these results we propose a mechanism for anaerobic bioleaching of covellite in the presence of ferric iron and speculate that it may occur in leach dumps where the oxygen concentration is, as reported elsewhere, very low.

Introduction

Thiobacilli are a group of gram-negative chemoautotrophic bacteria that can obtain energy for growth from the oxidation of a variety of inorganic sulphur compounds. *Thiobacillus ferrooxidans*, an obligately autotrophic species, can also oxidise ferrous ion. This organism is very important in many mineral-leaching operations, especially in bacterial leaching of sulphide ores for the recovery of several metals (Torma 1977). *T. ferrooxidans* may be involved directly or indirectly in the oxidation of sulphide (Donati et al. 1988; Sand et al. 1995).

T. ferrooxidans is generally assumed to be an aerobic organism, so oxygen would be required as the last electron acceptor for bacterial growth. However, the analysis of gases in the pore spaces of leach dumps (one of the most suitable methods in extractive metallurgy) revealed that 0.5 m below the surface level the oxygen concentration is extremely low, while the carbon dioxide concentration is high (Goodman et al. 1983). Some studies found that, under anaerobic conditions (Pronk et al. 1992; Goodman et al. 1983) or at extremely low pH values (Sand 1989), *T. ferrooxidans* is able to reduce ferric to ferrous ion in the presence of sulphur or sulphide ores. Pronk et al. (1992) reported that this organism grows anaerobically using ferric ion as the electron acceptor and suggested, in accordance with Brock and Gustafson (1976), that this could be the major biological process during oxidation of metal ores in leaching operations. However, there are no reports showing growth of *T. ferrooxidans* on insoluble sulphides (the common substrates found in metal ores) under anaerobic conditions to confirm the role of ferric ion as an electron acceptor. This study was conducted to investigate whether *T. ferrooxidans* is able to grow anaerobically on insoluble synthetic copper sulphide in the presence of ferric ion and whether an efficient metal recovery could be obtained under these conditions.

Materials and methods

Bacteria

The strain of *T. ferrooxidans* used was DSM 11477 (formerly Tf18, Donati et al. 1988). Bacteria were grown in 9 K medium (Silverman and Lundgren 1959) and harvested from the late logarithmic phase of growth. The culture suspension was filtered through a 0.22-μm filter (Nucleopore) and washed several times with acidified water (pH = 1.50) to eliminate ferric ion. Finally the bacterial pellet was suspended in medium lacking iron, and used as inocula.

Medium

9 K medium at pH 1.80, containing 6.0 g/l ferric ion [as $Fe_2(SO_4)_3$] instead of 9 g/l ferrous ion, was employed. In some cases, iron was not added (see below). Media were sterilised by filtration through a 0.22-μm bacterial filter.

Leaching experiments

Leaching experiments were carried out in 250-ml flasks (Fig. 1) with 200 ml medium containing ferric ion. Flasks were incubated in

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E. Donati (✉) · C. Pogliani · J. L. Boiardi
CINDEFI (Centro de Investigación y Desarrollo
en Fermentaciones Industriales), Departamento de Química,
Facultad de Ciencias Exactas, Universidad Nacional de La Plata,
47 y 115 (1900) La Plata, Argentina
Fax: 0054 21 25 4533

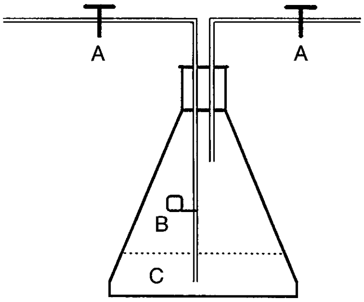

Fig. 1 Scheme of anaerobic flask. *A* valve; *B* sodium bicarbonate solution; *C* medium and copper sulphide

an orbital shaker at 130 rpm and 30 °C. In all flasks 0.4 g synthetic copper sulphide (99% pure, particle size less than 74 μm, from Strem Chemicals Inc.) was added (0.2% pulp density). A 1.0-ml sample of sterilised (by filtration) 1.0% w/v sodium bicarbonate was added to a small reservoir placed inside the flasks (see Fig. 1). Flasks were inoculated with *T. ferrooxidans* to an initial bacterial population of about 10^7 cells/ml. Cultures without ferric ion were carried out as controls to exclude the possibility that growth was due to leakage of oxygen into the flasks. Sterile controls were run under the same conditions as the inoculated flasks, except that the inocula were replaced by an equal volume of 2% w/v thymol in methanol. All treatments were carried out at least in duplicate.

In order to obtain anaerobic conditions, sterilised nitrogen (passed first through an alkaline pyrogallol solution to remove traces of oxygen) was sparged for about 15–20 min into the flasks. The flow of nitrogen through the flasks was stopped (closing both valves A of Fig. 1) when a negligible reading for dissolved oxygen was obtained in a galvanic electrode situated in a parallel control flask. Afterwards, by leaning the flasks to one of the sides, sodium bicarbonate was incorporated into the media. This ensured the presence of carbon dioxide during bacterial incubation.

Other leaching experiments, without iron, with 6 g/l ferrous ion (as $FeSO_4$) or with 6 g/l ferric iron [as $Fe_2(SO_4)_3$], were carried out under similar but aerobic conditions.

Analytical methods

Ferrous ion was determined by titration with potassium permanganate solution (Kolthoff et al. 1979). Soluble iron (ferric plus ferrous) and dissolved copper were analysed by atomic absorption spectrophotometry. Acid production was determined by titration with a sodium hydroxide solution (Kolthoff et al. 1979) and the pH of cultures was measured with a combined pH glass electrode. The bacterial concentration in the culture supernatants was determined by direct counting with a Petroff-Hausser chamber (Cooney 1981).

Results

Figure 2 shows copper release in the inoculated and sterile systems (with and without ferric ion addition) after 35 days. The highest extraction (68%) was obtained in the inoculated system containing ferric ion, while extraction was 19% in the sterile control. After 35 days 10.3 mmol copper was solubilized by anaerobic bacterial activity. That amount of copper was calculated, assuming an equal dissolution by acid in both cultures and subtracting the copper solubilized in the inoculated control without ferric ion.

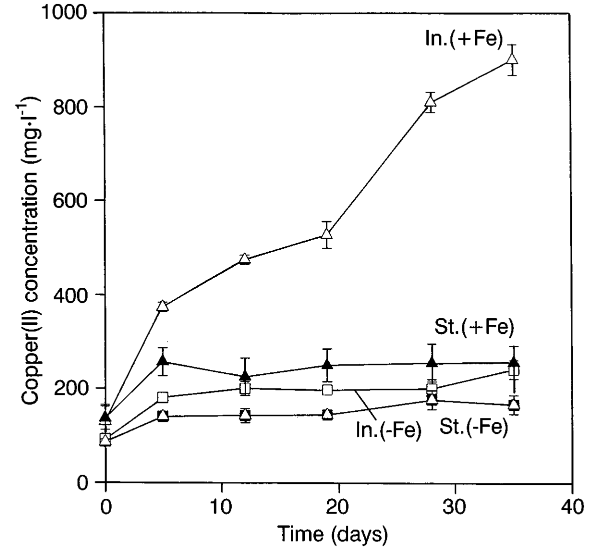

Fig. 2 Copper extraction in the leaching under anaerobic conditions. *In(+Fe)* cultures with ferric ion inoculated with *Thiobacillus ferrooxidans*; *In(−Fe)* cultures without ferric ion inoculated with *T. ferrooxidans*; *St(+Fe)* sterile control with ferric ion; *St(−Fe)* sterile control without ferric ion

In those cultures performed in the absence of ferric ion the dissolution level was 18% for the inoculated treatment and 13% for the sterile control. This behaviour confirmed that the cultures evolved under anaerobic conditions in the presence of ferric ion and, it can be assumed the extractions obtained in the absence of ferric ion were mainly due to acid attack (the initial culture pH was 1.80).

Figure 3 shows the development of the bacterial population in the inoculated flasks and the progress curve for ferrous ion concentration for the cultures

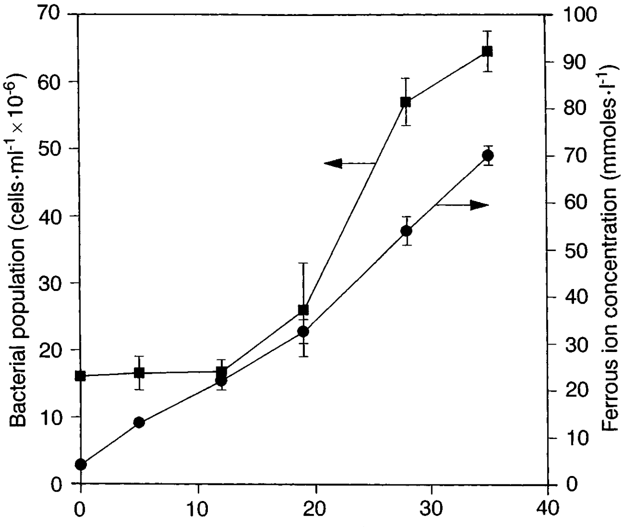

Fig. 3 Bacterial population and ferrous ion concentration in the bioleaching of covellite by *T. ferrooxidans* under anaerobic conditions in the presence of added ferric ion

growing at the expense of ferric ion. It can be seen that appearance of ferrous ion was concurrent with copper dissolution (shown in Fig. 2).

Discussion

Copper sulphide is spontaneously oxidised by ferric ion (in sterile and inoculated flasks) according to the well-known reaction (Kelly et al. 1981):

$$CuS + 2\ Fe^{3+} \rightarrow Cu^{2+} + S + 2\ Fe^{2+} \quad (1)$$

The rate of increase of copper and ferrous ions in solution (Figs. 2, 3) confirmed that the reaction 1 (Eq. 1) had occurred very fast. However, in the absence of bacteria, ferric ion reduction reached a maximum of 3 mmol at 5 days and thereafter remained constant along the experiment (Fig. 2). This behaviour could be explained because the elemental sulphur produced in reaction 1 would have covered the sulphide surface, protecting it from further chemical oxidation. By contrast, in the inoculated flasks, copper solubilization (parallel to ferric ion reduction) constantly increased during the experiment. This could be explained by further oxidation of copper sulphide, which previously required sulphur oxidation. This needed the presence of *T. ferrooxidans* (it did not occur in sterile flasks), probably using ferric ion as electron acceptor (there was no other oxidising agent under anaerobic conditions). This process could be represented by the equation:

$$S + 6\ Fe^{3+} + 4\ H_2O \rightarrow 6\ Fe^{2+} + SO_4^{2-} + 8\ H^+ \quad (2)$$

Our results supported the proposed mechanism because the ratio reduced ferric ion/solubilized copper is approximately 7:1 after 35 days in the inoculated flasks. This ratio is much higher than the ratio 2:1 of reaction 1. This higher ferric ion reduction could be explained by sulphur oxidation by *T. ferrooxidans* with ferric ion as the electron acceptor as proposed in reaction 2 (Eq. 2).

The mechanism, involving successive reactions (Eqs. 1 and 2), is suitable to explain the high reduction of ferric ion, and parallel copper dissolution, observed under anaerobic conditions. Moreover, the decrease of pH in the inoculated system (from 1.80 to 1.62) is also consistent with the acid production proposed in reaction 2. The growth of the bacteria in the cultures showed an initial lag phase, which was followed by an exponential growth phase (Fig. 3). The number of cells formed in liquid suspension was 1×10^9 cells/mmol ferrous ion. The initial prolonged lag phase suggests that reaction 1 was not bacteria-catalysed (there was no bacterial growth until the layer of sulphur was produced).

The copper recoveries under aerobic conditions with initial ferrous ion or initial ferric ion were 42% and 55% respectively. These percentages of solubilized copper were reached quickly after 10 and 8 days respectively and remained constant (data not shown). The copper solubilization did not continue in these aerobic systems because the surface of the covellite was covered by the precipitation of jarosites (Curutchet et al. 1992). The precipitation of jarosites in the cultures with initial ferrous ion is more important than in those with initial ferric ion because the bacterial oxidation of ferrous ion increases the pH. Furthermore, ferrous ion is mainly oxidised by the bacteria attached on the mineral surface allowing the precipitation of jarosites on the substrate (Donati et al. 1996).

Under anaerobic conditions the acid production (reaction 2), and the absence of iron oxidation, prevented a significant precipitation of jarosites. Copper recovery under anaerobic conditions (68% after 35 days) was almost as high as under aerobic conditions without iron (71% after 40 days).

These observations extend previous results where, as already mentioned, it was shown that *T. ferrooxidans* was able to grow on sulphur and zinc-containing sulphide ores under anaerobic conditions (Pronk et al. 1992; Sand 1989; Goodman et al. 1983). Our results indicate that *T. ferrooxidans* is also able to grow anaerobically on covellite with ferric ion as the electron acceptor. The dissolution of covellite by ferric ion under anaerobic conditions was only significant if *T. ferrooxidans* was present. In this way, an important bacterial action would be expected inside dumps where the oxygen concentration is negligibly low (Goodman et al. 1983). Anaerobic bioleaching would take place because of the presence of ferric ion, which is produced by the extensive aerobic bacterial oxidation of ferrous ion in oxygen-rich zones near the surface of dumps.

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References

Brock TD, Gustafson J (1976) Ferric ion reduction by sulfur and iron-oxidizing bacteria. Appl Environ Microbiol 32: 567–571

Cooney CL (1981) Growth of microorganisms. In: Rehm HJ, Reed G (eds) Biotechnology. VCH, Berlin, pp 73–111

Curutchet G, Pogliani C, Donati E, Tedesco P (1992) Effect of iron (III) and its hydrolysis products (jarosites) on *Thiobacillus ferrooxidans* growth and on bacterial leaching. Biotechnol Lett 14: 329–334

Donati E, Porro S, Tedesco P (1988) Direct and indirect mechanisms in the bacterial leaching of covellite. Biotechnol Lett 10: 889–894

Donati E, Curutchet G, Pogliani C, Tedesco P (1996) Bioleaching of covellite using pure and mixed cultures of *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. Process Biochem 31: 129–134

Goodman AE, Babij T, Ritchie AIM (1983) Leaching of a sulphide ore by *Thiobacillus ferrooxidans* under anaerobic conditions. In: Rossi G, Torma AE (eds) Progress in biohydrometallurgy. Associazione Mineraria Sarda, Iglesias, pp 361–376

Kelly DP, Norris PR, Brierly CJ (1981) Microbiological methods for the extraction and recovery of metals. In: Bull AT, Ellwood DC, Ratledge C (eds) Microbial technology: current state, future prospects. Cambridge University Press, Cambridge, pp 263–308

Kolthoff I, Sandell E, Meehan E, Brickenstain S (1979) Análisis químico cuantitativo. Nigar, Buenos Aires

Pronk JT, Bruyn JC de, Bos P, Kuenen JG (1992) Anaerobic growth of *Thiobacillus ferrooxidans*. Appl Environ Microbiol 58: 2227–2230

Sand W (1989) Ferric ion reduction by *Thiobacillus ferrooxidans* at extremely low pH-values. Biogeochemistry **7**: 195–201

Sand W, Gerke T, Hallmann R, Schippers A (1995) Sulfur chemistry, biofilm and the (in)direct attack mechanism -a critical evaluation of bacterial leaching. Appl Microbiol Biotechnol 43: 961–966

Silverman MP, Lundgren DG (1959) Studies on the chemoautotrophic iron bacterium *Thiobacillus ferrooxidans*. I An improved medium and a harvesting procedure for securing high cellular yields. J Bacteriol **77**: 642–647

Torma AE (1977) The role of *Thiobacillus ferrooxidans* in biohydrometallurgical processes. Adv Biochem Eng 6: 1–37