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Biooxidation of ferrous iron and sulphide at low temperatures: Implications on acid mine drainage and bioleaching of sulphide minerals

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ABSTRACT

Chilean copper production has been growing in the last 20 years reaching an annual production of 5,557,000 tons of Cu in 2007. For each ton of copper produced, about 200 tons of sterile and low grade ore and 100 tons of tailings are discharged in the environment. Most of these wastes contain significant amounts of sulphide minerals, mainly pyrite, that once submitted to weathering, may produce acid mine drainage. On the other hand, copper high prices raised the interest for processing the low grade ore deposited in large dumps by leaching. An important part of these mining wastes and low grade ores is located in the Andes, where the mean temperature is usually ~5 °C or less. The rate at which bioleaching reactions occur is directly related to the temperature at which the microorganisms (bacteria and archaea) develop. A temperature decrease causes both a decrease on the chemical reactions rates and a decrease or inhibition on microbial growth. The results for microorganism isolation from an old tailing deposit, exposed at low temperatures (5 °C) during most of the winter are presented in this work. Initially the isolated microorganisms showed a limited capacity in oxidizing Fe(II) sulfate (3 g L^{-1}) at pH 1.6, and tetrathionate (0.01 M), with an initial pH of 4.0 at 5 °C. However, after successive cultures, microorganisms showed a low capacity to oxidize both substrates, as well as the sulphide contained in the tailing samples. The terminal Restriction Fragment Length Polymorphism (tRFLP) of the isolated cells grown in basal medium containing Fe(II) showed a nearly pure culture of *Acidithiobacillus ferrooxidans*. The present study indicates that, even at very low temperatures, microorganisms play an important role in the acid mine drainage generation and also during the oxidation and leaching of sulphide ores.

1. Introduction

The oxidative activity of chemolitothrophic microorganisms (archaea and bacteria) in mesophilic environment, moderately high and extremely high temperatures has been widely studied fundamentally by their importance in bioleaching of sulphides (Goebel and Stakebrandt, 1994; Yahya et al., 1999; Rawlings, 2001). Bacterial species as *Acidithiobacillus ferrooxidans, L. ferriphillium, Acidithiobacillus thiooxidans*, etc., have been frequently isolated from sulphides minerals in heaps, dumps and also from flotation tailings at environment temperature (Southam and Beveridge, 1992; Schipper et al., 1995; Hallberg and Johnson, 2001). Flotation tailings are wastes generated during the flotation process, when copper sulphide minerals are concentrated by pyrometallurgical process, and they are one of the largest mining wastes. In Chile, the annual production of about 5,600,000 tons of copper generates more than 1,000,000 tons of tailings daily. These tailings are deposited in tailing ponds and exposed to environmental conditions which in some cases include low temperatures, about 0 °C during 6 or more months in the year. Due to their mineralogical characteristics Chilean copper ores present low carbonate content, so that 98% of these tailings would be silicates. The remaining 2% corresponds to pyrite (FeS_2), depressed by flotation. The pyrite present in these tailings is exposed to weathering and is slowly oxidized, generating sulphuric acid. When conditions of low pH are reached, tailings can be colonized by ferrous iron and sulphur oxidizing bacteria, accelerating the acid mine drainage generation. The most cited organisms implicated in acid mine drainage are** *A. ferrooxidans*, *A. thiooxidans*, *Thiobacillus thioparus, Acidiphilium acidophilus* and *Leptospirillum ferrooxidans* (Leduc et al., 2002). The reactions and their implications in acid water generation have been widely studied at ambient temperatures (Dold and Fontboté, 2001). Bacterial activity related with acid water mine generation and Fe(II) iron oxidation at a psychrophilic environment was demonstrated for the first time by Ahonen and Tuovinen (1989), from mine water samples. Solubilization and ferrous iron oxidation by a Thiobacillus-like bacteria from North Greenland (Langdahl and Ingvorsen, 1997; Eberling et al., 2000) and Canada (Leduc et al., 2002) had also been described. Recently, a psychrotolerant *At. ferrooxidans* strain able to

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grow on in Fe(II) iron at 5 °C was isolated from Siberia and Sweden (Kupka et al., 2007). The ability of chemolitothrophic ferrous or sulphur oxidizing bacteria to grow and operate at low temperatures could open a new face in the field of low grade sulphide ores bioleaching in heaps or dumps, even though in the case of Chile they are exposed to low temperatures during an important period during the year (Yañez et al., 2007). The aim of this work was to detect the presence of chemolitothrophic ferrous and/or sulphur oxidizing bacteria at low temperatures, in Chilean tailing pond samples exposed to winter temperatures lower or near 0 °C and to determine their capacity to oxidize pyrite at low temperatures.

2. Material and methods

2.1. Tailing samples

Two tailing samples of a Codelco-Andina old pond, presently out of operation, extracted at 25 cm (M1) and 75 cm (M2) deep were used in this work. Samples were analysed and acid consumption was determined according to the EPA Acid Base Accounting (ABA) methodology (Sobek et al., 1978). The results are summarised in Table 1. Sulphide sulphur was calculated from the difference between total sulphur and sulphate sulphur. The acid potential (AP) of each sample was calculated assuming that the sulphide sulphur corresponds to pyrite which could generate acid by complete oxidation. The low insoluble copper content indicates that most of the sulphide content corresponds to pyrite. NP is the neutralization potential calculated from the acid consumption and NNP is the net neutralization potential. AP, NP and NNP are expressed in g of equivalent $CaCO_3$ per kg of tailings. A negative value of NNP indicates that the sample is a potential net acid generator, which is the case of both samples. The paste pH values are slightly acidic, indicating that both samples have already partially reacted as confirmed by the significant sulphate content.

2.2. Microorganisms enrichment

For the enrichment of ferrous iron microorganisms, 10 g of the sample was added to 250 mL sterile shake flasks containing 100 mL basal medium with the following composition (g L^{-1}):$(NH_4)_2SO_4$, 0.4; $MgSO_4 \times 7H_2O$, 0.4 and KH_2PO_4, 0.056 and Fe (II) sulphate, 2 g L^{-1}, (Escobar and Godoy, 1999). Flasks were periodically monitored, pH, Eh, Fe(II) iron and total Fe contents in solution were determined. Total and ferrous iron in the solution were determined by o-phenanthroline colorimetric method (Muir and Anderson, 1977); or ferrous iron was determined by the modified o-phenanthroline colorimetric method (Herrera et al., 1989) if solutions presented a high ferric:ferrous ratio. For sulphur oxidizing bacteria, a basal medium (g L^{-1}) ($(NH_4)_2SO_4$, 6.0; $MgSO_4 \times 7H_2O$, 1.0; $Ca(NO_3)_2$, 0.02; K_2HPO_4, 1.0 and KCl, 0.2), containing potassium tetrathionate, 0.01 M, pH 4, as reduced inorganic sulphur compounds (RISCs), was used (Escobar and Godoy, 1999). Flasks were monitored by solution pH and incubated in an environmental shaker at 5 °C and 100 rpm. Sterilized tailing samples were used as negative control for all enrichment experiments. All the experiments were duplicated. Results shown correspond to the mean values of the duplicated experiments.

Table 1
Chemical composition and ABA results of tailing samples.

Sample	Paste pH	S_{tot}	SO_4^{2-}	S^{2-}	Cu_{tot}	Cu_{sol}	AP	NP	NNP
		%					g $CaCO_3$ equiv kg^{-1}		
M1 (0.25 m)	4.3	1.33	2.19	0.60	0.09	0.02	18.8	7.1	−11.6
M2 (0.75 m)	3.2	2.46	1.71	1.89	0.14	0.05	59.1	12.0	−47.0

2.3. Bioleaching experiments at low temperature

Bioleaching experiments were realized in 250 mL sterile shake flasks with 15 g of tailing sample, 95 mL basal medium (g L^{-1}):$(NH_4)_2SO_4$, 0.4; $MgSO_4 \times 7H_2O$, 0.4 and KH_2PO_4, 0.056 initial pH 2.8 and 5 mL of a suspension of microorganisms isolated from the culture in Fe(II) iron of each sample. The bioleaching of the pyrite present in the tailing samples was monitored for solution pH.

2.4. Microorganisms identification

To identify the microorganisms, DNA was extracted from cells growing in Fe(II) iron and then the tRFLP technique was applied using the universal primers 341f (Liu et al., 1997) and 1100r (Osborn et al., 2000) presented in Table 2, and the enzyme *Rsa*I, according to the software Probe Match of RDPII, Enzyme Resolution and Virtual Digest available in MICA (http://mica.ibest.uidaho.edu) (Escobar et al., 2008). Digested fragments were separated by capillary gel electrophoresis and detected in laser induction fluorescence Genetic Analyzer (ABI PRISM model 310, Applied Biosystems). Table 3 shows the tRFs lengths predicted for the most frequently found bacteria in bioleaching environments for the combination of these primers and the enzyme *Rsa*I.

3. Results and discussion

3.1. Biological activity at 5 °C

A slow activity of ferrous oxidizing bacteria was observed in the isolation flasks for both samples, which was demonstrated by a sustained increase of Fe(III) iron in solution starting to day 18 of incubation (duplicates Fig. 1). This coincided with a low increase of solution redox potential up to 600 mV (vs Ag/AgCl) on day 80 for both samples (data not shown). A small decrease of Fe(III) iron was observed after 50 days for M1 and after 60 days for M2 (Fig. 1), which can be explained by scarce $Fe(OH)_3$ precipitation. In sterile experiments, an increase of Fe(III) iron was observed after 120 days of incubation; possibly due to a deficient sterilization of the samples, which enable the survival of some viable microorganisms. The development of sulphur oxidizing microorganisms was still slower in the isolation flasks from the two samples, showing acidification of the culture medium only after 50 days in both samples (Fig. 2). Initial pH increase observed in samples M2 is probably due to the high acid consumption of the solid. In the sterile flasks, some acidification was also obtained after 100 days, probably also due to an insufficient sterilization of the samples used in the control experiments. The observed oxidation of Fe(II) to Fe(III) and of tetrathionate to sulphuric acid clearly demonstrates the presence in both samples of microorganisms growing at 5 °C and able to oxidize Fe(II) iron at initially pH 1.6 as well as RISCs.

According to the Arhenius equation, the effect of temperature on the reaction kinetics can be calculated by the following relation:

$$\ln(k_1/k_2) = -(Ea/R)(1/T_1 - 1/T_2) \quad (1)$$

where k_1 and k_2 are the specific kinetics constants at temperature T_1 and T_2 and T_1 and T_2 are the temperature expressed in Kelvin. Ea is the activation energy, expressed in Joule/mol.

Table 2
Universal primers used to tRFLP analysis.

Primer	Sequence (5′→3′)	Target site[a]
341f (sense)	CCTACGGGAGGCAGCAG	341–357
1100r (antisense)	GGGTTGCGCTCGTTG	1100–1114

[a] *E. coli* numbering.