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Mesophilic inoculation enhances primary and secondary copper sulfide bioleaching altering the microbial & mineralogical ore dynamics

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ABSTRACT

Bioleaching involves a chemical-microbial-driven dynamic process of oxidation and dissolution, as well as formation of surface secondary phases that change the copper sulfide exposure/occlusion profiles. This dynamic process determines the kinetics of copper sulfides bioleaching. Former studies have shown the microbiological dynamics of bioleaching solutions, but few have focused attention at the ore surface, while most mineralogical studies have been done with pure copper sulfide species under controlled conditions. In this work we aim to unravel the link between the microbiology and the mineralogy during bioleaching, and to determine the effect of mesophilic inoculation of a mainly primary copper sulfide ore. State of art molecular microbial community analysis showed that microbial dynamics in the leaching solutions is not representative of the bioleaching process since it differs significantly from the one established at the ore surface. Moreover, a single mesophilic inoculation permanently alters the microbial dynamics, both in the leachate and the ore surface, generating a major and fast alteration of the primary copper sulfide minerals chalcopyrite ($CuFeS_2$) and bornite (Cu_5FeS_4), as well as the transient formation of covellite (CuS), which was the unique secondary phase detected by advanced mineralogical analysis. Overall, mesophilic inoculation significantly enhanced copper recovery from a low-grade primary copper sulfide ore by means of alteration of the microbial and mineralogical dynamics.

1. Introduction

The non-ferrous metals mining industry is facing several challenges. Among them, the processing of refractory and/or low-grade ores is one of the greatest. In the case of copper, heap and dump acid leaching represent low-cost technologies that have been applied commercially for copper oxides and secondary sulfides. Although heaps and dumps representing natural habitats for acid adapted microorganisms (acidophiles), there is little information about the microbial dynamics of those microorganisms that colonize low-grade ores and leach copper from sulfide minerals. Bioleaching corresponds to the microbially-catalyzed process of conversion of insoluble metal sulfides into soluble forms. This process involves variations in the microbial populations and mineralogical transformations that determine the kinetics of copper extraction, which is a central element for its economical feasibility and commercial application. Clearly, copper sulfides bioleaching takes place at the ore surface. However, most of the former studies have shown the microbiological dynamics of the leaching solutions (Demergasso et al., 2010; Remonsellez et al., 2009), and very few have shown the microbial populations that colonize the ore (Hawkes et al., 2006; Watling et al., 2014), while most mineralogical studies have been done with pure copper sulfide species under controlled conditions (Córdoba et al., 2008).

It is known that the microorganisms that predominate in bioleach liquors are specialized acidophiles, and that heaps and dumps are highly heterogeneous environments, showing variations in their microbiology (Diaby et al., 2007; He et al., 2008). Hence it is important to study the microbial dynamics of the bioleaching process in order to understand the role of acidophiles during copper sulfide oxidation, and how to optimize the leaching kinetics. One aspect that has been most neglected is the effect of inoculation of low-grade ore with mesophilic consortia, due to low kinetics and poor copper recovery compared to higher temperature conditions employing thermophiles. Recent studies have shown the benefits of inoculation over natural rates of ore colonization, and particularly over the time required to reach an efficient regeneration of ferric ion as leaching agent in bioleaching systems (Tupikina et al., 2014; Watling et al., 2014). However, none has linked the microbial and mineralogical dynamics in order to understand surface phenomena and contribute from this perspective to optimize the process kinetics. In this line of thought, inoculation of ore with a specific species/consortium at a specific time could change the microbial dynamics towards faster extraction kinetics compared to the non-inoculated. Several studies have shown that species from the genus *Leptospirillum* predominate over *Acidithiobacillus* and especially *At. ferrooxidans*, (Mutch

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et al., 2010; Plumb et al., 2008; Wakeman et al., 2008), once thought to be a major player in bioleaching processes (Bosecker, 1997). Hence, the study of the ore microbial dynamics and the effect of mesophilic inoculation increasing the initial concentration of the predominant acidophilic species may result in a commercial strategy that enhances leaching kinetics. This added to the fact that low-grade ore thermophilic inoculation conducive to colonization and growth is difficult, since those conditions are unfavorable and sulfides are in short supply (Mutch et al., 2010), poses the question as to whether mesophilic inoculation effect on leaching kinetics can be enhanced towards a commercial application.

In this work we aim to go one step further and study the link of microbial and mineralogical dynamics during bioleaching, and assess at the same time the effect of mesophilic inoculation on a mainly primary copper sulfide low-grade ore through the determination of the surface ore microbial populations, and the mineralogical variations of the copper sulfides during the process.

2. Materials & methods

2.1. Microbial culture

The acidophilic consortium used in this study was obtained by enrichment from ore samples from north and central Andes mining regions in Chile. This consortium was maintained in batch aerated bubble column reactors at 30 °C in a minimal medium described before (Bobadilla Fazzini et al., 2011) containing soluble iron, elemental sulfur and chalcopyrite concentrate as energy sources. The microbial composition was analyzed via quantitative PCR (qPCR) showing a 98% abundance of *Leptospirillum* spp. with a minor proportion of *At. thiooxidans* (<1%) and 2% Archaea mainly belonging to the genus *Ferroplasma*.

2.2. Ore sample

A low-grade ore sample (0.36% copper and 0.94% iron) from a copper mine in the Antofagasta Region of Chile was used. The mineralogical ore composition comprises 0.36% chalcopyrite, 0.20% bornite, 0.15% pyrite, 0.10% chalcocite, 0.01% covellite, 43% quartz, 27% feldspar, 14% plagioclase, 9% sericite, 3% clay and 1% biotite. The sample was crushed and sieved with 4 Tyler mesh, which means that all particles were below 4.7 mm.

2.3. Bioleaching assays

From a single ore lot, samples of approximately 500 g were obtained after strict protocols to minimize sampling errors. Each sample was agglomerated and packed in 6 cm diameter acrylic columns and subject to diluted sulfuric acid conditioning at a feeding rate of 5 $L/h \cdot m^2$ in open circuit for 24 h until reaching pH 1.4 in the effluent. Later, columns were fed with diluted sulfuric acid solution pH 1.4 in closed circuit. No trace nutrients (basal salts, yeast extract or other), reduced inorganic sulfur compounds (elemental sulfur, tetrathionate or other) nor iron was included in the feeding solution at any time. In the case of mesophilic inoculation, the acidophilic consortium was added once to the solution to reach an initial density of 5,00E + 06 cel/g ore just after the beginning of the close circuit feeding. For both conditions (with and without inoculation) five column replicates were set up so that individual columns could be sacrificed at different times and the leached residues analyzed. All columns were incubated at 30 °C under non-sterile conditions. The pregnant leaching solution (PLS) obtained in closed circuit was periodically sampled and evaporation was compensated with pure water before each sampling time.

2.4. Microbial analysis

Measurements were done directly on the original and treated ore, and from the PLS exiting the columns and never from the accumulating solutions reservoirs. Cell concentrations in the ore and the PLS were done by direct chamber counting under phase-contrast microscope (Thoma Chamber, depth 0.010 mm). In the case of the ore, cell detachment was performed by sonication in a buffer solution (0.5 M NaH_2PO_4, 0.5 M Na_2HPO_4, Tween 0,05% w/v, ethanol 10% v/v, 100 mM $NaCH_3COO$, DMSO 5% v/v, 50 mM EDTA, glycerol 5% v/v). DNA extraction of both PLS and processed ores was performed separately at different times by the phenol/chloroform/isopropyl alcohol method. Quantitative PCR on extracted DNA samples was done for total bacteria, total archaea, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum* spp., *Acidiphilium* spp., *Sulfobacillus* spp. and *Ferroplasma* spp. previously described (Bobadilla Fazzini et al., 2011). In the case of processed ore samples, extracted DNA was subject to PCR amplification of the 16S rRNA region with primers described previously (Caporaso et al., 2011), sequenced using a MiSeq Reagent Kit v2 (300 cycles) in a MiSeq Illumina equipment (Illumina, USA) and subject to high-throughput community sequencing data interpretation with the open-source software pipe-line QIIME(Caporaso et al., 2010).

2.5. Chemical analysis

Total iron and copper were determined by Atomic Absorption Spectrometry (Aanalyst 400, Perkin Elmer), and Fe(II) by the *o*-phenantroline method (Kolthoff and Sandell, 1963). Free acid was determined by titration with 0.1 N NaOH.

2.6. Mineralogical analysis

Polished sections of the original ore as well as processed ore samples were analyzed at different times by particle mineralogical analysis (PMA) at 5 μm resolution with QEMSCAN Express® equipment (FEI, USA).

3. Results & discussion

This work first aims to describe the links between the microbial communities in leachates and the communities occurring on ore surfaces during copper-sulfides bioleaching in a low-grade ore. To do so, an initial assessment of the microbial dynamics was studied in a series of bioleaching assays in ore-packed column replicates comparing the microbial dynamics in PLS and processed ore samples. Previous studies have characterized sulfide mineral PLS indicating the predominance of archaea from the genus *Ferroplasma* in a chalcocitic ore process in Myanmar (Hawkes et al., 2006), or moderately thermophilic bacteria belonging to *Sulfobacillus* spp. at a gold mine in South Africa (Coram-Uliana et al., 2006). In Chilean mines, the PLS characterization of Escondida mine indicated the initial prevalence of *At. ferrooxidans* and a gradual increment of *Leptospirillum* species (Remonsellez et al., 2009).

In our bioleaching assays, the PLS of non-inoculated columns showed initial low concentrations with only detectable amounts of *At. thiooxidans*, that slightly rose on day 36 to later drop below detection limit. In parallel, *Sulfobacillus* spp. and *Leptospirillum* spp. were detected in the leachate from day 24 and 36 on, respectively, and intermittently prevailed until the end of the bioleaching assay (Fig. 1, left panel). Considering that copper sulfides bioleaching takes place at the ore surface, non-inoculated bioleaching columns were stopped at regular intervals, drained for 24 h, discharged and the processed ore was further used for microbial analysis. After DNA extraction from solid samples, qPCR community analysis was performed. The results of ore surface microbial dynamic characterization showed a completely different scenario. While *At. thiooxidans* initially appeared in low numbers at the PLS, the ore analysis show it as predominant species on the ore surface until day 36, where Sulfobacilli rise to reach their maximum. Despite the detection of *Leptospirillum* spp. simultaneously in the PLS and on the ore surface, this genus constitutes a minority at the ore surface while it was predominant in solution. Former studies have shown the

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