International Journal of Mineral Processing 138 (2015) 30–37

Contents lists available at ScienceDirect

International Journal of Mineral Processing

journal homepage: www.elsevier.com/locate/ijminpro

Zinc recovery during refractory ore biooxidation by an indigenous consortium

N. Marchevsky [a,b], M.S. Urbieta [a], C. Bernardelli [a], M. Mas [b], E.R. Donati [a,*]

[a] Centro de Investigación y Desarrollo en Fermentaciones Industriales (CINDEFI, CCT LA PLATA-CONICET, UNLP), Facultad de Ciencias Exactas, Universidad Nacional de La Plata, Calle 50 N° 227, 1900 La Plata, Argentina

[b] Departamento de Minería, Facultad de Ciencias Físico-Matemáticas y Naturales, Universidad Nacional de San Luis, San Luis, Argentina

ARTICLE INFO

Article history:
Received 1 February 2014
Received in revised form 12 November 2014
Accepted 13 March 2015
Available online 27 March 2015

Keywords:
Zinc bioleaching
Biooxidation
Gold recovery
Indigenous consortia

ABSTRACT

Two enrichment cultures (one iron oxidizing and one sulfur oxidizing) obtained from an acid mine drainage were physiologically and molecularly characterized; the first of them showed 99% 16S rRNA gene sequence similarity with *Leptospirillum ferrooxidans*, while the sulfur oxidizing species was highly related to *Acidithiobacillus ferrooxidans* and *Acidithiobacillus ferrivorans* sequences although, unlike them, the species in the enrichment culture does not seem to be able to oxidize iron. The consortium constituted by both enrichment cultures achieved a successful biooxidation of Hualilan ore allowing the increase of gold recovery up to 96.4% in the best culture condition (low pulp density in 1 K medium). At the same time, this condition showed an effective zinc bioleaching (up to 86%) although the recovery was much higher in cultures with initially supplemented iron; kinetics studies suggested that the bioleaching rate in 1 K medium at low pulp densities is controlled by diffusion through a layer mainly constituted by jarosite (rather than sulfur) which was detected in X-ray diffraction diagrams. The recovery of zinc as a subproduct of the pretreatment to optimize gold recovery would also allow the decrease of the metallic charge of the leachates from the biooxidation tanks which is an environmental advantage.

1. Introduction

In low grade refractory sulfide gold ore, gold is usually dispersed as microscopic particles within the pyrite and arsenopyrite matrices. Biooxidation is a well-known technology for the pretreatment of such ores that allows an efficient recovery of dispersed gold by the cyanidation method (Muravyov and Bulaev, 2013; Sun et al., 2012a, b). Mixed and pure cultures of acidophilic mesophiles and moderate and even extreme thermophiles have been used for the biooxidation of refractory gold ores; at high pulp densities (used in commercial applications) mesophiles showed better biooxidation potential compared to moderate and extreme thermophiles (Ciftci and Akcil, 2010). Even when there are some contradictory results in the literature, indigenous microbial communities usually seem to be superior to microbial consortia constructed using strains from other environments (Bryan et al., 2011; Chandraprabha et al., 2002).

During biooxidation processes, other valuable metals can be released to the solutions by microbial action (Dinkla et al., 2013; Kaewkannetra et al., 2009; Kondrat'eva et al., 2012). The recovery of such metals as subproducts of the main process would allow not only an economical advantage but also an environmental improvement, decreasing the metallic charge of the leachates from the biooxidation tanks. Recovery of zinc is still performed using conventional methods (pyrometallurgical smelting of concentrates) with bioleaching being only used at pilot scale (see below) but this procedure excludes low-grade or refractory ores. For these ores, heap bioleaching (and consequently a slower process) instead of tank bioleaching should be performed to avoid intensive grinding which would make the process uneconomical. Biooxidation is operated in stirred tanks for high-grade ores or gold concentrates; in this way high reaction rates are obtained and the capital and operating costs are justified by gold market price. The solubilization of zinc during the biooxidation process might imply that the recovery of this metal could be achieved using a less contaminating technology and without any additional investment. After biooxidation, residues might be leached by cyanidation and the solutions might be used in downstream processing for zinc recovery.

Hualilan area is considered one of the most important sources of gold in San Juan Province (Argentina) in the Southern Andes. The earliest recorded mining activity in the area predates the Spanish conquest and it intensified after. The chemical composition of the ore – although highly variable – reveals an average content of 10 ppm Au – partially refractory – 80 ppm Ag and up to 8% Zn mainly as sphalerite. Sphalerite is one of the most important sources for Zn production in the world. Zn

* Corresponding author.
E-mail address: donati@quimica.unlp.edu.ar (E.R. Donati).

http://dx.doi.org/10.1016/j.minpro.2015.03.009
0301-7516/

extraction from sphalerite using bioleaching has recently attracted more attention due to the economic and ecological advantages of this technology over traditional roasting and smelting (Deveci et al., 2004; Giaveno et al., 2007; Haghshenas et al., 2009; Mousavi et al., 2007; Rodriguez et al., 2003; Shi et al., 2006; Soleimani et al., 2011; Souza et al., 2007).

The aim of this investigation is to study the recovery of zinc during biooxidation of a refractory gold-bearing ore from Hualilan district. Enrichment cultures containing sulfur and iron oxidizing bacteria were obtained from different samples taken from an acid mine drainage. An artificial consortium mixing these enrichment cultures was used as inoculum in biooxidation experiments after characterization of both cultures by fluorescence in situ hybridization and 16S rRNA gene cloning and sequencing. In biooxidation experiments, additions of different alternative energy sources, different initial pH values and pulp densities were tested in order to find suitable conditions for recovering both metals.

2. Materials and methods

2.1. Mineral

Mineral samples from Hualilan mining area (San Juan province, Argentina) were used throughout this study. The main mineralogical components were pyrite, pyrrhotite, galena, sphalerite, and chalcopyrite. Besides, limestone and dolomitic limestone were detected in the sample. The chemical composition of the sample was 25.67 g/t Au, 190.2 g/t Ag, 11.53% Fe, 8.12% Zn, 1.3% Mn, and minor amounts of other metals (476 g/t Cd, 1403 g/t Cu, 698 g/t Pb). The fraction with size particle less than 74 μm was used in the biooxidation experiments.

2.2. Microorganisms and media

Several water and sediment samples were taken from the acid mine drainage near to La Carolina mining (San Luis province, Argentina). Environmental samples were directly inoculated in 9 K medium or in the same medium without iron (0 K) supplemented by elemental sulfur powder 10 $g \cdot L^{-1}$. The pH values were initially adjusted to 3 for 0 K medium and 1.8 for 9 K medium utilizing H_2SO_4 (1:10). Media with basal salts were sterilized by autoclaving (20 min at 121 °C and 1 atmosphere overpressure), while solution containing iron was sterilized by filtration through a 0.2 μm pore size filter. The enrichment cultures were made in Erlenmeyer flasks containing 100 mL final volume and inoculated at 10% (v/v) or 2 g for water samples and sediments respectively. The flasks were incubated at 30 °C and agitated at 150 rpm in an orbital shaker. Enrichment cultures that showed microorganism growth were maintained by successive transfer in respective medium at the same conditions. Ferrous iron oxidation and sulfur oxidation rates were evaluated for all the enrichment cultures. The two most efficient were selected to perform the biooxidation experiments. Iron(II) and sulfur oxidizing cultures were called LC-1 and LC-2 respectively and they were unable to oxidize the other energy source.

2.3. Fluorescence in situ hybridization (FISH)

Aliquots of 1 mL of active cultures of LC-1 and LC-2 were used for fluorescent in situ hybridization (FISH) assays. Samples were fixed with paraformaldehyde at 4% final concentration for 4 h at 4 °C and then filtered through GTTP 0.25 Millipore membranes (0.22 μm) using a filtration column. Filters were washed and neutralized with 20 mL of PBS buffer (130 mM NaCl, 7 mM Na_2HPO_4, 3 mM NaH_2PO_4, pH 7.2) and air dried. Hybridizations were done following Amann's protocol (Amann, 1995) using Cy3 labeled probes: TF539 (5′ CAG ACC TAA CGT ACC GCC 3′, 20% formamide in hybridization buffer) specific for *Acidithiobacillus ferrooxidans*, ATT223 (5′ AGA CGT AGG CTC CTC TTC 3′, 40% formamide in hybridization buffer) specific for *Acidithiobacillus thiooxidans* and LF665 (5′ CGC TTC CCT CTC CCA GCC T 3′, 35% formamide in hybridization buffer) specific for *Leptospirillum ferrooxidans*. 4′,6′-Diamidino-2-phenylindole (DAPI) stain was used in all hybridizations to evaluate total cell number. Dako Fluorescent Mounting Medium (Dako North America Inc., USA) was added to preparations in order to avoid fluorescence fading. A Leica DM 2500 epifluorescence microscope was used to visualize hybridization results. Images were taken using a Leica DFC 300 FX camera and its corresponding software (Leica Microscopy Systems Ltd, Heerbrugg, Switzerland).

2.4. DNA extraction, 16S rRNA gene cloning and sequencing comparison

Two microliters of each enrichment culture was filtered to retain solid residues and centrifuged 15 min at 13,000 rpm. Cell pellets were washed with pH 1.5 (H_2SO_4) water, resuspended in 1 mL TE buffer (10 mM Tris HCl pH 8.0, 1.0 mM EDTA). Cells were incubated with 250 μL of 10% sucrose in TE buffer and 250 μL of 5 μg/mL lizosime in TE buffer solution at 37 °C for 1 h. To improve cell lysis 100 μL of solution of protease K 5 mg/mL and SDS 10% in TE buffer were added and incubated 1 h at 37 °C. In order to separate aqueous and organic phases 70 μL sodium acetate 3.0 M (pH 3.4), 100 μL of chloroform and 200 μL phenol–Tris were added and centrifuged 15 min at 13,500 rpm. Aqueous phase was extracted using 100 μL of chloroform and centrifuged 15 min at 13,500 rpm for decanting cellular rests. Cold isopropyl alcohol was used for DNA precipitation in 1 h incubation at −20 °C and 20 minute centrifugation at 13,500 rpm using a refrigerated (4 °C) centrifuge. DNA pellet was washed with 70% cold ethanol and air dried. DNA was resuspended in 50 μL of TE buffer and incubated at 60 °C for 1 h.

Bacteria general primers 27F: 5′-AGAGTTTGATCCTGGCTCAG -3′ and 1541: 5′-AAGGAGGTGATCCAGCCGCA-3′ were used for amplifying DNA extracted from enrichment cultures (Achenbach and Woese, 1995). PCR conditions were as follows: initial denaturation at 95 °C for 5 min, followed by 38 cycles of denaturation at 95 °C for 1 min, annealing at 48 °C for 1 min and extension at 72 °C for 1 min and a final step of extension at 72 °C for 10 min. Amplification reactions contained 3 μL of DNA solution per 25 mL reaction volume, 1× PCR buffer (Promega Biotech), 2.5 mM of each of the deoxynucleotides, 2.5 mM $MgCl_2$, 500 mM of the forward and reverse primers and 0.025 $U \cdot mL^{-1}$ of Taq DNA polymerase (Promega Biotech). PCR amplification was checked by 1.5% agarosa gel electrophoresis stained with ethidium bromide.

Amplified 16S rRNA gene products (>1400 bp) were cloned using the Topo Ta Cloning Kit (Invitrogen. CA, USA) and sequenced using Macrogen services (Macrogen Inc., Seul Korea).

16S rRNA gene sequences of LC-1 and LC-2 enrichment cultures were checked for potential chimeras using Bellerophon Chimera Check program (http://greengenes.lbl.gov/cgi-bin/nph-bel3_interface.cgi) and Maillard software. Finally they were compared with NCBI database using BLAST (http://ncbi.nlm.nih.gov/BLAST). LC-1 and LC-2 16S rRNA sequences, together with their closest relatives were aligned using ARB package (http://www.arb-home.de). Alignments were corrected manually and phylogenetic trees were constructed using neighbor-joining (Saitou and Nei, 1987) and Jukes–Cantor correction. The robustness of individual branches was estimated by bootstrapping with 1000 replicates (Felsenstein, 1985).

2.5. Biooxidation experiments

Leaching experiments were conducted in 250 mL Erlenmeyer flasks containing 150 mL of 0 K medium at 2% of pulp density. H_2SO_4 0.5 M was added to adjust the initial pH value at 1.8. The inoculum was composed of a mixed culture of LC-1 and LC-2. LC-1 was previously cultivated in 9 K medium (pH 1.8) and LC-2 in 0 K medium with elemental powder sulfur at 0.67% (w/v). After bacterial growth reached exponential phase, cultures were filtered through blue ribbon filter paper to eliminate iron precipitates or sulfur. Cells were harvested by centrifugation, resuspended in basal salt