Minerals Engineering 48 (2013) 68–76

Contents lists available at SciVerse ScienceDirect

Minerals Engineering

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

Effects of pH, temperature and solids loading on microbial community structure during batch culture on a polymetallic ore

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ARTICLE INFO

Article history:
Available online 11 December 2012

Keywords:
Sulphide ores
Bioleaching
Bacteria
Archaea

ABSTRACT

The bioleaching of an organic-rich polymetallic ore was conducted under conditions intended to probe the boundaries of microbial activity using iron and sulphur oxidising microorganisms and heterotrophs enriched from self-heating pyritic coal. Solution chemistry parameters such as rapidly increased ORP and reduction in pH subsequent to inoculation point to the development of active microbial communities. The ease with which communities adapted to the organic-rich ore and the bioleaching systems indicated that the organic compounds were not present in leachates at toxic levels. Overall, extractions obtained in three series of inoculated tests were at 35 °C: 79–96% Zn, 48–82% Cu, 47–55% Ni and 79–86% Co; at 55 °C: 96–97% Zn, 72–80% Cu, 46–50% Ni and 82–83% Co. T-RFLP provided semi-quantitative estimates of species abundance. The greatest microbial complexity was observed with moderate pH and low solids loading. Microbial complexity was reduced significantly by low pH or increased solids loading. Nevertheless, efficient bioleaching was observed over a relatively wide range of operating conditions. Even under the more extreme conditions, the community profile was dominated by combinations of organisms not typically seen in most commercial operations.

1. Introduction

With the depletion of high grade ores, it is becoming necessary to extract metals from lower-grade and more complex ores to meet world demand for some metals. Where those ores contain mineral sulphides, the oxidative dissolution of the sulphides can be 'catalysed' via the actions of certain iron- and sulphur-oxidising acidophilic microorganisms. When agitated, air-sparged tank biotechnology is used, the selection of leaching conditions, particularly solution acidity, operational temperature and solids loading, to optimise metal extraction is an important part of process development.

The sensitivity of bioleaching microorganisms to these three parameters is well known. Acidophiles, like all microorganisms, exhibit highest growth and substrate utilisation rates at preferred solution pH values (pH_{OPT}) that differ for different species; microbial activity lessens markedly at solution pH values on either side of the preferred pH. This has been demonstrated for several individual species in growth media with elemental sulphur (Plumb et al., 2008), tetrathionate (Watling et al., 2012), iron(II) (Watling et al., 2008; Ojumu and Petersen, 2011) or pyrite (Yahya and Johnson, 2002). Similarly, different microbial species are most active within a narrow range of temperatures. They become inactive when temperatures rise by only a few degrees above their preferred temperatures (T_{OPT}) and exhibit reduced activity (Franzmann et al., 2005; Halinen et al., 2009; Ongendangenda and Ojumu, 2011) and lower attachment efficiency (Bromfield et al., 2011) at temperatures below T_{OPT}. Increased pulp density (up to 30% w/v) in agitated, air-sparged, bioreactors has been reported to decrease bacterial iron(II) oxidation (Liu et al., 2007), reduce cell viability (Deveci, 2002) and result in lower metal extractions (Olubambi et al., 2008; Pradhan et al., 2010). Archaea may be more sensitive to solids loading than bacteria (compare above citations with Nemati and Harrison, 2000).

The potential of bioleaching technology to process low-grade, polymetallic sulfidic ore deposits rich in organic carbon is a current topic of interest for governments and mining companies eager to realise the metal values (e.g., Puhakka et al., 2007; d'Hugues et al., 2008). The sensitivity of iron(II) and sulphur-oxidising acidophiles to organic compounds is well documented, from historic studies on the effects of surface active reagents and metal extractants on *Acidithiobacillus ferrooxidans* (Torma and Itzkovitch, 1976; Tuovinen, 1978) to studies using some of the more-recently identified microorganisms (Aston et al., 2009; Dopson et al., 2006; Okibe and Johnson, 2002; Watling et al., 2009).

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http://dx.doi.org/10.1016/j.mineng.2012.10.014

In this study, the bioleaching of a pyritic ore containing 8 wt.% organic shale content at three initial pH set points (not subsequently controlled), two temperatures (controlled) and four solids loadings (5–20%) was monitored over a 4 week period. Semi-quantitative changes in the microbial community structure during leaching were examined using T-RFLP. The results are discussed in relation to bioleaching data for four elements associated with the pyrite, Zn, Cu, Ni and Co, and the impact of the prevailing conditions in each test on community structure.

2. Materials and methods

2.1. Sulfidic ore

A complex ore, rich in organic carbon, with 8% pyrite and low concentrations of zinc, nickel, copper and cobalt was used for all tests. The particle size fraction used in the 35 °C tests with 5 wt.% solids loading was 100% −2 mm. In other tests a finer-ground sample (100% −1 mm) was used. The element composition of the ore was determined using inductively-coupled plasma atomic emission spectroscopy (ICP-AES) and ICP-mass spectrometry (ICP-MS) following acid digestion with nitric/perchloric/hydrofluoric acids (twice) and nitric/hydrochloric acid dissolution of the digest residue. Total and organic carbon and total sulphur were determined using a Leco carbon sulphur analyser. X-ray diffraction analysis and Rietveld refinement of the XRD patterns gave quantitative estimates of major mineral phases. These analytical methods were also applied to the insoluble residues from the bioleaching tests.

2.2. Microbial cultures

The 'iron(II) growth medium' (pH 1.8) contained: $FeSO_4{\cdot}7H_2O$ (10 g L^{-1}), $(NH_4)_2SO_4$ (0.4 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.4 g L^{-1}), KH_2PO_4 (0.4 g L^{-1}) and trace element solution (1 mL L^{-1}; for trace element solution see DSMZ medium 882, www.dsmz.de). Yeast extract was added (0.2 g L^{-1}) to the iron(II) enrichments incubated at 45 °C to encourage the growth of heterotrophically-inclined acidophiles such as *Sulfobacillus* spp. (Franzmann et al., 2005). The 'sulphur growth medium' (pH 1.8) contained: elemental sulphur (5.0 g L^{-1}), $(NH_4)_2SO_4$ (1.5 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.25 g L^{-1}), KH_2PO_4 (0.25 g L^{-1}), yeast extract (0.2 g L^{-1}) and trace elements, (1 mL L^{-1}). Heterotrophic microorganisms were enriched using DSMZ medium 269 (pH 3.0) with glucose as substrate. Microorganisms were adapted to the ore by replacing elemental sulphur with pulverised ore (P_{80} 38 μm, 10 g L^{-1}) in the 'sulphur growth medium' or by adding ore (10 g L^{-1}) to DSMZ medium 269 (pH 3.0, yeast extract 0.2 g L^{-1}).

Microorganisms were enriched from solid and liquid samples collected from the Collie Coal Mine, Western Australia where the pyrite-rich coal is subject to periodic heating and source material yields acidophilic mesophiles and moderate thermophiles suited to bioleaching (Robertson et al., 2002). Enrichments were prepared using the three nutrient media containing iron(II), reduced inorganic sulphur compounds or glucose at two temperatures, 30 and 45 °C. These stock laboratory mixed cultures were used for all experiments. Where particulate matter did not interfere, cells were enumerated using phase-contrast microscopy and a Helber Bacteria Counting Chamber (Thoma ruling, 5.0×10^{-5} mm^3 chamber volume). The mixed cultures with highest cell numbers were transferred to media containing ore. Mixed cultures grown in media containing ferrous ions, sulphur, glucose or ore were maintained over a 15 month period. Cultures with high cell numbers were selected and combined for the preparation of larger volumes of inoculum containing complex microbial populations when required for bioleaching testwork.

2.3. Bioleaching

Bioleaching tests were conducted in air-sparged, agitated, temperature-controlled tanks in 2 L medium prepared by substituting ground ore (50 g L^{-1}) for the elemental sulphur in the 'sulphur growth medium'. Tests were conducted at 35 °C and 55 °C with initial set points pH 2.0, 1.6 and 1.2, with solids loading 5%. The temperatures were chosen, partly informed by the pyrite content and the anticipated heap temperature at full scale, to be within the range of temperatures at which known bioleaching acidophiles grow, while at the same time creating conditions of mild heat stress that might influence community diversity. Solution pH values were chosen to be low enough to limit iron(III) compound precipitation but high enough to maintain viable cultures when acid was generated during pyrite oxidation. The effects of solids loading 10–20% were examined in tests at 35 °C and initial pH 2.5. The higher initial pH for these tests was selected in anticipation of progressively higher acid generation from pyrite oxidation with increased solids loading. The 35 °C tanks were inoculated with adapted mixed cultures of mesophiles (10% inoculum, to yield initial concentration 6×10^7 cells mL^{-1}) after the solution pH was stabilized at the designated set points by the dropwise addition of 18 M H_2SO_4. The 55 °C tanks were inoculated with mixed cultures containing mesophiles and moderate thermophiles. Abiotic tests at 5% (initial pH 1.6) or 10% (initial pH 2.5) solids loadings, not inoculated and with periodic addition of biocide (sodium benzoate; 0.05 g L^{-1}, final concentration), were conducted under similar conditions. The solution potential (ORP vs Ag/AgCl) and pH were measured periodically and solution samples filtered and analysed by ICP-AES and ICP-MS. Microbial cells were examined using phase contrast microscopy but could not be enumerated because of the presence of fine ore particles.

2.4. Molecular biology

Reactor samples were left to settle to separate the liquid from the mineral slurry. Liquid samples (5–15 mL) were filtered through 25 mm diameter, 0.22 μm pore size filters (Whatman), washed with 10 mL acidified (~pH 1.8; H_2SO_4) and filter-sterilised ultrapure water and again with 20 mL filter-sterilised TRIS–EDTA buffer (pH 8.0). The filter papers were then processed using the MoBio UltraClean Soil DNA Isolation kit (GeneWorks Ltd.) as per the manufacturer's instructions, with the following modification: cells were lysed using a Q-BIOgene FastPrep bead-beating instrument for 30 s at a speed of 6.5 m s^{-1} (MP Biomedicals). Slurry samples (~0.25 g) were processed directly using the DNA isolation kit and modified protocol.

Terminal restriction fragment length polymorphism (T-RFLP) analysis was used to determine the structure of the bacterial and archaeal communities using the extracted DNA as described previously (Bryan et al., 2006). Restriction enzyme digests were done using either CfoI or HaeIII and each digest was analysed in triplicate. Terminal restriction fragments (T-RFs) were compared to a T-RFLP database and the presence of particular organisms inferred through the identification of corresponding T-RF peaks for each enzyme, where each occurred at similar relative abundance in the profile. The relative abundance (%) values given are the average abundance of the T-RFs from the two digests for each organism. Certain organisms (e.g. *L. ferriphilum* and *L. ferrooxidans*) share the same T-RF for one enzyme, but can be differentiated on the basis of the other. In this case, the abundance values given are for the second enzyme. As a result of these calculations, it is possible to record greater than 100% combined abundance of all organisms in the community.