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Microbial population dynamics of inoculated low-grade chalcopyrite bioleaching columns

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ABSTRACT

This study investigates the effect of temperature on the population dynamics of microorganisms in bioleaching columns charged with a low-grade chalcopyrite ore. A mixed culture containing ten known bioleaching microorganisms was used to inoculate four bioleaching columns operated at 60, 50, 40 or 30 °C. Subsequently, Terminal Restriction Fragment Length Polymorphism (T-RFLP) was used to examine the diversity of bacterial and archaeal populations in the leachates and ores of the four columns. Similar results from samples collected from different locations in the columns give confidence in the reproducibility of the methods used.

Of the 10 microbial inoculants, only *Acidithiobacillus caldus*, *Leptospirillum ferriphilum* and *Ferroplasma acidiphilum* were identified from the leachate and the column solids. However, adventitious growth of a number of other species resulted in different microbial populations in the leachate and on the ore. The results bring into question the effectiveness of heap inoculation, a strategy proposed to overcome the paucity of thermophilic organisms occurring naturally, even in very long-term sulfide-leaching operations. The anticipated impact of temperature on the leachate population was ameliorated by the solution management regime used for the columns, which was chosen to imitate heap leach practice.

1. Introduction

As high-grade ore reserves are processed and in the absence of discoveries of new high-grade deposits, it is increasingly necessary to process ores of lower grade to meet demand. Mineral bioleaching is a method by which some low-grade ores can be processed economically. Metal extraction from low-grade sulfide ores and concentrates can be achieved with the assistance of acidophilic chemolithotrophic iron- and sulfur-oxidizing microorganisms (Bosecker, 1997; Watling, 2006).

A wealth of knowledge exists regarding bioleaching microorganisms grown in pure and mixed culture, in chemically defined media (Franzmann et al., 2005; Johnson, 1998). However, little is known about the dynamics of such microorganisms in the mixed populations of mineral extraction systems. A limited number of studies provide a qualitative description of bacterial populations associated with commercial bioleaching systems (He et al., 2008; Xie et al., 2007). However, only one study has been found (Plumb et al., 2008) in which the growth and activity of acidophilic microorganisms provided with a low-grade chalcopyrite ($CuFeS_2$) energy source are examined. These authors showed that in pure culture, the selected acidophiles were all capable of growth on the sterilised low-grade chalcopyrite ore, albeit at different rates, but that in mixed culture certain strains became dominant.

Microbial population dynamics alter with changing environmental temperature. Microorganisms such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum* dominate mesophilic (15–40 °C) bioleaching environments while *Acidithiobacillus caldus*, *Sulfobacillus thermosulfidooxidans* and *Acidimicrobium ferrooxidans* dominate moderately thermophilic (40–60 °C) bioleaching environments (Watling, 2006; Hallberg and Johnson, 2001). Archaea of the genera *Acidianus*, *Sulfolobus* and *Metallosphaera* are the dominant mineral sulfide oxidisers in engineered thermophilic (>60 °C) environments rather than bacterial species (Johnson, 1998).

Moderate thermophiles and thermophiles can be exploited in the higher-temperature oxidation of chalcopyrite (Fu et al., 2008; Marhual et al., 2008). However, optimising the temperature for thermophilic activity and/or creating growth conditions that generate the greatest cell numbers do not necessarily result in the greatest copper yields (Vilcáez et al., 2008). High copper extraction is the result of a combination of factors, including microbiological factors.

As in other environments, it may be assumed that acidophiles exist in various symbiotic associations, thus they assist bioleaching more ably when present in mixed cultures. The proportions of various

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microorganisms in mixed cultures depend on mineralogy and leaching conditions (Pradhan et al., 2008). Sulfide ore heaps represent heterogeneous microbiological habitats with gradients of pH, O_2, CO_2 and nutrients. Therefore, spatial variations in microbial populations within these systems would be anticipated.

Due to the difficulties of collecting solids materials from sulfide heaps during operation, most studies on the microbial diversity have been undertaken by analysing the leachate that exits the base of the heap. However, the formation of biofilms encasing microorganisms attached to the mineral surfaces is a well recognised phenomenon. Therefore, in order to obtain a complete picture of the biodiversity of a heap, it is necessary also to examine the microbial populations associated with the ore.

The purpose of this study was to investigate the impact of temperature on the survival of inoculant strains (both bacterial and archaeal) in both the leachate and ores of bioleaching columns charged with a low-grade chalcopyrite ore and operated at 60, 50, 40 or 30 °C. The adventitious growth of native microbial populations in the columns contributed further insights to the study.

2. Materials and methods

2.1. Bioleaching columns

Four experimental stainless steel columns were acid conditioned to leach any acid-soluble components arising from the internal acid-resistant coating. They were then charged with a non-sterile, low-grade chalcopyrite ore with approximately 0.5% copper and 0.4% sulfide contents. Chalcopyrite was the main copper-bearing phase with minor bornite (Cu_5FeS_4) also present. The ore had an iron content of about 3% but the pyrite content was <0.5%. The ore was acid conditioned (agglomerated) with 20 kg/t concentrated sulfuric acid. The mass of ore in each column was approximately 36 kg.

Each column was operated separately at the selected temperature using a heated water jacket. Solution management was via closed-cycle, drip feed irrigation (approximately 1.7 mL/min) from an ambient-temperature 17-litre reservoir to the top of the columns. The initial feed solution contained 500 mg/L Fe^{2+} and 500 mg/L Fe^{3+}. Solution residence time within the column was about 1.25 days and six days in the reservoir. Airflow was 500 mL/min from the column base. Periodic addition of concentrated acid to the reservoir maintained the feed to the desired pH 1.5 throughout the experiment.

2.2. Microbial inoculants

Each experimental column was inoculated with a consortium of ten microorganisms, seven bacterial species (*Acidimicrobium ferrooxidans* DSM 10331^T, *Acidithiobacillus caldus* DSM 8584^T, *A. ferrooxidans* DSM 584^T, *A. thiooxidans* DSM 14887^T, *Leptospirillum ferriphilum* DSM 14647^T, *L. ferrooxidans* DSM 2705^T, *Sulfobacillus thermosulfidooxidans* DSM 9293^T) and three archaeal species (*Acidianus brierleyi* DSM 1651^T, *Ferroplasma acidiphilum* DSM 12658^T, *Metallosphaera hakonensis* DSM 7519^T).

Prior to inoculation each strain was cultured at its optimum temperature and pH using an appropriate iron (II), sulfur or heterotrophic medium to achieve high cell numbers (Franzmann et al., 2005; Plumb et al., 2008). Strains were not adapted to the ore, as sufficiently high cell densities could not be achieved during growth on the ore. Cells from individual cultures were harvested using centrifugation (20 min at 48,000×g) and re-suspended in 9 K medium to achieve the desired cell density (2.1×10^8 cells/mL). Equal volumes of each single-strain culture were then mixed to form the inoculum. One litre of mixed inoculum was added to each column to give a cell count of 5.9×10^8 cells/kg ore.

2.3. Sampling of leachate

Column leachate (2 L) was collected aseptically from the column discharge after 275 days of operation. Leachates were examined by phase contrast microscopy to ascertain that viable cells were present. The microorganisms present in the leachate were harvested by centrifugation (1 h at 48,000×g) and then viewed again by phase contrast microscopy. Total DNA was extracted from the leachate, as described in Zammit et al. (2008).

2.4. Sampling of ores

Replicate ore samples were collected aseptically after 414 days at four depths in each column; top (0–300 mm), middle top (300–600 mm), middle bottom (600–900 mm) and bottom (900–1200 mm). Samples were dissected into approx 1 g (wet weight) subsamples and total DNA extracted using the FastDNA® SPIN Kit for Soil and the FastPrep® Instrument (MP Biomedicals, Santa Ana, CA, USA) as per manufacturers' instructions.

2.5. Clone library of amplified bacterial and archaeal 16S rRNA genes from leachate

Bacterial and archaeal 16S rRNA gene clone libraries were constructed using DNA extracted from the leachate samples for each of the four columns, giving eight libraries in total. PCR amplification was performed as described by Watkin et al., (2009) and the product ligated into the pGEM-T Easy vector system (Promega, Australia) as per manufacturer's instructions. Positive bacterial and archaeal clones containing the full length DNA insert were subjected to restriction fragment length polymorphism (RFLP) analysis using restriction enzymes *Msp*I and *Rsa*I respectively (Promega, Australia).

Representative clones of each of the dominant PCR-RFLP patterns were prepared for sequencing as described in Watkin et al. (2009). Contigs were checked and assembled using ContigExpress (Vector NTI Advanced 10.3.0, Invitrogen). The sequences were then compared to the sequences lodged in the GenBank database using BLAST. Sequence data have been submitted to the GenBank database and assigned accession numbers FJ216433 to FJ216449.

2.6. Terminal restriction fragment length polymorphism analysis of extracted DNA from leachate and ore

The diversity of microbes present in the leachate and ore at the end of the bioleaching experiment and their inferred approximate relative abundances was determined by T-RFLP analysis, as described in Hallberg et al. (2006) with the following exceptions. PCR amplification of the 16S rRNA gene was performed using bacterial (fluorescently labelled WellRED D2 (Sigma-Genosys, Australia) 27F and unlabelled 1492R) and archaeal primers (fluorescently labelled WellRED D3 (Sigma-Genosys, Australia) 20F and unlabelled 1392Gr). Restriction digests were performed on successfully amplified products (5 μL) using *Alu*I, *Cfo*I, *Hae*III for both bacterial and archaeal products and *Msp*I for only bacterial products.

Terminal restriction fragments (T-RFs) were determined by comparison of their mobilities with those of the size standard. The relative abundance of each T-RF was calculated using the peak areas for each T-RF relative to the total peak area.

T-Align (Smith et al., 2005) was used for comparisons of replicate T-RFLP profiles in order to generate consensus profiles to identify shared and unique T-RFs between leachate and ores of the four columns. Identification of microbial species using the individual T-RFs from the columns was deduced by comparing with the clones identified in this study and those T-RFs in a database held at Bangor University, UK (Wakeman et al., 2008; Johnson et al., 2008).