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Bioleaching of a low-grade copper ore, linking leach chemistry and microbiology

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ABSTRACT

Three largely-independent studies were undertaken on the same heap leach system during the period of transition from processing oxidised ores to sulfide ores: monitoring of heap solutions for microorganisms, analysis of samples from a spent heap, and column tests. Microbial cell numbers and diversity were monitored in process water samples from the transition heap over a four-year period. Cell numbers remained low throughout, 1–30 $\times$ 10^4 cells mL^{-1}, possibly reflecting growth inhibition by the high element concentrations in process water. High iron, magnesium and aluminium concentrations in spent heap pregnant leach solution (PLS) are attributed to siderite and clinochlore dissolution and would be expected to impact on microbial growth. Planktonic cell numbers in a column leachate declined rapidly by two orders of magnitude when concentrations of ferric ion and sulfate exceeded 30 and 75 g L^{-1}, respectively. Nevertheless, a variety of bacterial strains closely related to *Acidithiobacillus* (*At.*) *ferrooxidans*, *At. caldus*, *Leptospirillum* (*L.*) *ferriphilum*, *Acidimicrobium* (*Am.*) *ferrooxidans*, *Acidiphilium* (*Ap.*) *cryptum*, an *Alicyclobacillus*-related strain and *Sulfobacillus* (*S.*) *thermosulfidooxidans*, and the archaeon *Ferroplasma* (*F.*) *acidiphilum* were isolated, mainly from the more acidic intermediate leach solutions (ILS). Overall, the results obtained from the use of culture-dependent and culture-independent methods of community analysis were complementary and consistent. The majority of identified genera and species were present in both the process water samples from the operating heap and the solutions and ore samples from the spent heap. In the spent heap, distinct populations dominated different sample types. *Leptospirillum*- and *Acidithiobacillus*-like strains dominated PLS samples and *Leptospirillum* also dominated seven of eight spent ore samples and all of the heap sediment samples, making it the primary iron(II) oxidising species.

1. Introduction

Increasingly, the mining industry is faced with the need to process low-grade ores using low-cost technology to meet world demand for copper. Heap and dump leaching tend to be the technologies of choice for low-grade secondary copper sulfide ores and, to a much less extent, primary copper sulfide ores such as chalcopyrite (Domic, 2007). Sulfide minerals in heaps and dumps represent natural habitats for acidophilic microorganisms but there is limited information on those that colonise commercially-operated heaps and assist in the extraction of copper.

Historically, because of the inherent difficulties in the systematic collection of solid materials from operating heaps, microbial community analyses were undertaken by examining the process solutions fed to the top of the heap or exiting the base of the heap. The results of such studies indicated low overall species diversity and changes in community structures related to physical and chemical conditions (e.g., Demergasso et al., 2005; He et al., 2008; Readett et al., 2003), and that the microbial communities attached to mineral phases are different to those in the solution phase (Hawkes et al., 2006; Halinen et al., 2012). Thus, where possible, both solution and solid materials from heaps should be examined, preferably using a combination of culture-dependent and culture-independent methods, to obtain a more complete picture of microbial community structures. One common characteristic that emerged from these and related studies is the relatively small number of dominant strains in any one bioleaching system, though not necessarily the same strains in different systems.

The variety of methods available to describe communities of acidophilic, mineral-oxidising microorganisms in heaps and other environments, including PCR amplification-based techniques for

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the identification and (possible) quantification of species, are summarised by Johnson and Hallberg (2007). In the present study, the culture-independent technique T-RFLP of microbial DNA was used to describe microbial community structures in process water and ore samples. This technique was chosen because, in addition to inferring species identities by comparison with a database of terminal restriction fragments (T-RFs) for acidophiles, it is also possible to estimate approximate relative abundances of dominant species, as described by Hallberg et al. (2006). Both Wakeman et al. (2008) and Mutch et al. (2010) assembled mixed cultures of bacteria and archaea with which to inoculate leaching columns charged with low-grade polymetallic ore (0.57% Cu as chalcopyrite) and chalcopyrite ore (0.5% Cu), respectively, and used T-RFLP to investigate microbial community structures. In both studies, the microbial consortia that evolved from the original inocula were relatively simple. Wakeman et al. (2008) compared the evolution of communities on medium-grained (+2.0–6.5 mm) and coarse-grained ore (+6.5–12 mm) and noted changes in species and abundances as a function of time and size fraction. Communities analysed by Mutch et al. (2010) were generally representative of the optimum temperatures at which species were expected to survive, but there were clear differences between communities in leachate and on ore samples.

From mid-2005 to 2009, Straits Resources operated an open-pit–heap leach–solvent extraction–electrowinning plant at Whim Creek northern Western Australia, to extract copper from a 10 million tonne resource with 1 wt% copper grade. The strategy in re-opening the mine was to consider the Whim Creek deposit and Mons Cupri deposit (4 km south west of Whim Creek) as a combined resource. The copper bearing minerals, including malachite, cuprite, azurite, chrysocolla, chalcocite and chalcopyrite, were hosted within a quartz–chlorite–sericite–siderite matrix in both deposits, together with pyrite, pyrrhotite, sphalerite and galena (Downes et al., 1998; Collins et al., 2004a,b). The average ore grade in the initial heaps (2006–2007) was 0.87% Cu but had diminished to 0.30% Cu in samples collected from the stock pile at the time of closure in 2009.

Depletion of the oxidised ore was the driver for test work leading to the construction of heaps using mixed oxide-sulfide ores. Straits Resources had previously engineered successful transitions from oxide leaching to sulfide bioleaching at their Girilambone (NSW) and Nifty (WA) copper projects, including the introduction of heap aeration as the proportion of sulfide to oxide minerals increased (Readett and Sylwestrzak, 2002; Readett et al., 2003). As part of the test work, chalcopyrite-bearing sections of selected drill cores from Mons Cupri and Whim Creek were crushed, blended and leached in columns to assess whether the Mons Cupri deposit could provide additional resources for the Whim Creek operation. The column data showed the composite ore blend to be strongly acid consuming (>90 kg per tonne of ore) and unproductive with respect to copper, only 10–15% extraction in 180 days accounting for the acid- and cyanide-soluble copper minerals but not chalcopyrite. However, some of the results provided insights on microbial behaviour related to leach chemistry and are included in this paper.

Thus the data reported in this paper were derived from three, largely-independent activities: (i) the monitoring of microbial cell numbers in leachate samples collected 2006–2009 from transition heaps of mixed oxide and sulfide ore; (ii) comparison of the microbial community in transition heap leachate in the monitoring period and in near-surface samples from the spent heap (2010) after heap closure, and (iii) microbial behaviour in high-concentration column leachates. The links between solution composition and microbiology was explored. The application of T-RFLP to deduce which microbial strains were the main contributors to the heap microbial community was assessed by comparing 'identified T-RFs' from the spent heap with the identities of isolates from operating heap process water.

2. Materials and methods

2.1. Monitoring of transition heap process water

During heap operation (2006–2009), process water samples (500 mL) from ponds containing pregnant leach solution (PLS), intermediate leach solution (ILS) or raffinate were refrigerated and transported to the laboratory within 1 week of collection. The solutions were agitated briefly. ORP and pH were measured on 3 mL subsamples. Subsamples (1.5 mL) were centrifuged at 20,000 *g* for 30 min and the supernatant solutions discarded. The residual cell pellets were suspended in process water (50 μL) and the cells enumerated using a Nikon Alphaphot-2 phase contrast microscope and a Helber Bacteria Counting Chamber (Thomas ruling, 5.0×10^{-5} mm^3 chamber volume).

Enrichments at two temperatures (30 °C and 45 °C) were prepared using three media: (i) iron(II) ($FeSO_4{\cdot}7H_2O$, 20 g L^{-1}) with yeast extract (0.1 g L^{-1}); (ii) iron(II) without yeast extract and (iii) elemental sulfur (5 g L^{-1}) with yeast extract (0.1 g L^{-1}). These media were prepared by supplementing basal salts medium (BSM) with the substrate and growth factor. A 1-litre volume of BSM containing: $(NH_4)_2SO_4$, 1.5 g; KH_2PO_4, 0.25 g and $MgSO_4{\cdot}7H_2O$, 0.25 g and trace element solution (0.5 mL) in deionised water, was adjusted to pH 1.8 with 18 M H_2SO_4. The trace element solution was prepared separately and contained: $CoSO_4{\cdot}7H_2O$ (2.49 g), $CuSO_4{\cdot}5H_2O$ (2.81 g), $MnSO_4{\cdot}H_2O$ (1.69 g), $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (1.77 g), $NiSO_4{\cdot}6H_2O$ (2.62 g) and $ZnSO_4{\cdot}7H_2O$ (2.87 g) in 1 L deionised water adjusted to pH 2.5 with 18 M H_2SO_4. The BSM was sterilised in an autoclave at 121 °C, 100 kPa for 20 min. Enrichments were incubated for 2 weeks with periodic microscopic analysis and cell enumeration. For the iron(II) enrichments, the colour change to vivid orange was an indirect indicator of iron(II) oxidation. Putative identifications of bacteria in process water samples were made on the basis of iron- and sulfur-oxidising ability.

2.2. Isolation of microorganisms from transition heap process water

Samples of ILS, PLS or raffinate were used to inoculate a variety of media designed to encourage the growth of different bioleaching microorganisms, specifically: the three media described above and, in addition, 'modified 9 K medium' (M9 K) (Silverman and Lundgren, 1959) at pH 1.6 and pH 1.8; and DSM M874 *Ferroplasma* medium (www.dsmz.de). The constituents of M9 K were: $(NH_4)_2SO_4$ (0.4 g); $MgSO_4{\cdot}7H_2O$ (0.4 g); KH_2PO_4 (0.4 g); $FeSO_4{\cdot}7H_2O$ (20 g) and trace element solution (1 mL) in 1 L deionised water, adjusted to pH 1.6 or pH 1.8 with 18 M H_2SO_4. For M9 K medium, the trace element solution was prepared separately and contained: $MnCl_2{\cdot}2H_2O$ (62 mg); $ZnSO_4{\cdot}7H_2O$ (68 mg); $CoCl_2{\cdot}6H_2O$ (64 mg); H_3BO_3 (30 mg); Na_2MoO_4 (10 mg); $CuCl_2{\cdot}2H_2O$ (66 mg); $NaVO_3$ (30 mg) in 1 L deionised water, adjusted to pH 1.8 with 18 M H_2SO_4.

All enrichments were incubated in an orbital–shaking incubator at 180 rpm at 30 °C. Pure strains of iron(II)- and/or sulfur-oxidising bacteria were isolated from the enrichments using modified iron(II)–sulfur–yeast extract solid-medium plates (Johnson, 1995) and single colonies were transferred back to liquid media. Only one cell type was enriched on DSM M874, and one strain with morphology similar to *Leptospirillum* spp. was purified from M9 K at pH 1.6 using decimal serial dilution in a micro-titre plate. Isolates were identified by 16S rRNA gene sequencing.

2.3. Spent heap sampling and enrichments

Microorganisms from spent ore and sediment samples were enriched at 30 °C, 50 °C and 60 °C. Enrichment cultures (20 mL)