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Investigating the effect of acid stress on selected mesophilic micro-organisms implicated in bioleaching

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ABSTRACT

During start-up of heap bioleaching, low grade ores may be treated with acid for agglomeration and to combat the acid neutralising capacity of the gangue minerals. This may stress the bioleaching inocula, particularly upon inoculation during ore agglomeration. Acid addition for agglomeration varies across operations, ore types and their neutralising capacity, with limited information published on recommended concentrations. The initial pH in the agglomeration mix is typically below pH 1.0 and may be as low as pH 0.5.

This paper investigates the effect of acid stress in terms of initial acid concentration and duration of exposure in submerged culture on mesophilic micro-organisms typically implicated in mineral sulphide bioleaching and critical for heap colonisation at start-up. Following acid stress, cultures were returned to standard operating conditions in batch stirred slurry reactors and their performance assessed in terms of mineral leach rates, ferrous oxidation and the rate of microbial growth. Increasing acid stress resulted in an increase in the lag period before onset of microbial growth and iron oxidation due to decreased viable cell numbers, specific metabolic activity or both. Following adaptation, typical growth and ferrous iron oxidation rates were observed under low stress conditions while reduction in the rate and extent of microbial growth and ferrous iron oxidation persisted at extreme conditions. A reduction in yield (microbial cells produced per kg iron oxidised) was observed with increased acid concentration over comparative times. Microbial speciation analysis indicated a substantial decrease in the diversity of the microbial species surviving.

1. Introduction

Heap bioleaching is a hydrometallurgical process used to recover valuable metals from low grade ores containing minerals such as chalcocite (Cu_2S), covellite (CuS) and, more recently, chalcopyrite ($CuFeS_2$) (Brierley 2008). Nickel, cobalt and zinc containing mineral sulphides are also amenable to bioleaching. Micro-organisms facilitate the dissolution of sulphide minerals by regenerating the ferric iron and protons responsible for mineral attack through oxidation of the ferrous iron and reduced sulphur species produced during mineral dissolution. This provides a continuous and potentially well-distributed source of leaching agent. Ideally, the rate of microbial regeneration of these leach agents ensures their plentiful supply such that mineral availability is rate limiting. However, effective microbial colonisation of heaps is required to achieve optimum microbial activity. Microbial colonisation is affected by the availability of naturally occurring micro-organisms, the effective introduction of micro-organisms, their adaptation to new conditions, such as humidity, acidity, aeration, energy and carbon source, their rapid attachment to the mineral and their metabolic activity (Africa et al., 2012; Chiume et al., 2012; Watling, 2006).

During the heap preparation process the ore body is crushed to reduce particle size and increase cracks and fissures, thereby liberating the mineral of interest and exposing it to solution carrying the leach agent, thus increasing the dissolution of the metal (during the leaching step) and reducing extraction time. This, however, increases the amount of fine material. The fine materials affect percolation through the constructed heap, solid–liquid–gas contacting and the associated gas–liquid mass transfer. Poor percolation can lead to low metal extraction due to solution channelling or the development of impermeable (dead) zones within the heap (Kappes, 2005; Schlitt, 1992). Several heap leach operations are reported to have experienced problems associated with poor

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recovery due to percolation issues caused by low-grade complex ores, tailings and clayey deposits (Dhawan et al., 2013). This has been further demonstrated by investigation of leaching efficiency where layers of fines were allowed to develop (Vries, 2013).

Agglomeration may be introduced subsequent to crushing as a pre-treatment to allow adhesion of the fines to the coarse material by addition of liquids such as the leaching solution or binders or both to produce strong and stable agglomerates in which fine particles coalesce onto larger rock particles via liquid bridges (Dhawan et al., 2012; Kodali et al., 2011; Bouffard, 2005). By increasing the uniformity of the resultant effective agglomerate size, agglomeration ensures more uniformly permeable heaps, improving the percolation of solution through the ore.

The second role of crushed ore agglomeration is to provide an opportunity for the thorough application of the leaching solution to initiate the leaching process itself prior to building the heap (Dhawan et al., 2012; Bouffard, 2005; Purkiss and Anthony, 2004). Agglomeration solutions such as sulphuric acid are used for copper, nickel and uranium bearing ores and cyanide solutions used for gold and silver bearing ores, to improve the agglomeration process and facilitate the leaching of minerals (Bouffard, 2005). Acid agglomeration, used largely in the bioleaching of mineral sulphides, including copper-containing ores, also combats the acid neutralising capacity of the gangue mineral and prepares the ore for microbial attachment.

Micro-organisms experience stress when they are introduced into the operation due to changes in environmental conditions. This is especially pronounced if they are introduced during the agglomeration process. The effect of high acidity on microbial growth and leaching of mineral sulphides has been reported previously (Song et al., 2013, 2011; Tupikina et al., 2011; Plumb et al., 2008; Deveci et al., 2008). Plumb et al. (2008) studied the effect of the pH range 0.5–3.5 in a batch stirred tank reactor of micro-organisms implicated in bioleaching and demonstrated the varying pH optima for the six organisms studied (*Acidithiobacillus thiooxidans, Acidithiobacillus caldus, Acidiphilium brierleyi, Leptospirillum ferriphilum, Sulfolobus thermosulfidooxidans, Metallosphaera hakonensis*) across the range pH 1.0–2.5. Their work is supported by the findings of Deveci et al. (2008) and Song et al. (2013). While several organisms showed metabolic activity at pH 0.5, in all cases it was reduced with respect to optimum pH. In well-colonised heap leaching systems, the microbial oxidation rate exceeded the mineral leaching rate of low grade ore across the pH range 1.1–2.0 (Plumb et al., 2008). Negative effects were observed at pH less than 0.9 in moderately thermophilic and thermophilic systems, with growth and leaching seriously impaired at pH 0.7 (Tupikina et al. 2013, 2011).

While most bioleaching operations run at pH values in the range 1.2–2.5 (Plumb et al. 2008), the higher acidity levels used in industry during the agglomeration process are poorly documented and vary considerably from one operation to another. Further, little is reported in literature on the contribution of high acidity to microbial stress and its subsequent impact on the overall leaching performance.

In this paper, the effect of acidity and the duration of exposure on a mixed mesophilic culture comprised of organisms typically implicated in mineral bioleaching are investigated. The culture was subjected to increasing concentrations of sulphuric acid for one, three and 24 h, prior to inoculation into a batch stirred tank reactor under typical operating conditions (initial pH of 1.4). On cultivation, the time of onset of microbial growth and ferrous iron oxidation and their rates were monitored. Performance was compared to that of a control test, not subjected to acid stress. In addition, using microbial species analysis conducted by qPCR, the relative tolerance of different microbial species to acid stress was considered.

2. Experimental method and materials

2.1. Microbial cultures

Microbial cultures used in this study were maintained as a mixed mesophilic stock culture containing 1% *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*), 5% *Acidiplasma cupricumulans* (*A. cupricumulans*), 3% *Ferroplasma acidiphilum* (*F. acidiphilum*), and predominantly 90% *Leptospirillum ferriphilum* (*L. ferriphilum*), confirmed by qPCR. The micro-organisms were grown on a pyrite concentrate in a 1 L batch stirred tank reactor at 35 °C. The stock was sub-cultured on a weekly basis by removing 150 mL slurry and replacing it with 3.5 g of pyrite concentrate and 150 mL of Norris media (Norris et al., 1996) to allow the micro-organisms to remain active. The cell concentration was maintained at 1×10^9–4×10^9 cells mL^{-1}.

2.2. Mineral

The pyrite concentrate provided by BHP Billiton (Randburg, South Africa) was used for energy source throughout the study. The milled concentrate was wet sieved to obtain a 38–75 μm size fraction. Size analysis was performed on the fraction using a Malvern Particle Size Analyser and 90% passed 53.18 μm and 31% passed 10 μm. The composition of the concentrate was 41% sulphur and 50% iron. The relative density of the pyrite concentrate was 4.49 kg dm^{-3} (measured using pycnometer).

2.3. Reactor set-up and experiments

Identical 1.0 L glass jacketed stirred tank reactors (STR) of 0.18 m height and 0.10 m internal diameter were used at a liquid working volume of 0.7 L. The reactor was agitated with a 4-bladed pitched blade impeller of diameter of 57 mm at an agitation rate of 550 rpm (tip speed of 1.64 m s^{-1}). The batch culture slurry contained media (Norris et al., 1996), a mixed mesophilic culture inoculum and 3% (w/v) pyrite concentrate. The operating temperature was maintained at 35 °C by circulating heated water through the jacket. Aeration through a line sparger was controlled at 2 L compressed air per minute using a rotameter. Evaporation was minimised by passing a coolant maintained at 2 °C through the condensers. A set of four reactors were run at any given time: one as a control and three as stress experiments.

For each experiment, 150 mL of inoculum containing 3×10^9 cells mL^{-1} was stressed at a specified acidity for the exposure time specified in Table 1. Following stress exposure, the inoculum was added to the bioleaching reactor at operational conditions for optimal leaching. The reactor contained 550 mL Norris media and 21 g (3% w/v) of pyrite concentrate equilibrated at 35 °C. The reactor pH was adjusted to 1.40 using Na_2CO_3. The stressed culture was agitated at 550 rpm (impeller tip speed of 1.64 m s^{-1}) and aerated at 2 L min^{-1}.

Table 1
Experiments conducted in sets of four, varying time exposure and the solution volume required to adjust the initial pH.

Test conditions				Volume of pH adjusting agent upon addition to reactor post stress (mL)
Acid concentration (M)	Exposure time (h)			
Control	0	0	0	1.13 (H_2SO_4 99%)
0.34	1	3	24	5.60 (4 M Na_2CO_3)
0.51	1	3	24	10.40 (4 M Na_2CO_3)
0.68	1	3	24	15.40 (4 M Na_2CO_3)