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Effect of inoculum size on the rates of whole ore colonisation of mesophilic, moderate thermophilic and thermophilic acidophiles ☆

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ABSTRACT

Bioheap leaching of low grade copper sulphides has been applied successfully at the commercial scale for the extraction of copper from secondary sulphide minerals. It is important to optimise the inoculation of heaps in order to minimise the residence time required for the heap and to maximise extraction.

Thermophilic bioleaching of the primary sulphide chalcopyrite poses an additional challenge of rising temperatures inside the heap demanding microbial succession. After heap start up, rising heap core temperatures make conditions less favourable for mesophilic microbial species, and the moderately thermophilic community succeeds them in the consortium. In turn, thermophilic microorganisms succeed the moderately thermophilic microbes as the higher temperatures are reached.

A detailed understanding of the microbial colonisation of whole ore is necessary to optimise microbial succession during thermophilic bioleaching, as is that of microbial growth kinetics on whole ore. Most published research is focused on microbial growth rates of bioleaching organisms in liquid cultures; little work is reported on microbial colonisation of whole ore and subsequent microbial activity. To extend the information available on the microbial diversity and succession in a bioleaching habitat, a study of bioleaching microbes colonising the ore body is required.

The aim of this work was to explore aspects of colonisation of low grade chalcopyrite ore at 23 °C, 50 °C and 65 °C by acidophilic micro-organisms. Laboratory column packed bed reactors were designed to simulate heap leach environments and to provide a systematic way of studying microbial dynamics on whole ore. The effect of inoculum size and inoculation strategies on microbial activity established and the subsequent leaching performance were investigated under conditions that support mesophilic, moderately thermophilic and thermophilic micro-organisms. A relationship was shown between the inoculum size and the culture time required to achieve Eh values greater than 700 mV, especially at 23 °C and 65 °C. However, the culture time required to establish an active iron- (and sulphur-) oxidising culture was also influenced by ore type, irrigation rate and inoculum adaptation. The effect on effluent Eh, pH and dissolved iron levels is also discussed.

1. Introduction

Bioleaching is a process making use of microorganisms for the extraction of specific metals from ores (Pradhan et al., 2008; Suzuki, 2001; Watling, 2006) such that the microbial activity ensures the regeneration of leach agents such as Fe^{3+} and H^{+}. The use of bioleaching for copper extraction has been applied commercially for the extraction of copper from secondary sulphide minerals. Heap and run-of-mine stockpile leaching practices has been used for more than a hundred years to treat oxides and secondary sulphides (Marsden, 2008). Currently more than 10% of the copper produced in Chile is done by bioleaching, compared to about 5% in 2002 (Demergasso et al., 2010).

Mesophilic bioleaching has been shown to be very effective for oxidising secondary sulphide minerals; however, it has not been used successfully for the extraction of copper from primary copper sulphide minerals, particularly chalcopyrite (Clark et al., 2006; Van Staden et al., 2008). The oxidation of chalcopyrite with mesophilic cultures only achieved relatively low oxidation values (30 – 60%) and prolonged leach time has not improved the recovery (Clark et al., 2006). In 1999, Billiton showed that good copper extraction rates could be achieved from chalcopyrite concentrates with thermophilic micro-organisms in a stirred tank reactor, coupled with acceptable residence times and solids contents (Clark et al., 2006).

Thermophilic bioleaching for the extraction of copper from chalcopyrite in a bioheap poses an additional challenge of rising temperatures inside the heap. These impact the microbial population. After heap start

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up, rising heap core temperatures make conditions less favourable for mesophilic microbial species, and the moderately thermophilic community takes over in consortium succession. In turn, thermophilic microorganisms succeed the moderately thermophilic microbes as thermophilic temperatures are reached (Pradhan et al., 2008).

Industrial bioheaps can reach tens of meters in height and kilometers in width. Their large volume, and associated inventory holdup, implies minimising residence time while maximising extraction is critical, hence it is important to optimise the inoculation and colonisation process to minimise start up time. These microorganisms are normally autotrophic, they grow in low pH environments (pH 1.5 to pH 2) and obtain energy for growth from oxidising reduced forms of iron and sulphur present in the mineral. Various aspects of the inoculation process e.g. to the need to inoculate, how to inoculate and when best to inoculate are not fully specified and need further investigation (Rawlings and Johnson, 2007).

Inoculation for thermophilic heap bioleaching is particularly challenging as it is necessary to achieve active microbial communities throughout the heap operation across the various temperature ranges. Van Staden et al. (2008) also highlighted the importance of optimizing inoculation with extreme thermophiles for heap bioleaching at high temperatures and raise important issues that still need to be addressed.

Optimisation of microbial succession during high temperature bioleaching requires a fundamental understanding of microbial growth and colonisation of whole ore at temperatures specific to microorganisms involved in bioleaching. The majority of the published research on microbial growth rates of bioleaching organisms is focused on liquid cultures, with limited work reported on microbial colonisation of whole ore. The focus of this study was specifically to explore aspects of establishing sufficient microbial activity of acidophilic microorganisms, associated with colonisation, on low grade chalcopyrite ore at 23 °C, 50 °C and 65 °C as a function of inoculum size.

2. Materials and methods

2.1. Microbial cultures

Mixed stock cultures of acidophilic autotrophs growing at 23 °C and 35 °C were used in equivalent proportions as inoculum for the column experiments conducted at 23 °C. The 50 °C and 65 °C mixed stock cultures were used as inoculum for the 50 °C and 65 °C columns, respectively. The stock cultures were maintained in batch aerated stirred tank reactors containing pyrite (P) or pyrite and chalcopyrite (P/C) mineral as energy source (Table 1) and were sub-cultured weekly.

The microbial composition of the stock cultures was analysed via quantitative real time polymerase chain reaction (qRT PCR) analysis (Tupikina et al., 2013; Dew et al., 2011). The liquid samples containing the cells were filtered to concentrate cells prior to DNA extraction and qRT-PCR analysis. All samples were processed using the High Pure PCR Template Preparation Kit (Roche). Primers for the following species and strains were used for the qRT PCR analyses: *Acidithiobacillus ferrooxidans*, *At. ferrooxidans* D2, *At. thiooxidans*, *At. caldus*, *Leptospirillum ferrooxidans*, *L. ferriphilum*, *Sulfobacillus disulfidooxidans* G1, *S. thermosulfidooxidans*, *Ferroplasma acidiphilum*, *F. cupricumulans* JTC3, *Metallosphaera hakonensis* and *Sulfolobus metallicus* (Tupikina et al., 2013). Where the primers used did not allow all the DNA extracted to be accounted for, cloning and sequencing were used to identify any additional species present.

All glass columns, except one, were inoculated after packing with the agglomerated ore. Each column was filled with 3 L of acidic water (pH 1.7) containing the calculated amount of cells to yield the desired final concentration of cells. The flooded column was left standing for approximately 18 h, after which the solution was drained slowly from the bottom before commencing with irrigation. The final column was inoculated during agglomeration. Here the inoculum was added directly to the agglomerated ore after the acid addition to yield the final desired cell concentration and a moisture content of 4.5 to 5.5%. A summary of operational conditions is shown in Table 1.

2.2. Ores

Low grade ore samples from two different copper mines in Chile were used in the experiments. Ore type 1 was comprised of 0.2% chalcocite, 0.5% chalcopyrite, 0.3% covellite, 4.0% pyrite, 28.6% muscovite, 7.4% kaolinite, and 44.8% quartz. Cu and Fe form 0.46% and 2.48%, respectively. Ore type 2 was comprised of 0.09% chalcocite, 1.66% chalcopyrite, 0.05% covellite, 4.69% pyrite, 0.59% Fe oxides and 91.41% gangue. Cu and Fe form 0.68% and 3.74%, respectively. The ore samples were crushed so that the samples were 80% less than 12.5 mm.

Table 1
Summary of the operational conditions for the different column experiments.

	Temp (°C)	Inoculum size (cells/ton)	Inoculum type[a]	Ore type[b]	Irrigation rate (L/m^2/h)[c]	Inoculum addition	Fe^{2+} (g/L)[d]
A	23	None		1	3	Flooded	0
B	23	3×10^8	P	1	3	Flooded	0
C	23	3×10^9	P	1	3	Flooded	0
D	23	3×10^{11}	P	1	3	Flooded	0
E	23	3×10^{12}	P	1	3	Flooded	[0, 1 & 2][e]
F	23	2×10^8	P/C	1	3	Flooded	0
G	23	3×10^8	P/C	1	3	Flooded	0.5
H	23	2×10^8	P/C	1	6	Flooded	0
I	23	3×10^8	P/C	2	3	Flooded	0.5
J	23	3×10^{10}	P/C	2	3	Flooded	0.5
K	50	None		2	3	Flooded	0.5
L	50	3×10^8	P/C	2	3	Flooded	0.5
M	50	3×10^{10}	P/C	2	3	Flooded	0.5
N	50	3×10^{12}	P/C	2	3	Flooded	0.5
O	65	None		2	3	Flooded	0.5
P	65	3×10^7	P/C	2	3	Flooded	0.5
Q	65	3×10^8	P/C	2	3	Flooded	0.5
R	65	3×10^9	P/C	2	3	Flooded	0.5
S	65	3×10^{10}	P/C	2	3	Flooded	0.5
T	65	3×10^{10}	P/C	2	3	Agglome-ration	0.5

[a] Pyrite mineral adapted — P; Pyrite/Chalcopyrite mineral adapted — P/C.
[b] Refer to Materials and Methods for description of Ore type 1 and Ore type 2.
[c] Inoculum addition via flooding the column for 18 h prior to irrigation, or during agglomeration.
[d] Ferrous iron addition to the raffinate.
[e] This column was started with 0 g Fe^{2+}/L, increased to 1 g Fe^{2+}/L on day 15, and 2 g Fe^{2+}/L on day 22.