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Quantification of growth and colonisation of low grade sulphidic ores by acidophilic chemoautotrophs using a novel experimental system

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ABSTRACT

Microbial colonisation of low grade sulphidic ores and subsequent growth in heap bioleaching systems has not been quantified rigorously. In this study, an experimental system simulating the sub-processes that occur at the agglomerate scale was used to quantify the colonisation, growth and propagation of *Acidithiobacillus ferrooxidans* in an unsaturated bed of crushed and agglomerated low grade chalcopyrite ore. The relative distribution of the microorganisms in the flowing leachate solution ('PLS'), the stagnant interstitial liquid and weakly and strongly attached to the mineral surfaces was determined at various time points during the leach. There was a distinct difference in population dynamics in each of these discrete phases. The microbial cells present in the interstitial phase dominated the microbial population in the ore bed. Particularly, the microbial concentration in the free flowing PLS was found to be a poor representation of the ore-associated microbial population. The calculated growth rate of *At. ferrooxidans* in the PLS was unreasonably high when modelled as a continuous system, indicating that change in cell concentration in the PLS was dominated by transfer from the mineral ore associated population. However, the transfer rate was not correlated directly to changes in either the interstitial or attached population sizes. Therefore, unless transfer rates can be accounted for, PLS population dynamics do not accurately represent those in the column as a whole. Growth rates of microorganisms in the interstitial, weakly mineral-attached and strongly mineral-attached phases better predicted growth of *At. ferrooxidans* on the whole ore system owing to the dominance of the microbial location in these phases.

1. Introduction

Heap bioleaching is recognised as a useful unit operation for the extraction of metals from low grade sulphidic ores, for which the energy costs of mineral liberation and concentration are not easily justified or transportation to a conventional treatment plant is not practical. Heap bioleaching has been demonstrated commercially for secondary sulphide minerals such as chalcocite and is increasingly of interest for the extraction of metals from primary sulphide minerals, especially low grade chalcopyrite ore. The latter is currently the most abundant copper-bearing sulphidic mineral (Watling, 2006). The refractory nature of chalcopyrite under bioleaching conditions necessitates high temperature processes for efficient extraction, requiring an active microbial bioleaching environment to enable accumulation of metabolic heat for progression to a high temperature environment.

Iron and sulphur oxidising acidophilic microorganisms regenerate the ferric iron and acid necessary for mineral sulphide dissolution by chemical attack. Leaching performance is affected by the colonisation of the ore and subsequent microbial activity which influences, and is influenced by, the surrounding physicochemical environment. The effect of physicochemical conditions on bioleaching of low-grade chalcopyrite ore by *Acidithiobacillus ferrooxidans* has been studied previously (Cordoba et al., 2008; Fu et al., 2008; Halinen et al., 2009a, 2009b; Kodali et al., 2004; Plumb et al., 2008; Xia et al., 2008) and microbial attachment to the mineral surface is shown to contribute directly to mineral dissolution (Crundwell, 2003; Kinzler et al., 2003; Sand and Gehrke, 2006). Although the influence of various minerals, including chalcopyrite, on microbial attachment and mechanisms of attachment has been investigated (Africa et al., 2010, in press; Bromfield et al., 2011; Escobar et al., 1996; Harneit et al., 2006; Rodriguez et al., 2003) *in situ* studies of growth rate kinetics on whole chalcopyrite ore are limited. They are typically restricted to systems that do not mimic the fluid flow regime within a heap bioleach process effectively. Furthermore, experimental observations of microbial growth, activity and attachment in the whole ore environment have been subject to limitations such as the sterility of whole ore and purity of the microbial cultures, as well as inadequate techniques for quantification of microbial population dynamics within a single ore body.

In the heap bioleach process, crushed ore is agglomerated prior to stacking, to enable a suitable bed porosity to be maintained.

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Agglomerates contain some 8% moisture (Petersen, 2010). The irrigation solution flows through this partially saturated packed bed, typically simulated as a plug flow system. The resulting agglomerates consist of a range of particle sizes and a stagnant liquid phase through which mass transport is largely diffusion controlled. On studying the microbial colonisation of the bioheap, it is necessary to consider the sub-regions that are formed within the agglomerated ore bed. In this study, the presence and growth of microorganisms in four regions or phases within a heap bioleaching ore bed are considered. Some microorganisms are assumed to attach to the mineral surface (sessile cells) either as a biofilm or through electrostatic interaction, some remain in the stagnant interstitial liquid trapped within the agglomerate and some report to the flowing leach solution (typically termed pregnant leach solution (PLS) in the heap system). Microorganisms may be weakly attached to the mineral surface, either in transition to firm attachment or in contact with the EPS layer (Africa et al., 2010; Sand and Gehrke, 2006; van Loosdrecht et al., 1990) or strongly attached to the mineral surface via an EPS layer which is assumed to occur with time (Sampson et al., 2000; van Loosdrecht et al., 1990). A simplified representation of the configuration of the phases within the ore bed is presented in Fig. 1.

This study investigated the colonisation and growth rate of *Acidithiobacillus* (*At.*) *ferrooxidans*, used as model microorganism, on crushed and agglomerated whole chalcopyrite ore using a novel experimental system. This system simulates heap bioleach conditions at the agglomerate scale, and allows quantification of *At. ferrooxidans* in the flowing liquid ('PLS'), interstitial, weakly attached and strongly attached phases within the ore bed. These data are analysed in terms of growth kinetics in each phase and across the ore bed. Ferric iron was included in the feed to promote mineral dissolution by ferric attack. Thus, the origin of ferrous iron in this system is at the mineral surface, better simulating the chemical conditions within a commercial heap.

2. Methodology

2.1. Experimental system

The experimental rig, shown in Fig. 2a, was designed to simulate agglomerate scale heap reactor systems. The experimental system consisted of 30 mini-heap columns (upper level), irrigated by intermittent pulses from the spray valves that traversed the columns shown in Fig. 2a and b. Effluent from each column was collected into individual collection vessels (lower level). A single experimental run could be separated into up to four subsets of mini-heap columns, each operating under differing conditions. Each subset consisted of a single aseptic and 7 biotic mini-columns. A high degree of reproducibility between the individual columns has been found (Govender, unpublished data).

2.2. Ore

The low-grade ROM chalcopyrite ore used in these experiments contained 4 wt.% pyrite (FeS_2) and the following copper bearing minerals (wt.%): chalcopyrite ($CuFeS_2$; 0.5), covellite (CuS; 0.3), chalcocite (Cu_2S; 0.2), bornite (Cu_5FeS_4; 0.1) and enargite (Cu_3AsS_4; 0.1) (SGS Lakefield). Approximately 60 kg of ore was pre-leached in 0.1 M acidified solution (96% H_2SO_4) over a 24 h period. The ore was washed, pressure-filtered and dried at 30 °C. The dried, pre-leached ore was processed into size fractions (Table 1). Thereafter, ore samples of 150 g were reconstructed to be representative of the particle size distribution of the ROM ore and sterilised by γ-irradiation (50 kGy). Therefore, each 150 g ore sample was assumed to be identical, representing grab samples of a larger heap.

2.3. Microbial culture

A pure culture of *Acidithiobacillus ferrooxidans* DSM 14882 was grown on sterile autotrophic basal salts (ABS) medium containing 0.5 g L^{-1} Fe^{2+} ($FeSO_4{\cdot}7H_2O$), trace elements (Kolmert and Johnson, 2001) and γ-irradiated (50 kGy) fine grain pyrite (1%) at pH 1.7 (H_2SO_4). The ABS solution was developed by Johnson's laboratory at Bangor University (UK) and is the same as the heterotrophic basal salts medium except the final $(NH_4)_2SO_4$ concentration is 0.15 g L^{-1} (Johnson et al., 2008). The stock culture was incubated at 30 °C on an orbital shaker (150 rpm). Cell numbers were determined using a Thoma counting chamber and phase contrast microscope. Viable cell counts were determined using the Miles-Misra method (Miles et al., 1938). Briefly, liquid samples were serially diluted in ABS and 10 µL of each dilution spotted onto an iron overlay agarose plate (Feo; Johnson, 1995) in duplicate. Viable cells

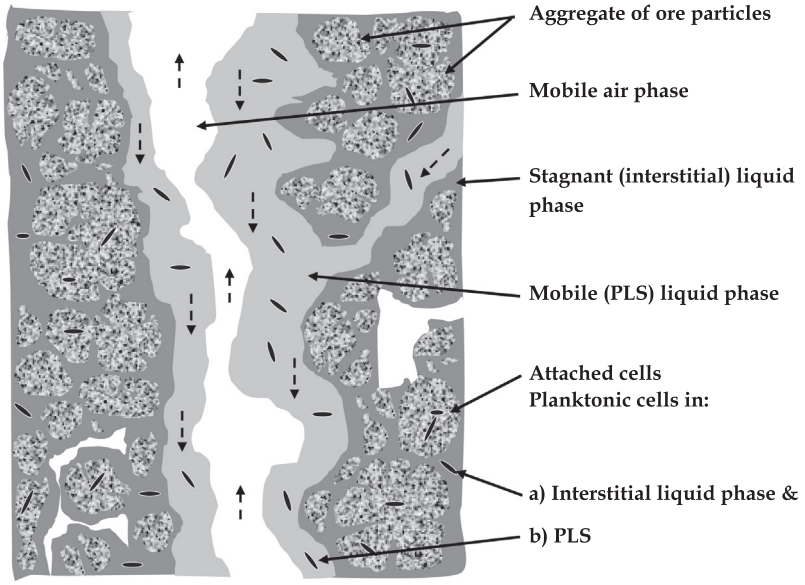

Fig. 1. Schematic diagram of the solid, liquid and gas phases within the agglomerate ore bed. Hashed lines show the flow of the mobile liquid and gas phases around and between aggregated mineral particles.