Minerals Engineering 75 (2015) 14–25

Contents lists available at ScienceDirect

Minerals Engineering

journal homepage: www.elsevier.com/locate/mineng

Effect of physico-chemical and operating conditions on the growth and activity of *Acidithiobacillus ferrooxidans* in a simulated heap bioleaching environment

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ARTICLE INFO

Article history:
Received 11 September 2014
Accepted 6 February 2015
Available online 2 April 2015

Keywords:
Heap bioleaching
Acidithiobacillus ferrooxidans
Microbial growth
Ferrous iron oxidation
Microbial adaptation
Cupric ion stress

ABSTRACT

Recent understanding of microbial retention within heap bioleaching systems has highlighted the importance of quantifying microbial growth and activity in both the bulk flowing solution and in the ore-associated phases. Typically, industrial heap bioleaching operations report variations in process conditions such as inoculum preparation and concentration and elevated copper concentrations in the recycled irrigation solution. In this paper, a mini-column reactor system containing pre-constructed and agglomerated, low-grade ore samples representing grab samples from a larger heap, were used to investigate the effect of a selection of physico-chemical and operating conditions on microbial growth, colonisation and substrate utilisation kinetics, considering both the planktonic and sessile populations of *Acidithiobacillus ferrooxidans*. The factors studied included inoculum size, inoculum cultivation conditions, availability of ferrous iron in the bulk flowing solution and copper concentration in the bulk flowing solution. The microbial population in the interstitial phase, i.e. associated with, but not bound to, the ore, remained the most abundant within the heap under all physico-chemical conditions considered. A comparison of the tests with different inoculum sizes found that a smaller inoculum size resulted in an increased delay in microbial growth and ferrous iron oxidation, but similar apparent maximum specific growth rates and iron oxidation rates. In contrast to the microbial culture grown on pyrite, a delay in microbial activity was observed for the culture grown on ferrous iron. However, greater microbial cell densities were reached, in the interstitial and attached phases compared with the pyrite-grown culture. The introduction of 6 g L^{-1} cupric ions into the feed solution containing 0.2 g L^{-1} ferric iron resulted in decreased microbial growth rate in the interstitial phase but not in the attached phase. Where the pyrite culture was pre-exposed to cupric ion, the microbial growth rate in the interstitial and attached phases was significantly enhanced. Nevertheless, the presence of cupric ion in the irrigation solution resulted in a decrease in microbial ferrous iron oxidation rate, irrespective of pre-culture conditioning. This study emphasises the important role played by the stagnant interstitial phase during the colonisation of a low-grade heap, particularly under adverse conditions for microbial growth and activity. It also highlights the role of inoculum culture conditions on the potential trade-off between increased heap colonisation and increased lag periods in microbial activity during heap start-up.

1. Introduction

Current research is focused on the application of heap bioleaching technology for the extraction of base metals from refractory, low-grade copper-bearing sulphide minerals. Commercial heap bioleaching operations have the advantage of lower capital and operating costs, and lower environmental impact over direct smelting and pressure leaching technologies; particularly when treating lower grade ores (Norgate and Jahanshahi, 2010; Watling, 2006). A rapid increase of the temperature within the heap to the desired operating temperature is crucial for optimal heap performance. This may be achieved by minimising the period of microbial adaptation to the heap environment; specifically the interval between heap start-up and increased rates of microbial oxidation of sulphur species (Dew et al., 2011), a reaction which contributes significantly to heat generation.

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http://dx.doi.org/10.1016/j.mineng.2015.02.006
0892-6875/

Heaps typically experience variations in inoculum size, availability of ferrous iron and feed copper concentration (Pradhan et al., 2008). In a recent study, Tupikina et al. (2014) studied the effect of inoculum size on microbial activity using a simulated heap system, loaded with ore from the same bulk sample used in the present study (ore type 1). The authors observed an increase in the lag phase of microbial growth and activity with decreasing inoculum size.

Initial and rapid dissolution of easily soluble copper-bearing minerals, such as chalcocite and covellite, typically result in cupric ion concentrations between 6 g L^{-1} (Watling et al., 2010) and 30 g L^{-1} (Paipa et al., 2005) in the leach solution percolating through the heap. The ability of *Acidithiobacillus (At.) ferrooxidans* to adapt to cupric ion concentrations as high as 25 g L^{-1} has been demonstrated in shake flasks studies (Das et al. (1997). This cupric ion-adapted culture was found to exhibit similar ferrous iron oxidation rates in the presence of high cupric ion concentrations as a cupric-free, wild-type culture. In this same study, it was observed that the ability of the cupric-adapted culture to oxidise ferrous iron was hindered in the presence of 5 and 10 g L^{-1} ferric iron in a single metal solution.

Microbial adaptation has also been found to enhance inoculum retention within bioleaching systems. *At. ferrooxidans* grown on pyrite concentrate showed higher attachment on concentrates of pyrite and chalcopyrite, and milled low-grade ore, than a ferrous grown culture (Africa et al., 2012). The reactor system used in the aforementioned study, consisted of finely-milled concentrate or low-grade ore, coated onto spherical beads of 6 mm diameter, such that no free fine particles were present. While this reactor configuration provided a basis for comparison with respect to surface area, it did not simulate the fluid flow dynamics between the bulk flowing and stagnant solutions within a heap adequately. The presence and proportion of fines has been shown to affect the relative volumes of flowing and stagnant liquid within a heap (Bouffard and West-Sells, 2009).

In this study, *At. ferrooxidans* cultures grown on ferrous iron, pyrite concentrate, as well as on pyrite concentrate in the presence of 6 g L^{-1} cupric ion, were introduced into agglomerate-scale, mini-column reactors (Govender et al., 2015). These mini-column reactors were loaded with low-grade ore and operated with consistent ore surface area, ore mass and feed flow rate; owing to the use of agglomerates "constructed" to give consistent particle size distribution representative of a grab sample from the heap. The mini-column reactors were irrigated with feed containing either 0.2 g L^{-1} ferric iron or 0.2 g L^{-1} ferric iron with 6 g L^{-1} cupric ion in solution. The effects of microbial cultivation conditions and physico-chemical conditions on microbial colonisation, growth and ferrous iron oxidation are presented. The corresponding effect on relative abundance of micro-organisms in the phases previously identified in Govender et al. (2013), i.e. the bulk flowing solution (PLS), stagnant interstitial phase associated with the ore and ore-attached phases, is also discussed. Three comparative data sets are presented and interpreted in an integrative manner, addressing: the effect of inoculum size, the effect of ferrous iron in the feed solution, the effect of inoculum culture conditions, and the effect of cupric ion concentration as a function of inoculum culture conditions.

2. Methodology

2.1. Ore characteristics and sample preparation

The characteristics of the crushed low-grade ore and preparation of ore samples have been described previously in Govender et al. (2013). The ore contained 4.0 wt.% pyrite and the copper-bearing minerals (wt.%): chalcopyrite (0.5), covellite (0.3), chalcocite (0.2), bornite (0.1) and enargite (0.1). The gangue minerals, muscovite and quartz were found to be most abundant at 29 and 45 wt.%, respectively. The ore contained 2.48% Fe_{total}, 2.45% S_{total} and 0.46% Cu. Ore samples of 150.3 ± 1.4 g (dry weight) and identical particle size distributions (Table 1) were constructed as per the method detailed in (Govender et al., 2015). The samples were sterilised with a 50 kGy dose of γ-irradiation, which was specific to the sample volume.

2.2. Microbial culture and inoculum preparation

An *At. ferrooxidans* stock culture (DSM 14882) was grown in sterile autotrophic basal salts medium (ABS) containing 0.15 g L^{-1} $(NH_4)_2SO_4$, 0.15 g L^{-1} $Na_2SO_4{\cdot}10H_2O$, 0.05 g L^{-1} KCl, 0.05 g L^{-1} $MgSO_4{\cdot}7H_2O$, 0.05 g L^{-1} KH_2PO_4 and 0.014 g L^{-1} $Ca(NO_3)_2{\cdot}4H_2O$, with trace elements solution (Kolmert and Johnson, 2001) and supplemented with either 0.5 g L^{-1} ferrous iron ($FeSO_4{\cdot}7H_2O$) or 1% (w/v) γ-irradiated (50 kGy) pyrite concentrate. A further pyrite-grown culture was exposed to increasing concentrations of cupric ions (0.5, 1, 3 and 6 g L^{-1}) over successive sub-culturing. All cultures were grown in 250 mL shake flasks (150 rpm) at 30 °C and an initial pH of 1.7 (H_2SO_4, 96%).

Inocula were grown to late exponential phase. Microbial numbers were estimated using a Thoma counting chamber and phase contrast microscope. Viable microbial population numbers were determined as colony forming units (CFU) on iron overlay plates (Johnson, 1995) using the Miles–Misra method (Miles et al., 1938), described in detail in Govender et al. (2013). For each of the experiments presented in Table 2, the relevant inocula were diluted in sterile ABS (pH 1.7) to the required cell concentration per ton of dry ore (cells ton^{-1}).

2.3. Mini-column reactor set-up

A detailed description of the mini-column reactor system is provided in Govender et al. (2015). An experiment consisted of thirty mini-columns, separated into two sets of fifteen. Each set was irrigated from a different feed source. Each set of fifteen mini-columns was further sub-divided into two sets of tests of seven columns, with an abiotic control test. Each mini-column was loaded with acid-agglomerated, pre-constructed ore samples (sterile solution at pH 0.6, 2 kg H_2SO_4 per ton dry ore). The experimental system was placed in a 30 °C temperature-controlled room. The ore in the mini-column reactors was acid washed with sterilised 0.1 M acidified solution (H_2SO_4, 96%) for 24 h, then left to drain for an hour prior to inoculation. A single, 2 mL pulse of inoculum was injected onto the top of each ore bed, as specified in Table 2, with the exception of the abiotic controls.

An hour after inoculation, irrigation of feed solution commenced at 10 mL h^{-1}, equivalent to 2 L m^{-2} h^{-1}. The solution comprised ABS and trace element solution (pH 1.7) with either ferrous iron ($FeSO_4{\cdot}7H_2O$), ferric iron ($Fe_2(SO_4)_3{\cdot}xH_2O$) or ferric iron and cupric ion ($CuSO_4{\cdot}5H_2O$) as specified per test in Table 2. All solutions were heat-sterilised (121 °C, 20 min).

Table 1
Particle size distribution of constructed agglomerate scale ore samples.

Particle size (mm)	>16.00	16.00–8.00	8.00–5.60	5.60–2.00	2.00–1.00	1.00–0.25	<0.25
Weight%	6.71	48.15	10.57	13.71	4.83	4.11	11.91