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Using isothermal microcalorimetry to measure the metabolic activity of the mineral-associated microbial community in bioleaching

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ABSTRACT

Microbial colonisation of mineral ore is critical in efficient mineral solubilisation for metal recovery in heap bioleaching processes. A better understanding of the processes and behaviour by which microorganisms attach to, and colonise, mineral surfaces may help to enhance bioleaching processes. The direct quantification of metabolic activity of mineral associated cells is difficult and has not been demonstrated adequately in ore beds typical of heap leaching. In this investigation, isothermal microcalorimetry (IMC) was used to measure metabolic activity of a mixed mesophilic culture colonising the mineral surface. IMC is non-destructive and measures continuous heat flow from chemical or biological processes. Our results showed an increase of heat output from the colonised surfaces of mineral concentrate in the biotic system with respect to the abiotic system, caused by oxidative reactions facilitated by the mineral-microbial biofilm. This confirmed that the attached microorganisms were metabolically active and facilitated ongoing mineral leaching through regeneration of lixiviants. The progression of mineral colonisation in a mini-column system was monitored using IMC, scanning electron microscopy and conventional wet chemistry measurements.

1. Introduction

Bioleaching exploits the metabolic activity of a consortium of iron and sulphur oxidising microorganisms to recover valuable metals from mineral ore. The role of microorganisms in the mineral dissolution process is generally described by the indirect contact mechanism (Sand et al., 2001). This mechanism involves the initial attachment of the microorganisms to the mineral surface, followed by colony formation and the production of extracellular polymeric substances (EPS). The EPS provides a reaction space for microbial activity and mineral dissolution through the regeneration of ferric ions and subsequent oxidation of sulphur compounds (Rohwerder et al., 2003; Rawlings 2004). Effective attachment and growth of leaching microorganisms on the mineral surface is a key factor in enhanced mineral dissolution (Sand and Gehrke, 2006).

A number of studies have investigated microbial colonisation of mineral surfaces, EPS formation as well as factors influencing both colonisation and EPS on the mineral surface. Africa et al. (2013) and Bromfield et al. (2011) quantified attachment to mineral surfaces in a flow through system as a function of microbial species and mineral surface. Chiume et al. (2012) investigated the role of irrigation rates on colonisation of sulphidic ores and observed that lower irrigation rates lead to enhanced surface colonisation. Govender et al. (2013) and Tupikina et al. (2014) investigated colonisation and cell growth on low grade sulphidic ores as well as population dynamics in the whole ore system. Govender et al. (2015a) extended this study to account for the impact of physiological conditions on colonisation. These studies relied on detachment of microbial cells from the mineral ore surfaces to quantify them. Isothermal microcalorimetry (IMC) provides a direct, complementary method applicable for use in measurement of metabolic activity of microorganisms colonising mineral surfaces in place on the surface. IMC is widely applied to monitor cell growth in many industrial processes such as food production (Galindo et al., 2005; Kabanova et al., 2013), biomedical applications (Bonkat et al., 2012; Borens et al., 2013), drug development (Baldoni et al., 2009; Braissant et al., 2014) and environmental research (Lescure et al., 2013).

In biomineral processes, IMC has been used previously to show that heat flow from microbial oxidation of pyrite can be measured directly and correlated to the iron oxidation rate, thus allowing estimation of the degradability of ores (Schröeter and Sand, 1993). Furthermore, a relationship between heat flow and the number of iron-oxidizing bacteria was established (Schippers et al., 1995). Schippers et al. (2007) used IMC to measure the potential average oxidation rate of pyrrhotite containing mine tailings dam within a 25 m unsaturated zone. Based on these

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oxidation rates it was deduced that it would take between 80 and 140 years to oxidise the pyrrhotite tailings completely, assuming the oxidation rates remained constant. IMC has also been used to measure the activity of lithotrophic and organotrophic microorganisms associated with pyrite containing tailings at lower temperatures (Elberling et al., 2000). Rohwerder et al. (1999) measured the activity of pure *Acidithiobacillus ferroxidans* (previously known as *Thiobacillus ferrooxidans*) leaching pyrite-containing ore at mesophilic and low temperatures. The leaching rate and activity was faster and higher at mesophilic conditions compared to low temperatures. Sodium dodecyl sulphate (SDS) and layers of crushed concrete were also shown to be efficient in mitigating the effects of ARD using IMC (Schippers et al., 1998). In a different study, long term assessment of ARD mitigation in large lysimeters was done and SDS was shown to only reduce the activity temporarily (Sand et al., 2007).

In this study, IMC was used to measure quantitatively the metabolic activity of a mixed mesophilic culture colonising the surface of pyrite mineral concentrate-coated beads. IMC was used in conjunction with traditional methods to present the progression of microbial-mineral interaction. The integration of IMC with traditional analytical methods allowed for a relationship to be established between (1) metabolic activity on the ore surface and number of colonising cells; (2) metabolic activity and the mineral surface coverage by colonising cells i.e. on the basis of mineral surface area; and (3) metabolic activity and leaching performance of the flow-through mini column leaching system to be established.

2. Materials and methods

2.1. Microbial cultures and growth media

The mixed mesophilic culture consisting of *Leptospirillum ferriphilum, Acidithiobacillus caldus* and archaea (*Ferroplasma acidiphilum* and *Acidiplasma cupricumulans*) was grown on a 3% (w/v) pyrite concentrate in 0 K basal salts medium made up of 3 g L^{-1} $(NH_4)SO_4$, 0.1 g L^{-1} KCl, 0.5 g L^{-1} K_2HPO_4, 0.5 g $L^{-1}$$MgSO_4{\cdot}7H2O$, and 0.01 g L^{-1} $Ca(NO_3)_2$ and 1 mL of 1000 × stock of trace elements (Kolmert and Johnson, 2001) in a 1 L batch stirred tank reactor at 35 °C. The stock reactor was maintained on a basis of a weekly draw and fill in which 15% (v/v) was replaced with fresh media and associated concentrate. The microbial community in the reactor was maintained in the range of 1×10^9 to 4×10^9 cells mL^{-1} (Ngoma et al., 2015).

2.2. Mineral

Pyrite mineral concentrate from Gamsberg was used as an attachment substrate and source of energy in this study. The mineral concentrate was pulverized and wet sieved to obtain a 38–75 μm size fraction. Size analysis was performed using a Malvern Particle Size Analyser and a d_{10} of 7.3 μm, d_{50} of 39.2 μm and d_{90} of 88.8 μm determined. The composition of the concentrate was 34.8% sulphur and 47.45% iron as determined by Leco and ICP-OES respectively.

2.3. Preparation of mineral coated glass beads

The use of mineral coated beads allowed a quantifiable and uniform surface area for attachment and subsequent colonisation. Following Africa et al. (2013), glass beads of 6 mm in diameter were coated with pyrite concentrate using a clear one-part acetoxy-curing silicone sealant (Bostik™) and subsequently air dried for a minimum of 24 h. The mineral coated beads were sterilized using irradiation (45 kGy) as described by Govender et al. (2015b).

2.4. Flow-through column reactor

Glass column reactors (2.5 cm diameter, 19 cm working length) were constructed (Glasschem, Stellenbosch) (Africa et al. 2013). Each column was loaded with 300 mineral- coated beads, and operated as a continuous flow through system. Prior to inoculation, the loaded column reactors were washed and conditioned with 0 K media (pH 1.6) at 1 mL min^{-1} for 24 h to remove readily leachable materials and create an environment that is conducive for microbial attachment to the ore surface. The columns were inoculated by saturation (Tupikina et al., 2014) using an upflow of 100 mL 0 K media supplemented with 2×10^9 mixed mesophilic microbial cells per kilogram of ore and 0.5 g L^{-1} of Fe^{2+} as $FeSO_4{\cdot}7H_2O$ at a rate of 1 mL min^{-1} in a closed circuit. The inoculum suspension was recycled for 18 h to allow microbial-mineral contacting. Thereafter, the columns were drained and the liquid fraction collected. The effluent solution was analysed for the quantity of planktonic cells remaining using microscopic cell counts. A continuous downflow of fresh feed containing fresh 0 K media (pH 1.6) supplemented with 0.5 g L^{-1} Fe^{2+} ($FeSO4{\cdot}7H_2O$) was introduced and the columns operated as flow-through unsaturated beds for the duration of the experimental run. The column experiments were operated at 30 °C for 20 days. Daily effluent or pregnant leach solution (PLS) samples were taken and analysed for pH, redox potential, ferrous and total iron concentration and cell concentration.

Six (6) columns were ran and sacrificed at regular intervals (one on days 1, 7, 12, 15 and 20 respectively), with one column treated as an un-inoculated control. The mineral coated glass beads with microbial cells attached on the surface were transferred into a sterile beaker and mixed gently. Two and five beads were randomly selected using sterile forceps and transferred into IMC vials for subsequent metabolic activity measurements using isothermal microcalorimetry (Section 2.6). Another bead was transferred into an Eppendorf tube and fixed with 2.5% (v/v) glutaraldehyde at 4 °C for subsequent scanning electron microscopy (SEM) (Section 2.8). In addition, for the determination of cell concentrations colonising the mineral surface, 101 beads were transferred into a sterile 250 mL shake flask, in duplicate, for cell detachment (Section 2.7).

2.5. Solution sampling and analysis

All pH measurements were performed using a Metrohm 704 pH metre and probe, calibrated at pH 7.0, pH 4.0 and pH 1.0 before use. All redox potential readings were determined using a glass electrode with a built-in silver/silver chloride (Ag/AgCl, 3 M KCl) reference electrode, connected to a Metrohm 704 pH/Eh meter. The precision of the measurements was tested using a Crison standard redox solution having a potential of 468 mV at 25 °C. The ferrous iron concentration as well as total iron in solution were measured spectrophotometrically using the colorimetric method described by Komadel and Stucki (1988). Microbial cell counts were determined using a Thoma counting chamber and an Olympus BX40 Microscope at 1500 fold magnification [oil phase, phase contrast optics, 15× magnification eyepiece, 100× magnification objective, detection limit of the Thoma counting chamber of 3×10^5 - cells mL^{-1} as described by Chiume et al. (2012)].

2.6. Isothermal microcalorimetry

In this study, isothermal microcalorimetry (IMC) was used to quantify microbial metabolic activity of cells colonising the mineral surface. Microcalorimetric experiments were carried out using TAM III instrument supplied by TA Instruments, USA. This instrument is designed to monitor both endo- and exothermic reactions over a temperature range of 15–150 °C continuously. It is equipped