Developing a Flow-Through Biokinetic Test to Characterize ARD Potential: Investigating the Microbial Metabolic Activity on Pyrite-bearing Waste Rock Surfaces in an Unsaturated Ore Bed

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Abstract

ARD generation from mine waste rock associated with hard rock sulfide and coal mining poses environmental concerns over time. Therefore, it is important to characterise waste rock material for its acid-forming potential, prior to disposal. In this paper, column reactors simulating the flow-through, unsaturated packed bed environment of waste dumps were used to assess acid-forming potential of two pyrite-bearing waste rocks. Additionally, the activity of mineral-associated microbial populations was visualised and measured using isothermal microcalorimetry. The work forms the basis for development of a representative flow-through system, a modification of the UCT Biokinetic test that considers microbial-waste rock surface interaction.

Keywords: Acid rock drainage, metabolic activity, waste rock surface, microbial colonisation

Introduction

Current day mining operations typically generate large amounts of waste rock or pulverized mine tailings or both from the mining and metal extraction processes. These waste stockpiles may contain significant amounts of sulfidic minerals which undergo oxidation through contact with air and water (Dold et al., 2009). Their oxidation is exacerbated by the presence and activity of naturally occurring iron- and sulfur oxidizing microorganisms (Egiebor and Oni, 2007). These generate acid-laden liquid effluents stream, known as acid rock drainage (ARD), containing toxic metals and sulfates that pose a threat to surrounding water sources (Parbhakar-Fox et al., 2015).

To quantify the ARD risk, it is important to test mine waste for its potential to form acid prior to its disposal to ensure an appropriate disposal approach. This enables adequate control and management of ARD to minimise environmental impact and reduce costs associated with environmental remediation during life of the mine, through mine closure and persisting as legacy after life of mine (Parbhakar-Fox et al., 2015). A suite of tools is available to characterise the acid forming potential of waste material. These can be classified into laboratory, field-based and whole-rock geochemical assessments alongside mineralogical evaluations (Morin and Hutt, 1998). New or extended characterisation methods are sought to better address limitations caused by complex microbiological, hydrological, mineralogical, and geochemical processes and their interactions within a mine waste environment (Dobos, 2000). Characterising ARD generation potential of mine wastes through static and kinetic tests is critical for effective mitigation through treatment and disposal. Static tests are common and rapid, providing "snapshot" data under extreme conditions of acidity and oxidative potential to measure 'net' acid generation potential. However, they fail to account for relative rates of acid consuming and generating reactions or for the key role microorganisms play in ARD generation. Kinetic tests typically include humidity cell tests and field tests; these are slow and expensive (Hesketh et al 2010; Parbhakar-Fox et al 2013).

The UCT Biokinetic Test has been introduced to provide meaningful data on long term ARD generating potential of waste rock and tailings and its kinetics in a relatively short space of time (± 3 months) and is relatively inexpensive to operate (Hesketh et al.,

2010; Broadhurst et al., 2013). It recognises the key role of microbial activity and microbial association and colonisation of the mineral surface by iron- and sulfur oxidizing microorganisms in the generation of ARD from waste rock dumps. However, limitations of the batch biokinetic test include poor representation of typical contacting mechanisms of liquid flow or leach solution with waste rock and failure to account for washout of neutralising capacity typical of a flow-through system such as a waste rock dump and the associated impact of differing kinetics of acid formation and neutralisation by ore components. Further development of the biokinetic test to provide a flow-through test is desired. The draw-and-fill flask based biokinetic test (Golela et al., in prep) addresses the first limitation while the flow-through biokinetic test under development here aims to remove both limitations.

In this study, we investigate the potential of a flow-through biokinetic test protocol, using two pyrite bearing low-grade waste rock fractions. Recognising the importance of microbial activity and its association and colonisation of the mineral surface in ARD generation, acidification potential, microbial activity and the community implicated in waste rock weathering and leaching were used as critical indicative variables for monitoring the potential of a flow-through system.

Methods

Waste rock samples

Two pyrite-bearing low grade or waste rock samples, namely low sulphur metapelite (PEL-LS) and high sulphur metapelite (PEL-HS) were used in this study. The samples were milled and wet sieved to obtain -75 µm particles. After air drying at 37 °C, representative samples were prepared by using a riffle splitter. The bulk mineralogical compositions of the waste samples were acquired using QEMSCAN and are shown in fig 1. Acid generating minerals present consist predominantly of pyrite (PEL-LS:13.99 wt. %, PEL-HS: 33.43 wt. %) and pyrrhotite (PEL-LS: 2.35 wt. %, PEL-HS: 3.9 wt. %). Acid consuming minerals consist predominantly of calcite (PEL-LS: 0.22 wt. %, PEL-HS: 0.03 wt. %), fast and intermediate weathering minerals predominantly contain garnet (PEL-LS: 2.83 wt. %, PEL-HS: 4.52 wt. %) and slow weathering minerals consist predominantly of muscovite (PEL-LS: 14.77 wt. %, PEL-HS: 12.42 wt. %). The waste rock samples contain approx. 35% inert quartz (PEL-LS: 36.67 wt. %, PEL-HS: 34.76 wt. %).

The pulverised waste rock samples were coated onto 6 mm glass beads, using Bostic glue (Africa et al. 2013), to provide a uniform and quantifiable surface area. The coated glass beads were air dried for a minimum of 24 hours before they were sterilised by irradiation at 45 kGy dosage.

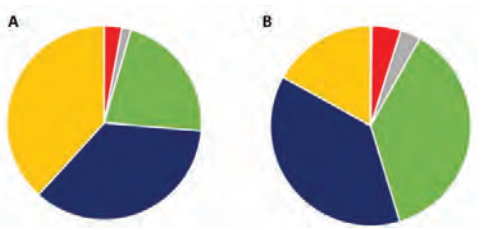

Figure 1 *Mineralogical analysis of the two waste rock samples acquired using QEMSCAN. A (PEL-HS) and B (PEL-LS) are results of the waste samples showing abundant acid forming minerals (pyrite and pyrrhotite;), dissolving mineral (calcite;), fast- (garnet;), intermediate- (Mn-Fe silicate and augite;) and slow-weathering (K-feldspar and muscovite;) respectively, and quartz as an inert mineral ().*

Microbial cultures

A mixed mesophilic culture, comprising *Leptospirillum ferriphilum*, *Acidithiobacillus caldus* and archaea (*Ferroplasma acidiphilum* and *Acidiplasma cupricumulans*), was used in this study. The culture was grown on a 3% (w/v) pyrite concentrate in 0K basal salts medium (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 mineral concentrate. The culture was regularly assessed through direct microscopic cell counts (Thoma counting chamber; Olympus BX40 Microscope at 1500 fold magnification using phase contrast optics; detection limit of the Thoma counting chamber: 3×10^5 cells mL^{1}). Routine moni-

toring included measurement of the redox potential using a Ag/AgCl reference electrode connected to a Metrohm 704 pH/Eh meter and pH measurement. 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).

Reactor operation

For the flow-through biokinetic test, twelve columns were operated, six of each waste rock sample. Each column was loaded with 300 waste rock mineral-coated glass beads and operated as a continuous flow-through system. Prior to inoculation, the loaded column reactors were washed and conditioned with 0K media (pH 1.6) at 1 mL min^{-1} for 24 h to remove readily leachable materials and create an environment conducive for microbial attachment to the ore surface by initiating neutralising reactions on the waste rock. One column of each waste rock type served as an un-inoculated control. The remaining 10 columns were inoculated by saturation (Tupikina et al., 2014) using up-flow of 100 mL 0 K media supplemented with 10^{10} mixed mesophilic microbial cells per kilogram of ore and 0.5 g L^{-1} of Fe^{2+} as $FeSO_4.7H_2O$ at 1 mL min^{-1} in closed circuit. The inoculum suspension was recycled for 18 h to allow microbial-mineral contacting and attachment. Thereafter, the columns were drained and the liquid fraction collected and analysed for planktonic cells by microscopic cell counts. A continuous down-flow of fresh feed containing sterile fresh 0 K media (pH 1.6) supplemented with 0.5 g L^{-1} Fe^{2+} ($FeSO_4.7H_2O$) was introduced at 1 mL min^{-1} and the columns operated as flow-through unsaturated beds for the duration of the experimental run for 20 days at 30 °C. Daily effluent pregnant leach solution (PLS) samples were taken and analysed for pH, using a Metrohm 704 pH metre and probe, calibrated at pH 7.0, pH 4.0 and pH 1.0 before use, redox potential, and Fe^{2+} and total Fe concentration using a colorimetric assay described by Komadel and Stucki (1988).

Individual columns were sacrificed on days 1, 7, 12, 15 and 20, respectively for each rock type. The microbial phase attached to the ore within these columns was analysed morphologically using electron microscopy, quantified and characterised by detachment and direct microscope cell counting, and characterised in terms of metabolic activity using isothermal microcalorimetry.

Microbial detachment and surface visualisation

A modified detachment protocol, described by Makaula et al. (2017), was used to recover microorganisms from waste rock surfaces on coated beads. In this current study the washing step was repeated 3 times to detach firmly attached microbial communities. For microbial-mineral surface visualisation, samples of colonised mineral surface obtained during each column sacrifice were processed as described by Makaula et al. (2017) prior to scanning electron microscopy.

Measurement of activity on mineral associated microbial populations

Isothermal microcalorimetry (IMC) was used to quantify the microbial metabolic activity of cells associated with the waste rock surfaces, as described by Makaula et al. (2017). Two microbially colonised waste rock coated beads from each sample were loaded aseptically into an IMC vial. Each sample was analysed in duplicate and maximum heat flow of each ampoule was recorded.

Results and Discussion

After the 24-hour conditioning period, the pH increased from pH 1.6 to 1.77 (± 0.025) for HS and 1.7 (± 0.012) for LS respectively. Following inoculation during which pH decreased, the pH of the experimental samples increased from pH 1.38 to 1.77 for PEL-HS and 1.6 for PEL-LS. The pH in the control samples increased from pH1.6 to 2.01 for PEL-HS and 1.69 for PEL-LS (fig 2A). The pH increase in the PLS suggests an initial dissolution of acid neutralising minerals such as calcite. During the complete flow-through operation phase, the average pH for the experimental PEL-HS and PEL-LS samples were 1.61 (± 0.035) and 1.59 (±0.011) respectively and the average pH for control samples was 1.63 (±0.024) for PEL-HS and 1.61 (± 0.022) for PEL-LS. These remained relatively stable and similar to the pH 1.6 media fed to the column reactors continuously in flow-through operation. This suggests that there

was a depletion of readily available neutralising agents in the waste rocks or that the residence time in the column was too low to liberate these compounds significantly. The relatively constant pH also suggests a lack of effective activity in the leaching of the available sulfidic mineral in both waste rocks.

The redox potential of the experimental samples decreased from 499 to 401 mV for PEL-HS and from 504 to 430 mV for PEL-LS during the 18-hour inoculation period. The redox potential of the control samples remained the same at 301 mV for PEL-HS and increased from 301 to 335 mV for PEL-LS. The redox potential of both PEL-HS and PEL-LS experimental samples remained relatively low, circa 300 to 500 mV, throughout the continuous flow-through operation (fig 2B). This suggests either a lack of effective and detectable iron-oxidizing microbial activity that would result in the catalysed regeneration of Fe^{3+} and associated increased redox potential, or that the microbial activity generating Fe^{3+} is insignificant with respect to the ferric leaching of the mineral and the flowrate of the solution. This lack of effective activity corresponded with continued presence of Fe^{2+} (fig 2C) and no evidence of its conversion into Fe^{3+} (fig 2D). When the flow rate was lowered to 4 mL h^{-1} (within the industry standard), microbial activity was easily detectable from solution chemistry measurements (data not shown).

The SEM micrographs in fig 3 show leached waste rock surfaces sampled at day 1 and 20. On day 1, single cells were observed on the precipitate covered waste rock surfaces (fig 3A and B). On day 20 the surface of PEL-LS was pitted with microbial footprints (fig 3C). This indicates that microbially facilitated degradation took place and the cells detached. The microbial detachment may be caused by depletion of energy on the attached position or a high liquid flow rate (shear) or a combination of these factors. Cells of various morphologies were present, including spiral and rod-shaped cells that were consistent with the morphology of the microorganisms in the inoculum. Both single cells and colonies of embedded cells were also observed on the PEL-HS.

Cells on the waste rock mineral surfaces were mechanically detached and microscopically counted. Firmly attached cell numbers, as well as the percentage of mineral surface that is covered by these microorganisms in a monolayer are presented in fig 4A. Assuming a monolayer, the approximate number of cells required to saturate the surface is calculated at 1.32×10^{12} cells m^{-2}. After the 18-hour in-

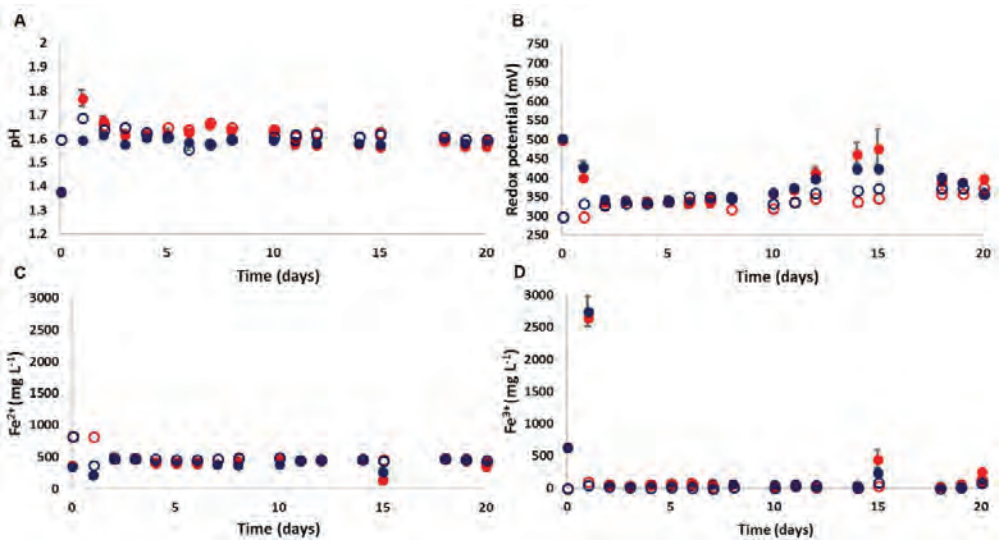

Figure 2 *A) pH, B) redox potential, C) Fe^{2+} and Fe^{3+} of the HS experimental (●) and LS experimental (●) and the HS control ( ) and LS control ( ) column PLS values over the course of the experimental period. Error bars represent the standard deviation from the mean pH across the experimental columns.*

oculation period, 9.21×10^{10} cells m^{-2} or approximately 7% coverage for PEL-LS and 1.08×10^{11} cells m^{-2} or 8.2% coverage for PEL-HS was achieved. Microbial growth was observed on the mineral surface and a total of 2.36×10^{11} cells m^{-2} (17.9% coverage) on PEL-LS and 2.85×10^{11} cells m^{-2} (21.6% overage) on PEL-HS were observed on day 20.

The observations of microbial growth on waste rock surfaces in terms of cell number was complemented by measurement of metabolic activity resulting from the oxidative processes, facilitated by the colonised cells (fig 4B). After 18 hours, the measured maximum heat output was 63 mW m^{-2} for PEL-LS and 123 mW m^{-2} for PEL-HS. At day 20 this increased to 157 mW m^{-2} for PEL-LS and 293 mW m^{-2} PEL-HS. This increase in metabolic activity corresponds with an increase in the active microbial populations associated with the waste rock surfaces.

Conclusions

This study thus far has demonstrated the feasibility of flow-through biokinetic system to provide a representation of microbial activity and leaching of liberated waste rock to provide an indication of acid generating potential. Solution chemistry analysis showed minimal changes regarding the performance of the system, owing to the high flowrate used which facilitated a stable oxidation environment. Through analysis of the mineral-microbe surface environment, progressive microbial growth on the mineral surface was shown through surface visualisation and mechanical cell detachment, together with increasing metabolic activity of surface associated microbial populations. This information is crucial in the understanding of microbial mineral interactions during characterisation of waste material for ARD and its ultimate mitigation. The results obtained provide a

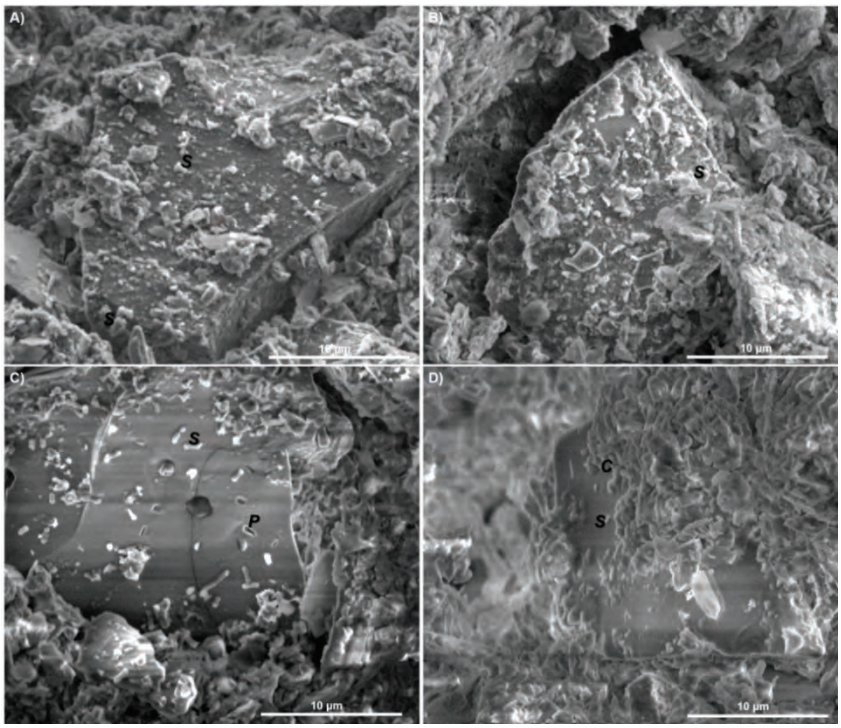

Figure 3 *SEM images of colonised pyrite bearing waste rock surfaces coated onto glass beads and leached over 20 days. Microbial mineral interactions on PEL-LS surfaces are shown in micrograph A (day 1, 18 hrs) and C (day 20) and interactions on PEL-HS surfaces are shown in micrograph B (day 1, 18 hrs) and D (day 20). Observed surface features including single cells (S), colonies (C) and pits (P), are labelled. A scale bar (10 µm) is shown on each image.*

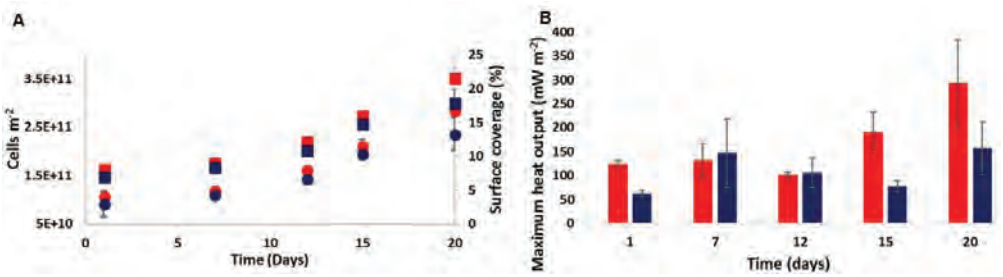

Figure 4 *A) Microorganisms firmly attached to the waste rock mineral surface of PEL-LS (●) and PEL-HS (●) at each time point, as well as the calculated percentage of surface microbial coverage for PEL-LS (O) and PEL-HS (O). The degree of surface coverage was determined from the number of cells firmly attached to the surfaces. Error bars represent the standard deviation from the mean of the mineral associated and firmly attached cells across the wash repeats. B) Maximum heat flow per unit surface area for PEL-LS (O) and PEL-HS (O) over 20 days measured using the IMC. Error bars represent the standard deviation from the duplicates of each material.*

platform on which to develop a standard protocol for the flow-through biokinetic test. This, together with acid-base accounting, mineralogy and data on dump hydrology, is proposed to provide improved insight into ARD characterisation and prediction.

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