A novel experimental system for the study of microbial ecology and mineral leaching within a simulated agglomerate-scale heap bioleaching system

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Abstract

Heap bioleaching systems are complex, with multiple sub-processes interacting at various scales within the heterogeneous reaction environment. This provides a challenge to determining the growth characteristics of micro-organisms and reaction characteristics of the mineral ore in a representative environment. The experimental system presented in this paper was designed to simulate heap bioleaching conditions using multiple, identically constructed agglomerate-scale mini-column reactors. Ore samples were prepared representatively as grab samples of a larger heap. Particle size distributions and agglomerate masses of the prepared ore samples were shown to be similar within acceptable variance and provided comparable surface areas for microbial colonisation and chemical reaction. The microbial abundance within the whole ore system was determined from effluent sampling for the planktonic population and the systematic and sequential sacrifice of identically operated mini-column reactors to determine the change in the ore-associated microbial population with time. Microbial colonisation and growth rate kinetics were determined from analysis of these populations. The growth curves obtained for the bulk flowing solution and ore-associated populations at the base case operating conditions were reproducible, within a 95 % confidence interval.

Keywords: Heap Bioleaching; Packed Bed Reactors; Design; Heterogeneous Reaction; Microbial Growth; Immobilised Cells

1. Introduction

The treatment of low-grade, copper-bearing mineral sulphides by heap and dump leaching has emerged as a promising alternative process for metal extraction, responsible for around a quarter of global copper production [1]. Current studies are aimed at understanding the dissolution of low-grade chalcopyrite ores using heap bioleaching [2-5]. Although chalcopyrite has been identified as the most abundant copper-bearing mineral sulphide, low-grade chalcopyrite ores are difficult to treat using chemical dissolution because of the refractory nature of the mineral. Both ferric iron and hydronium ions have been found to leach chalcopyrite, with the rate of these reactions dependent on the ferric to ferrous iron ratio [6], galvanic interactions between mineral sulphides [7, 8] and temperatures in excess of 50°C [9, 10].

Despite this, there is a lack of fundamental understanding of the role and interaction of key sub-processes that drive the mineral dissolution reactions. Micro-organisms play a vital role in the regeneration of the leaching agents for mineral sulphide dissolution, which may either be ferric iron or hydronium ions or both, with the rate of microbial oxidation of ferrous iron and sulphur species directly proportional to microbial abundance and activity. Typically, commercial bioheaps experience a significant lag period from the time of heap inoculation until enhanced mineral dissolution rates through microbial activity are observed [2]. This lag period may be due to microbial adaptation to the heap environment, determined by the extent of mineral-microbe interactions through microbial attachment to the mineral surface, microbial growth and colonisation of the heap [11].

In earlier studies of microbial population dynamics within copper mine dumps [12] and pilot scale heap leach columns [13], authors found significant variation in microbial abundance along the depth of the packed bed. At present, however, the PLS microbial concentration is assumed to indicate the microbial abundance and diversity within commercial heaps [14, 15]. As a result, understanding of the microbial population dynamics within a heap is limited; with key questions regarding inoculation strategy, the choice of designed over natural inoculum and the adaptation and tolerance of inoculum, remaining unresolved [5, 11, 16].

Table 1 provides a summary of design conditions for selected lab-scale studies focused on microbial attachment and colonisation of low-grade ore in a heap environment. Microbial attachment studies on low-grade ore have been performed at the particle scale, in a biofilm reactor system [17, 18], and at the agglomerate-scale, in glass column reactors loaded with geo-coated glass beads [19, 20]. Authors observed preferential attachment to sulphide minerals; more specifically, pyrite over chalcopyrite and low-grade ore, and localised attachment to surface defects irrespective of the mineral, for mesophiles *Acidithiobacillus (At.) ferrooxidans* and *Leptospirillum (L.) ferriphilum* and the moderate thermophile, *Metallosphaera (M.) hakonensis*. For *At. ferrooxidans*, the observed attachment rates

were enhanced by enrichment of the culture on sulphur over ferrous iron as a growth substrate and increasing temperature [18, 20-22]. Although the aforementioned studies were performed with maximum solution-ore contacting as opposed to an unsaturated environment, these findings may inform inoculation preparation and the role of pyrite in inoculum retention as well as heat generation through acid and ferric production.

Numerous studies have investigated the abundance and diversity of microorganisms within the whole ore heap bioleaching system; specifically, the characterisation of microbial colonisation associated with the mineral has recently been studied using agglomerated crushed low-grade ore [23-30]. In the study by Tupikina et al. [23], multiple columns were each loaded with *ca*. 5 kg of crushed low-grade chalcopyrite ore which had been agglomerated with 0.1 M sulphuric acid. The reactors were then inoculated with a consortium of micro-organisms prepared from stock tank reactors, operated at temperatures ranging from mesophilic to moderately thermophilic (25 - 65°C). In the earlier study [30], the authors monitored the diversity of the microbial populations in the PLS and those associated with the ore, as the temperature within the heap progressed from ambient to 60°C, by sequentially sacrificing individual columns at pre-determined intervals throughout the experiment. The heating jacket surrounding the glass reactor was assumed to distribute heat uniformly throughout the ore bed, with a negligible effect on the temperature profile from solution and gas transport. A similar column reactor system together with a unique in-bed sampling technique and a larger scale, box reactor configuration (loaded with *ca*. 135 kg of dry ore) were used to study the effect of solution flow dynamics on microbial colonisation of the heap [28, 31]. However, ore samples removed using the in-bed sampling technique were not representative of the bulk ore bed, with the removal of ca. 200 g of ore from a 4 kg ore bed. The technique was biased towards sampling the lower portions of the bed where fine particles may have accumulated, rather than allowing for random sampling. Collectively, the aforementioned studies of microbial colonisation in bioheaps have highlighted the significant difference in microbial diversity and population abundance between the PLS and ore-associated phases, the importance of the interstitial phase where microbial population and growth appear to be highest and the impact of fluid flow dynamics on the rate and extent of colonisation of a heap. The extension of these studies under fully representative conditions in a manner that facilitates the alteration of local conditions is therefore desirable.

Details of the design and commissioning of an experimental system capable of simulating heap bioleaching conditions at the agglomerate-scale, are presented in this paper. The experimental system, including the equipment, experimental approach, sampling protocol and analytical techniques, allowed for independent study of microbial attachment, colonisation of crushed low-grade ore and subsequent microbial growth kinetics in the flowing PLS and ore-associated phases. Validation of the underlying assumptions of the design of the experimental system were undertaken and case study

experiments were performed in duplicate to determine the reproducibility of the results obtained. The agglomerate-scale experimental system described in detail in the current paper was used previously for the study of microbial growth rates and colonisation by *At. ferrooxidans*, used as a model micro-organism, on low-grade chalcopyrite ore [27].

Table 1: Selected lab-scale reactor systems used for the study of microbial attachment and colonisation on low-grade ore, with suggested key limitations for the study of microbial growth

<table>
<tr><th></th><th>Reactor description</th><th>Reactor scale</th><th>Study focus and limitation for use in microbial growth studies</th><th>Test parameter</th></tr>
<tr><td rowspan="2">Attachment</td><td>Biofilm
[17, 18]</td><td>particle</td><td rowspan="2">Study of microbe-mineral contacting for microbial attachment, not growth. Representative of submerged culture conditions over unsaturated heap bioleach conditions.</td><td>mineral type</td></tr>
<tr><td>Geo-coat, glass column
[19, 20]</td><td>300 glass beads
(ϕ=6mm)</td><td>mineral,
substrate</td></tr>
<tr><td rowspan="6">Microbial colonisation on whole ore</td><td>Insulated with element, PVC column [28]</td><td>4 kg</td><td>Microbial retention as a function of solution flow rate with non-representative in-bed sampling.</td><td>flow rate</td></tr>
<tr><td>Jacketed, glass column
[23, 30]</td><td>5 kg</td><td>A column in a test set sacrificed for representative sampling. Non-uniform solution contacting and temperature profiles may affect growth study.</td><td>inoculum size</td></tr>
<tr><td>Jacketed column with element [25, 26]</td><td>9 kg</td><td rowspan="3">Non-sterile ore samples. Ore-associated population analysed at termination only.</td><td>temperature, pH</td></tr>
<tr><td>Segregated PVC column
[29]</td><td rowspan="2">36 kg</td><td>ferric iron concentration</td></tr>
<tr><td>Jacketed, stainless steel column [24]</td><td>temperature</td></tr>
<tr><td>Perspex box [31]</td><td>135 kg</td><td>Dimensions of ore bed allow study of differing wetting and colonisation patterns across ore body, however non-representative sampling of ore bed</td><td>flow rate,
irrigation scheme</td></tr>
</table>

2. Proposed experimental approach

Heap bioleaching systems may simply be described as unsaturated packed beds in which micro-organisms contribute to the gas-liquid-solid phase reactions and influence the physico-chemical reaction environment. However, each low-grade ore system is unique and dependent on dynamic components such as varying mineral compositions from separate deposits, non-uniform gas distribution through the heap that flows counter-current to irrigation solution dispersing down the heap via gravity and capillary forces, as well as diverse and dynamic microbial communities that are inherent to the ore body or have been introduced to the system. The resulting reaction environment is difficult to predict and therefore control.

The primary aim of the designed experimental system was to create identical heap bioleaching conditions within multiple agglomerate-scale column reactors such that the reaction kinetics and microbial population dynamics within each column were comparable to each other. Irrespective of the scale of experiments, the influence of sub-processes such as gas-liquid mass transfer, solution-ore contacting and mineral surface availability are expected to impact on microbial attachment, growth and propagation studies. In the design of this novel experimental system, a bottom-up approach was proposed in order to eliminate or minimise these effects on the observed microbial trends.

The bulk low-grade ore sample was pre-treated and processed into various size fractions. Each ore sample was then re-constructed to represent the particle size distribution (PSD) of the bulk sample. To minimise the effects of gas-liquid mass transfer, solution contacting and the resulting non-uniform heat balance across the ore bed, the mass of each ore sample was chosen to occupy only a small volume (one-fifth) of the mini-column reactor. In this way, multiple agglomerate-scale ore samples of identical PSD and mass were created, essentially grab samples of a larger heap.

A single experiment consisted of multiple mini-column reactors. The ore samples were sterilised using γ-irradiation (50 kGy) to prevent the indigenous microbial populations from influencing the outcome of the experiments. As such, upon addition of a well-characterised inoculum, in this case a pure *At. ferrooxidans* culture, subsequent microbial growth and activity may be directly associated with that of the known culture. In addition, since micro-organisms favour initial attachment to mineral surface defects [18], and heat sterilisation may alter the mineral surface either through thermal stress or the deposition of secondary mineral phases [32, 33], gamma (γ-) irradiation was chosen over autoclaving for ore sterilisation. Each ore sample was acid agglomerated, creating a narrower particle size distribution and uniformly wetted environment for microbial colonisation, and thereafter loaded into the mini-column reactors between two sets of glass beads. The mini-column reactor configuration was also designed to enhance solution-ore contacting by uniformly dispersing irrigation solution over the top of the packed bed. This irrigation scheme served to minimise the effects of poor gas-solution-ore contacting on microbial population dynamics. The residence time distribution (RTD) study was used in conjunction with compartment models to diagnose the behaviour of solution within the packed bed reactor, providing insight into the extent of mixing and the proportion of stagnant to flowing solution present.

The smaller ore samples were well suited for representative sampling and were subdivided for moisture content analysis and extraction of microbial populations in each of the phases associated with the mineral (i.e. interstitial, weakly attached and strongly attached to the mineral surface). Sampling of the populations associated with the ore bed, however, required the termination of a mini-column reactor from a set of reactors in a single test, consisting of multiple mini-column reactors

running at identical physicochemical conditions. The experimental system was therefore able to provide data to describe: inoculum retention, initial attachment, and microbial growth and colonisation in all phases within the whole ore environment.

3. Description of equipment

Mechanical and electronic design considerations were applied to the concept of a consolidated agglomerate-scale reactor system to construct an experimental rig, meeting the final design specifications given in Table 2. The equipment consisted of a stainless steel frame housing multiple mini-column reactors, effluent collection vessels, the spray irrigation system connected to pressurised feed vessels, electronic control system and the motorised belt and pulley mechanism, as illustrated in Figure 1 (i).

Table 2: Design specifications for the consolidated mini-column reactor system

Frame and trays	Material of construction	304 SS
	Dimensions	2000 x 400 x 1000 mm
Mini-columns	Number of columns	30
	Material of construction - columns	High density polyethylene (HDPE)
	Dimensions	ϕ = 80 mm, H = 100 mm
	Effluent aperture	ϕ = 2 mm
Effluent collection vessels	Number of effluent collection vessels	30
	Material of construction – collection vessels	Polyethylene terephthalate (PETE)
Pressurised feed vessels	Number of feed vessels	2
	Material of construction	304 SS
	Dimensions	ϕ = 400 mm, H = 520 mm
	Gauge pressure (p_g)	0.5 bar
Irrigation system	Number of valves	2
	Medium	Aggressive - Acid-resistant
	Valve description	2 way, ¼ inch 24 VDC Solenoid
	Tubing	Masterflex Viton L/S 16
	Spray mechanism	Micro mist nozzle ϕ = 1.8 mm
Irrigation system mobility	Motor	0.55 KW, 3 phase
	Timing belt	25 mm
	Pulley	16 & 25 mm, 19 teeth

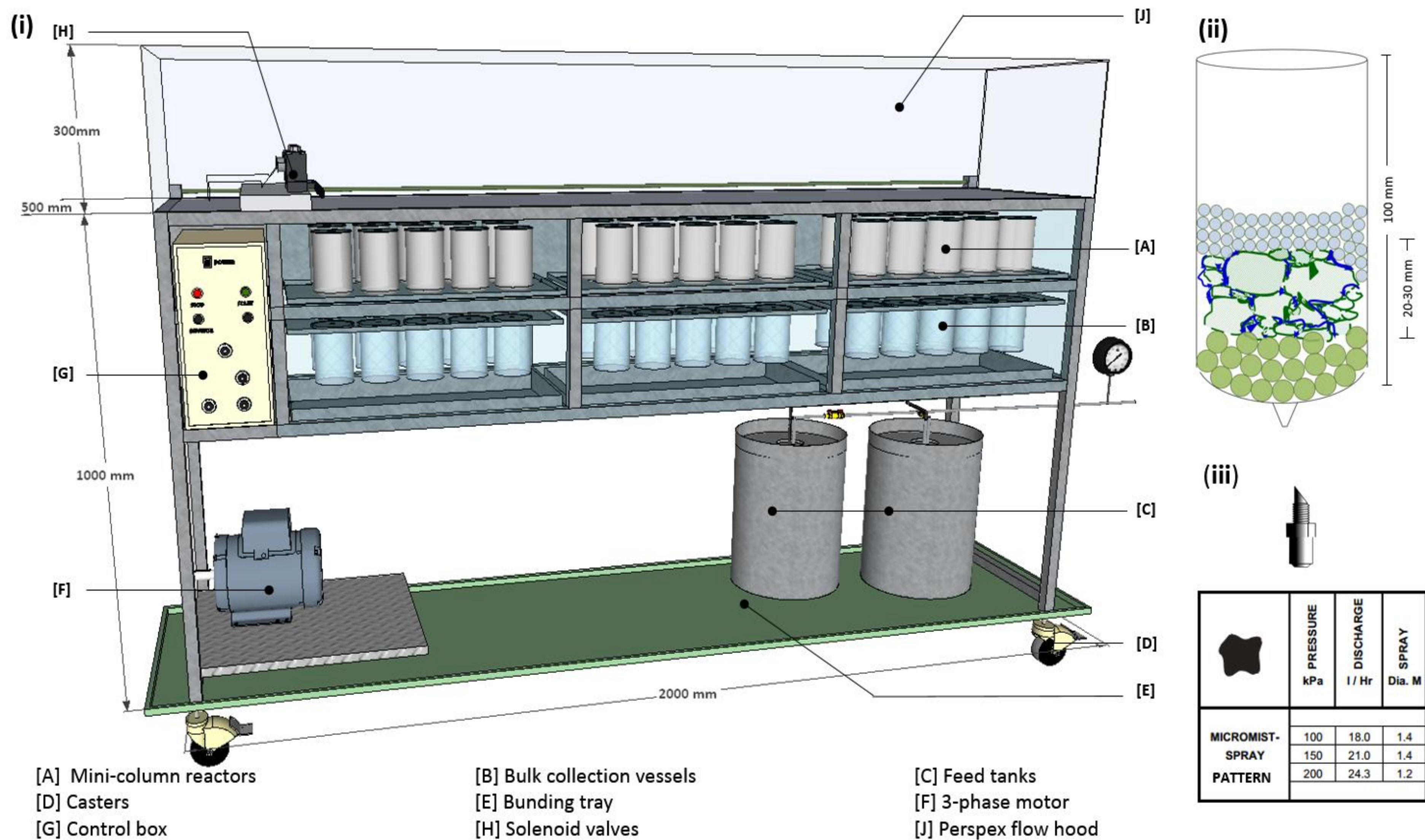

Figure 1: 3D Schematic of the designed experimental system (i) with illustration of HDPE mini-column reactor (ii) and Microjet ® spray nozzle and specifications

3.1. Rig components

The stainless steel frame, constructed from square tubing, included stainless steel trays upon which the HDPE mini-columns [**A**] and effluent collection vessels **[B]** were secured. Each column was loaded with the agglomerated ore sample packed between two sets of glass beads. An illustration of the bead-ore-bead configuration within the columns is presented in Figure 1(ii). The upper bed of beads promoted an even distribution of feed solution over the ore bed, thus minimising solution. The lower bead bed prevented the loss of smaller ore particles which may have obstructed the aperture through which effluent exited the column. The columns were covered with custom made silicon lids to minimise contamination and moisture loss. Each column was accessible during an experiment, allowing for easy removal from a test set without interfering with the remaining reactors.

The stainless steel trays position the effluent collection vessels **[B]** directly beneath the mini-column reactors. Each set of collection vessels was accessible by sliding the tray along a single groove mechanism, facilitating an effluent sampling strategy that did not interfere with the mini-column reactor system. During sampling, a secondary set of trays with empty effluent collection vessels were used to immediately replace those that were removed for analysis.

The spray irrigation system consisted of two solenoid valves **[H]**, mounted onto a PVC platform attached to a timing belt pulley system powered by a 3 phase motor **[F]**. The platform then slid along two adjacent panels, moving the valves along the top of each of the column reactors. Each valve was connected to a 50 L feed tank **[C]** containing sterilised feed solution, pressurised to 0.5 bar (gauge) using filtered (0.22 μm) compressed air. Attached to each valve was a micro-mist spray nozzle (Microjet® Irrigation Systems), capable of producing a fine mist of 1.4 m diameter at 1 bar (gauge). The resulting gentle mist was assumed to have minimum physical impact on the primary glass bead bed and ore surface. An illustration of the components of the irrigation system is shown in Figure 1 (iii), which also provides further specifications of the micro mist spray nozzle. A PVC bunding tray **[E]**, was attached to the main frame and served to contain and isolate any spills or overflow of solution from the mini-column reactors, collection vessels or feed tanks.

A Perspex hood **[J]** was constructed over the mini-column reactors, effluent collection vessels and irrigation system, isolating these components from the surroundings. Filtered air (0.22 μm) was introduced into the hooded compartment, creating a positive pressure environment. This minimised exposure of the system to airborne contaminants. Casters **[D]** were attached to the steel frame to provide mobility to the entire experimental rig. The mini-column reactors did not have localised temperature control systems, therefore the ability to easily transport the reactor system to different constant temperature rooms was a necessary design consideration. Two such rooms were available for the purpose of this study; one controlled at 30°C and another at 37°C.

3.2. Automated control system

The circuit boards and electronic components responsible for controlling the movement and positioning of the valves were housed in the control box **[G]**. A schematic of the control logic system is shown in Figure 2. The function of the system was to automatically dispense feed solution into the reactor vessels according to a timed sequence. This required a carriage onto which two solenoid valves (SV1and SV2) were mounted to be moved along an aluminium track. The carriage was paused above each reactor as the valves were opened to discharge the feed via a spray nozzle. The carriage transport belt drive mechanism was powered by a 3-phase motor via a reduction gear-box and driven by an inverter which allowed for the speed to be controlled.

The feed sequence was initiated by a pulse from a *cycle timer*, adjusted to a 6 minute cycle, which then initiated forward travel of the carriage. A LED/Photo transistor pair optical devices, attached to the carriage, travelled along the aluminium rail punctuated with slits. Each slit was aligned with the centre of each mini-column reactor. For each detection, the transport stopped and the two timers (SV timers 1 and 2) were triggered to open the two solenoid valves, typically for 1 to 2 seconds. The adjustable time interval over which the solenoid valves remained open over each mini-column, determined the irrigation feed rate.

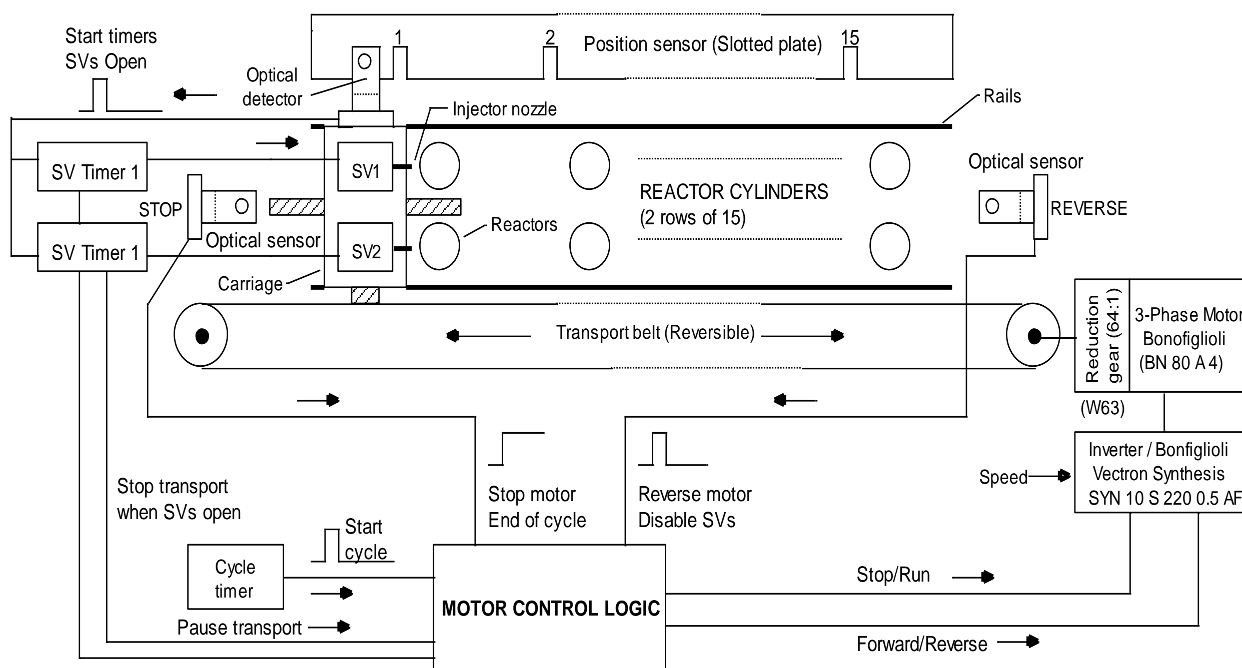

Figure 2: Schematic of control logic and timing of reactor irrigation system

The timers were analog devices (NE555) and may be set independently by potentiometers on the control unit. The forward transport of the carriage resumed after both timers had completed their 3cycles. When the carriage reached the end of the aluminium track, as sensed by the *reverse optical*

sensor, the motor reversed into the start position, detected by the *stop optical sensor*. The solenoid valves were disabled during the reverse travel. The system remained static until the next cycle was triggered by the primary cycle timer. This sequence of operation continued indefinitely or until interrupted by the operator.

4. Description of variable operating parameters

The multiple mini-column reactors were designed to be identical to each other with respect to particle size distribution, fluid flow dynamics and initial biomass concentration. With the independent feed tanks, the experimental system may be used to investigate the effect of varied feed compositions; separated into 15 columns per test condition. Each set of tests may be further divided into two sets of 7 mini-column reactors in addition to a control column reactor; each testing variable process conditions, such as inoculum size and microbial culture adaptation.

Irrigation rates for industrial heap operations are a function of the surface area that must be covered by the irrigation solution, typically 4 – 18 L m^{-2} hr^{-1} [9, 34, 35]. The independent timing of each valve and variable motor speed may be controlled to obtain the desirable irrigation scheme. In addition, the direction and distribution of the atomised spray may easily be manipulated by replacing the type of spray nozzle.

The spray irrigation system may also be employed to distribute the inoculum culture over the ore bed over a specific time interval. This may be achieved by replacing the feed tank with a vessel containing the inoculum sample. In general, the inoculum is introduced as a single pulse over the top of the ore bed, however the ore may also be inoculated during the agglomeration process prior to loading the mini-column reactors.

5. Experimental approach and methodology

5.1. Selected operating parameters for base case study

Replicate experiments were performed independently of each other under identical physico-chemical conditions (0.2 g L^{-1} Fe^{3+} in feed solution; pH 1.7; 30°C) and over different durations. Each test consisted of 8 mini-column reactors, 7 inoculated column reactors with a single abiotic control. Test A was performed over 690 hours and Test B over 330 hours. For test A, a column was sacrificed every 96 hours beginning at Hour 72 whilst for Test B, a column was sacrificed every 48 hours beginning at Hour 48.

In this study, the ore bed surface area was *c.a.* 0.005 m^2 and so a corresponding irrigation rate of

2 L m^{-2} hr^{-1} was used. Each valve was programmed to disperse 1 mL of feed over the top of each mini-column reactor, every 6 minutes. Thus the irrigation rate per column was 10 mL hr^{-1}. The timing belt pulley system was set to actuate valve motion every 3 minutes and rest for 3 minutes after each pulse irrigation step, at a motor speed of 30 hertz.

5.2. Ore mineralogy and ore bed preparation

The crushed low-grade ore used in these experiments was acquired from Escondida, Chile. A bulk mineralogical analysis performed by SGS Lakefield, found the gangue minerals, muscovite and quartz, to be most dominant at 28.6 and 44.8 wt %, respectively. The ore also contained 4 wt. % pyrite (FeS_2) and the following copper bearing minerals (wt.%): chalcopyrite (0.5 %), covellite (0.3 %), chalcocite (0.2 %), bornite (0.1 %) and enargite (0.1 %). Elemental analysis found the ore to contain 2.48 % Fe^{total}, 2.45 % S^{total} and 0.46 % Cu.

The bulk ROM low-grade chalcopyrite ore sample was pre-leached in 0.1 M acidified solution (96 % H_2SO_4). After 24 hours, the bulk sample was filtered, washed twice with distilled water, re-filtered, dried and separated into various particle sizes using woven metal sieves. The particle distributions are presented in Table 3. Ore samples of 150.3 ± 1.4 g (dry weight) and identical particle size distributions were constructed. The samples were sterilised with a 50 kGy dose of γ-irradiation, which was specific to the sample volume.

Table 3: Particle size distribution of the re-constructed agglomerate-scale ore samples [27]

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

At the start of an experiment, randomly selected reconstructed ore samples were agglomerated with distilled water, acidified to a pH 0.6 (96 % H_2SO_4). Each mini-column reactor was then loaded with *ca*. 110 g of 16 mm diameter glass beads followed by the agglomerated ore sample and another set of *ca*. 80 g of 8 mm diameter glass beads. The height of the ore bed was between 20 and 30 mm.

The experimental setup was situated in a temperature controlled room; regulated at 30°C. Sterilised 0.1 M acidified solution (96 % H_2SO_4) was connected to the feed system. Prior to inoculation, the mini-column reactors were acid washed with sterilised 0.1 M acidified solution (96 % H_2SO_4) for 24 hours at 10 mL h^{-1}. The acid wash was performed to settle the ore bed and remove easily soluble acid-consuming materials to allow subsequent maintenance of an acidic environment.

5.3. Media and feed preparation

For the feed solution, acidified deionised water (pH 1.7, H_2SO_4) containing 0.2 g L^{-1} Fe^{3+} ($Fe_2(SO_4)_3.4.5H_2O$) was made-up per L with 20 mL autotrophic basal salts (ABS) medium and 1 mL trace element solution. The ABS stock solution, adapted from Johnson et al. [36], had the following composition: 7.5 g L^{-1} $(NH_4)_2SO_4$, 7.5 g L^{-1} Na_2SO_4, 2.5 g L^{-1} KCl, 25 g L^{-1} $MgSO_4.7H_2O$, 2.5 g L^{-1} KH_2PO_4 and 0.7 g L^{-1} $Ca(NO_3)_2.4H_2O$. The trace element solution consisted of 10 g L^{-1} $ZnSO_4.7H_2O$, 1 g L^{-1} $CuSO_4.5H_2O$, 1 g L^{-1} $MnSO_4.4H_2O$, 1 g L^{-1} $CoSO_4.7H_2O$, 0.5 g L^{-1} $Cr_2(SO_4)_3.15H_2O$, 0.6 g L^{-1} H_3BO_3, 0.5 g L^{-1} $Na_2MoO_4.2H_2O$, 1 g L^{-1} $NiSO_4.6H_2O$, 1 g L^{-1} $Na_2SeO_4.10H_2O$ and 0.1 g L^{-1} $Na_2WO_4.2H_2O$ [37]. Both the ABS and trace element stock solutions were acidified to pH 1.7 using 96 % H_2SO_4. All prepared solutions were sterilised in laboratory scale autoclaves at 121°C for 20 minutes. Large volumes of feed solution (ca. 45 L) were sterilised using an *in-situ* autoclaving technique.

5.4. Microbial culture and inoculation strategy

The *At. ferrooxidans* (DSM 14882) culture was grown on γ-irradiated pyrite concentrate (1 % w/v), ABS medium, trace elements and 0.5 g L^{-1} Fe^{2+} ($FeSO_4.7H_2O$) at pH 1.7. A single batch of inoculum with *ca*. 7.5 x 10^5 cells per mL was prepared as described in Govender et al. [27]. With the exception of the control column, a single 2 mL pulse of inoculum was spread across the bed of glass beads at the top of each mini-column, with the aim of achieving 10^{10} cells per ton of dry ore. Irrigation of the columns was initiated an hour after inoculation. This point in time is considered time zero (t = 0 h). Prior to inoculation, direct counting of stock culture was performed using a Thoma counting chamber and phase microscopy. Thereafter, inoculum samples were analysed for viable cell counts using the Miles-Misra method [38]. In this study, *At. ferrooxidans* was chosen as a model micro-organism to determine the feasibility of the experimental system for the purpose of quantifying microbial growth and colonisation in a simulated heap environment.

5.5. Solution sampling protocol and analysis

Bulk collection vessels were replaced every 24 to 48 hours, with a 1-hour sample collected at that point in time. The bulk sample was analysed for total soluble iron and copper using atomic absorption spectroscopy (AAS). The 1-hour samples were used to obtain real-time measurements for pH, redox potential, ferric and total iron concentrations and microbial concentration. The pH was measured using a combination glass electrode with a built-in silver/silver chloride (Ag/AgCl, 3 M KCl) reference electrode, connected to a 713 pH meter from Metrohm. A glass electrode, with a combination of a platinum (Pt) indicator and a silver chloride (AgCl) reference electrode, was used together with a 704 Metrohm meter, to measure the redox potential of the PLS. Soluble ferric and total iron concentrations were measured using the modified ferric chloride assay [39]. The microbial population was quantified as viable cell numbers (cfu) using the methodology described in Section

5.7.1. To prevent microbial growth and activity in the bulk collection vessels between sampling times, 50 g L^{-1} sodium benzoate ($NaC_6H_5CO_2$) was added to each new bulk collection vessel, using 1 µL per mL expected sample volume. Sodium benzoate acts as a bacteriostatic and fungistatic agent under acidic conditions.

5.6. Detachment protocol for whole ore samples

A column from each test was removed at regular intervals to provide microbial population dynamics on the whole ore. The column was drained thoroughly to capture the void volume retained within the ore bed. A detachment protocol was used to extract microorganisms from each of the phases associated with the ore, *viz*. interstitial, weakly attached and strongly attached. An illustration of the sampling protocol and location of each of these phases is represented in Figure 3.

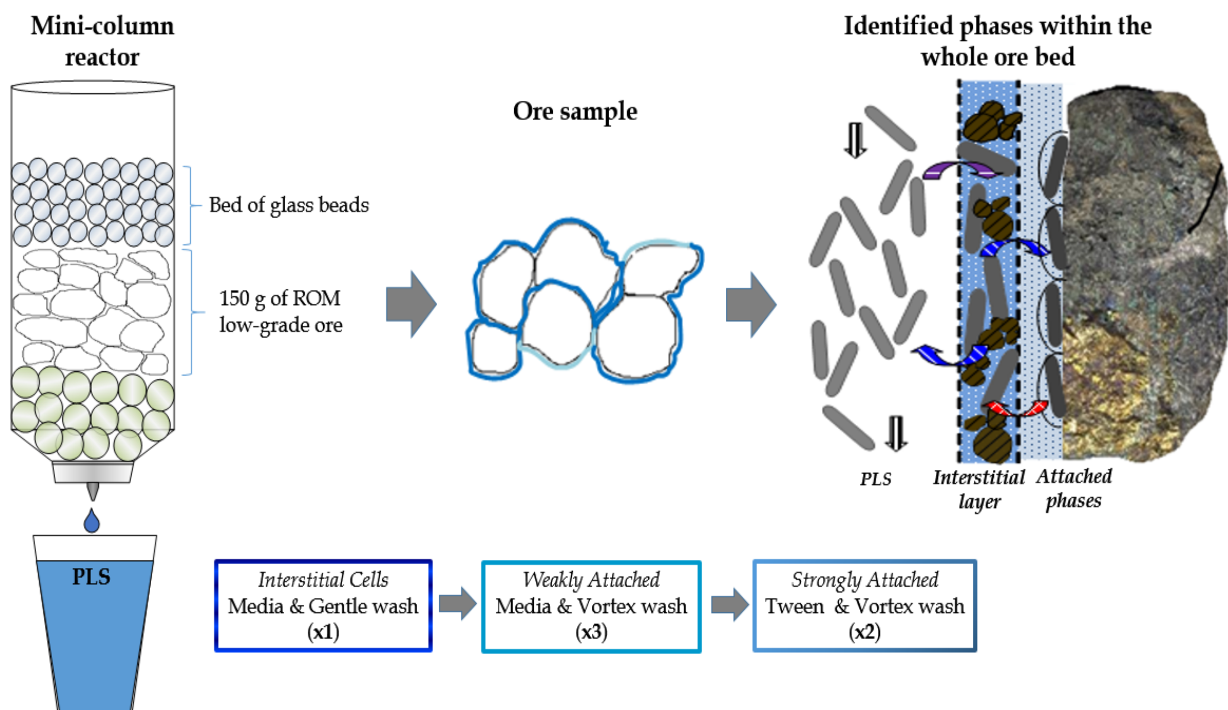

Figure 3: Illustration of detachment protocol for identified ore-associated phases

The ore bed was mixed and a grab sample of *ca*. 50 g was transferred to an Erlenmeyer flask. The remaining sample was analysed for moisture content and mineralogy. The solid sample was then suspended in 25 mL ABS-feed solution, using a 2:1 ratio of solids to solution, and gently mixed to integrate the stagnant interstitial liquid with the wash solution; effectively extracting the interstitial population from the solid sample. The solution was than decanted into 100 mL centrifuge tubes. The solution and suspended fines were separated by low speed centrifugation for 2 minutes at 800*g* and the supernatant was carefully recovered and analysed for the interstitial cell population. The fines and washed solid sample were reconsolidated in the Erlenmeyer flask, re-suspended in 25 mL ABS-feed

solution and then thoroughly agitated using a bench-top vortex for 2 minutes. The solution sample and suspended fines were decanted and separated by centrifugation. This process with ABS-feed and agitation was repeated twice more. The resulting three supernatants were mixed together and analysed for the weakly attached microbial community. In the final detachment stage, the fines from the centrifuge tube and the solids were reconsolidated in the Erlenmeyer flask, re-suspended in 25 mL Tween-feed solution and thoroughly agitated. The Tween solution was made up of ABS and 0.4% (v/v) Tween ® 20 (Merck). The solution was decanted and the fines separated by centrifugation. This stage was repeated once more. The combined supernatants were analysed for the strongly attached microbial population. Wash solution volumes added and recovered were carefully noted. The Miles-Misra plating technique was used on iron overlay plates, described in detail in Section 5.7.1, to quantify microbial populations in terms of viable colony forming units (cfu). At regular intervals random samples were also analysed for microbial population using a Thoma counting chamber and phase contrast microscopy, to verify accuracy of cell enumeration. Culture purity was ascertained throughout the experiment using 16S qPCR analysis.

5.7. Analytical techniques

5.7.1. Iron overlay plates for the Miles-Misra enumeration technique

The iron overlay Miles-Misra plating technique for viable cfu counting was selected as it was less susceptible to interference by fines than the SYBR Gold method (Bryan *et al.*, unpublished data) and has a lower limit of detection than direct cell counting by phase microscopy, given as 1 x 10^2 cfu mL^{-1} and ~3 x 10^5 cells mL^{-1}, respectively. Also, the methodology allowed for a large number of samples to be processed rapidly.

The iron overlay plates ($Fe\underline{o}$) were prepared in advance according to Johnson [40]. The overlay plates were allowed to cool and stored at 4°C. Prior to use, the plates were kept at room temperature for at least 24 hours. Each sample requiring microbial analysis was serially diluted to extinction using sterilised ABS solution in pre-sterilised 96-well plates. A 10 µL sample of each dilution was spotted onto an iron overlay plate in duplicate. The plates were incubated at 30°C for at least one week before being counted. Viable cell concentrations were counted as colony forming units per mL sample volume (cfu mL^{-1}). An image (100x magnification) of the observed ‘fried egg’ shaped colonies, typical of *At. ferrooxidans*, may be found in the supplementary material.

5.7.2. Modified ferric chloride assay

The modified ferric chloride assay [39] was used for the determination of ferric and total iron concentrations in the leach solutions, in preference to the phenanthroline assay as a result of the interference of cupric ions on the absorbance spectrum of the orange-coloured tris (1, 10-phenathroline) iron complex.

5.8. Residence time distribution study

A non-reactive, colorimetric tracer was used in the residence time distribution study to characterise solution flow through the mini-column reactor system. The intensity of the red-pink colour of a cobalt sulphate heptahydrate ($CoSO_4.7H_2O$) solution, was measured at 510 nm using a HELIOS UV-Visible Spectrophotometer v4.04. A 2 mL pulse of standard solution of 50 g L^{-1} Co^{2+} at pH 1.7, was introduced to each mini-column reactor at the same stage of the experiment at which a pulse of inoculum is usually introduced; i.e. an hour after the 24-hour acid wash was discontinued. Effluent samples were collected at 10 and 20 minutes intervals over the 6 hours over which the experiment was performed. These residence time distribution experiments were carried out in triplicate. In addition to colorimetric tracer studies, an *At. ferrooxidans* stock culture was also used as a tracer. The aim of this part of the study was to determine the microbial retention efficiency, using the single pulse inoculation strategy.

6. Characterisation of experimental system

6.1. Identical irrigation rates

Each valve was programmed to deliver a fixed and equal volume of solution to each mini-column reactor. To test this assumption, initial feasibility experiments were performed to compare the dispensed irrigation volumes across all the columns, with results presented in Figure 4. A comparison of measured flow rates between mini-columns fed from the same valve showed a relative error of less than 5%. The variation in solution flow rates between the two valves was found to be less than 1%. Aside from external electrical disturbances that were found to interfere with the signals, the irrigation control mechanism produced an acceptable variation between dispensed volumes.

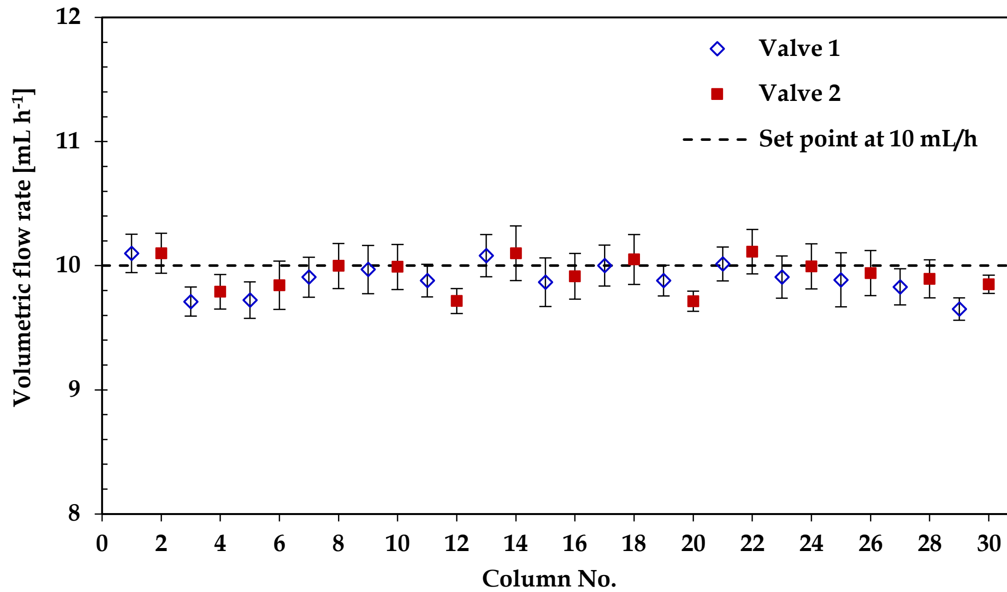

Figure 4: A comparison of volumetric flow rates between each of the columns fed by the different valves. Error bars represent the variance between multiple mini-columns fed by a valve (n = 15).

6.2. Retention of inoculum using current inoculation strategy

The single pulse inoculum was introduced in a drip-wise manner over the upper bed of glass beads within each mini-column reactor. This was assumed to permeate evenly throughout the clusters of particles, with a significant portion of the inoculum being retained within the column to contribute to subsequent microbial growth. To test this hypothesis, multiple columns were inoculated and sampled at regular intervals using a similar procedure to that described for the residence time distribution studies. The percentage of the initial microbial population retained within the replicate mini-column reactors is presented in Figure 5, assuming no microbial growth over the 5 hour period.

After a relatively rapid loss of *c.a.* 6.8 x 10^4 cells per hour over the first 3 hours, the microbial concentration began to stabilise, with less than 4% of the cells leaving over the next 2 hours. At the end of the 5 hour study, just over 59 % of the cells were retained within the agglomerated ore body. This result was in agreement with the study by Harneit et al. [21] were 60 % of an *At. ferrooxidans* culture was shown to attach to low-grade chalcopyrite ore, within the first few hours of the experiment. The comparable microbial retentions suggested that the chosen inoculation strategy was adequate for the purpose of investigating microbial growth and colonisation on whole low-grade ore.

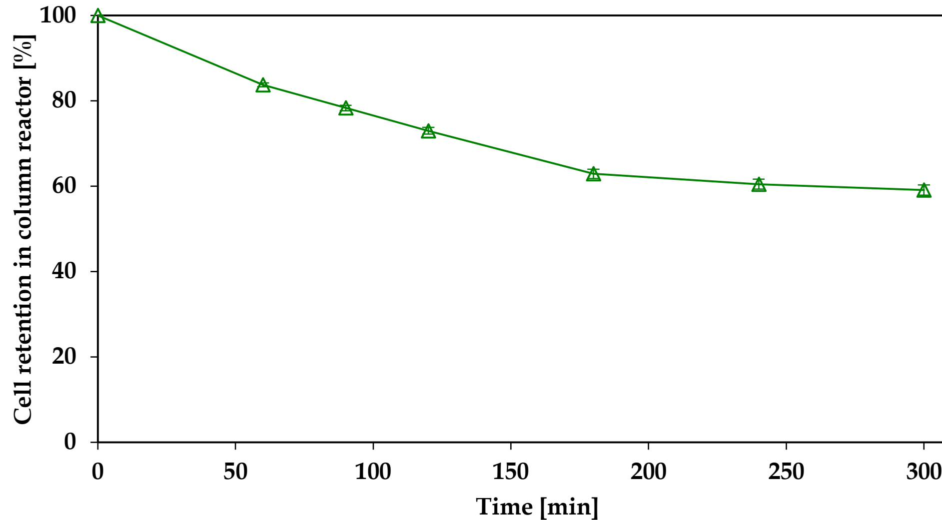

Figure 5: Microbial retention within the ore bed as a percentage of the inoculum introduced. Error bars represent the variance between multiple mini-columns in the same test (n = 3).

6.3. System characterisation using residence time distribution (RTD)

RTD experiments were performed to characterise the solution flow behaviour within the designed mini-column reactors. The size of the ore bed, its placement between two sets of glass beads and the use of a spray irrigation system were chosen to minimise solution channelling and promote uniform distribution of solution within the ore bed.

The resulting $E(\theta)$ curve, shown in Figure 6, suggested that solution flow through the designed mini-column reactor system was relatively well mixed. This behaviour was informed by the spread or variance of the $E(\theta)$ curve. The presence of dead volume or stagnant regions within the ore bed was represented by the long-tail observed as the experiment progressed. The observed single peak instead of multiple peaks of decaying magnitude, suggested the absence of internal recycle flow streams [41].

Space time, τ, a function of reactor volume and volumetric flow rate, was found to be *ca.* 84 minutes and the mean residence time, $\bar{t}$, determined from the tracer concentration with time data, was found to be *ca.* 75 minutes. As the empirically determined mean residence time is lower than the calculated space time, this supports the presence of a stagnant volume within the ore bed.

Using a similar approach to the study by Bouffard and West-Sells [42] at various heap scales, compartment models were used to further characterise the fluid flow behaviour within the ore bed (Figure 7). Model 1 assumed that the mini-column or packed bed reactor behaved as an ideal, well mixed continuous stirred tank reactor (CSTR) and Model 2 proposed that the system behaved as a plug flow reactor (PFR), followed by a CSTR with dead volume in parallel with a by-pass stream. Each model was fitted using a total volume of 14 mL (obtained empirically from drainage and

moisture content data not shown here) and sum of least squares regression analysis. Even though neither of the models mentioned above account for recycle flow behaviour, the assumptions made are sufficient to provide estimates of the working or active volume ($V_p + V_m$) and the dead or stagnant volume (V_d) within the ore bed.

A comparison of the predicted RTD curves for each of the proposed models to that determined from experiments, shown in Figure 6, suggests that although the designed mini-column reactor showed the characteristics of a well-mixed system, the plug flow behaviour and the effect of stagnant regions within the ore bed affected the flow of solution through the ore bed. This can be observed by the deviation of the experimental RTD curve from that of Model 1. Therefore, Model 2, was the preferred compartment model to use for the determination of the working and stagnant volumes; estimated to be 11.7 mL and 2.3 mL, respectively.

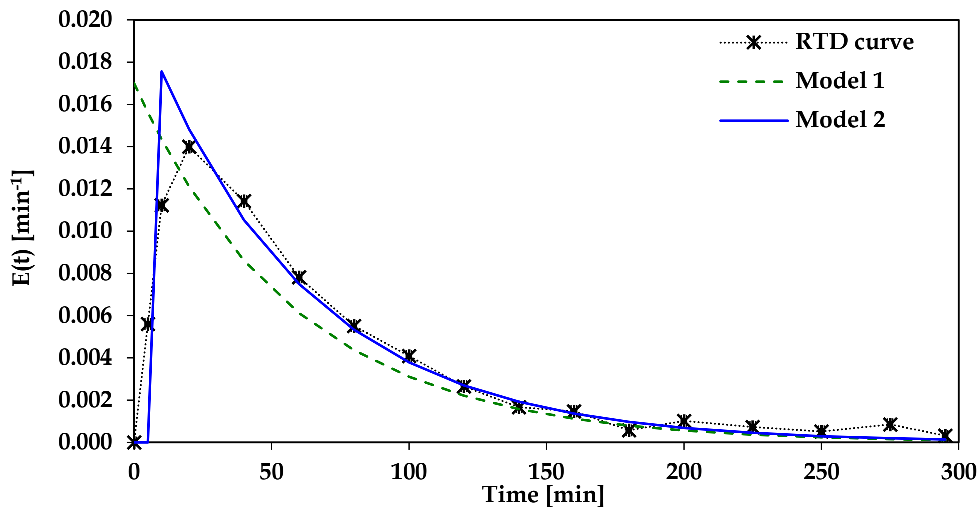

Figure 6: A comparison of the experimental and modelled RTD curves

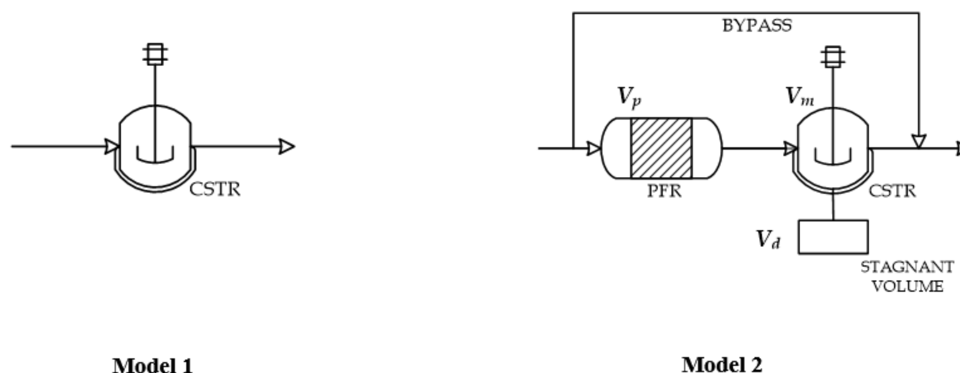

Figure 7: Proposed compartment models used to describe solution flow behaviour

For this study, the stagnant volume was found to represent ca. 20 % of the working volume within the mini-column reactor. This may be compared to the study by Bouffard and West-Sells [42], where the stagnant volume in an ore bed was found to vary between 0% in a 350 kg column test and 57 % in a 3150 ton demonstration scale heap, with the proportion of stagnant volume being influenced by particle size distribution and the presence of fines.

6.4. Reproducibility of results at base case conditions

Experiments under base case conditions were duplicated, with the results analysed to determine reproducibility. Aside from the difference in the durations of Test A and B, the differences in microbial concentration in the inoculum and initial sample are presented in Table 4. The microbial concentration remaining in the mini-columns, two hours after inoculation was taken as the initial microbial concentration. The initial microbial concentration in Test B was approximately 30% higher than that of Test A. When compared to results of the preliminary microbial retention study described earlier, equivalent microbial populations of 65 and 59 % for Test A and B respectively were obtained (Table 4).

Table 4: A comparison of the initial microbial populations introduced to and retained within Tests A & B

		Test A	Test B
Inoculum culture		*Acidithiobacillus ferrooxidans*	
Mean no. of cells inoculated into each column	cells	9.8×10^5	1.4×10^6
Mean no. of cells in each column after 2 hours	cells	6.4×10^5	8.3×10^5
Inoculum media for 2mL pulse		Sterile ABS at pH 1.7	
Test duration	hours	690	334

Typically, leach performance is based on rate of extraction of the metals of interest from the low-grade chalcopyrite ore. Total soluble iron and copper concentrations measured in the effluent samples were used to produce the iron and copper extraction curves shown in Figure 8. Each curve shows the sample mean with upper and lower confidence intervals per sample.

A non-parametric test was used to compare the unpaired data sets statistically, with the null hypothesis assuming that if the data sets had identical distributions, random sampling from the mean ranks of both sets would be significantly different. The resulting two-tailed p-value was greater than 0.05; suggesting that the iron and copper extraction data for the repeated tests are comparable and did not differ significantly from each other. The statistical tests suggested that deviations may be from errors related to sampling technique and analytical methods but did not rule out the possibility of

systematic errors associated with the experimental system. Duplicate experiments, carried out independently of each other, were found to be comparable to each in terms of leaching performance.

Most importantly for the current study, a measure of the confidence in the observed microbial population data was provided. Since the microbial population associated with the mineral may only be measured when a reactor was sacrificed, the reproducibility of the result could only be determined when the experiment was repeated. In contrast, the microbial concentration in the effluent sample may be compared to that of other reactors in the test. Aside from the reproducibility between independently performed experiments, the measurements of microbial concentration as colony forming units (cfu) using the Miles-Misra plating method was subject to a maximum relative error of 13% (results not shown).

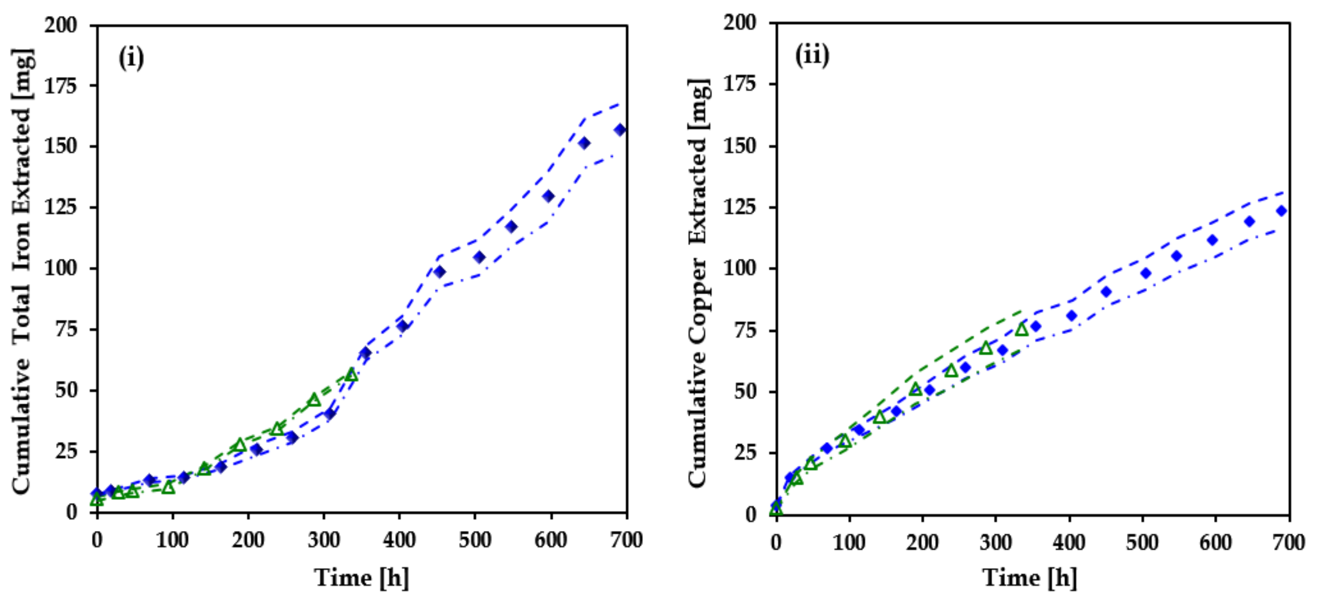

Figure 8: Cumulative extraction curves for iron (i) and copper (ii) for Test A [♦] and Test B [Δ] with upper [- - - - -] and lower [- · · · · · · -] confidence intervals (95 %), where 2≤n≤7.

In Figure 9(i), the error bars for each concentration profile represented the standard deviation between samples taken from multiple mini-column reactors in a single test. A statistical analysis of the difference between the repeated tests suggested that the mean concentration curves for each test were within the 95% confidence interval of each other and the difference was not significant. Figure 9(ii) is representative of a combined concentration profile for the ore-associated (i.e. interstitial and attached) microbial populations and incorporates the results from the 7 sacrificed reactors from each test over a single time series. Aside from the graphical fit of both data sets into a single concentration profile, the difference between each concentration profile was found to be less than 4%. The acceptable differences in microbial data from the repeated experiments suggest good reproducibility of the

observed outcomes and provided confidence in the results of kinetic calculations. Therefore, the proposed research approach enabled the representative monitoring of the ore-associated microbial population, through the sacrificing of individual mini-column reactors from a test set of identical reactors.

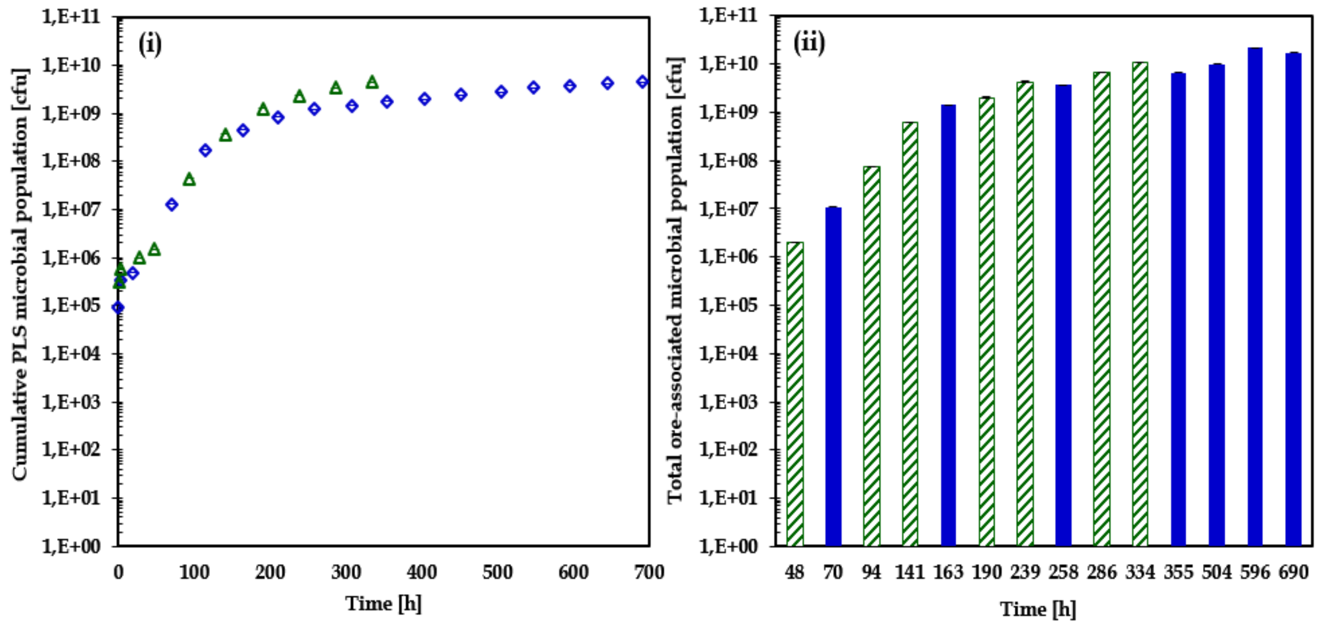

Figure 9: The microbial populations in the effluent PLS (i) and ore-associated phase (ii) for repeated tests A [◊ , shaded] and B [Δ , striped], where 2≤n≤7 for each test.

The results of the attachment studies [17-20] presented in Table 1 may be used to inform and compare the initial microbial retention on low-grade ore, however, these studies do not provide microbial abundance or leaching data. The larger scale column work on low-grade ore [24-26, 29] presents microbial abundance and diversity data at the termination of the experiments. Nevertheless, these results support the findings of this study where the ore-associated microbial population was significantly larger than that in the bulk flowing solution. Few studies listed in Table 1 have sampled and performed analysis on the ore at specific intervals during an experiment [23, 28, 30, 31]. These studies also found that microbial abundance on the ore dominated the overall microbial population in a simulated heap environment. However, a direct comparison to the results of this study cannot be made since mixed cultures and higher irrigation rates were used; with varying heap irrigation rates shown to affect microbial abundance and diversity in each of the phases [28, 43].

Complete analysis of the microbial population dynamics data for Test A, has been presented previously in Govender et al. [27]. In the same paper, the maximum specific growth rates and doubling times for *At. ferrooxidans* using the combined test data sets were also presented.

7. Conclusions

The designed experimental approach together with the methodology employed has allowed for the study of microbial population dynamics in both the PLS and ore-associated phases. The ore bed configuration and spray irrigation mechanism facilitated reproducible microbial retention within the mini-column reactors. Metal extraction curves, for both iron and copper, were shown to be comparable between the repeated experiments, within acceptable error. Multiple mini-column reactors in a single test were shown to be representative of each other with respect to chemical and microbial speciation in the effluent PLS, and microbial population dynamics in the ore-associated phases. The mini-column reactor system thus has potential to enable rigorous study of microbial colonisation and growth kinetics across a range of operating conditions, such as irrigation rate, solution composition, ore type and preparation, and inoculum composition and preparation. The impact of environmental conditions and process inhibitors on the microbial phase can therefore be elucidated. Feasibility studies as a function of ore type as well as studies to better characterise the agglomerate-scale micro-environment will be conducted, leading to improved understanding of the heap environment.

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