Investigating Microbial Colonisation in Bioheaps with Varying Irrigation Rate

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ABSTRACT

Microbial colonisation plays an important role in mineral dissolution in heap bioleaching of low grade ore. To date, colonisation studies have focused on microbial attachment of single species to mineral concentrate under batch conditions, not representative of the heap leaching environments, with recent extension to flow systems (Africa *et al*. 2010, Minerals Engineering, 23, 486; Bromfield *et al*. 2010, Bio- & Hydrometallurgy conference proceedings). Hydrology and soil engineering investigations have suggested significant interactions between microbial colonisation and fluid flow in porous systems. Therefore, heap hydrology is expected to affect microbial colonisation through solution-ore and microbe-mineral contacting.

The influence of the irrigation rate on microbial colonisation was assessed using columns packed with acid agglomerated low grade copper-containing ore. The systems were inoculated via irrigation with iron and sulphur oxidising mesophilic microorganisms (10^{12} cells/ton ore), whilst operating under continuous flow through the unsaturated, aerated bed, using three different irrigation rates (2**,** 6 and 18 $l/m^2/h$). A novel in-bed sampling technique allowed for the extraction of ore samples at intervals during the leaching process to give novel insight into the microbial growth and the interstitial, weakly and strongly attached microbial population.

Increasing bacterial adherence and cell number retained in the ore bed was clearly seen over the 32 day leaching period. Average specific growth rates of ore-associated micro-organisms of 0.0053, 0.0052 and 0.0043 h^{-1} were found for 2, 6 and 18 $L/m^2.hr$, showing faster colonisation under low flow regimes. At higher irrigation rates, higher detachment and cell removal are postulated, as the total number of cells exported from the ore bed was $2.2x10^{10}$, $6.5x10^{10}$ and $7x10^{10}$ cells for irrigation rates of 2, 6 and 18 $L/m^2.hr$ respectively. For all conditions, the interstitial cells from the stagnant zone of the ore bed were the most dominant form of cells accumulated within the heap systems.

KEYWORDS

Bioleaching, sulphide ores, shear forces, sampling, colonisation

1 INTRODUCTION

The need for alternative methods to those conventionally used for the extraction of valuable metals e.g. smelting, has become increasingly important to extract metals from ores of lower grade, and reduce the energy footprint and the environment burden of extraction. The development of heap

bioleaching as an alternative technology was prompted by the increasing complexity and low grade of ores mined (Watling, 2006). In the hydrometallurgical heap leaching process, crushed ore is inoculated with a consortium of micro-organisms to catalyse iron and sulphur oxidation reactions providing leach reagents in the form of ferric iron and acid to assist the mineral dissolution of sulphide minerals (Brierley, 2001).

To optimise the performance of the heap bioleach system a comprehensive awareness of the contributing processes and sub-processes is required. Watling (2006) highlighted the cause of lack of in-depth understanding of bioleaching to the separated and independent focus of investigations on different aspects such as chemistry, microbiology and hydrodynamics. The aim of this study is to add to the bioleaching knowledge base through integrated study of microbiological and hydrological aspects of heap bioleaching. Particularly, the impact of irrigation application rate on microbial colonisation is investigated.

Microbial colonisation of the mineral surface occurs through the following steps:

(i) The transport of microbial cells occurs as the liquid phase is transported throughout the heap to the mineral surface (Rossi, 1990; van Loosdrecht *et al*., 1990). The microorganisms can either be eluted in the leachate, or accumulated within the heap in the stagnant zones, or attached to the ore in response to surface interactions, illustrated by Figure 1. Microorganisms present in the free-flowing fluid are referred to as planktonic cells, whilst those planktonic cells accumulating in the stagnant regions are known as the interstitial cells.

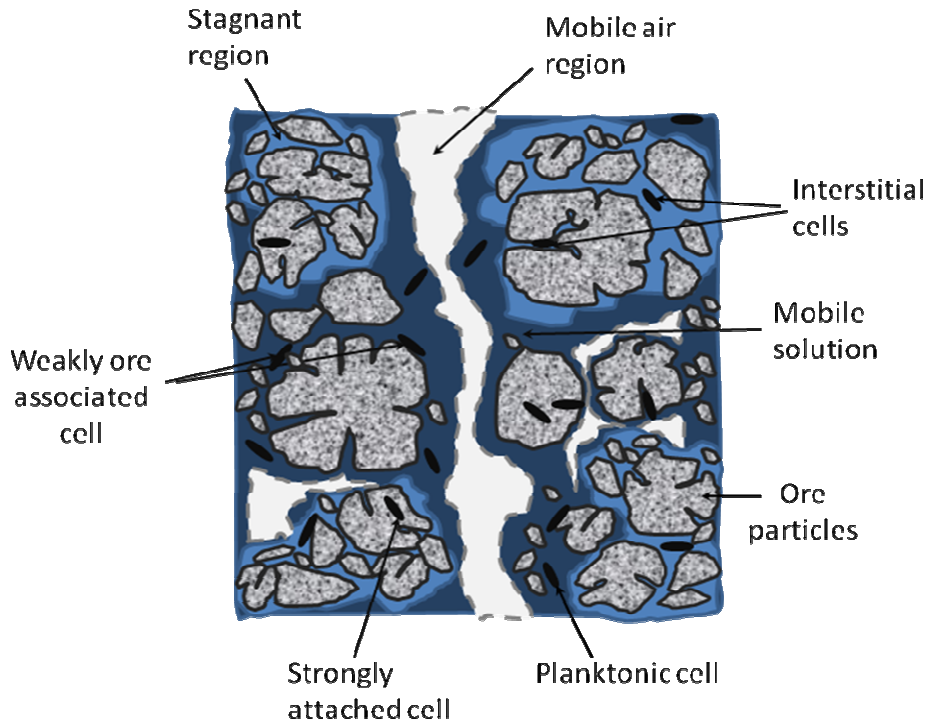


Figure 1: Schematic of the inside of the heap, showing planktonic cells in the flowing mobile liquid phase, microorganisms weakly and strongly associated with the ore surfaces, and microorganisms accumulating within the stagnant regions of the porous rocks.

(ii) There is selective reversible and irreversible attachment to the mineral surface (Rossi, 1990; van Loosdrecht *et al*., 1990, Rockhold *et al*., 2002). Reversible attachment accounts for the

microorganisms loosely associated with the ore surface, whilst the irreversible attachment accounts for the microorganisms strongly associated. The loosely attached microorganisms can be detached from the ore surfaces by repulsive electrostatic forces (Ghauri *et al.*, 2007; Rodriguez *et al.* 2003) and mild shear forces (Rossi, 1990; van Loosdrecht *et al*., 1990; Rockhold *et al*., 2002; Pintelon *et al*., 2009). The potential for detachment of microbial cells from the ore also creates an exchange between planktonic cells and the sessile microorganisms (van Loosdrecht *et al*., 1990).

(iii) The growth and multiplication of microorganisms results in the formation of microbial cultures which contribute to biooxidation (Rossi, 1990; van Loosdrecht *et al*., 1990, Rockhold *et al*., 2002).

Studies of microbial colonisation reported to date mainly focus on the degree of attachment of microorganisms to the mineral concentrates (Gehrke *et al*., 1998; Sampson *et al*., 2000; Kinzler *et al*., 2003; Sand and Gehrke, 2006), the mechanisms behind microbial attachment (Rossi, 1990; van Loosdrecht *et al*., 1990, Rockhold *et al*., 2002; Rodriguez *et al*., 2003; Ghauri *et al*., 2007), EPS formation, and more recently the location and specificity of microbial attachment to mineral (Africa *et al*., 2010; Bromfield *et al*. 2010, Bio- & Hydrometallurgy conference proceedings). These studies are important in understanding the microbial sub-processes occurring within the leaching environment. These observations have limited applications because most experimental findings were derived from systems with conditions which were not analogous to actual heap bioleaching environments. Additionally, studies relating microbial colonisation of low-grade ore bioheaps to the influence of irrigation conditions have not been reported extensively.

Bioheaps are typically irrigated at an application rate which does not cause saturation (Brierley, 2001), to ensure oxygen and carbon dioxide transfer to microbes. Researchers have investigated the effect of irrigation rate on temperature in the heap, sulphide oxidation rate and metal recovery (Cooper and Dixon, 2006; Bouffard and Dixon, 2009), largely using heap leaching simulations rather than experiments. Few researchers have assessed the fate of the bioleaching microorganisms under the different irrigation rates employed during heap leaching operations. Lizama *et al.* (2005) focused on heap leaching of pyrite and sphalerite at irrigation rates ranging from 1.8 to 21.6 $l/m^2/h$ in columns at heights varied from 1 to 8 m. They concluded that increased irrigation rates had no effect on the initial colonisation period. However, the postulated detachment of microbes from the heap system by fluid shear forces (Rossi, 1990; van Loosdrecht *et al*., 1990; Rockhold *et al*., 2002; Pintelon *et al*., 2009) and the reported preferential sorption of microbes onto the gas-water interface over the solid-water interface (Wan *et al*., 1994) suggests otherwise. From these studies, it can be postulated that employing low irrigation rates facilitates good microbe-mineral contacting, enhancing colonisation rates while preventing detachment of microbes due to fluid shear. The objective of this study was to examine the effect of irrigation rates on microbial attachment, growth rates and removal of microbes in systems that mimic heap conditions, through a comparative study.

2 METHODOLOGY

2.1 Ore, Microbial Cultures and Growth Media

Low grade copper-bearing ore, containing 0.69% copper, 2.95% iron and 2.02% sulphur was utilised. The crushed ore was agglomerated using 50 ml deionised H_2O and 3.7 ml conc. H_2SO_4 / kg ore to secure the fines fraction.

A mixed mesophilic stock culture containing *At. ferrooxidans*, *At. caldus*, and predominantly *L. ferriphilum*, grown on pyrite concentrate in a batch stirred tank reactor at 30°C, was used. A preliminary study was conducted to determine the inoculum concentration at which the concentration of the cells eluting without attachment was within the detection limit of the total microscopic cell count ($3x10^5$cells/ml). The range 10^8 to 10^{13} cells/ton of ore was considered and 10^{12} cells/ton of ore chosen in this investigation.

The irrigation feed composition was: 0.5 g/l $FeSO_4.7H_2O$, 183.3 mg/l $(NH_4)_2SO_4$, 60.5 mg/l $NH_4H_2PO_4$, and 111.2 mg/l K_2SO_4 in deionised water. The pH was adjusted to pH 1.15 using 96-98% concentrated sulphuric acid (H_2SO_4). All reagents used were of analytical grade.

2.2 Column Operation

Figure 2 represents the system used in this investigation. The experiments were conducted in small scale heap leach columns of 100 mm diameter and 360 mm height. The columns were packed with approximately 4 kg acid agglomerated ore as described by van Hille *et al.* (2010). Liquid irrigation rates of 2, 6 and 18 $l/m^2/h$ were used. Prior to inoculation, the systems were acid washed at the same rate (6 $l/m^2/h$) for 1 day to remove readily leachable materials and create an environment conducive to microbial attachment to the ore surface. The columns were operated under ambient temperature conditions and a feed pH of 1.15. The columns were aerated from the base at 200 ml/min and drip irrigated from above with acidic feed solution resulting in counter-current flow systems. The experiments ran for 32 days, with in-bed sampling conducted at intervals during the leaching process. Sampling of the liquid effluent (PLS) took place daily.

KEY:

A – Air Outlet
B – Column
C – PLS Collection vessel
D – Temperature sensor
E – Rotameter
F – Inlet feed point
G – Air inlet
H – Pump

Figure 2: Illustration of column experimental set-up

2.3 In-bed Sampling Technique

The in-bed sampling technique was developed for characterisation and quantification of the microbial community associated with the mineral ore bed as a function of time, following inoculation and subsequent colonisation. This provided a novel approach to the attainment of time progression data at the laboratory scale, not reported previously. Figure 3 provides a schematic representation of the in-bed sampling equipment. Figure 4 illustrates the progression of the sampling procedure used. The bottom of the column was opened, carefully removing the supporting perforated plate to avoid out spillage of the packed bed. The in-bed sampler was attached to the column (Figures 3 and 4). The packed bed of ore was lowered slowly using the screw, until it reached section B of the apparatus. The metal slicing plate was inserted into the slicing slot, cutting through until the narrow tip ejected out the other side of the opening. The screw was lowered completely to allow the bottom half of the packed bed to drop into the section B of the in-bed sampler. Section B was detached. A slice of the ore remaining in section B was removed manually for analysis. Section B was reattached to section A and the screw lifted until the ore bed touched the metal slicing plate. The slicer was removed and the ore bed slowly inserted back into the column. The in-bed sampling apparatus was removed, the bottom column plate replaced and operation continued as normal.

2.4 Analytical procedures

The pregnant leach solution (PLS) volume collected was recorded daily. Variation in pH was measured using a Metrohm 691 pH meter. The redox potential was measured with a Crison GLP 21 Redox meter relative to a reference solution of potential 468 mV at 25°C. The ferrous iron concentration was measured spectrophotometrically using the colorimetric 1-10 phenanthroline method described by Komadel and Stucki (1988). Total iron in solution was determined using both atomic absorption spectroscopy (AAS) and spectrophotometric method following conversion of all iron to the ferrous form. Copper concentration in solution was also obtained from AAS. The analytical measurements were performed in triplicates. Microbial cell counts were determined using a Thoma counting chamber and an Olympus BX40 Microscope at X100 magnification (oil phase, phase contrast optics detection limit of direct counting method of $3x10^5$cells/ml, inherent 30% error margin (Bryan *et al.* (submitted for publication))). Cell counts were performed on the eluted PLS as well as the samples obtained from the mechanical detaching of the microorganisms from the ore using the practical but still robust detachment protocol developed in CeBER and reported by Bryan *et al.* (submitted for publication). The detachment protocol was developed to be able to distinguish between the planktonic, interstitial, weakly-associated and strongly attached microorganisms present within heap systems. The limitations of this technique include the inability to remove all the cells from the ore within the number of washing steps prescribed, and the difficulty in determining the protocol's efficiency (Bryan *et al.* (submitted for publication)).

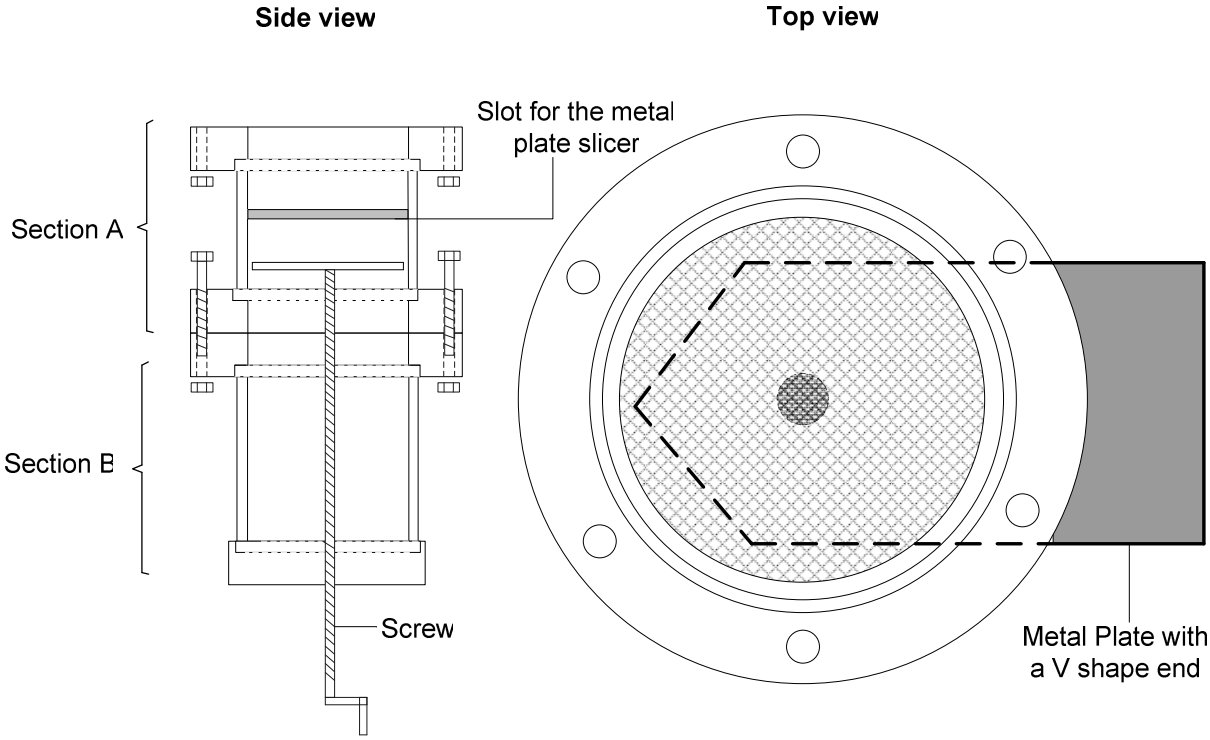


Figure 3: In-bed sampling apparatus

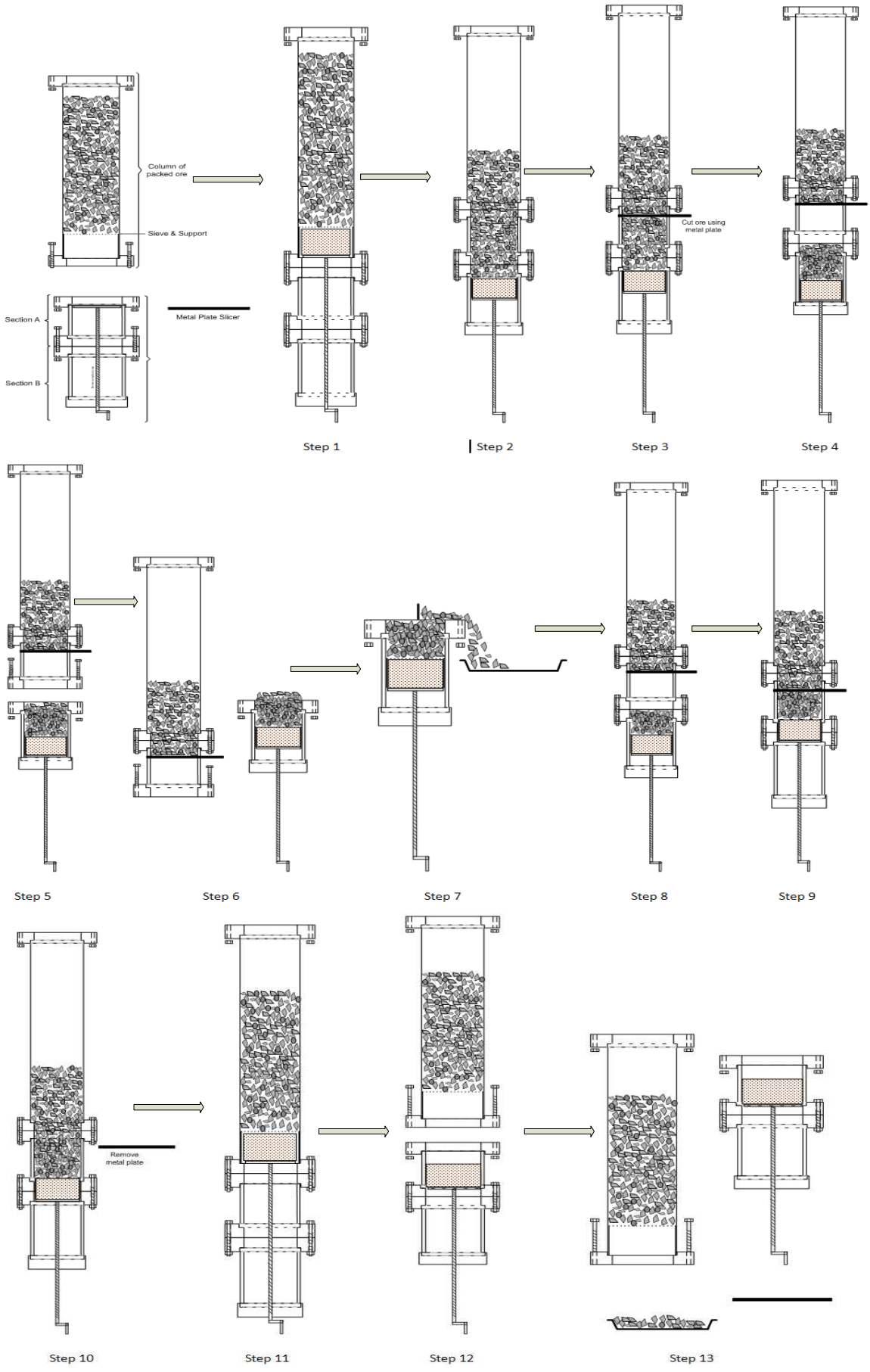
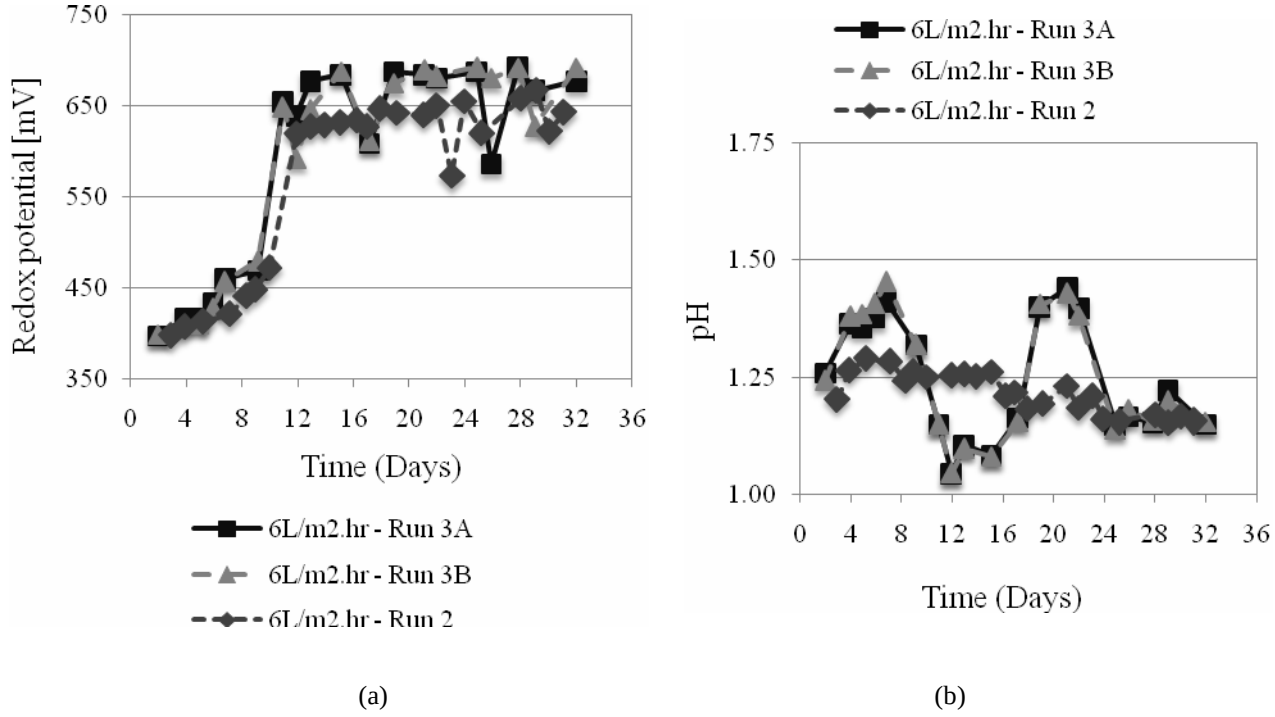


Figure 4: Illustration of the step by step in-bed sampling procedure.

3 RESULTS AND DISCUSSION

The data generated over multiple runs at an irrigation rate of 6 l/m^2/h are first presented to illustrate the typical findings obtained using the new in-bed sampling technique. This is followed by a comparison of the systems operating under the different flow conditions.



(a) (b)

Figure 5: Trend in (a) the redox potential and (b) pH, of the eluted solution passing through the column heap at an irrigation rate of 6 l/m^2/h, over replicate experimental runs.

The volumetric flow rates out of the system were constant within 20% for the duration of the leach run (data not shown), indicating low influence of the in-bed sampling technique on the heap systems. Figures 5 (a) and (b) show the redox and pH profiles of the eluted leachate as a function of leach time at an irrigation rate of 6 l/m^2/h. The redox potential of the feed solution was ~380 mV. The redox potential of the leachate increased slowly from 400 to 470 mV during the first 10 day phase. The redox potential increased rapidly from 470 to 650 mV between days 10 and 12, due to enhanced microbial growth and biooxidation of ferrous to ferric iron. Thereafter, the redox potential fluctuated between 580 and 700 mV. The feed solution was at a pH of 1.15. The pH of the eluted solution fluctuated between 1 and 1.5 for the duration of the leach run.

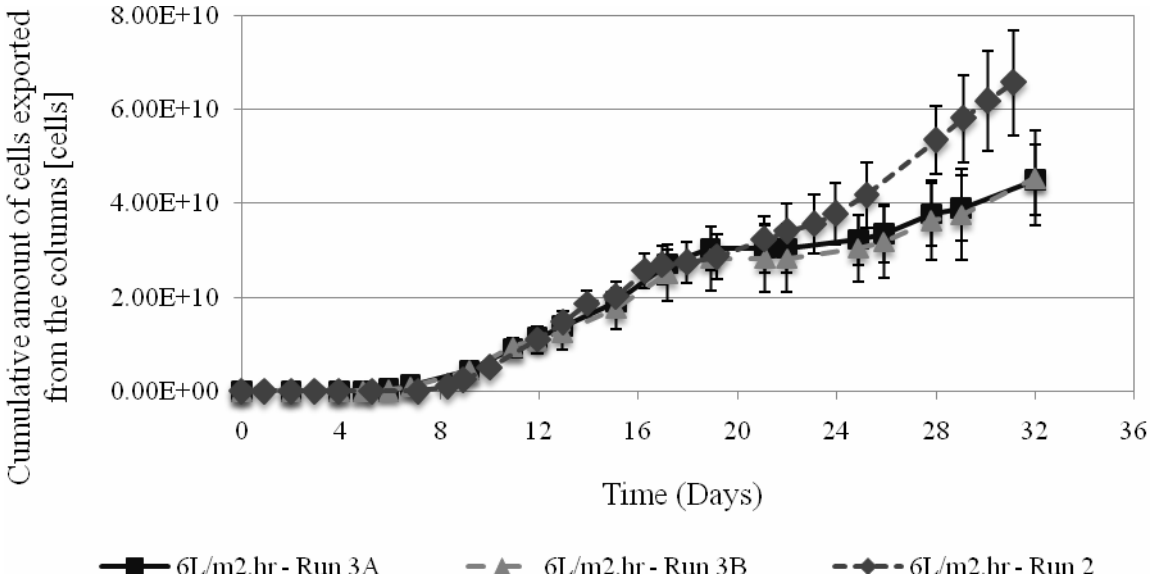


Figure 6: Cumulative removal of microbial cells in column effluent at an irrigation rate of 6 l/m^2/h, over replicate experimental runs. Error bars represent the propagated error determined from the standard deviation of the mean cell count within each experimental run.

Figure 6 shows the cumulative planktonic cells leaving the ore bed in the PLS over time, at an irrigation rate of 6 l/m^2/h. Due to the low detection limit of the microscopic cell counting technique, no cells were observed in the eluted solution during the first 8 days of leaching. This could also be attributed to the cell attachment to the ore followed by a period of low growth in which the microorganisms adapted to the new heap leaching environment. Microbial cells were detected in the eluted leachate on day 8 at a concentration of ~7x10^{5}cells/ml, and an increase in cell number exported from the columns observed, corresponding to the increased redox potential. After 32 days of leaching, the total number of cells exported was in the range 4.5x10^{10} to 6.5x10^{10}cells, and the PLS cell concentrations fluctuated around 2x10^{6}cells/ml (data not shown).

Figure 7 shows the growth curve of the combined interstitial and attached cells determined by in-bed sampling and detachment during the leach runs, at an irrigation rate of 6 l/m^2/h. Figure 7 also shows the reproducibility across the experimental runs. Increased accumulation of microbes within the systems was observed over the leaching period. Between 1.2x10^{11} and 1.9x10^{11}cells translating to 3x10^{13} and 4.8x10^{13} cells/ton ore remained in the columns after 32 days across replicate runs. The error bars represent the error within each experimental run, taking the standard deviation across triplicate cell counts as a percentage of the mean. The variations between replicate runs may be attributed to the limitations of the detachment technique in the removal and recovery of the microbial cells from the ore samples analysed.

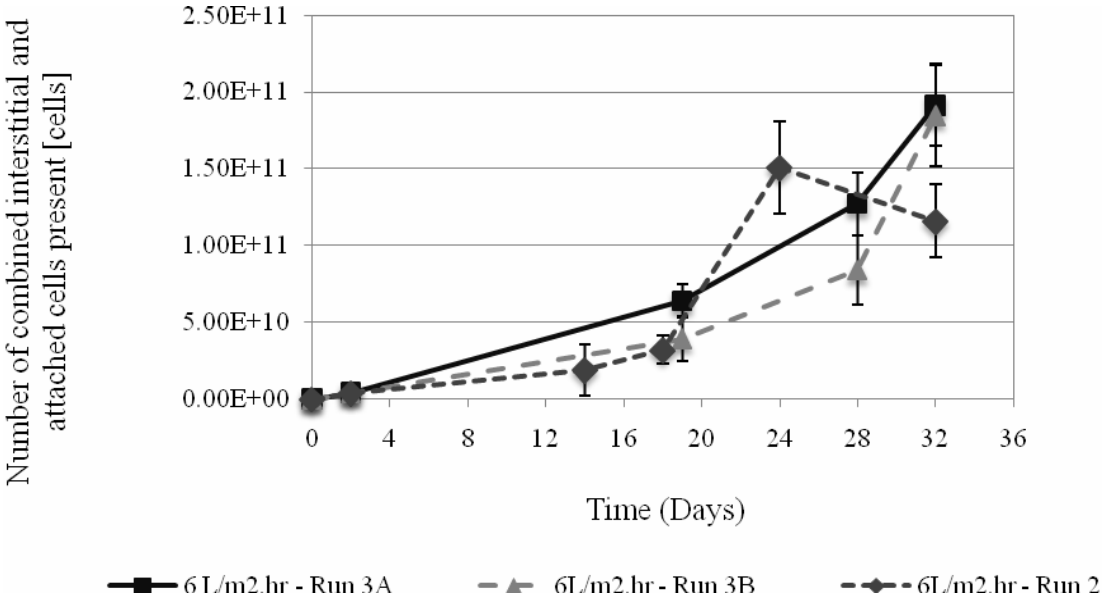
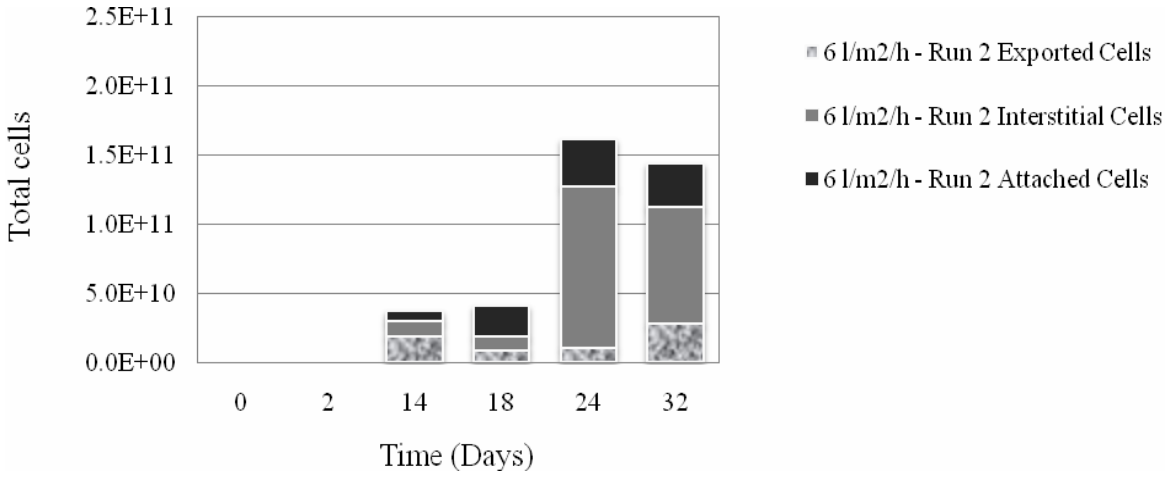


Figure 7: Microbial growth curve obtained by combining the interstitial and attached cells accumulated in the ore bodies, determined from the mechanical detachment of cells from the ore samples periodically removed from the heap systems using the in-bed sampling technique, given for replicate runs at an irrigation rate of 6 l/m^2/h. Error bars represent the standard deviation of the combined interstitial and attached cell counts.

Figure 8 shows the progression of the microbial growth whilst identifying the difference between the exported planktonic cells (accumulated within the time intervals between the in-bed sampling points), interstitial and attached cells during leach runs, at an irrigation rate of 6 l/m^2/h. The data was obtained from the microscopic analysis of the cells mechanically detached from the ore extracted using the in-bed ore sampler. Increased bacterial adherence to the ore was observed over the 32 day leaching period across all runs. Increasing cell numbers in stagnant fluid (interstitial phase) were also observed. The interstitial cells were the most dominant form of cells accumulated within the heap systems (58 %), compared to 20 % exported (in the interval between 24 to 32 days) and 22 % attached cells after 32 days leaching. Where data are considered over the whole time period, 26.9 % were attached, 54.1 % in the interstitial phase and the remaining 19 % eluted from the column over the overall time period.

(a)



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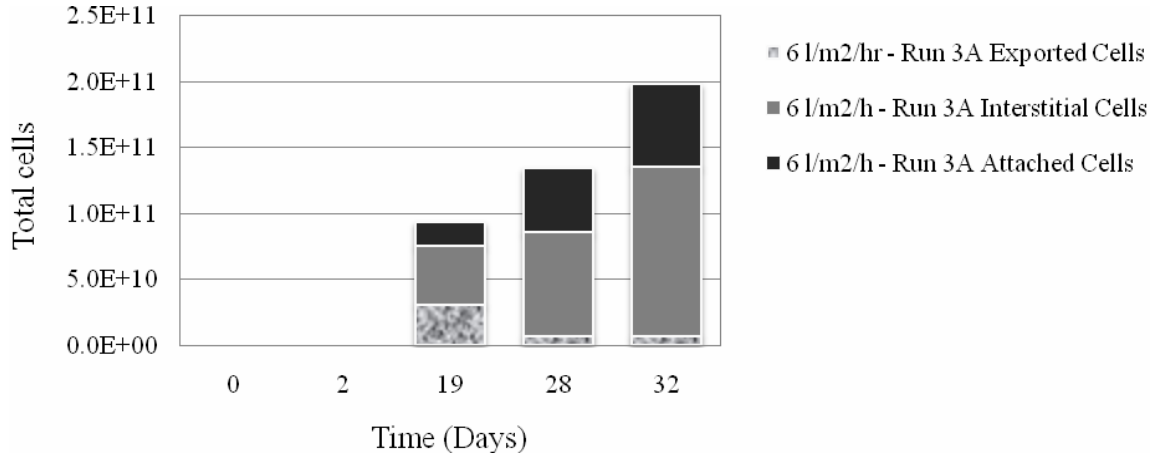

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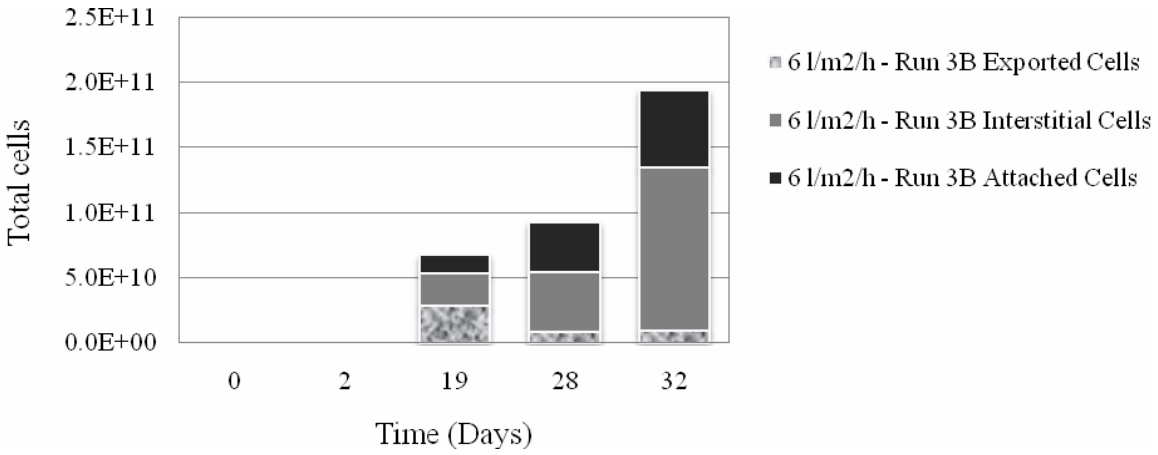
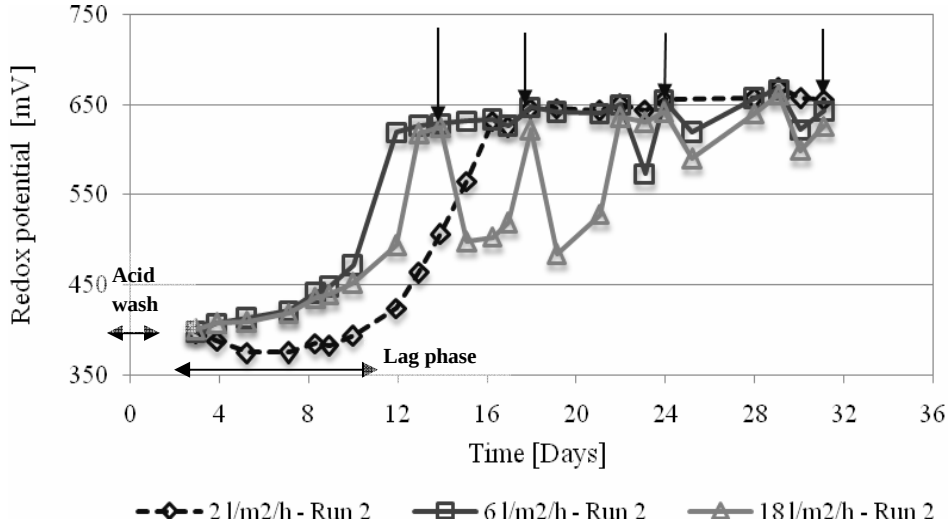
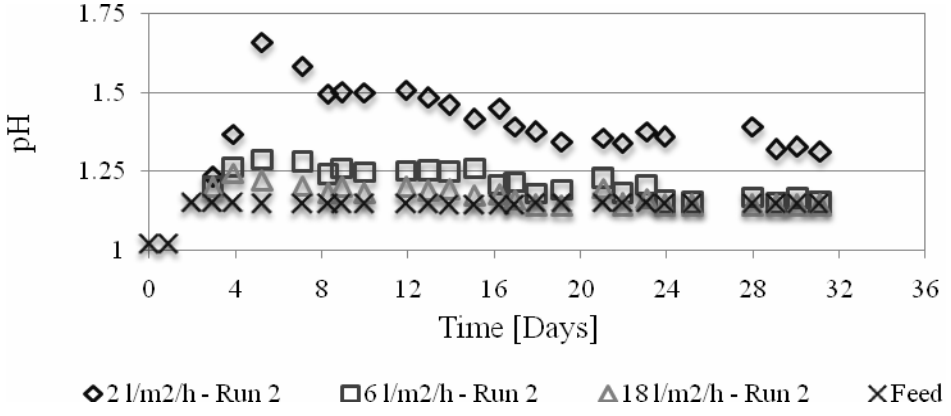


Figure 8: Comparison of planktonic cells (removed within each time period), interstitial, and attached microorganisms present in the column reactors, determined from the mechanical detachment of cells from the ore samples periodically removed from the heap systems using the in-bed sampling technique, given for replicate runs **(a)** Run 2 **(b)** Run 3A and **(c)** Run 3B, at an irrigation rate of 6 l/m^2/h.

A comparison of data generated in experimental run 2 at irrigation rates of 2, 6 and 18 l/m^2/h is presented in Figures 9 to 12. Figure 9 shows the trend in redox and pH profiles of the eluted leachate. The arrows indicate the days on which in-bed sampling was conducted. The fluctuations in the redox potential at 18 l/m^2/h correspond to the days immediately after each in-bed sampling point, indicating greater susceptibility of the high flow system to changes in the leaching environment caused by the in-bed sampling procedure technique. However, in the later runs such fluctuation was not observed. The redox potential of the leachate increased slowly from 400 to 470 mV during the 10 day lag phase at irrigation rates 6 and 18 l/m^2/h. From day 10 to 12, there was a significant increase in redox potential from about 450 to 650 mV, indicating the rate of microbial oxidation of ferrous iron was greater than ferric leaching of the sulphide minerals present. Thereafter, the redox potential fluctuated between 600 and 650 mV. Under low flow conditions (2 l/m^2/h), the redox potential took longer to climb to 600 mV (16 days) than the other flow conditions, before stabilising at 650 mV. This occurrence can be attributed to slower transport of microorganisms into the ore bed. Additionally, the slower rise in redox potential could be a result of mass transfer limitation within the low flow system.



(a)



(b)

Figure 9: Trend in (a) the redox potential and (b) the pH, of the eluted solution passing through the column heap given for different irrigation rates ◊ - 2 l/m^2/h, □ - 6 l/m^2/h and Δ - 18 l/m^2/h, during run 2. Arrows are indicative of in-bed sampling days.

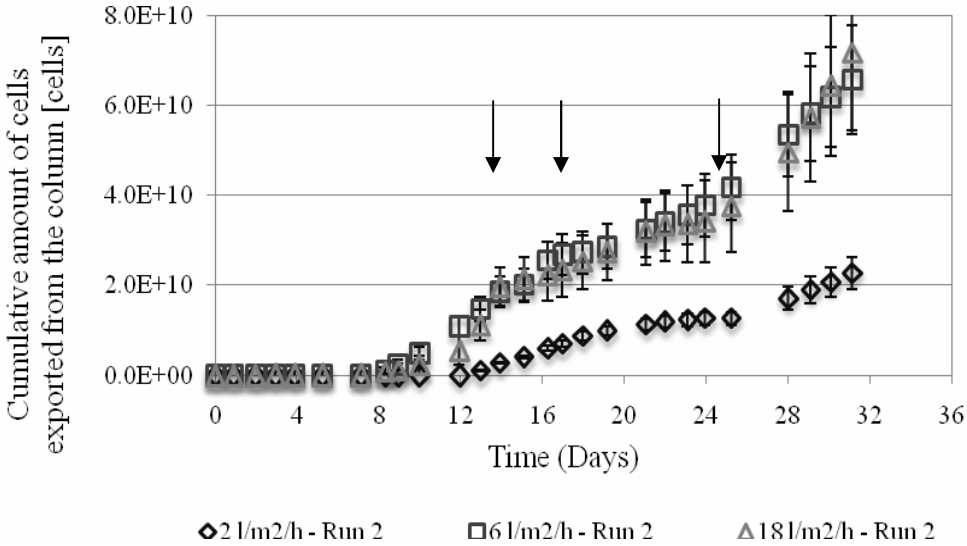


Figure 10: Cumulative removal of microbial cells in column effluent at irrigation rates ◊ - 2 l/m^2/h, □ - 6 l/m^2/h and Δ - 18 l/m^2/h, during run 2. Error bars represent the propagated error determined from the standard deviation of the mean cell count within each experimental run. Arrows are indicative of in-bed sampling days.

The pH of the leachate fluctuated between 1 and 1.5 for the duration of the leach run (Figure 9b). There was no significant difference between the pH at the different flow rates 6 l/m^2/h and 18 l/m^2/h. The profile indicated that the pH was higher at 2 l/m^2/h than for the other irrigation conditions. This could be due to acid consumption via gangue dissolution occurring at a faster rate than the delivery of acid to the leaching environment at low flow conditions and the microbially assisted acid regeneration.

Figure 10 shows the cumulative planktonic cells observed in the leachate over time, at irrigation rates of 2, 6 and 18 l/m^2/h, during run 2. No cells were detectable in the effluent during the first 8 days of leaching. Thereafter, the removal of cells through the PLS was observed indicating microbial growth. The microbial cell concentrations in the PLS from all three columns fluctuated, with cell concentrations reaching as high as $6.0x10^6$ cells/ml (2 l/m^2/h) and $3x10^6$ cells/ml (18 l/m^2/h) (data not shown). After 32 days of leaching, high cell exportation from the column was observed for 6 and 18 l/m^2/h removing approximately $6.5x10^{10}$ and $7.0x10^{10}$ cells respectively, compared to $2.2x10^{10}$ cells at 2 l/m^2/h, with the propagated cumulative error being < 25 %. The larger cell removal at the higher flow conditions could be attributed to the detachment of cells as a consequence of the greater shear stress induced, suggesting a lower colonisation of the heap at higher irrigation rates. In this data, there was little variation between the planktonic cell data for irrigation rates of 6 and 18 l/m^2/h. However, one of the three repeat runs showed higher cell removal from the column for high flow (18 l/m^2/h) with a total of $2.2x10^{11}$ cells exported after 32 days leaching, compared to $1.1x10^{10}$ and $5.7x10^{10}$ cells for 2 and 6 l/m^2/hr respectively (Table 1).

Table 1: Total cumulative exported cells after 32 days leaching for runs 2 and 3, and the preliminary run 1.

	Cells ($x10^{10}$)		
Irrigation rate (l/m^2/h)	2	6	18
Run 1	1.1	5.7	22
Run 2	2.2	6.5	7.0
Run 3	1.2	4.2	5.9

Figure 11 shows the growth curve of the combined interstitial and attached cells at irrigation rates of 2, 6 and 18 l/m^2/h, during run 2. Increased accumulation of microbes within the systems was observed under all flow conditions resulting in final concentrations of ore associated cells of 4.3x10^{13} (2 l/m^2/h), 3.0x10^{13} (6 l/m^2/h) and 2.0x10^{13} (18 l/m^2/h) cells/ton ore remained in the columns after 32 days. A greater number of ore associated bacteria accumulated in the systems under low flow conditions (2 and 6 l/m^2/h).

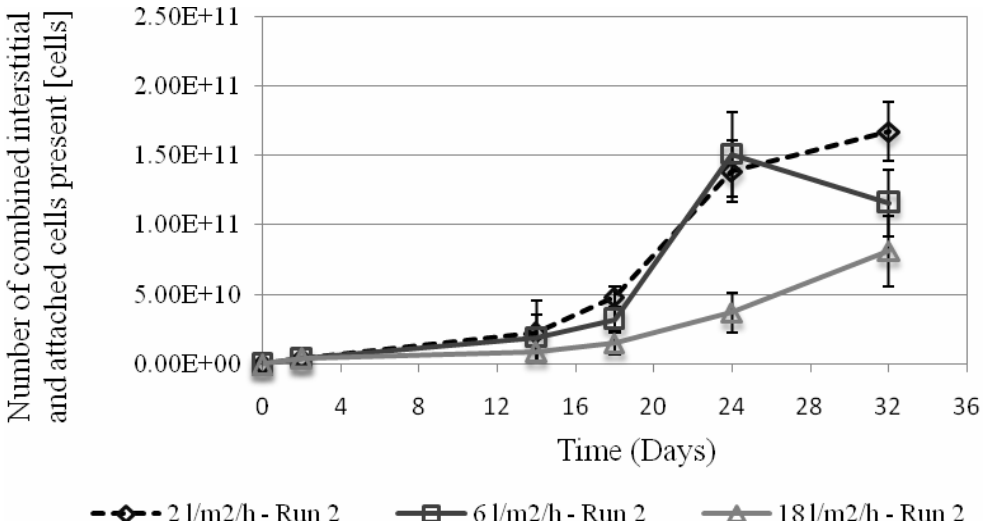


Figure 11: Microbial growth curve obtained by combining the interstitial and attached cells accumulated in the ore bodies, determined from the mechanical detachment of cells from the ore samples periodically removed from the heap systems using the in-bed sampling technique, given for different irrigation rates ◊ - 2 l/m^2/h, □ - 6 l/m^2/h and Δ - 18 l/m^2/h, during run 2. Error bars represent the standard deviation of the combined interstitial and attached cell counts.

The rate at which the bacteria multiplied, r_x, is a function of the available microbial population, X, at a particular time, t.

$$r_x = dX/dt = \mu X$$

The growth rates were calculated using the concentration, X, represented by the sum of the interstitial and attached cells, whilst neglecting the planktonic cells which were removed from the column. The specific growth rates obtained from the gradient of ln(X) as a function of time are shown in Table 2. Investigations of the growth kinetics of mesophilic bioleaching microorganisms have been carried out in continuous culture systems (Breed and Hansford, 1999; Dempers *et al.,* 2003). Dempers *et al.* (2003) reported growth rates ranging from 0.038 to 0.119 h^{-1} for experiments conducted with temperatures varying from 30 to 40°C and pH from 1.1 to 1.7, using a mixed culture of mesophilic bacteria under conditions typical of a *Leptospirillum* dominated culture. Further unpublished growth rates on whole ore suggest specific growth rates of the mesophiles in the range 0.01 to 0.08 h^{-1} (Minnaar *et al*. 2010). The growth rates determined in this study are less than 15% of those reported by Dempers *et al.* (2003) and approximately 50% of the lower rates demonstrated on whole ore using qPCR to quantify cell growth (Minnaar *et al*. 2010), showing a clear difference in microbial growth within the various leaching environments. Further it is recognised that by neglecting the planktonic cells the growth rate may be underestimated. However, the relative proportions suggest that this error should be less than 20%.

Table 2: Growth rates (μ^{max} = bacterial specific growth rate (h^{-1})) calculated based on the combined interstitial and attached microbial population within the columns

Condition ($l/m^2/h$)	μ^{max} (h^{-1}) determined based on ore-associated ore population		
	Run 2	Run 3A/B	Combined run 2 and 3
2	0.0055	0.0051	0.0053
6	0.0052	0.0054/51	0.0052
18	0.0043	0.0043	0.0043

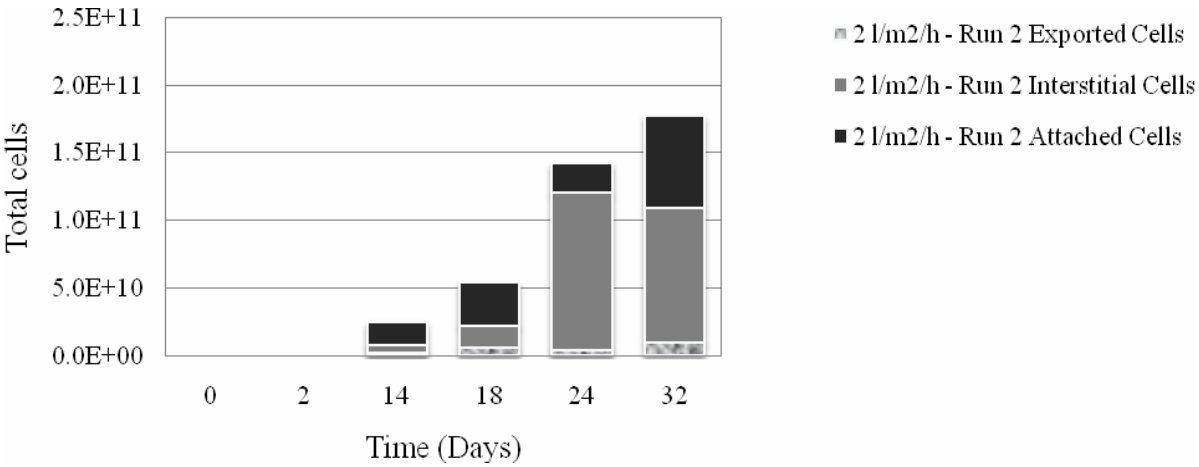
Figure 12 shows the progression of the microbial growth and the relative proportions of exported planktonic cells, interstitial and attached cells at the three irrigation rates, during run 2. Increasing bacterial association with the ore was observed over the 32 day leaching period across all conditions. A greater number of bacteria adhere to ore under low flow conditions (2 and 6 $l/m^2/h$), due to reduced detachment of microorganisms by the fluid shear and slower exportation of microorganisms from the systems. Higher attachment of microorganisms to the ore at low flow rates could also be a response to nutrient and ferrous iron limitations due to the low availability as a consequence of slower supply to the system. Additionally, the higher surface attachment and colonisation observed for lower flow rates can be attributed to the control of the available void space by the irrigation rate (Bouffard and West-Sells, 2009) and gas saturation of the system allowing for higher microbial retention within the system (Wan *et al*., 1994). Under low flow conditions, microorganism-ore contact time increased within the leaching system, allowing the cells to form stronger surfaces bonds and remain attached for longer periods.

Increased cell numbers in stagnant fluid were also observed. A cell balance over the system is shown in Table 3. For all conditions, the interstitial cells were the most dominant form of cells accumulated within the heap systems. The total quantity of cells retained in the ore bed for an irrigation rate of 18 $l/m^2/h$ was much lower than for the other flows, inferring that under higher flow conditions the detachment and transport of microbial species out of the column affected the microbial growth.

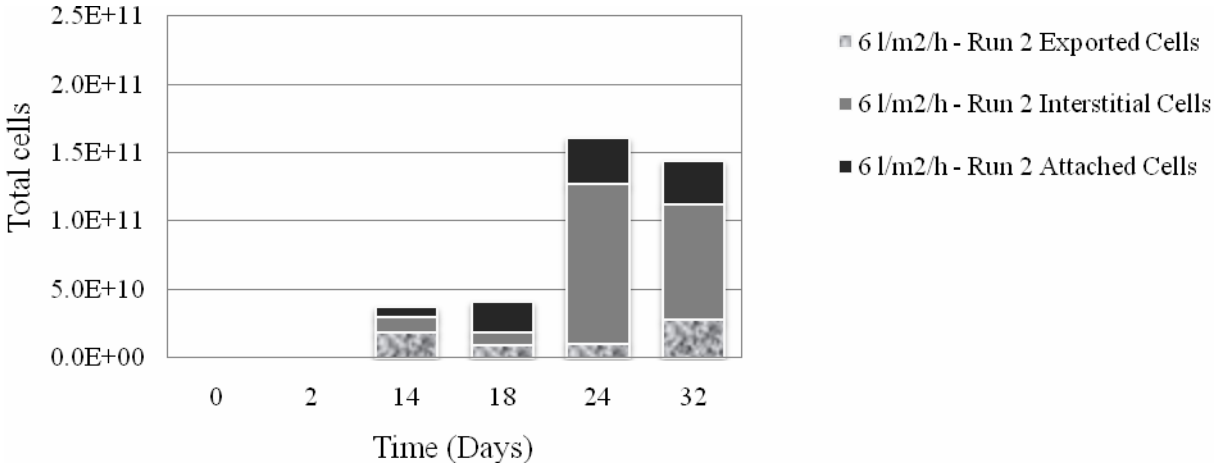
Table 3: Cell balance over the 3 flow systems during leaching run

	Irrigation rate ($l/m^2/h$)		
	2	6	18
Inoculated (cells)	4.00E+09	4.00E+09	4.00E+09
Total planktonic (cells)	1.18E+10	4.50E+10	5.88E+10
Total interstitial (cells)	1.13E+11	1.28E+11	7.29E+10
Total attached (cells)	6.02E+10	6.35E+10	2.32E+10
Total cells (cells)	1.85E+11	2.36E+11	1.55E+11
% planktonic	**6.4**	**19.0**	**38.0**
% interstitial	**61.0**	**54.1**	**47.0**
% attached	**32.6**	**26.9**	**15.0**

(a)



(b)



(c)

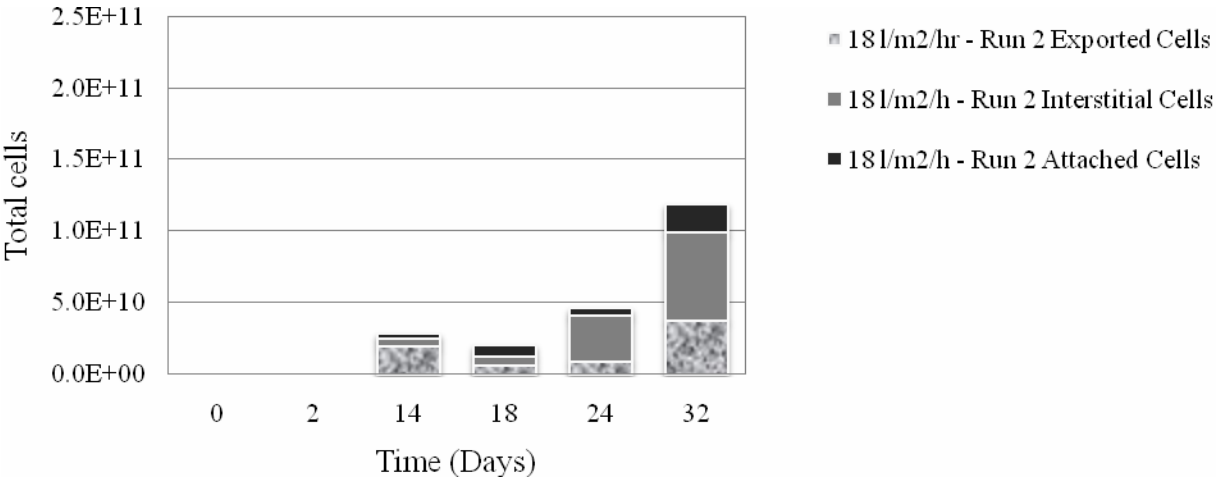


Figure 12: Microbial attachment to the low grade copper bearing ore body in the column reactors, determined from the mechanical detachment of cells from the ore samples periodically removed from the heap systems using the in-bed sampling technique, given for different irrigation rates ◊ - 2 l/m^2/h, □ - 6 l/m^2/h and Δ - 18 l/m^2/h, for run 2.

4 CONCLUSIONS

Investigations assessing the impact of irrigation rates on microbial colonisation have not been presented previously. This study has shown that the rate at which microbes multiply and attach to ore in simulated bioheap leaching systems was influenced by the irrigation rate.

The new in-bed sampling technique demonstrated the ability for ore samples to be obtained without significant disruption of the ore bed under low to moderate flow conditions. The data collected from this provided useful information on the relative degrees of attachment of the microbes accumulated within the heap structures. This is the first analysis and report of the data obtained from the in-bed sampling technique. This instrument can also be extrapolated and used to assess colonisation at different heights within the bed of a particular leaching system.

In particular, the enhancement of microbial surface colonisation at lower irrigation rates was observed, as illustrated by the increase in attached and interstitial cell numbers with time and with respect to similar experiments. There was preferential accumulation of the microbes in the stagnant regions over both those in the PLS and on the ore surfaces, as illustrated by the dominant interstitial population. Growth rates on whole ore in the column geometry were shown to be significantly lower than those measured in submerged continuous culture studies.

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