INVESTIGATING THE GEOCHEMISTRY AND MICROBIAL ECOLOGY OF HEAP BIOLEACHING SYSTEMS AT THE AGGLOMERATE SCALE

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I certify that all material in this dissertation which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other university.

Signature:

ABSTRACT

Heap bioleaching is an alternative processing method for low-grade copper ores. Microbial iron oxidation produces ferric iron which solubilises copper-bearing minerals while microbially generated acid maintains a pH consistent with copper solubility. The microorganisms can inhabit the liquid within the heap or attach to the ore particles. Temperature, pH, and iron content of the heap effluent (leachate) are typically monitored to examine heap health, however, the leachate has recently been disproven as an accurate analogue of the cell distribution within the ore-bed during microbial colonisation. Three ore-associated phases are now recognised, the interstitial, weakly attached and strongly attached. This dissertation examined the cell distribution, community structure, and the distribution of iron and copper between the four phases past heap colonisation. 150 g samples of non-sterile low-grade enargite ore were used to create agglomerate-scale ore-beds that were inoculated with a mixed mesophilic culture and monitored over 192 days at 27ºC. Cell distribution was at least 2500x higher in the ore-associated phases in comparison to the leachate. *Leptospirillum ferriphilum* was the dominant species in every phase. Iron and copper concentrations were 10x and 100x higher in the ore-associated phases compared to the leachate, respectively. Batch bioreactor tests using enargite-concentrate provided an analogue to potential conditions in the ore-associated phases in a functional heap, as only 6% of the total copper was solubilised from the agglomerate-scale beds. Microbial growth and copper dissolution decreased with pH and growth rates decreased at iron concentrations over 20g/L. On a large scale, a better understanding of the microbial environment with regards to cell and metal distribution leads to improved heap modelling and sampling procedures which examine all phases within the heap instead of the leachate only. Improved bioleaching technology then has the potential to provide a feasible method for mining refractory low-grade, large-scale deposits.

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CHAPTER 1. INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Today's mining industry faces many challenges such as unpredictable metal prices, increasing operating costs, declining reserves, water supply and energy use restrictions, and changing environmental legislation (Brierley 2010). There are currently a variety of extraction and metallurgical methods that are helping to address some of these issues. One such technology is bioleaching, a biohydrometallurgical process that employs the microbially-mediated solubilisation of metals (Watling 2006). An advantage of bioleaching is that it can be implemented in scenarios where deposits may not have the size, transport access, or appropriate mineral assemblages for conventional mining methods to be economically viable (Watling 2006; Brierley 2010; Watling 2016). Additionally, heap bioleaching operations show significantly lower energy costs and lower emissions than traditional methods and can therefore be used to extract low-grade refractory ores in lieu of processes such as smelting (Watling 2006; Brierley 2010).

As with most scientific studies, this work focuses on a small piece of a much larger puzzle. Although this study targets a deeper understanding of microbial distribution and ecology, it would be short-sighted to imply that microbiology is the single most important factor in bioleaching productivity (Govender-Opitz et al. 2017). The success of a heap bioleaching system is dependent on many factors, including but not limited to permeability, temperature, site specificity, and acidity (Brierley 2010; Chiume et al. 2012; Govender et al. 2013; Watling 2016). Without an effective fluid flow, for example, microbial colonisation of the heap can be difficult to accomplish and to maintain (Govender-Opitz et al. 2017). The wide range of variables and interdisciplinary nature associated with the operation of these systems is a constant challenge in the successful development of heap bioleaching technology (Brierley 2010).

Metal recovery is also often too slow for industrial use, highlighting the additional obstacles presented by economics and recovery rates in the industrial development of heap bioleaching operations (Watling 2006; Brierley 2010). Greater knowledge of the poorly understood relationship between microorganism and ore can help to optimise the heap and better predict the overall system productivity (Watling 2016).

Another challenge to overcome with regards to heap bioleaching has to do with the magnitude of the heaps. Bioleaching heaps are large operations (kilometre-scale) and often have limited sampling access. This means that when measuring heap health on the industrial scale, microorganisms are quantified using only the cell numbers present in the leachate (Govender et al. 2013). This widespread sampling protocol led to a novel experimental study in 2013 by Govender et al., designed to investigate the microbial distribution within a laboratory (agglomerate) scale ore-bed.

1.2 PREVIOUS WORK

The Govender et al. (2013) experiment targeted a more comprehensive analysis of the microbial environments within heap bioleaching systems. This was due to the notion that bioleaching heaps consist of both liquid and substrate, meaning that the microbial communities are exposed to different environments within the ore-bed. In short, the hypothesis was that the leachate would not be an accurate representation of the whole-ore system during heap colonisation (Govender et al. 2013). The Govender et al. (2013) study separated the microbial habitats within an ore-bed into four distinct micro-environments. These are called “phases” and are defined as:

1) Leachate: The flowing liquid percolating through the heap which is collected as an effluent. The leachate carries the metal ions from the ore-bed to the processing plant.
2) Interstitial: The stagnant liquid present between particle interfaces.
3) Weakly attached: The microorganisms which are attached to the ore but not in a biofilm.
4) Strongly attached: The microorganisms which make up the biofilm, directly on the surface of the ore particles.

The Govender et al. (2013) assessment of the phases was carried out by harvesting replicate samples from the agglomerate scale ore-beds at regular intervals over the colonisation period (700 hours). The experiment used a pure culture of *Acidithiobacillus ferrooxidans* and a sterile ore, answering fundamental questions about the variations in microbial concentrations between phases. Most importantly the results suggested that the leachate cannot provide an accurate analogue of the whole-ore environment (all four phases) during heap

colonisation. For example, cell concentrations in the leachate stabilised 200 hours after inoculation, whereas the concentrations in the ore-associated phases (the interstitial, weakly attached, and strongly attached) continued to increase until 355 hours. In other words, the leachate showed no indication after 200 hours that the ore-associated populations were still increasing.

However, the Govender et al. (2013) study also left many questions unanswered in terms of non-sterile systems. The previous work left a gap in the research pertaining to the cell concentrations as the Govender et al. (2013) study examined the heap colonisation stage (700 hours) exclusively. This period constitutes only a fraction of the lifespan of any heap leaching operation, as they can be active for months to years (Watling, 2006). Presently, the notion that the leachate is not an accurate analogue to the whole-ore environment may not be applied past the 700-hour mark as there has been no testing past this point.

In industry, heaps consist of non-sterile ore inoculated with mixed cultures. To maintain a sterile environment on the kilometre scale would be extremely difficult as biological contaminants containing different strains of bacteria and archaea can be introduced in a multitude of ways. For instance, to keep the agglomerate-scale heaps sterile in the Govender et al. (2013) study all the input (liquid medium) needed to be autoclaved to remove any microorganisms that were introduced during preparation (in the water, on the lab-ware, the laboratory surfaces, in the chemicals, etc.). Therefore, in industry mixed cultures are more practical because only the organisms suited to the environment will survive, in time becoming more adept at life within the heap by building resistance to toxic concentrations of different elements (Watling, 2006). Additionally, using a pure culture did not provide insight into variation in community structure between the phases and the changes in the communities during the colonisation period.

Finally, the Govender et al. (2013) study focused only on the disparity between the cell concentrations within the system, meaning that there was no insight into the variation in metal concentration between the leachate and the ore-associated phases. A thorough understanding of the effects of metal concentrations on the organisms within a bioleaching heap is critical to a healthy system. This is because elevated metal concentrations can be detrimental to microbial health, which is directly related to extraction rates (Watling 2016).

1.3 AIMS

The main aim of this study was to quantify the cell and dissolved metal distributions within agglomerate-scale low-grade copper ore-beds over a six-month period, which is past the initial colonisation of the system. Cell distribution was monitored between each phase, that is, the leachate, the interstitial, the weakly attached and the strongly attached. Metal distributions were monitored between the leachate and the ore-associated phases which is a blanket term to describe the interstitial, the weakly attached, and strongly attached phases all at once.

Additional questions that stem from this central research target include:

» How do the results compare with previous work using the same agglomerate-scale technique but with a non-sterile ore and a mixed culture?

As discussed in Section 1.2, sterile ores are not a practical analogue of industrial-scale bioleaching systems as microbial contaminants are too easily introduced. This means that pure cultures (one species of bacterium such as *At. ferrooxidans*, for example) are not typically maintained in an industrial setting. By examining a mixed culture and a non-sterile ore using the same technique as the previous work by Govender et al. (2013), the disparity in the cell and metal distributions are examined in a setting closer to what may be found to an industrial heap. This allows for a more accurate comparison to be made between the laboratory scale studies and operational heaps.

» Does the microbial community in the leachate have the same structure as the communities present within the ore-bed?

Understanding community structure is important for insight into the environmental conditions and the ecological drivers in each phase. For example, if the community in the leachate has the same structure as those within the ore-bed, it means that the leachate is a diluted window into the community structure of the overall system. If this is the case, the leachate could be used as a proxy for the community structure of the ore-associated phases, provided higher cell concentrations are accounted for. Alternatively, an incongruence between the communities across the phases within the ore-bed means that each phase is

distinct, and that the environments which the organisms inhabit are variable. This also implies that sampling methods would need to be tailored to examine all phases within the bioleaching heap separately rather than as one entity.

» Does the indigenous microbial community remain intact throughout the experiment, or is the ore-bed colonised only by the mixed mesophilic culture?

The ore used in this study was non-sterile and therefore is likely to host its own indigenous microbial community. Although this may not necessarily inhibit bioleaching productivity, it may be that the community living on the ore is best adapted to life in that particular environment (Watling 2006, 2016). Understanding the relationship between the indigenous microbial community and the inoculum is a crucial factor in overall heap productivity and improved inoculation techniques.

» In a high temperature batch test experiment using stirred tank reactors containing the same ore but a thermophilic culture – what are the effects of lowered pH and increased iron concentration on cell growth?

Previous studies have shown that the interstitial phase is dominant in terms of cell numbers within agglomerate-scale ore beds (Govender et al. 2013; Bryan et al. 2016). Recognition of the limitations of this phase helps to improve overall understanding of the heap. The reactor batch tests will examine the effects of high metal concentrations and low pH on the microbial culture to help provide context as to any inhibition of the community in the interstitial phase. As the ore used in this study does not leach effectively at ambient laboratory temperatures (Escobar et al. 2000; Lee et al. 2011; Takatsugi et al. 2011) the batch reactor study will be performed at 63ºC to provide insight into the dissolved copper concentrations in a high temperature setting. Although it is important to note that while metal recovery is the driver for industrial bioleaching operations, this work is not focused directly on maximising copper extraction.

1.4 IMPORTANCE OF THE WORK

From a global economic standpoint, this work is valuable due to an ever increasing demand for copper (Lee et al. 2011). About 65% of the global copper supply is extracted from porphyry copper deposits, however these are typically

low-grade and cannot always be processed economically by traditional methods (Watling 2006; Lee et al. 2011; Ridley 2013). Bioleaching is an effective method for processing low-grade ores, however ores prominently associated with porphyry copper systems such as chalcopyrite ($CuFeS_2$) and enargite (Cu_3AsS_4) are not yet amenable to bioleaching at the commercial scale (Watling 2006, Brierley 2010). This suggests that the development of a bioleaching methodology for chalcopyrite and enargite bearing deposits would be of great economic interest as there are millions of tons of these ores awaiting processing (Watling 2006). Bioleaching is an ever-growing, interdisciplinary field, combining mineralogy, hydrometallurgy, microbiology, chemistry, and engineering to harness processes with huge economic and environmental importance. Information provided by microbiological scale biohydrometallurgical studies can be used to close the gap between laboratory studies and commercial operations (Brierley 2010; Chiume et al. 2012). Improving commercial operations then leads to a more widespread use of the technology by allowing the processes within heaps to become more predictable and to be optimised for a wider range of deposit types (Brierley 2010).

That said, reproducing industrial scale conditions within the laboratory is very challenging due to a multitude of physical factors that make heaps difficult to reconstruct at the agglomerate scale, especially the size of the industrial operations themselves (Watling 2006). For example, this study examines 150 g samples, while a bioleaching heap can easily be stacked up to six metres high (Watling 2006). Laboratory studies may also use a sterile ore or a concentrate to examine microbial relationships which is not practical in a heap bioleaching operation (Watling 2016). In addition to this, ground truth results pertaining to microorganisms working within heaps outside of cell numbers are difficult to obtain. As microorganisms and ions are too small to be seen, a degree of uncertainty is a common fixture in biohydrometallurgical studies. To address this, new experimental protocols are constantly being developed to help answer these questions such as the novel experimental system described in Govender et al. (2013).

This dissertation builds on the preliminary knowledge gained in the 2013 work by extending insight into the microbial ecology past the initial colonisation stage to a six-month period. By using a mixed culture and a non-sterile ore, the scope of the

disparity between phases will be indicated. This will add to previous findings related to the relationship between the leachate and the ore-associated phases, which on an industrial scale demonstrates the necessity of a specialized sampling protocol.

This study also examines the metal concentrations in the ore associated phases and the leachate, providing evidence for vastly differing conditions between the phases within an unsaturated system. It is crucial to understand the distribution of metals because the microbiological environment is extremely important to consider when assessing the health and productivity of a heap. Some metals in increased concentrations can be toxic to microbial cultures (Watling 2006). To better ascertain the degree to which microbial communities are affected, the growth of a thermophilic community in a bioreactor will be measured. This will examine the limitations of a community when faced with varying acidity and iron concentrations, as these conditions are a result of the bioleaching process (Schippers and Sand 1999). This in turn builds a greater understanding of the different environments in which these organisms can live. An improved grasp on the limitations of these communities also allows microbiologists to tailor cultures to withstand more adverse conditions (Watling 2016).

Furthermore, this work investigates the links between geochemistry and microbial community structure. The community structure is very closely related to the resistance of the microbial population to elevated metal concentrations (Watling 2016; Li and Sand 2017). Additionally, examining the community structure may provide better insight into the relationship between the leachate and the ore-associated phases. For example, if the community structure is the same in each phase, it means that the leachate is an accurate depiction of the whole ore environment, albeit a diluted one. Alternatively, variable community structure between phases implies that sampling protocols cannot rely on the leachate alone to understand the entire system. On a much larger scale, improved understanding of the community structure in a heap bioleaching operation can help provide erudition as to the resilience of communities in the face of increased metal concentrations.

Finally, this study uses a non-sterile ore, which may provide a more extensive depiction of the indigenous population. As industrial operations are so large, it is

not viable to sterilise the ores used in heap construction. The relationship between the indigenous community and the inoculum is variable between deposits and is rarely discussed in the literature. This is due to the secretive and competitive nature of the mining industry, which does not allow for data pertaining to tailored inocula to be published (Brierley 2010). It is, therefore, unclear as to how the indigenous culture affects the inoculum and their role in the community structure of a bioleaching heap. An investigation into the indigenous organisms is a useful tool as these populations are often best suited to their natural environments (Watling 2016).

CHAPTER 2. LITERATURE REVIEW

2.1 INTRODUCTION

This literature review provides background information on the nature and importance of copper and insight into why production from low-grade copper deposits is a critical driver in the mining industry. A summary of the geology of porphyry copper and high sulphidation epithermal deposits will also help to set the context for this study.

Central to the current thesis will be a review of heap bioleaching processes and the mechanisms which drive them, followed by a description of reactor bioleaching. This will be followed by an outline of fluid flow, metal concentration, and cell movement between the leachate, interstitial, and weakly and strongly attached phases. The novel agglomerate-scale heap bioleaching process will then be discussed in detail, along with the microbial distribution of an agglomerate-scale leaching experiment. This chapter will conclude with a summary of studies that have examined the bioleaching of enargite ores.

2.2 COPPER

Copper is a base metal present at an average of around 55 parts per million (ppm) in the Earth's crust (van Loosdrecht et al. 1990; Ridley 2013). In ore deposits, copper is generally present in low-grades at or below 1% (Ridley 2013). The extraction of copper from low-grade deposits is an important area of research and development as there are a wide-range of low-grade deposits and waste dumps that are not currently economically exploitable due to technological, economical, and environmental limitations (Watling 2006; Brierley 2010). Worldwide copper production is 15.5 million tonnes per year, with 3% growth over the last 20 years (Ridley 2013). When prices are approximately $7,000 USD per ton, copper production generates $108.5 billion in revenue per year globally (Ridley 2013). Porphyry copper deposits are the dominant source of copper produced worldwide at roughly 65% (Sillitoe 2010; Ridley 2013).

2.3 ORE DEPOSITS

2.3.1 PORPHYRY COPPER DEPOSITS

Porphyry copper deposits are large, low-grade ore deposits centred on large volumes (up to 100 km^3) of hydrothermally altered rock (Sillitoe 2010; Ridley

2013). Most are found in continental arcs such as the Andes which hosts a 3000 km long, 20+ km wide system of deposits parallel to the continental margin starting in Northern Peru and going South (Ridley 2013). Porphyry copper deposits are related to felsic to intermediate porphyritic intrusions that lie at depths of 1-10 km below the base level of a volcano and within a few kilometres of the top of the underlying intrusion (Ridley 2013). Some well-known deposits are El Teniente, Chile (CODELCO), Bajo de Alumbrera, Argentina (Minera Alumbrera), and Bingham, USA (Rio Tinto). The grades and tonnages of these deposits are listed in Table 2.1 (Dicken et al. 2016). Ore minerals associated with porphyry copper systems are copper sulphides, molybdenite (MoS_2), native gold (Au), and tin (Sn) tungsten (W) bearing oxides (Ridley 2013). In porphyry copper systems, the minerals are typically disseminated or occur in veins and veinlets that form dense stockworks (Sillitoe, 2010). Copper grade typically increases towards the centre of the deposit (Ridley 2013).

Table 2.1: Grades and tonnages of some examples of porphyry-Cu deposits, information from USGS Mineral-Resources online spatial data (Dicken et al. 2016).

Mine	Owner	Cu Grade (%)	Tonnage (Mt)	Au Grade (g/t)	Mo Grade (%)
El Teniente, Chile	CODELCO	0.62%	20,731	0.005	0.019
Bajo de Alumbrera, Argentina	Minera Alumbrera	0.53%	806	0.64	N/A
Bingham, USA	Rio Tinto	0.88%	3,230	0.38	0.053

Another significant aspect of porphyry copper deposits is that they can be overlain by high-sulphidation epithermal gold deposits, as shown in Figure 2.1 (Simmons et al. 2005; Sillitoe 2010; Teal & Benavides 2010; Ridley 2013).

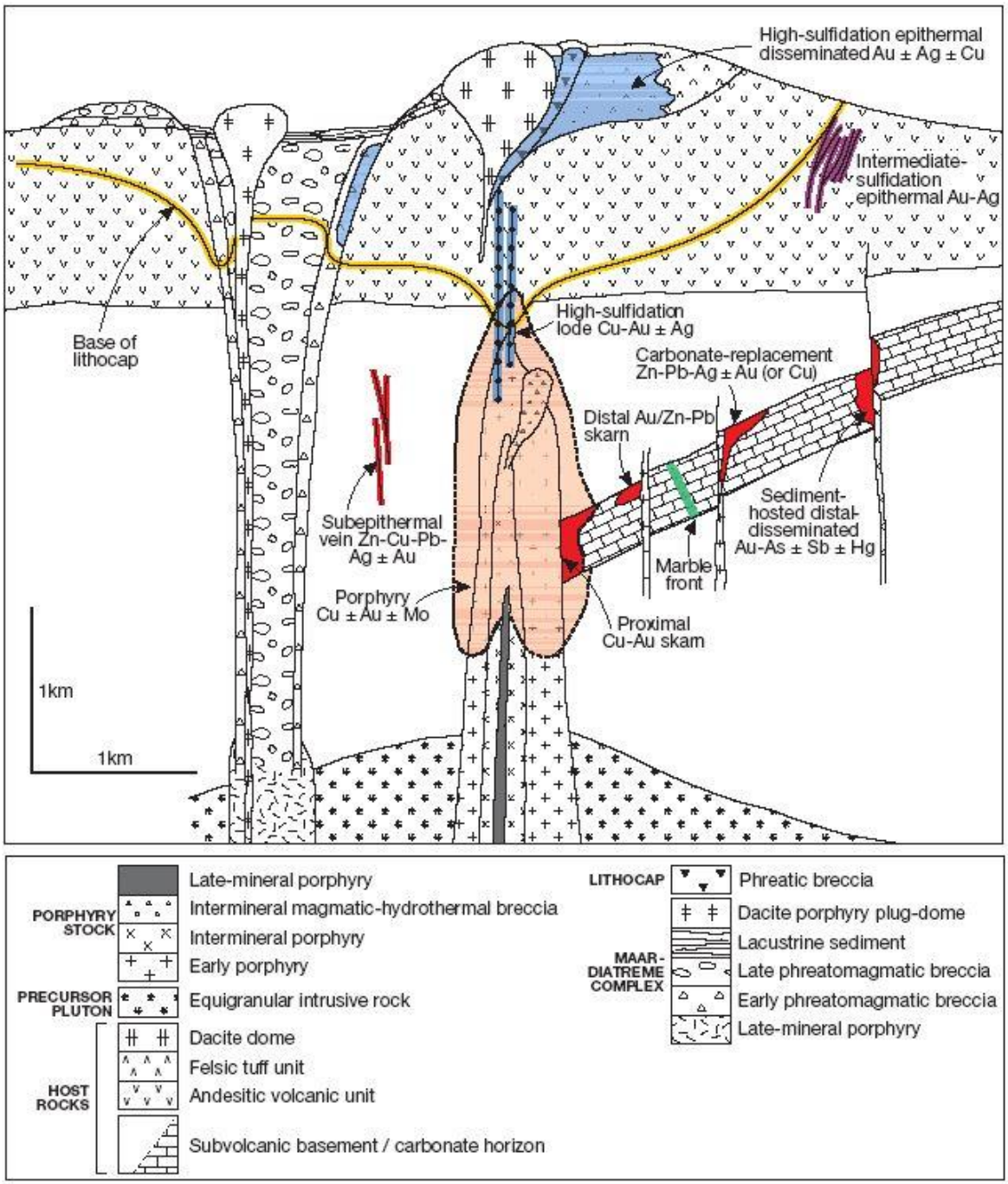


Figure 2.1: Diagram of porphyry copper and epithermal gold mineralisation from Sillitoe (2010). Note the base of the lithocap within the andesitic volcanic unit. This is the transitional zone between porphyry copper and high-sulphidation Au-Ag deposits.

2.3.2 High-Sulphidation Au – Ag Deposits

Epithermal deposits are low-temperature ore formations associated with volcanic centres that yield base metals such as copper or precious metals such as gold (Ridley 2013). They form close to the surface, therefore preservation typically limits deposits to Cenozoic age or younger (Ridley 2013). They can be separated into two groups based on their sulphidation state which refers to the sulphur

fugacity (fS_2) of the system, a term describing the gas pressure of molecular sulphur in the hydrothermal ore forming fluid (Ridley 2013). Fugacity affects the ore mineralisation as it controls the valence states of chalcophile elements which readily combine with sulphur, including gold, arsenic, and copper (Ridley 2013).

High-sulphidation epithermal gold deposits are closely related to porphyry copper deposits, lying between 0.5 - 1.5 km from the surface and no less than two kilometres from the volcanic centre (Sillitoe 2010; Ridley 2013). They often form in a lithocap, an area barren of ore and highly silicified (Sillitoe 2010; Ridley 2013). Yanacocha (Cajamarca, Peru) is currently the best-known example of this high-sulphidation porphyry-copper relationship (Gustafson et al. 2004; Simmons et al. 2005; Sillitoe 2010). Some additional deposits that also show the transition between porphyry copper and high sulphidation deposits, are the Hauraki goldfields in New Zealand, Mount Polley in British Columbia, and El Abra in Chile (Sillitoe 2010).

High sulphidation deposits typically contain ore minerals such as pyrite, enargite, bornite and covellite, and the rocks surrounding the deposit (intermediate to felsic volcanic rocks or intrusive rocks) are leached of non-silicate elements (Simmons et al. 2005; Ridley 2013).

2.4 Geology of the Yanacocha region

Yanacocha is a world class high-sulphidation epithermal gold deposit underlain by shallow porphyry copper gold systems in northern Peru (Simmons et al. 2005; Sillitoe 2010; Teal & Benavides 2010). It was discovered in 1984 as a potential gold deposit with production commencing in 1993 and peaking in 2005 at 3.33 million ounces of gold (Teal & Benavides 2010). In 2016, 560 thousand ounces of gold were produced at Yanacocha (Newmont 2017). It is currently under the ownership of Newmont Mining (51.35%), Minera Buenaventura (43.65%), and the International Finance Corporation (5%).

The shallow porphyry deposit is known as the Kupfertal Au-Cu porphyry deposit and consists of a supergene (downward mobile fluid) sulphide blanket composed of covellite (CuS) and chalcocite (Cu_2S) (Teal & Benavides 2010). This is mixed with hypogene (originating below surface) enargite (Cu_3AsS_4) formed from upward mobile fluid (Teal & Benavides 2010). Enargite is a refractory mineral

which is not amenable to biohydrometallurgical techniques at ambient laboratory temperature and will be discussed further in Section 2.5 and 2.8. The chalcocite and covellite are secondary assemblages and are easily leachable while the hypogene enargite is considered a refractory compound (Watling 2006; Lee et al. 2011). The enargite can be linked to the upper regions in the Kupfertal deposit as pyrophyllite is a clay mineral which generally occurs in the argillic alteration zone of porphyry copper deposits (Figure 2.1) and the deeper zones of high-sulphidation epithermal gold deposits (Ridley 2013). In addition, enargite was not seen in drill cores below 325 m (Gustafson et al. 2004).

Teal & Benavides (2010) describe the discovery of the Kupfertal porphyry Au-Cu deposit as "a new chapter of deeper exploration beneath gold-bearing high sulphidation (now oxidized) caps". This statement reflects how deposits like Yanacocha highlight an important aspect in the development of copper exploitation. Deposits unknown or unrelated to the original exploration program and mine development plan that are not the first exploration target have the capacity to become profitable with the appropriate technology (Gustafson et al. 2004; Watling 2006). In other words, minerals like enargite or chalcopyrite, that have previously been put aside as waste, could become economically important if the technology to process them efficiently and profitably is available (Watling 2006). As heap bioleaching technologies become more extensively understood they become more dependable. This increases the likelihood that the technology will be employed to fill the existing gaps in ore-processing of low-grade systems with multiple refractory minerals (Brierley 2010).

2.5 ENARGITE

Enargite is often a constituent of high-sulphidation deposits (Lee et al. 2011; Ridley 2013), frequently occurring with other copper minerals such as chalcocite (Watling 2006). Enargite is not amenable to traditional processing methods as it does not separate from other copper sulphides during flotation or results in highly toxic concentrates, due to the arsenic content (Watling 2006; Corkhill et al. 2008; Takatsugi et al. 2011). Bioleaching enargite is not yet viable at the industrial scale, however, several studies have examined the process at the laboratory scale with varying success which will be discussed in detail in Section 2.8

(Escobar et al. 1997; Escobar et al. 2000; Corkhill et al. 2008; Lee et al. 2011; Takatsugi et al. 2011).

Aside from economic driving factors, a more intricate understanding of enargite precipitation and oxidation pathways could potentially be used to develop methods for remediating acid mine drainage systems rich in dissolved arsenic (Escobar et al. 2000; Takatsugi et al. 2011).

2.6 BIOLEACHING

2.6.1 OVERVIEW

The challenges faced by the mining industry to process waste rock or low-grade ores creates a niche for technologies such as bioleaching (Watling 2006; Brierley 2010). The benefits of bioleaching are widespread, especially the ability to process refractory ores (Watling 2006; Brierley 2010; Lee et al. 2011). Bioleaching can be applied commercially using four primary methods, heap-, dump and *in-situ*-leaching, and stirred tank reactors (Rawlings 2004). It can be used to solubilise or concentrate a variety of metals such as copper, cobalt, uranium, nickel, and gold (Rawlings 2004). There are a host of patented bioleaching techniques that have been, or are currently being used industrially, including but not limited to BIOPRO™ (Newmont Mining Corporation) which uses heap leaching to extract gold, GEOCOAT® (Geobiotics) which uses a combination of heap leaching and stirred tanks to recover gold, BIOX™ (Outotec) which uses stirred tanks to pre-treat ores before they are processed for gold recovery, and BioNIC® and BioCOP® (Billiton) which use stirred tanks to extract nickel and copper, respectively.

In Brierley (2010), the commercial challenges of heap bioleaching are discussed, one of which is the risk companies take in implementing the technology. Another obstacle discussed is the complexity of developing a suitable process guarantee whilst industrial-scale processes are in their infancy. This allows for a range of innovative interdisciplinary research to take place focusing on a wide variety of parameters.

2.6.2 THE HEAP BIOLEACHING PROCESS

Heap bioleaching relies on the percolation of fluids through large stacked heaps of crushed ore (Govender-Opitz et al. 2017). As outlined in Watling (2006), preparation for heap leaching is a three-step process starting with curing, when crushed ore is mixed with concentrated acid to extract acid soluble metals and waste materials. The particles are then consolidated in a rotating drum for wetting and acidification. The system is semi-saturated through agglomeration with acidified water. The agglomerated ore is then stacked onto an impermeable liner and then acid leached, meaning acid (pH <1) is dripped or sprayed on the heap and allowed to percolate through the material for a fixed period. The flowing liquid (leachate) then carries the liquid containing the readily-dissolved metal ions liberated by leaching from the heap into a collection pool. This step can recover 80% of the copper from copper oxides and about 40% of the copper from minerals such as chalcocite in a short duration leach. After the acid leach, the heap is typically inoculated. There are a variety of existing inoculation strategies, mainly input of liquid from a pond with a microbial population or inoculation during acid agglomeration followed by stacking of the ore. In both methods, the culture may be tailored specifically to the ore minerals or the heap conditions. After inoculation, a higher pH leaching reagent (lixiviant), usually between pH 1.0 and 2.0 is then sprayed or dripped on top of the ore so it can percolate through the heap allowing remaining metals to be solubilised by chemical attack of the microbially-oxidised ferric ions. The mechanisms and reactions are described in detail in Section 2.6.5 and Section 2.6.6.

The heap is aerated either passively (via leaching solution) or air is blown upwards into the heap using pipes (Rawlings 2004; Govender-Opitz et al. 2017). This increases bacterial productivity as the microorganisms can be facultatively anaerobic, meaning they are capable of using oxygen if it is available but they can also adjust to anaerobic conditions if necessary (Rawlings 2004). Within the system, oxygen is used as an electron acceptor and CO_2 is used as a carbon source (Rawlings 2004). The pregnant solution, now containing soluble metals, is collected and refined by solvent extraction and electrowinning (SX-EW), and the raffinate, which is the leachate after the metal ions are removed (post SX), is recycled back to the top of the heap as shown in Figure 2.2 (Rawlings 2004; Watling 2006).

The bioleaching process can be defined in short as the microbial oxidation of iron (and sulphur) resulting in the solubilisation of ores (Schippers & Sand 1999; Rawlings 2004; Watling 2006). The microorganisms at work in a bioleaching system are typically a combination of acidophilic (acid-loving) bacteria and archaea; both of which use CO_2 as a carbon source and derive energy from oxidizing inorganic materials such as iron and sulphur (Schippers & Sand 1999; Rawlings 2004). The microorganisms associated with heap bioleaching are found at sites of acid mine drainage or geothermal areas (Watling 2016). The microbially-supplied ferric iron (Fe^{3+}) in the system then chemically solubilises metals, as shown in Section 2.6.5 and Section 2.6.6 (Rawlings 2004; Sand & Gehrke 2006). Meanwhile, sulphur oxidizing bacteria generate sulphuric acid by oxidising reduced inorganic sulphur compounds which maintain acidic heap conditions (Rawlings 2004). This process will be discussed further in Section 2.6.4, Section 2.6.5, and Section 2.6.6.

Microorganisms are affected by four main physiochemical factors, as described in Watling (2016): temperature, pH, the concentrations of cations (e.g. Cu and As), and the concentrations of anions (e.g. SO_4, Cl). Attachment of the microorganisms to the ore-bed is affected by fluid flow (convection), surface mineralogy, and concentration gradients, all of which are important to comprehend as ore colonisation also plays a direct role in the efficiency of heap bioleaching (Govender et al. 2013).

Overall leaching performance is affected by fluid flow within the heap and can cause many challenges in engineering and design as heap systems are often irrigated from the top of the bed (Fagan et al. 2014). In addition to this, heaps need to be fluid unsaturated because oxygen is relatively depleted in saturated areas (Chiume et al. 2012; Fagan et al. 2014). Modelling of heaps is therefore challenging, also due in part to the particle size distribution of the ore and decreased permeability of the bed (Fagan et al. 2014). Prokaryotes (bacteria and archaea) are only about 1-2 µm in diameter (van Loosdrecht et al. 1990), meaning that visual clues cannot be relied upon (Chapter 1, Section 1.4).

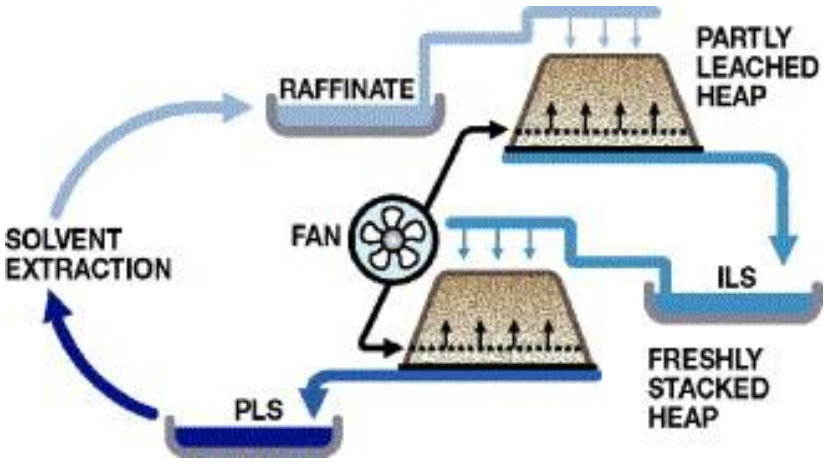


Figure 2.2: A schematic of a heap bioleaching operation (Watling 2006). "ILS" stands for Intermediate leachate solution. PLS refers to the Pregnant Leach Solution which is leachate that is carrying dissolved metals. In this dissertation leachate will be used to describe the liquid flowing from the heap due to its low copper content.

2.6.3 BIOLEACHING USING STIRRED TANK REACTORS

Stirred tank reactors (Figure 2.3) are typically used in the mining industry for biooxidation of gold-bearing concentrates (Acevedo 2000). This is because they are not amenable to processing large quantities of ore and rely on a high commodity price to be economical (Rawlings and Johnson 2007; Bryan et al. 2011). Stirred tank reactors are constantly agitated for homogeneity and to prevent settling (Rawlings and Johnson 2007). In addition, stirring provides an increased rate of transfer between the nutrients and the cells, the metabolic products from the cells and the liquid, and the solubilised species from the mineral surface and the liquid (Acevedo 2000). Aeration (addition of oxygen and carbon dioxide) is also actively added to stirred tank reactors where oxygen acts as the electron acceptor and carbon dioxide as the carbon source (Acevedo 2000). Stirred tank reactors are also advantageous as unlike bioleaching heaps, they can be controlled in terms of temperature and pH (Rawlings and Johnson 2007). Figure 2.3 shows a generic depiction of a stirred tank reactor.

Continuous stirred tank reactors (CSTR) are considered to be in steady state and homogenous at any point within the vessel (Fogler 2006). These reactors are generally non-sterile, with little or no monitoring or control over the microbial community at work (Bryan et al. 2011). CSTRs can also be used in series to process higher quantities of ores in different stages with a variety of temperatures and cultures (Acevedo 2000).

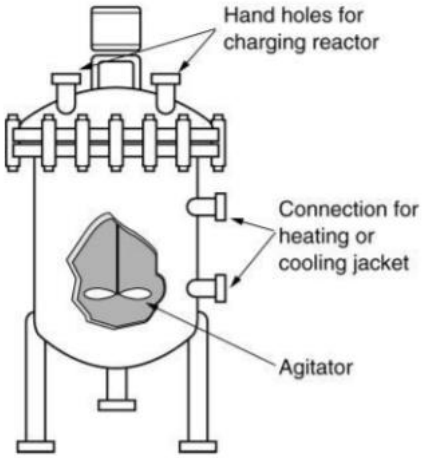


Figure 2.3: Diagram of a stirred tank reactor (Fogler 2006).

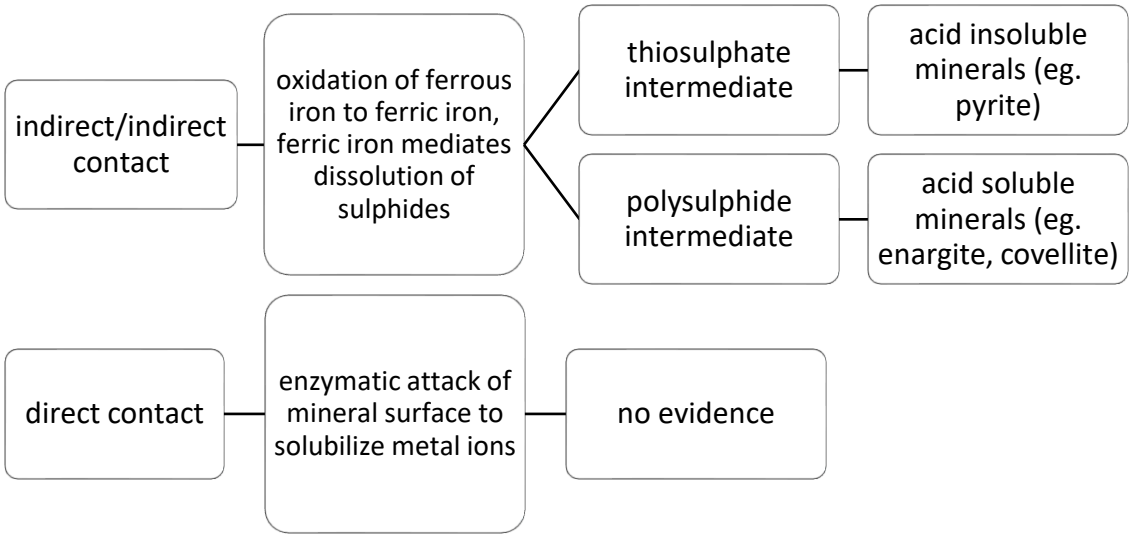
2.6.4 BIOLEACHING MECHANISMS

Bioleaching mechanisms can be separated into two groups depending on the acid solubility of the mineral: the thiosulphate intermediate or the polysulphide intermediate pathways (Schippers & Sand 1999). They can be further divided based on whether solubilisation is a product of attached or unattached microorganisms; a brief outline of this is shown in Figure 2.4 (Schippers & Sand 1999; Tributsch 2001; Crundwell 2003). Indirect contact leaching implies that microorganisms attached to mineral surfaces create favourable conditions for leaching, and indirect non-contact leaching refers to the same process by unattached microorganisms (van Loosdrecht et al. 1990; Schippers & Sand 1999). The terms indirect and indirect contact were proposed by Tributsch (2001) who stated that “direct” implies bioleaching is occurring due to a direct enzymatic process, and therefore the term “contact” leaching should be used instead. This idea has been largely accepted, and was further refined by Crundwell (2003). Therefore, even though a microorganism may be attached to a mineral surface, there is no transfer of metal ions directly from the microorganisms to the solution (Sand & Gehrke 2006). This also means that even though minerals are leached faster in the presence of bacteria, they are not using an alternate mechanism of dissolution, but simply accelerating the existing chemical dissolution process of the mineral by providing an increased level of ferric iron to the system (Crundwell 2003). It’s important to remember that bacteria oxidise iron continuously without considering consequence, and can create lethal environments for themselves

through runaway conditions such as excessive heat, low pH, and increased dissolved ion concentrations (Watling 2016).

Cells that are growing on the mineral surface such as those seen in a biofilm are thought to house the leaching reactions within their extracellular polymeric (EPS) layer (Figure 2.5), between the cell wall and the mineral surface (Rawlings 2004; Sand & Gehrke 2006). The cell itself oxidises the iron and then the EPS layer provides a venue for chemical attack of the sulphide by the microbially-generated ferric iron (Rawlings 2004; Sand & Gehrke 2006). Electrochemistry is also thought to affect the dissolution rates in some instances, as different sulphides with varying electric potentials can become polarised, resulting in a slower dissolution rate of the mineral (Sand & Gehrke 2006). Direct contact (i.e. enzymatic attack to free metal ions) has not been proven at this time (Crundwell 2003; Watling 2016).

Figure 2.4: Table showing the mechanisms and intermediates associated with bioleaching, based on the classification from TRIBUTSCH (2001)*, and* CRUNDWELL (2003)*.*



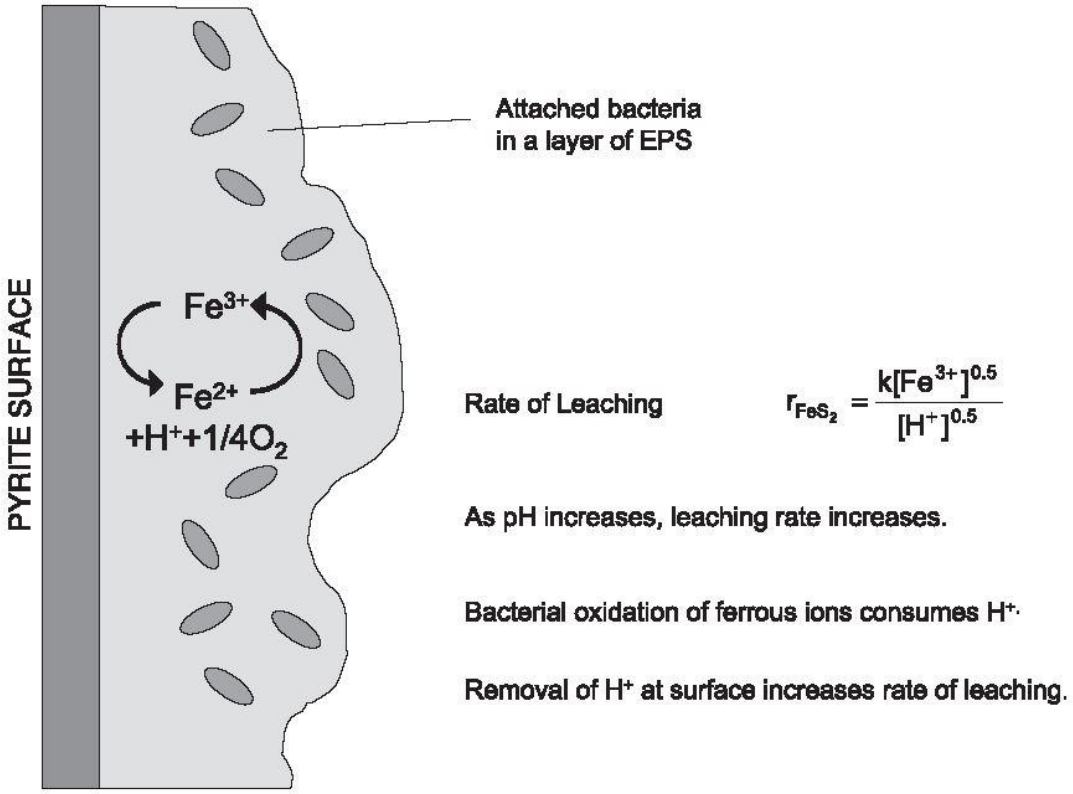


Figure 2.5: Diagram from Crundwell (2003) showcasing the indirect nature of bioleaching. See reactions in Section 2.6.5 and 2.6.7.

2.6.5 THIOSULPHATE INTERMEDIATE

Acid-insoluble sulphides (such as pyrite) are oxidized to metal ions and sulphate via the thiosulphate intermediate (Equations 2 and 3), seen in Figure 2.6, as proposed by Schippers & Sand (1999).

$$FeS_2 + 6Fe^{3+} + 3H_20 \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+ \quad (2)$$

$$S_2O_3^{2-} + 8Fe^{3+} + 5H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+ \quad (3)$$

The 2003 study by Crundwell showed that pyrite leaching occurs faster in the presence of bacteria than in the absence of bacteria (where the same ferric iron concentrations were maintained electrochemically). It was suggested that this is due to pH changes on the microbial scale, as the bacteria enabled a rise in pH at the mineral surface. Increased pH in proximity to the mineral surface increases the rate of pyrite leaching (Crundwell 2003). This finding provided evidence for the argument that leaching is induced indirectly by bacterial processes.

2.6.6 POLYSULPHIDE INTERMEDIATE

Minerals such as enargite and covellite that are (technically) acid-soluble are bioleached via the polysulphide mechanism (Equations 4, 5, 6*, and 7*) seen in Figure 2.6, from Schippers & Sand (1999).

* where MS is a metal sulphide and ⇨ implies microbial activity

$$MS + Fe^{3+} + H^{+} \rightarrow M^{2+} + 0.5H_2S_n + Fe^{2+} \ (n \geq 2) \quad (4)$$

The Fe^{2+} from Equation 4 (as well as Equations 2 and 3) is then available to be oxidized by microorganisms to produce Fe^{3+}, which is used as a reactant in Equation 4 (and 2 and 3).

$$2Fe^{2+} + 0.5O_2 + 2H^{+} \Rightarrow 2Fe^{3+} + H_2O \quad (5)$$

Equation 6 produces elemental sulphur, which can in turn be oxidised by microbial activity (Equation 7).

$$0.5H_2S_n + Fe^{3+} \rightarrow 0.125S_8 + Fe^{2+} + H^{+} \quad (6)$$

$$0.125S_8 + 1.5O_2 \Rightarrow H_2O + SO_4^{2-} + 2H^{+} \quad (7)$$

In a bioleaching system there are also bacteria present that oxidise sulphur (Equation 7) which is crucial to the overall process as they release protons into the system to allow further dissolution of sulphides (Rawlings 2004). Aside from that, acid generation is an important factor in terms of reducing costs as it reduces the need for acid addition (Brierley 2010).

Overall leaching of enargite by ferric iron is shown in Equation 8, as seen in Lee et al. (2011).

$$Cu_3AsS_4 + 4.5Fe_2SO_4 + 2H_2O \rightarrow 3CuSO_4 + 9FeSO_4 + 4S + HAsO_2 + 1.5H_2SO_4 \quad (8)$$

Equation 9 shows the dissolution of covellite.

$$CuS + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2FeSO_4 + S^0 \quad (9)$$

Although the chemistry can be explained by a series of reactions, it's important to reiterate that there are many additional factors at play on the microbial scale (therefore affecting the rate of iron oxidation) in a heap bioleaching system such as electrochemical cell interaction with the mineral interface and the processes ongoing within the cell (Watling 2016; Govender-Opitz et al. 2017).

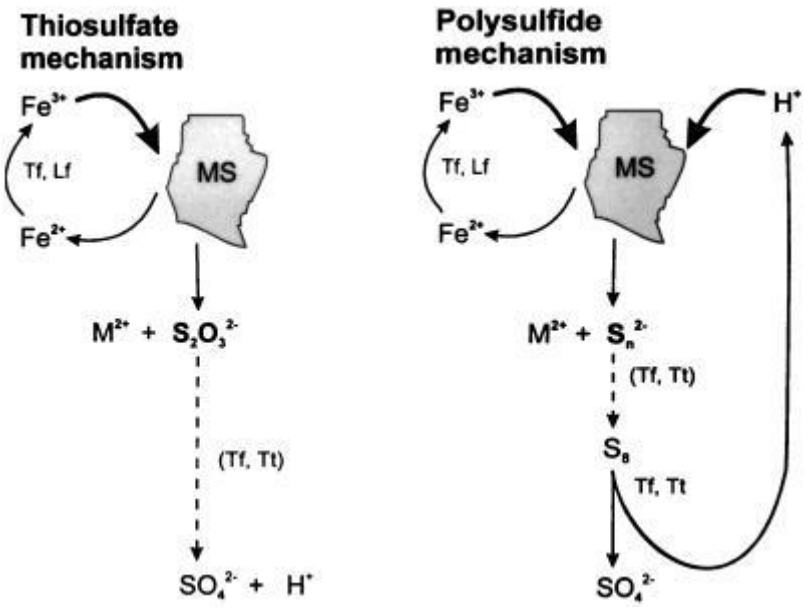


Figure 2.6: Schematic diagram depicting the thiosulphate and polysulphide pathways (Schippers & Sand 1999). Tf is Thiobacillus ferrooxidans, Lf is Leptospirillum ferrooxidans, and Tt is Thiobacillus thiooxidans.

2.6.7 FLUID FLOW

Fluid flow and mineral-fluid interaction is the backbone of bioleaching systems (Govender-Opitz et al. 2017). Understanding how and why fluid moves helps to provide an idea of how metals are distributed and how they travel within the system. Fluid flow can be examined by performing a residence time distribution study, as discussed in Fogler (2010). Residence time distribution studies provide insights into the hold-up areas of the system and the quantity of liquid

sequestered in different phases, but they do not provide an in-depth look at the flow within the heap and which areas are wet and dry (Fagan et al., 2014).

As a result of this, a study by Fagan et al. (2014) used magnetic resonance imaging (MRI) to map the flow of fluid through a laboratory-scale heap. The heap was drip irrigated at the top of the bed and fluid flow was controlled by gravity and capillary suction (lateral movement of fluid). Although drip irrigation is common in industry, it had not been previously examined in detail. Results showed that capillary suction was less prominent in wetted zones, where gravitational movement was dominant. In the upper section of the heap the bed was not wetted, meaning that there was limited microbial and metal transport in this area. The highest concentrations of metals and cells were found to be in the areas with the highest volume of fluid passing through. The wettest zones were directly under the irrigation feed and other gravitationally and channelled wet areas (in the top two thirds of the heap).

2.6.8 METAL CONCENTRATION

Metal concentrations within a heap also play a substantial role in microbial performance, as increased metal concentrations can be toxic to microbial populations (Watling 2006). This means that the bioleaching process itself can be detrimental to the organisms that catalyse it as metal ions enter solution (Brierley 2010; Watling 2016). Different organisms respond differently to increased metal concentrations, for example *Acidithiobacillus ferrooxidans* effectively oxidises ferrous iron at Cu concentrations up to roughly 50 g/L whereas *Sulfobacillus thermosulfidooxidans* is no longer metabolically active at Cu values of 0.4 g/L (Dopson et al. 2003).

There are some methods that combat the detrimental effects of high metal concentrations on the microbial populations in a bioleaching system. Indigenous bacteria, for instance, often possess a higher tolerance to increased metal concentrations in their environments (Watling, 2006). Additionally, successive culturing of non-indigenous cultures allows microorganisms to thrive in environments that would normally be harmful to them (Watling 2016). Although some strains of organisms (indigenous or artificially adapted) are more resistant to increased metal concentrations it does not mean that the strain will be more effective at leaching metals (Watling 2016).

Metal concentrations were examined using the agglomerate-scale technique (Govender et al. 2013) in a study by Bryan et al. (2016). This study found that metal concentrations were incongruent between the interstitial phase and in the leachate, with copper being 150 times higher in the interstitial phase and iron 10 times higher. In addition, Bryan et al. (2016) examined the community structure between the effluent and the interstitial stagnant liquid. Results were not statistically significant likely as a result of the sample size. More research needs to be done to clarify the relationships between metal concentrations and the ecology of microbial communities.

2.6.9 Bioleaching and acid mine drainage

Volant et al. (2014) examined the microbial community structure at six sites within an acid mine drainage system in Carnoulès, France. They found that the community structure changed depending on varying physiochemical conditions such as temperature, pH, and dissolved ions. This highlights that variations in microbial ecology are found on a large scale in such systems. This large-scale difference in community structure may be seen in the ore associated phases as discussed in Govender et al. (2013) and Bryan et al. (2016). This is because both the large-scale acid mine drainage study and the agglomerate-scale studies operate on the same theory – that different physiochemical conditions result in rearrangements of microbial communities. There are a few key differences in the Volant et al. (2014) study and the agglomerate-scale work, most importantly that the 2014 study used filtered water samples, meaning that the attached ore-associated phases were not observed. Considering results from Govender et al. (2013) and Bryan et al. (2016) this is then unlikely to be accurately representative of the whole ore environment and the bacteria responsible for metal solubilisation, as will be further discussed in Section 2.9.

2.7 Cell movement

Cell movement between particles is critical in understanding the relationships between the phases within an ore-bed. van Loosdrecht et al. (1990) discussed the methods of transport within a microbial environment: diffusive transport, convective transport, and active movement. Convective transport is tied to fluid flow, whereas diffusive transport and active transport are related to metal concentration. Diffusive transport means that cells only encounter particles by

chance, whereas active transport is when cells seek out areas of high concentration and actively swim towards it, a process called chemotaxis (Sand & Gehrke 2006; Chiume et al. 2012; Govender et al. 2013). Chemotaxis is one of the proposed mechanisms of microbial movement discussed in Govender et al. (2013) although it must be noted that the type strain of *At. ferrooxidans* used in this study is non-motile.

Transport allows microorganisms to come into contact with mineral surfaces where they may attach reversibly or irreversibly, however it can be hard to distinguish whether cells are permanently attached or not (van Loosdrecht et al. 1990). Cells then attach to the mineral before colonising the surface in a biofilm, which is a self-produced matrix of extracellular polymeric substances or EPS (Sand & Gehrke 2006; Li & Sand 2017). Sand & Gehrke (2006) discuss the involvement of the extracellular polymeric substance (EPS) layer in cell to mineral attachment and mineral dissolution. They found that EPS formation facilitated attachment to metal sulphides and tried to concentrate metal ions needed as energy sources.

Planktonic (free floating) cells such as those found in the leachate, as opposed to cells that are attached to the ore surface, are not the same. Attached cells have less protein and polysaccharides but more dead cells and glucuronic acids (Li & Sand 2017). The colonised cells are thought to accumulate dead organic material in order to improve survival rates while the increased glucuronic acid is associated with cell growth and is especially important in terms of biofilm development (Li & Sand 2017).

The proposed colonisation of the surface is important to consider with regards to this study because the attached phases are thought to be an integral part of the bioleaching process (Govender et al. 2013). This is due to higher cell numbers in the attached phases compared to those seen in the leachate (Govender et al. 2013). Understanding the reasons why more cells attach and how that occurs is a critical factor in the interpretation of these systems.

van Loosdrecht et al. (1990) states that reversible adhering cells are released back into the medium. This makes up the strongly attached (biofilm) and weakly attached phase (Govender et al. 2013). The interstitial phase may be the cells

that are released back into the medium or cells that move from the leachate into the stagnant zones (Govender et al. 2013).

2.8 Overview of previous work - bioleaching enargite

Enargite is a refractory sulphide which contains arsenic and is often associated with large, low grade deposits (Section 2.3). The arsenic content of enargite makes its stability important from an environmental perspective (Escobar et al. 1997; Escobar et al. 2000; Watling 2006; Lee et al. 2011; Takatsugi et al. 2011). Escobar et al. (1997) showed that enargite was not solubilised in shake flasks at 30°C, with only 9% dissolution reported in a bioleaching experiment using ferric iron solution. The study also showed that in a flask without ferric iron, the number of active bacteria declined as solubilisation took place. This means that the dissolved arsenic caused an inhibitory effect on the microorganisms in the absence of ferric iron. Escobar et al. (1997) then came to the conclusion that the dissolved arsenic bonded with ferric iron and was precipitated, thus removing the toxic arsenic cation from the system. Corkhill et al. (2008) similarly demonstrated that enargite does not leach well at 30°C using *Leptospirillum ferrooxidans* in shake flasks. This may also have been due to a lack of iron in the system, as enargite does not contain iron. Iron must be present in another mineral or in the feed in order to fuel the bioleaching mechanism (Escobar et al. 1997).

In Escobar et al. (2000), the mechanisms and kinetics of chemical and bacterial leaching of enargite were examined using *Sulfolobus* sp. BC at 70°C in shake flasks. The experiment ran for 550 hours and showed a significantly higher leaching capacity in the flasks inoculated with bacteria than in the chemical leach only. In addition to this, the study examined the difference between the microorganisms leaching with and without ferric iron in the medium. The solubilisation of copper was three times greater in the flasks containing ferric iron because, as discussed above, it allowed for the precipitation of ferric arsenate, removing dissolved arsenic from the system. This meant that the bacteria in the flasks with ferric iron medium were protected against elevated levels of dissolved arsenic. These two experiments highlight that the presence of dissolved arsenic in the system (without ferric iron) can have an inhibitory effect on microbial life.

A study by Lee et al. (2011) used 6 different composite samples to determine the leaching potential of Newmont's ore from Yanacocha Verde both at ambient

laboratory temperature (20°C) and at 65°C. The cultures used were a mesophilic culture containing *Acidithiobacillus* sp. and *Leptospirillum* sp. and a thermophilic mix of consortia composed of *Acidianus* sp, *Metallospheara* sp, and *Sulfolobus* sp. The composites were made up of an enargite-rich ore, a covellite-rich ore, and a chalcocite-rich ore. The chalcocite was effectively leached at mesophilic temperatures. In the enargite and covellite samples, 7.3-21% leaching was measured at mesophilic temperatures and 62-98% at thermophilic temperatures over a one-year period. The enargite composites (95% enargite) were the least amenable to leaching at ambient temperature, showing less than 15% copper extraction, with no solubilised copper attributed to enargite leaching at mesophilic temperatures. At thermophilic temperatures the percentages in solution were 80.5% and 90.4% (Figure 2.7). Additionally, the columns were drained and re-inoculated at day 160, which is thought to boost resilience to elevated arsenic concentration as is also discussed in Escobar et al. (1997). The presence of arsenic compounds was not thought to affect the leaching capacity of the system in Lee et al. (2011). In the mesophilic composites containing chalcocite, a small portion of covellite and enargite remained in the residue and was not leached. In backscattered electron (BSE) images taken post leaching, there is some evidence of microbial oxidation on the outside of the enargite grains but otherwise they are largely intact under mesophilic conditions, so there is no source of dissolved arsenic present. The other ore minerals were almost completely leached from the system.

An experiment done by Takatsugi et al. (2011) showed that *Acidianus brierleyi* effectively leached 90.9% of copper from an enargite bearing ore over 27 days at 70°C in shake flasks. Only a 5.9% recovery of arsenic was reported due to precipitation of iron arsenic compounds (ferric arsenate) and cupric arsenic. The enargite was coated in a secondary layer of sulphur via a solid-solid reaction, and then jarosite was precipitated on the surface due to electrostatic interactions between cations (Fe^{3+}) and the EPS layer formed by the bacteria. Jarosite is known to inhibit the productivity of bioleaching systems by clogging pore spaces and blocking fluid flow (Leahy & Schwarz 2009). The results imply that cultures like *A. brierleyi* may be suitable for treatment of hot arsenic-rich wastewaters (up to 70°C) by precipitation.

The studies by Escobar et al. (1997), Corkhill et al. (2008), and Lee et al. (2011) all show that enargite is not amenable to leaching at ambient laboratory temperatures.

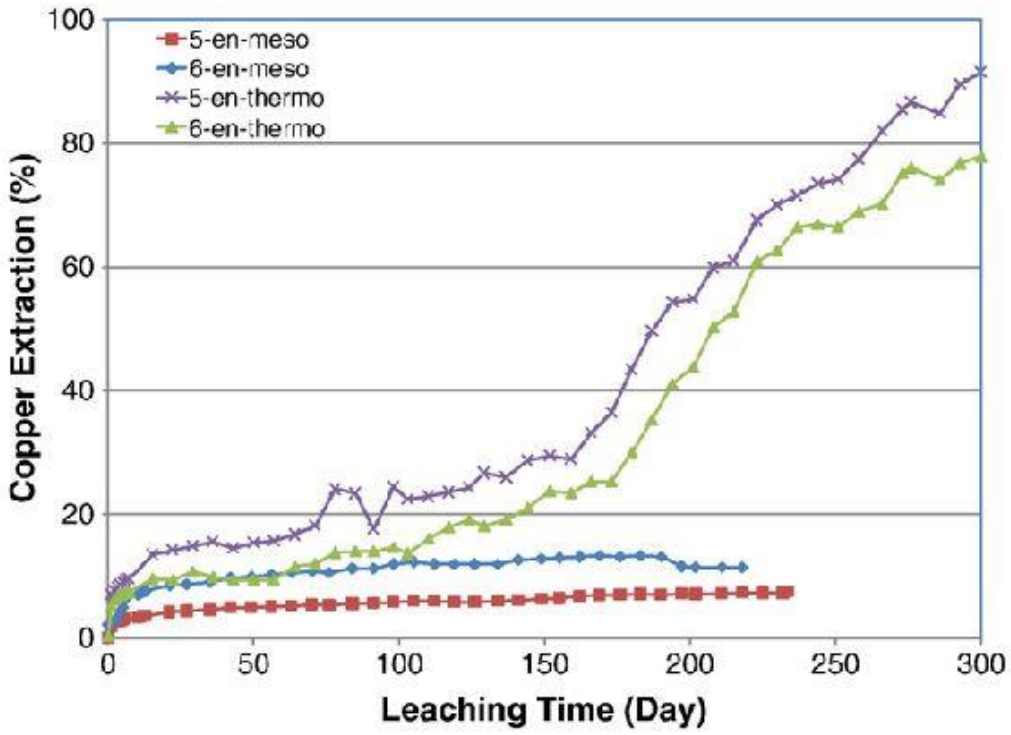


Figure 2.7: Enargite leaching from Lee et al. (2011). Note that the thermophilic cultures showed much higher copper extraction overall. The legend describes the composite number and the culture used for leaching. Composites 5 and 6 were both enargite rich ores. Meso refers to a community leaching at ambient lab temperature while thermo refers to a community leaching at 70º C.

2.9 AGGLOMERATE-SCALE HEAP BIOLEACHING

2.9.1 INTRODUCTION

Previous studies that quantified cell concentrations and metal distributions using agglomerate-scale heap bioleaching have been done and are described in Bryan et al. (2016), Chiume et al. (2012), and Govender et al. (2013). This section is split into two parts – first, a discussion of the phases within the ore-bed followed by a comparison of the results of the agglomerate-scale bioleaching studies.

2.9.2 PHASES

The phases discussed in the 2013 study are the pregnant leach solution (PLS; i.e. leachate), the interstitial phase, and the weakly and strongly attached phases (Chapter 1, Section 1.2).

The interstitial phase refers to the stagnant zones between or within particles. Both the interstitial phase and the leachate house planktonic (free floating) cells (Figure 2.8). The weakly and strongly attached phases are physically attached to the ore, the weakly attached is semi-attached to the ore, and the strongly attached phase is presumed to be the biofilm (Sand & Gehrke 2006; Takatsugi et al. 2011). Few studies have been done which correlate to the weakly attached phase, however, in Takatsugi et al. (2011) there was organic material (an EPS layer) found in a surface defect on the substrate. The area on the ore surface on which this was found was not thought to be thick enough for cell growth at about 100 nm, shown using TEM-EDS (Transmission Electron Microscopy – Energy Dispersive X-ray Spectroscopy). This may have been an indication of weakly attached cells, part of the EPS layer, but not in an area large enough to be part of the biofilm. The relationship between the weakly attached cells and the biofilm (or the strongly attached cells) is otherwise unclear at this point, more research is needed.

Biofilms have been researched more extensively, meaning that the strongly attached phase is slightly better understood. In 2017, Li and Sand found that *Sulfobacillus thermosulfidooxidans* showed changes in the mechanical and chemical properties of cells during biofilm formation. In contrast, the planktonic cells in the system were mainly composed of proteins and polysaccharides. The biofilms exhibited an increased number of dead cells. The dead cells were thought to help decrease the negative effects of high concentrations of toxic metal ions on the biofilm (Govender et al. 2013; Li & Sand 2017). The planktonic cells did not have this advantage, as they are not located in such proximity to the mineral surface. This means it would be reasonable to suggest that the metal concentration is lower in their environments, and they are adapted to such conditions. This study was done using a pure culture, but shows that there is an indisputable difference between planktonic and attached cells (Govender et al. 2013).

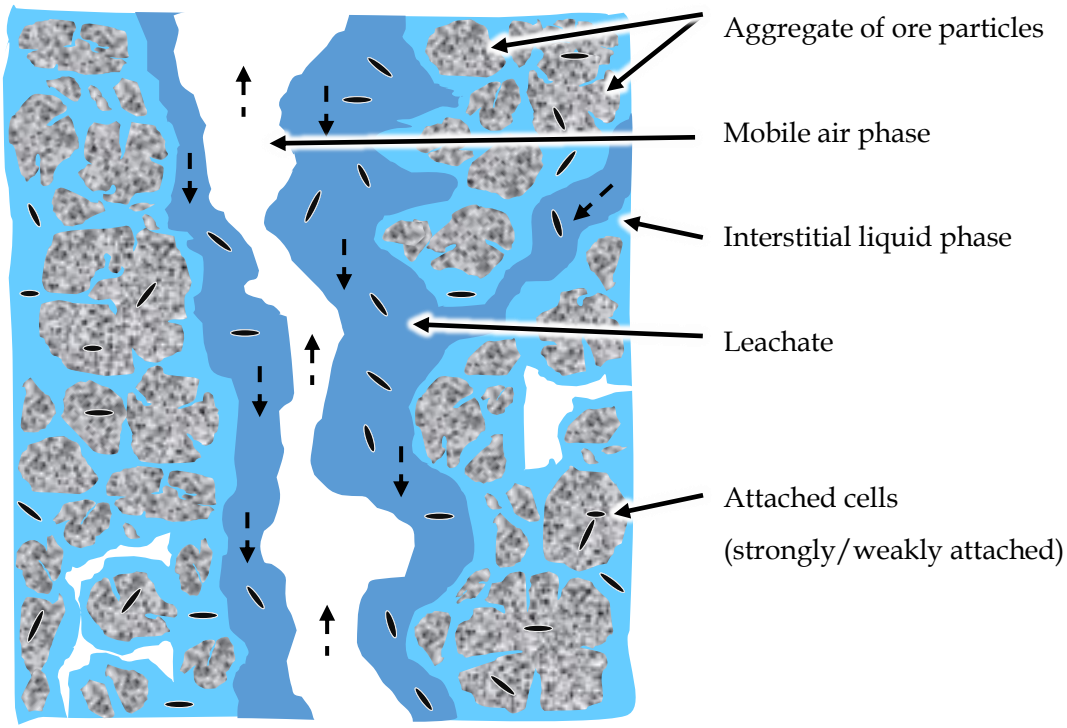


Figure 2.8: Modified from Govender et al. (2013), a diagram showcasing the four phases within the ore bed.

2.9.3 Previous studies

Govender et al. (2013) used agglomerate-scale columns, a sterile ore, and a pure culture of *Acidithiobacillus ferrooxidans* to examine the cell concentrations in four distinct phases within the heap bioleaching system during colonisation of the ore-bed (700 hours). The ore was predominantly pyrite (4 wt. %), chalcopyrite (0.5 wt. %), and enargite (0.1 wt. %). The experimental method was unique, with a carefully engineered novel set-up. Starting with 16 columns, the method was to harvest one at regular intervals to provide a snapshot of that point in time of the intricate microorganism-ore relationships. The remaining columns were left to run until they too were harvested.

Govender et al. (2013) showed that the leachate is distinct from the ore-associated phases with regards to cell concentrations and population dynamics and was not an accurate analogue for the rest of the system. The interstitial phase was found to be dominant in terms of cell concentrations throughout the colonisation of the ore-bed (Govender et al. 2013). Cell movement was also discussed, with several different proposed mechanisms such as chemotaxis, electrostatic and hydrophobic forces at the mineral surface, mineral surface

limitations, and diffusion or convection (Section 2.7). The interaction between cell and interface is still not well understood (Watling 2016).

Chiume et al. (2012) also used the agglomerate-scale method to study the effects of irrigation on microbial colonisation of the ore bed. Irrigation rate was found to influence the rate of microbial growth and ore colonisation, with increased surface colonisation measured when irrigation rates were lower. As shown in Fagan et al. (2014), increased cell numbers are found in the leachate during tests using a higher irrigation rate. The ore in Chiume et al. (2012) was not sterile and the culture was mixed, however no DNA sampling was done to show the microbial community structure between phases. The interstitial phase was dominant in terms of cell numbers (54%), followed by the attached phases (26%) and the leachate (19%), as seen inGovender et al. (2013)..

Bryan et al. (2016) showed that there was a difference in the interstitial community structure when compared with the leachate after a three-month leaching period using the same sterile low-grade chalcopyrite ore with a mixed culture (Figure 2.9). The results were, however, statistically insignificant, possibly due to the sample size (n = 3). The community structure was thought unlikely to be driven by different growth rates in each phase, but rather due to microbial transport, possibly chemotaxis; the movement of microorganisms in response to chemical stimuli is discussed above, in Section 2.7. There were no definite conclusions drawn as to whether increased cell concentrations are related to higher metal concentrations or if both can be attributed to the physiochemical state of the system. The experiment described in Bryan et al. (2016) was also set-up in the same fashion as the novel experimental system described inGovender et al. (2015), but using a drip feed rather than a spray irrigation. The metal concentrations were much higher in the interstitial phase than the leachate (10x to 150x), and the cell concentrations were also highest in the interstitial phase.

Quantifying metal concentrations in addition to cells is important because high concentrations of different certain can have negative effects on microbial communities (Watling 2016). A better understanding of the intricate relationships between phases is important as in industry, the leachate is typically the phase measured to determine the health of the heap (Govender et al. 2013).

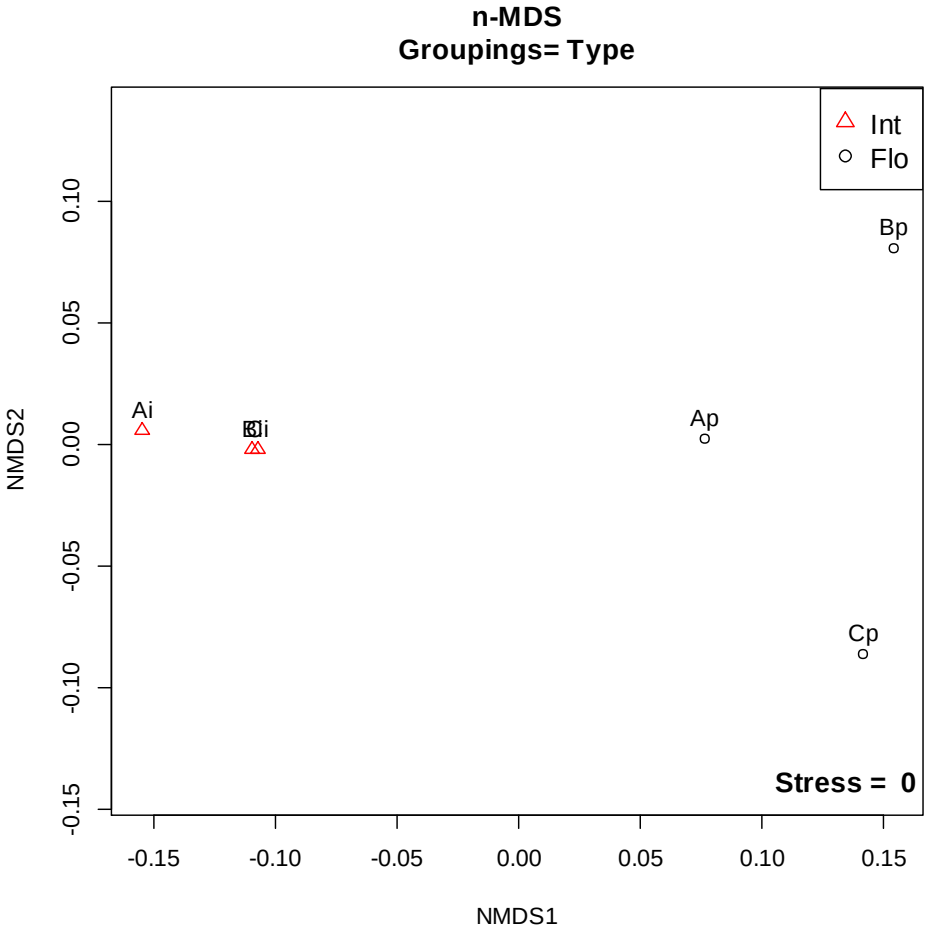


Figure 2.9: Diagram showing the differences in community structure between the leachate (circles) and interstitial phase (triangles), from Bryan et al. (2016).

2.10 DEVELOPMENTS IN BIOLEACHING

The efficacy of bioleaching is affected by a multitude of factors including but not limited to mineralogy, temperature, type of microbial culture and microbial limitations, pulp density, and precipitation (Sand & Gehrke 2006; Watling 2006; Fagan et al. 2014). Biohydrometallurgy doesn't yet compare with other extraction techniques (namely pyrometallurgy) because pyrometallurgical techniques are already widespread and are much faster (Watling 2016).

Innovation in biohydrometallurgy has been driven by industry during times of low metal prices, presumably as a coping mechanism to maintain production when profits are low (Brierley 2010). Developments in biohydrometallurgy are widespread and can be separated into commercial or technical challenges and are discussed in detail in Brierley (2010). Commercial challenges include leaching time, site variation, competing technologies, start-up risk, investment, intellectual property limits between companies, and process guarantees

(unpredictable technology – heaps). Technical challenges involve leaching certain metals – primary sulphides, complex polymetallic ores, massive sulphides, and silicate bound minerals. The most prominent factor in the limited utilisation of commercial bioleaching is the time it takes to leach metals (Watling 2006; Brierley 2010). Depending on the target ore, metal solubilisation can occur on different time scales, see Table 2.2 from Watling (2006).

The biohydrometallurgical implications tied to the ore mineralogy of each deposit are an extremely prominent factor economically, but chemically are not often described in detail in the literature (Brierley 2010). This is because the laboratory-scale work is done in a more fundamental setting, with concentrates in a controlled environment with consistent temperatures and flow-rates (van Loosdrecht et al. 1990) or with pure cultures and sterile ores (Govender et al. 2013). The fact that the discipline is linked to mining presents some limitations in itself, for example, bioleaching research and development is often under strict regulation with regards to intellectual property (Brierley 2010). Fluctuations and the cyclical nature of the industry also causes skilled researchers to lose their jobs, projects, or funding when stocks plummet. This is especially detrimental due to the duration of bioleaching processes with tests often taking years to complete (Brierley 2010).

Table 2.2: Dissolution and oxidation reactions of some copper-containing minerals in heaps and dumps modified from Watling, 2006.

Mineral	Leaching and oxidation reactions	Time
Atacamite	$Cu_2Cl(OH)_3 + 3H^+ \rightarrow 2Cu^{2+} + Cl^- + 3H_2O$	Hours to days
Chrysocolla	$CuSiO_3 \cdot 2H_2O + 2H^+ \rightarrow Cu^{2+} + SiO_2 \cdot 3H_2O$	
Neotocite	$(Cu,Mn)2H_2Si_2O_5(OH)_4 \cdot nH_2O + 4H^+ \rightarrow Cu^{2+} + Mn^{2+} + 4SiO_2 + n\ H_2O$	
Tenorite	$CuO + H_2SO_4 \rightarrow CuSO_4 + H_2O$	
Malachite	$Cu_2(CO_3)(OH)_2 + 2H_2SO_4 \rightarrow 2CuSO_4 + CO_2 + 3H_2O$	
Azurite	$Cu_3(CO_3)_2(OH)_2 + 3H_2SO_4 \rightarrow 3CuSO_4 + 2CO_2 + 4H_2O$	
Brochantite	$Cu_4(SO_4)(OH)_6 + 6H^+ \rightarrow CuSO_4 + 3Cu^{2+} + 6H_2O$	
Native copper	$Cu + 0.5O_2 + H_2SO_4 \rightarrow CuSO_4 + H_2O$	Days to months
Cuprite	$Cu_2O + 0.5O_2 + 2H_2SO_4 \rightarrow 2CuSO_4 + 2H_2O$	
Chalcocite	$Cu_2S + 0.5O_2 + H_2SO_4 \rightarrow CuS^* + CuSO_4 + H_2O$	
	$Cu_2S + Fe_2(SO_4)_3 \rightarrow CuS^* + CuSO_4 + 2FeSO_4$	
	$^*CuS$ is a reaction product rather than the mineral covellite	
Bornite	$Cu_5FeS_4 + 2Fe_2(SO_4)_3 \rightarrow 2CuS + CuFeS_2 + 2CuSO_4 + 4FeSO_4$	Months to years
Covellite	$CuS + 2O_2 \rightarrow CuSO_4$	
	$CuS + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2FeSO_4 + S^o$	
Enargite	$Cu_3AsS_4 + 4.5Fe_2(SO_4)_3 + 2H_2O \rightarrow 3CuSO_4 + 9FeSO_4 + 4S^o + HAsO_2 + 1.5\ H_2SO_4$	
Chalco-pyrite	$CuFeS_2 + O_2 + 2H_2SO_4 \rightarrow CuSO_4 + FeSO_4 + 2S^o + 2H_2O$	Years
	$CuFeS_2 + 2Fe_2(SO_4)_3 \rightarrow CuSO_4 + 5FeSO_4 + 2S^o$	

CHAPTER 3. MATERIALS AND METHODS

3.1 INTRODUCTION

This chapter describes the detailed experimental set-up, and materials and methods used in this study. It will begin by describing the initial preparation of the ore prior to arrival, and additional preparation done at the University of Exeter. The six-month agglomerate-scale column leaching experiment set-up will then be described, including the preparation of the ore-beds, inoculation methods, a description of the culture used, and an outline of the methodology behind the microbial retention study. This will be followed by a description of the experiment and the operating parameters.

The chapter will also outline the methodology of several peripheral studies performed based on the six-month heap leaching experiment. These include the un-inoculated detachment experiment, the DNA extraction methods, and the high temperature batch reactor tests. The chapter will close with the calculations performed to analyse the data.

3.2 ORE

The low-grade porphyry copper ore used in this study contained 0.8% enargite as the primary copper mineral. The ore was supplied by Newmont Mining Corporation, and came from 50 kg test bioleaching columns, outlined in Lee et. al (2011). Particle size distribution (PSD) tests and assays were completed prior to delivery to the University (Table 3.1 and Table 3.2).

Table 3.1: PSD determined by Newmont Mining Corporation (Roberto 2017, pers. comm.).

Screen size, Tyler	Screen size, mm	Weight, kg	Weight %
-3/4+1/2	**+9.5**	3.75	25.01
-1/2+1/4	**+6.3**	4.42	29.49
-1/4+6M	**+3.4**	2.12	14.12
-6M+10M	**+1.7**	1.15	7.69
-10M	**+1.6**	3.56	23.70
		15.0	**100.0**

3.2.1 ORE PREPARATION

The ore used for this study had been crushed to 100% passing ¾" (+9 mm) using a laboratory jaw crusher (Labtech/FL Smidth Automation) and screened to determine the particle size distribution (PSD) using a SWECO Vibro-Energy Separator (Roberto 2017, pers. comm.). The PSD of the ore is shown in Figure 3.1.

For assay, 500 g splits (-10 mesh, 1.7 mm) from the composites had been used. Copper values ranged from 10110 ppm to 4710 ppm, averaging 6200 ppm. Arsenic values ranged from 1540 ppm to 850 ppm, averaging at 1025 ppm, and iron values ranged from 97500 ppm to 86060 ppm, averaging at 91250 ppm (Appendix A).

Below is the detailed assay preparation method as described in a personal communication with Roberto (2017). The described assays provided the data for Table 3.2 below.

"*Five hundred gram charges (minus 10 mesh; 1.7 mm) were also prepared from splits of the master composite for head assays. Chemical and mineralogical composition of the composite is provided in Table 2. Carbon and sulfur analyses were performed on a LECO furnace and included total C and S, acid-insoluble C (CAI), HCl-soluble S (SHCL), and sulfide sulfur (sodium carbonate-insoluble S; SCIS). Sequential Cu assay and "quick leach test" assays were performed to determine the acid-soluble (CuAS), cyanide-soluble (CuCN), and water-soluble (CuWS) amounts of Cu in the composite. Total Cu was also obtained by ICP-OES, along with quantification of 24 other elements after digestion in 5% nitric acid. Mercury was determined by combustion according to ASTM Standard Test Method D-6722 with a NIC MA-3000 analyzer (Nippon Instruments, College Station, Texas, USA). Semi-quantitative mineralogy was performed on pulverized powder (75µm nominal particle size) by X-ray diffraction with Rietveld refinement on an X'Pert Pro diffractometer (PANalytical,Inc., Westborough, Massachusetts, USA).*"

Table 3.2: Mineralogy of low-grade copper ore (Roberto 2017, pers. comm.)

Quartz	Pyrite	Pyrophyllite	Diaspore	Alunite	Enargite	Rutile	Kaolinite	Zunyite	Galena
59.00%	19.90%	9.50%	6.60%	1.90%	0.80%	1.80%	0.10%	0.10%	0.10%

3.3 SIX-MONTH LEACHING EXPERIMENT SET-UP

The design and theory behind this experiment builds on previous work by Govender et al. (2013). The main difference is the duration for which the columns were run, as this study ran for 4615 hours (192 days), rather than 700 hours as was done in the Govender et al. (2013) study. Initially, the experiment was set to run for six-months (180 days, 4,320 hours) to provide insight into the conditions within an agglomerate-scale heap past the initial colonisation phase. The six-month period was chosen as an arbitrary period that would continue well past heap colonisation. As the six-month period approached, there was less variation in results between column harvests so the time between harvests was extended to include almost two additional weeks. The experiment is still referred to as a "six-month" experiment, however, it should be noted that the actual time frame is closer to six months and two weeks.

In addition to the duration of the experiment, another crucial difference from Govender et al. (2013) is that the ore for this experiment was non-sterile and used a mixed mesophilic culture instead of a pure culture of *Acidithiobacillus ferrooxidans*. The columns were also irrigated using a drip feed as opposed to a spray feeder system and kept at 27°C instead of 30°C due to availability of temperature-controlled rooms.

3.3.1 PARTICLE SIZE DISTRIBUTION STUDY

A particle size distribution study was completed upon the arrival of the material to the University of Exeter, to separate the sizes from a representative sample. The PSD study was then used to prepare the agglomerate scale ore beds in the six-month leaching experiment.

This study was performed in the Mineral Processing Lab at the Camborne School of Mines. The ore was split into two halves, one of which was riffled into ~250g sample sizes for the batch reactor tests (Section 3.9). The other half was separated into size fractions using nested brass sieves ranging from 16.00 mm to <0.25 mm, the parent sample weighing an average of 1867 g. Sieves were

shaken on a mechanical shaker for 20 minutes. The experiment was performed in triplicate (Figure 3.1) to ensure the bed was represented accurately by each PSD test.

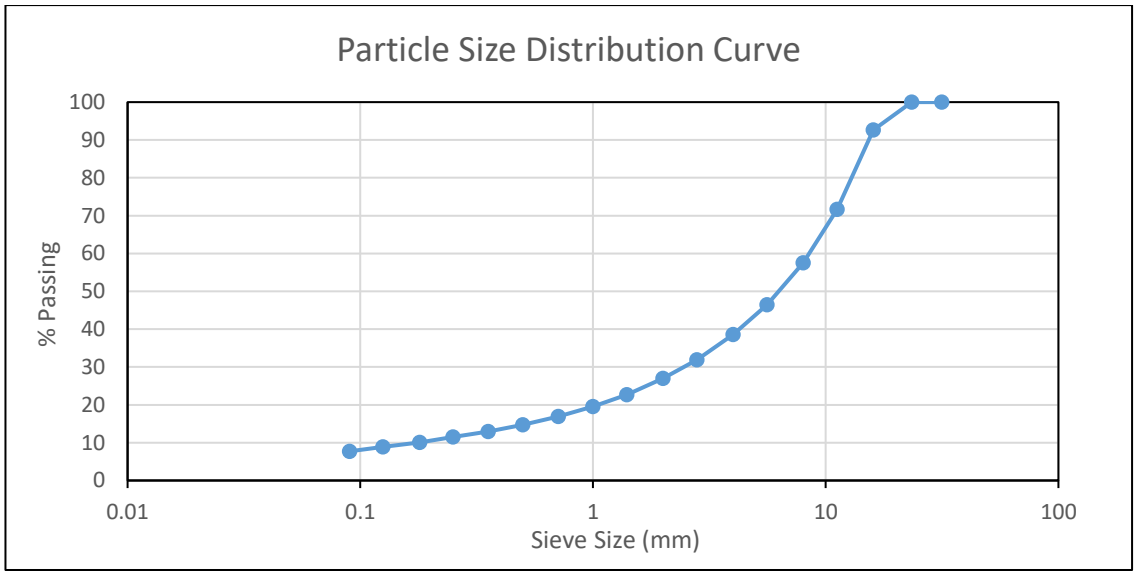


Figure 3.1: Particle size distribution curve for parent material.

3.3.2 Ore bed preparation

The agglomerate-scale ore beds were composed of grab samples which were reconstructions of the bed prepared based on the particle size distribution curve (Figure 3.1). Size fractions were the same as those described in Govender et al. (2015) and are listed in Table 3.3. Grab samples weighed an average of 150 g ± 3.03 g (dry) once reconstructed. Variations in sample weight were due to size differences in ore particles larger than 16 mm.

Table 3.3: Grab sample weights of each size fraction per 150 g sample.

Grab Sample Weights								
Size	(mm)	>16	16 to 8	8 to 5.60	5.60 to 2	2 to 1	1 to 0.25	<0.25
Weight	(g)	11.0	50.7	17.1	29.9	11.4	12.3	17.7

Samples were originally separated into 17 size fractions, but then were re-combined according to the size fractions seen above (Table 3.3). This was to allow sufficient weights of each fraction for the 150 g grab sample. Size fractions were mixed only if they came from the same particle size distribution test. The tests were run in triplicate, PSD 1, PSD 2, and PSD 3. For example, the 16 mm to 8 mm splits were separated into 16 mm, 11.2 mm, and 8 mm fractions during

the test. All three sizes were then mixed back together to make up the 50.7 g needed for the grab sample. The size fractions were only mixed from PSD 1. If there was not sufficient ore from each size fraction whilst constructing grab samples, then the size fraction from PSD 2 was combined and used.

Prior to the commencement of the experiment, grab samples were agglomerated in dilute sulphuric acid (H_2SO_4) at pH 0.6, using approximately 8 mL per agglomerate sample for a 5 % moisture content of the ore bed. Acid agglomeration was done in order to help consolidate the material (Govender et al. 2015).

Once agglomerated, the ore was packed into the columns, shown in Figure 3.2, as described in Govender et al. (2015). Columns were constructed in 500 mL polypropylene bottles (73 mm diameter). Each column also contained 110 g of 16 mm glass beads, and 160 g of 8 mm glass beads. The glass beads were washed in an acid bath (25% HCl) and then baked in an oven for 48 hours at 70ºC to ensure there were no metals present. The large glass beads on the bottom of the column were put in place to prevent the loss of fine particles. Smaller beads at the top of the column were emplaced to help distribute fluid flow and prevent feed channelling. The beads were not sterile as the mixed mesophilic culture was undefined. This means that any microorganisms introduced during set-up that could survive the conditions within the agglomerate-scale heap would be included in the experiment. A sterile sponge (Fisher Scientific) was also placed on top of the 8 mm beads at the top of the column to further prevent channelling within the agglomerate ore bed. Once agglomerated and packed into the column, the height of the bed within the column ranged from about 15 mm to 25 mm.

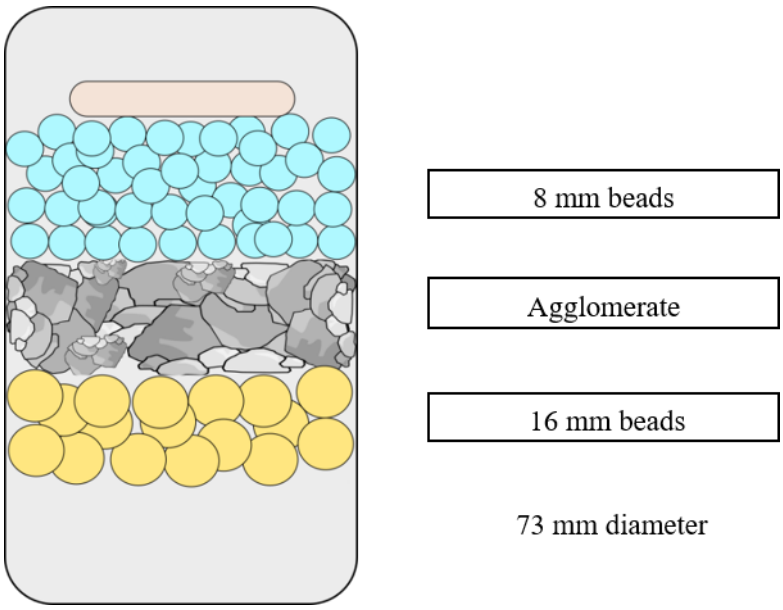


Figure 3.2: Cross sectional view of columns showing layers.

Once the columns were assembled, a 24-hour acid leach was done using acidified water with a pH of 0.6 (H_2SO_4) at a flow rate of 10 mL per hour (Ismatec IP-N Microprocessor delivery pump). This was done to remove all easily leachable minerals from system, and to further consolidate the bed (Govender et al. 2013).

3.3.3 Inoculation

After the 24-hour acid leach, the ore was inoculated with 2 mL per column of inoculum, a mixed mesophilic culture (described in Section 3.3.4). The stock culture was counted directly before inoculation, using a Helber counting chamber ($7.25x10^8$ cells/mL). Inoculum was placed directly onto the glass beads as a pulse input (below the sponge), for an inoculation target of 10^{13} cells per dry ton of ore. The inoculum in the Govender et al. (2013) study, on the other hand, was diluted to 10^{10} cells per dry ton of ore. The difference in inoculum is due to an experimental error, as the inoculum in this study was not diluted.

3.3.4 Culture

This study used a mixed mesophilic culture, enriched from mine waste at the former Wheal Maid mine site, Cornwall, UK. The enrichment medium comprised autotrophic basal salts (ABS; $(NH_4)_2SO_4$ [0.15 g/L], $Na_2SO_4 \cdot 10H_2O$ [0.15 g/L], KCl [0.05 g/L], $MgSO_4 \cdot 7H_2O$ [0.05 g/L], KH_2PO_4 [0.05 g/L], $Ca(NO_3)_2 \cdot 4H_2O$ [0.014 g/L]), at a pH of 1.7 (H2SO4), with a concentration of 1 % (w/v) pyrite. The

undefined culture was known to contain: *Acidiferrobacter* sp., *Acidithiobacillus albertensis*, *Acidithiobacillus ferrooxidans*, *Alicyclobacillus disulfidooxidans*, *Leptospirillum ferriphilum*, *Sulfobacillus* sp. L15, and an uncultured *Acidithiobacillus* sp. (Bryan et al. 2016).

3.3.5 Microbial retention study

To allow time for cells to attach to the ore particles, the columns were not irrigated for the first hour after inoculation. After one hour, the pump connected to the medium was switched on, this point is considered time 0. A microbial retention study was then completed over a period of 5 hours. Cell counts were done each hour using a cell counting chamber, quantifying the cells leaving in the leachate. This study was done in triplicate.

3.4 Experimental description and operating parameters

16 columns were run at 27°C with the longest running column (Column 1) running for 4615.32 hours (192.3 days). A column was removed every 48 to 500 hours, depending on the stage of the experiment. This was done to provide a window into the ore-bed at that specific point in time, with time points placed closer together to provide additional data during earlier stages of the experiment. As all columns were set up to be identical to one another (albeit discrete), each one therefore acted as a representative snapshot of the system at the time of the removal. Column 16, for example, was removed 43.1 hours into the study, providing insight into the system at that time only. Once a column was removed it was not re-assembled and the overall number of columns was reduced by one. This continued until there were no columns remaining.

At the outset of the experiment columns were removed at shorter intervals to closely observe the colonisation of the ore bed, as during this time it was expected that there would be more fluctuations in cell numbers. After the initial colonisation stage (~40 days or 1000 hours), the cell counts stabilised. From that point onwards, the columns were harvested at wider intervals (up to 3 weeks) to provide insight over a longer period.

3.4.1 PUMPING

Feed was administered using a drip irrigation system at a flow rate of 9 -10mL per hour (Ismatec IP-N Microprocessor delivery pump). No air or gas was pumped into the columns. Fluid flow was driven by gravity and the continuous input of fluid at the top of the column.

3.4.2 FEED SOLUTION

Feed was composed of basal medium, 0.4 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_20$, 0.2 g/L KH_2PO_4, acidified to pH 1.7 with 96% H_2SO_4. Ferric iron was added (200 ppm ferric iron from iron(II) sulphate heptahydrate) to encourage mineral dissolution.

3.4.3 LEACHATE MEASUREMENTS

Throughout the duration of the experiment, the leachate was analysed once a week. Collection of the leachate was ongoing for the entirety of the study, either in bulk (3 to 5 days) or as a point sample (30 minutes to an hour). The samples were not filtered and dripped directly into pre-weighed flasks. The flasks were kept in the 27ºC controlled temperature room until they were weighed (bulk) or analysed (point). Sample weights were used to calculate the flow rate (Appendix C). The point samples were collected over a short time period and provided measurements of the metal concentrations at a specific point in time only. These also allowed the determination of volatile measures such as iron speciation and pH. The bulk measurements were effectively composite samples and gave only the average values over an extended period. The bulk samples did not provide insight into any short-term fluctuations in values over those periods (Appendix D). It is important to consider that the bulk values would likely change over time while in the collection vessel due to continued microbial action.

Cell counts were performed only on the point samples using a Helber counting chamber. Total and ferric iron measurements were done (also only on the point samples) using the ferric chloride assay (Govender et al. 2012) and a spectrophotometer (Jenway 7315). Iron and copper values were collected for

both point and bulk samples and were analysed using Atomic Absorption Spectrophotometry (AAS) (Appendix D).

These measurements were then compared to the cell counts and metal concentrations seen in the leachate during the detachment protocol (Section 3.5). However, monitoring was also done simply to ensure that each column was representative of the system, and that there were no largescale changes in the system in terms of cell concentrations or dissolved metal concentrations over time.

3.5 DETACHMENT PROTOCOL

Table 3.4 gives a concise description of the detachment protocol; however, it will be described in detail with each section below representing a step in the procedure. The detachment of the ore associated phases was done using a 2:1 solids:liquids ratio, 50 g of material was mixed for each step with 25 mL of sterilised medium.

Prior to each detachment, leachate (in this case, effluent only) measurements were performed on 3 columns, the one that was running the longest (Column #1), a column chosen at random, and the column to be harvested that day. This was to ensure that there were no detectable anomalies (in the leachate) within the system and to provide an estimate of systematic error.

The goal of the detachment was to separate the four phases within the system (see Chapter 2, Section 2.9.2) from one another, the leachate, the interstitial, and the weakly and strongly attached. The interstitial, weakly attached, and strongly attached phases will be referred to collectively herein as the “ore-associated phases”. Results and all associated calculations pertaining to the column harvests can be found in Appendix C.

3.5.1 LEACHATE

The leachate was collected as effluent from the three columns chosen for that date. Collection was ongoing over a minimum of period of 30 minutes, and then analysed as described in Section 3.4.3. The column which was being harvested was then detached from the pump and left for one hour to allow any remaining liquid inside the column to flow out.

Note that previous studies (Govender et al. 2013, 2015) reference “pregnant leach solution”. This will be referred to as leachate in this study, as the term “pregnant leach solution” implies a confirmed dissolved metal concentration. Leachate flowing within the column is also sometimes referred to as the flowing phase, but in this study the flowing phase and the leachate will be referred to collectively as leachate.

3.5.2 Separation of the column and agglomerate preparation

Once the leachate had been collected the column was physically dismantled. The beads were then removed and weighed, dried for one week in an oven at 65°C (8 mm and 16 mm sized beads were kept separate), and then weighed again to calculate the percentage of moisture held within them.

A representative 50 g sample of the ore bed was then removed from the agglomerate bed and put aside – this 50 g is the portion that was used for the detachment procedure itself. The remainder of the ore was weighed, and then set aside to dry before being weighed again for calculations of the hold-up liquid within the bed.

3.5.3 Interstitial phase

The first phase to be separated from the ore bed itself was the interstitial phase. The 50 g sample was added to a sterile flask with 25 mL sterile basal medium and mixed gently by hand. The supernatant was then poured off and centrifuged for 2 minutes at 800 × *g* (gravitational force) (Beckman Coulter Avanti© J-E). The sample was approximately 25 mL in total.

3.5.4 Weakly attached phase

The ore sample (and remaining fines from the centrifuge) were then mixed together with 25 mL sterile basal medium to collect the weakly attached phase. The flask was mixed vigorously for 2 minutes on high speed using a bench top vortex, and then allowed to settle. Once settled, the supernatant was centrifuged for 2 minutes at 800 × *g*. The whole process was repeated in triplicate, with all 3 separations making up the weakly attached liquid (approximately 75 mL in total).

3.5.5 STRONGLY ATTACHED PHASE

To collect the strongly attached phase, the same procedure as described for the weakly attached was repeated twice but with an addition of 100 µL of Tween 20 (Merck) solution (0.4%v/v final concentration). Tween 20 is a detergent and removes attached cells from the ore. This produced approximately 50 mL of strongly attached liquid.

3.5.6 ANALYSIS OF THE PHASES

After the liquids had been separated into the four distinct phases, the supernatant (or combined supernatant), post-centrifugation, was poured into a sterile flask and weighed to allow for calculation of the volume of each phase in the column. Each phase was diluted using a sterile medium which was added during harvesting. Some liquid would have carried over throughout detachment. This carryover (cells, metals) was accounted for mathematically using residence time distribution data from Govender et al. (2013), and is outlined in Appendix C.

The liquids were then analysed in a similar fashion to the leachate measurements described above in Section 3.4.3, and included cell counts, iron measurements, and reserve samples taken for Atomic Absorption Spectroscopy. The metal concentrations were separated into the leachate and the ore-associated rather than into four distinct phases. In addition to this, up to 20 mL of liquid was filtered using 0.2 µm Whatman cellulose nitrate filter membranes. The filters (at this point, containing cells) were stored in bead solution and frozen with the filter at -20°C for later DNA extraction (Section 3.8).

Table 3.4: Overview of the experimental method for separation of phases.

	Vortex for 2 minutes	**Centrifuged 2 minutes at 800 × *g***	**100µL Tween 20**
Leachate	Drain only		
Interstitial		✓	
Weakly attached	✓	✓	
Strongly attached	✓	✓	✓

3.6 UN-INOCULATED DETACHMENT

As the ore was not sterile, an un-inoculated detachment experiment was also completed to quantify the indigenous community. In this experiment, prior to the step where inoculation of the columns would have taken place, the columns were dismantled using the detachment protocol described in Section 3.5.

3.7 DNA

Up to 20 mL of liquid from each phase during each takedown was filtered onto 0.2 µm cellulose nitrate filter membranes (Whatman). DNA was extracted and then analysed using terminal restriction fragment length polymorphism (T-RFLP) by Suzanne Kay (Environment and Sustainability Institute) as per Bryan et al. (2016), and data was processed by Dr. Chris Bryan. It was presented to the author in the format seen in Appendix B. DNA samples from Column 14 – 16 and from the un-inoculated detachment experiment were not able to be analysed.

3.8 REACTOR EXPERIMENT

Batch testing was done in one to two-week intervals to examine the effect of pH and iron concentrations on cell numbers (Appendix E). At the end of each batch reactors were subbed using the culture from the previous test. This allowed microorganisms to build resistance to increased pH and iron concentrations (Acevedo 2000).

3.8.1 ORE PREPARATION

The ore was crushed using a jaw crusher then sieved, reserving particles > 2 mm. Ore was then rod milled to 100% passing 100 µm (18 minutes milling). Particle size was verified using a Mastersizer 3000 lazer analyser (Malvern).

3.8.2 REACTOR SET-UP

The reactors were double jacketed for temperature control using an oil bath at 63ºC. The rotors were set to stir at 415 rotations per minute (rpm). CO_2-enriched (2% v/v) air was bubbled under an impeller through a metal tube welded into an L-shape to distribute aeration evenly. Baffles were added to increase even agitation and prevent the concentrate from settling at the bottom of the reactor. The experiment was designed so that the reactors were homogenous.

The culture used was an undefined thermophilic culture. It was grown in a shaker at 65ºC on a pyrite concentrate (2% pyrite). Reactors held 700 mL total liquid composed of 70 mL inoculation from the previous batch test and 630 mL basal medium apart from the 200 ppm additional ferric iron (described in Section 3.4.2) at pH 1.7. There was 14 g of milled enargite ore (Table 3.2) added to each reactor for a total of 2% (w/v) solids.

pH was maintained using 96% H_2SO_4 and iron concentrations were adjusted (where required) using iron(II) sulphate heptahydrate. One reactor was used as a control at the previous batch conditions (Table 3.5). For example, if Batch 1 had no adjustments, Batch 2 would have one control reactor with no adjustments, and then two reactors operating at the Batch 2 conditions (pH 1.4). Batch 3 would then be sub cultured with the adjusted reactors from Batch 2, in this case at pH 1.4. This was done to allow some microbial resistance to build in adverse conditions. Batches were generally run for one week but were run longer or repeated if the cell numbers were lower than anticipated.

Table 3.5: Outline of the batch reactor tests, including the total time and the conditions within each reactor.

Batch	Total time (h)	Reactor conditions
Batch 1	151	no pH adjustment
Batch 2	160	pH 1.4
Batch 3	160	pH 1.2
Batch 4	165	pH 1
Batch 5	162	pH 0.8
Batch 6	240	pH 0.9
Batch 7	169	pH 0.9
Batch 8	163	pH 0.8
Batch 9	359	pH 0.7
Batch 10	165	pH 0.6
Batch 11	195	20g/L iron (unadjusted control)
Batch 12	332	30 g/L iron
Batch 13	324	30g/L iron
Batch 14	367	40g/L iron

3.9 CALCULATIONS

3.9.1 CALCULATION OF INTERSTITIAL VOLUME

As the interstitial volume could not be measured, it was calculated. To calculate the interstitial volume, hold-up liquid was measured first by drying the ore in an oven at 65°C for one week (Section 3.5.2). Dry weight was then subtracted from wet weight, which gave the volume of the hold-up liquid (composed of both leachate and interstitial liquid).

The percentage of the interstitial liquid based on the concentration values from the residence time distribution study described in Govender et al. (2015) was then multiplied by the hold-up liquid to give the interstitial volume. These calculations are outlined in Appendix C.

3.9.2 CALCULATED REDOX

Redox values were calculated from the equation shown in Figure 3.3 in (Yue et al. 2014), using ferric to ferrous iron ratios measured with a spectrophotometer (Jenway) and the ferric chloride assay as described in Govender et al. (2012). The Fe^{2+} values were obtained by subtracting the Fe^{3+} values from the total iron measurement. The redox calculations can be found in Appendix C. Redox values for the ore-associated phases were calculated using the sum of the Fe^{2+} and Fe^{3+} values of the interstitial, weakly attached, and strongly attached phases (see Section 3.5.6).

$$E(mv) = -1\ x\ 10^{-3} x\ [T(K)]^2 + 0.91\ x\ T(K) + \frac{2.303R}{nF}\ x\ T(K)\ x\ 10^3\ x\ log\frac{C_{ferric,nominal}}{C_{ferrous,nominal}} + 492$$

Figure 3.3: Equation from Yue et al. (2014) for calculating redox values from Fe^{2+} and Fe^{3+} concentrations in solution.

CHAPTER 4. RESULTS

4.1 INTRODUCTION

This chapter summarises the results from this study to provide context for the discussion in Chapter 5 which will interpret the outcomes. It will begin with the tests performed during the column set-up, the microbial retention study and the uninoculated detachment experiment, as this data was vital for interpreting the results from the six-month leaching experiment itself. The outcomes from the six-month leaching experiment will then be given, starting with the flow rate of each column and the standard deviations of the test columns during column harvests. Cell distribution and metal dissolution results will follow, answering the primary research questions. After this, the calculated redox values will be given followed by the microbial ecology within the ore bed. Finally, the growth curve and metal dissolution results from the reactor batch test experiment will be provided.

4.2 MICROBIAL RETENTION STUDY

After the columns were inoculated ($7.25x10^8$ cells/mL), the system was left for one hour to allow the microorganisms time to attach to the ore. Once the pumps were switched on, cells were counted in the leachate each hour for the first 5 hours of the study (Figure 4.1, Appendix D). Any cells leaving would have to have exited via the leachate as this is a closed system (Fogler 2010). Time zero is one hour after the columns were inoculated, when the pump was switched on at a flow rate of 9 mL per hour (Section 4.4.1).

During the first hour of the study, $6.75x10^8$ cells/mL were lost (Figure 4.1, 4.2). The number of cells leaving then steadily decreased until hour 4 ($1.34×10^7$ cells/mL), with a small increase at hour 5, $5.61×10^7$ cells/mL.

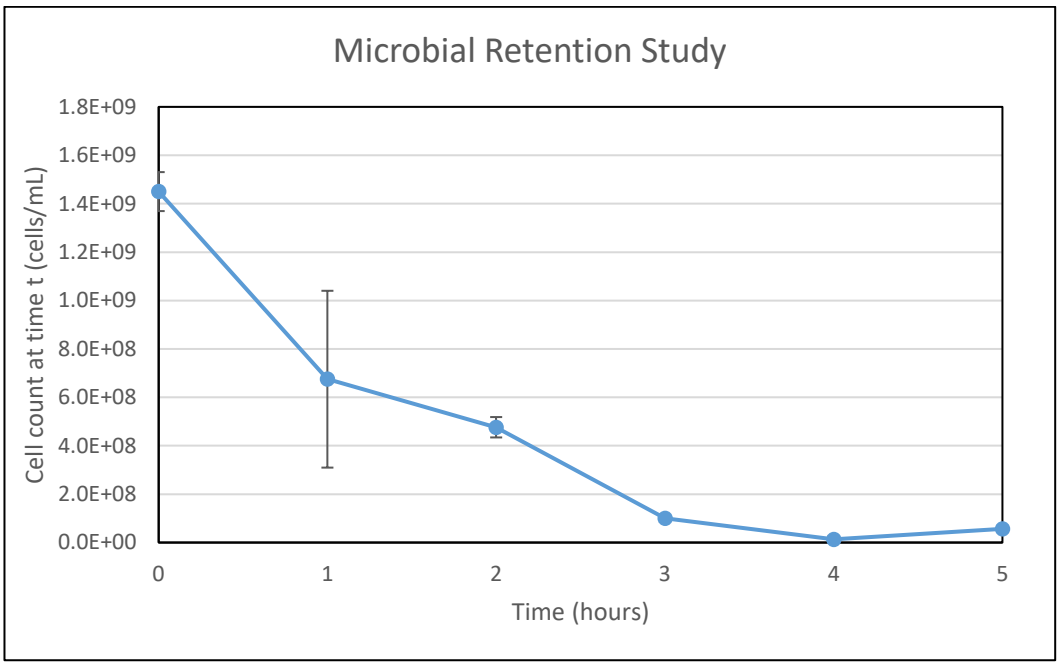


Figure 4.1: Microbial retention study results. Error bars represent the difference between triplicate measurements. The cell concentration of the inoculum is shown at t=0. Cell counts from hour 1 – hour 5 were performed on the leachate.

The percentage of the inoculum lost was also calculated as a percent at 91.1% of the inoculum added leaving over the 5-hour duration of the test (Figure 4.2). Over the first hour, 46.6% of cells were lost. The percentage lost then steadily decreased until a low point at hour 4 (0.92%), and then increased again at hour 5 to 3.87%. Some of the cells lost during this experiment may be attributed to the indigenous population on the ore, so the percentages of inoculum lost should be considered an estimation only. This will be discussed further in Section 4.3 and Chapter 5.

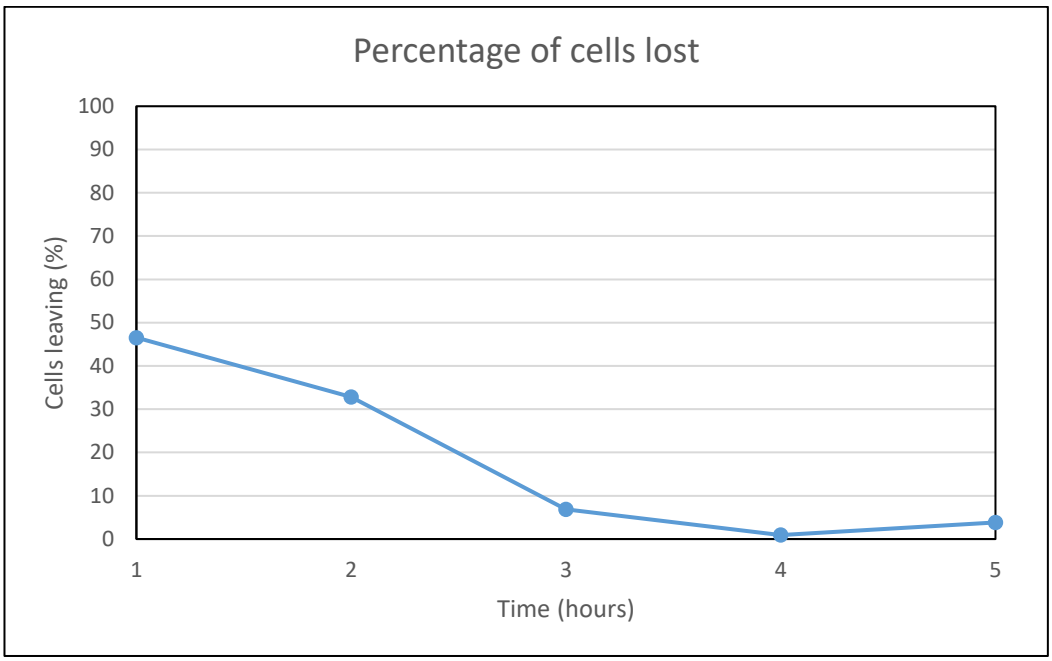


Figure 4.2: The percentage of cells lost from hour 1 to hour 5 of the microbial retention experiment, without accounting for the indigenous population or cell growth.

4.3 UNINOCULATED DETACHMENT EXPERIMENT

In the uninoculated detachment experiment, two uninoculated columns were harvested as outlined in Section 3.6. The results showed that there was an indigenous population present on the ore at the outset of the study. Table 4.1 shows the breakdown of the cell numbers in each phase (Appendix C). All numbers are an average of the duplicate measurements. The total concentration of cells recorded in the detachment experiment was 6.53×10^{9} cells/column. There were no cells counted in the leachate. The interstitial phase had the lowest cell concentration at 8.78×10^{7} cells/column, followed by the weakly attached phase at 2.58×10^{8} cells/column, and then the strongly attached phase at 6.23×10^{9} cells/column. This distribution is visually represented in Figure 4.3.

Table 4.1: Cell concentrations from the abiotic detachment experiment. Numbers are an average of duplicate columns.

Phase	Cell concentration
Interstitial	8.78×10^{7} cells/column
Weakly attached	2.58×10^{8} cells/column
Strongly attached	6.23×10^{9} cells/column

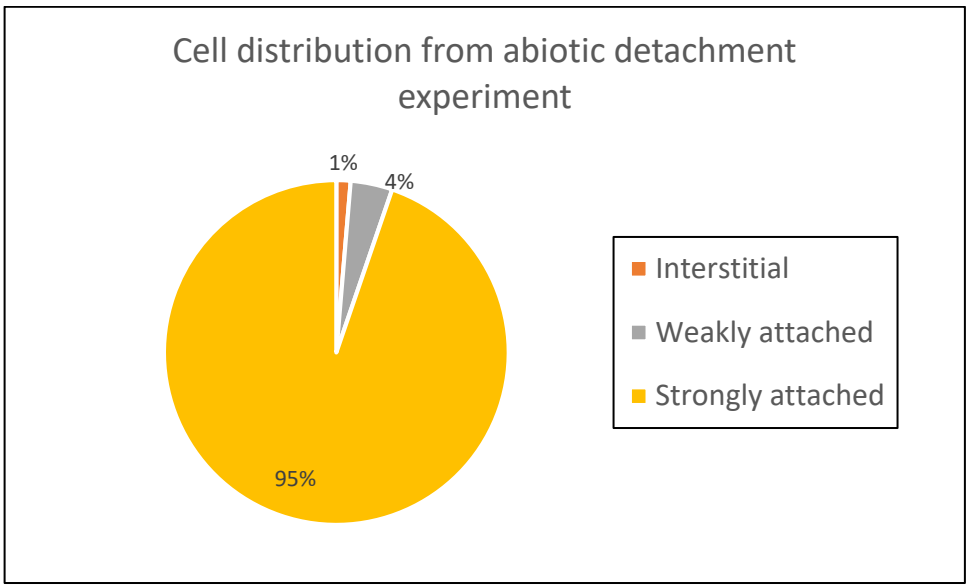


Figure 4.3: Pie chart showing the distribution of cells between phases (cells/column). Total cell concentration was 6.53×10^{9} cells/column.

4.4 Six-month leaching experiment

4.4.1 Flow rate

All leachate during the six-month experiment was collected, weighed and examined to provide flow rate, and iron and copper data (Appendix D). The cell numbers in the bulk samples were not recorded (Chapter 3, Section 3.4.3).

The average flow rate recorded was roughly 9 mL/h for the duration of the experiment (Figure 4.4). Spikes in flow are seen early in the experiment (during adjustments to the pump and columns) and in the middle of the experiment due to a mechanical issue, at which time the highest average flow rate was 19.6 mL/hour measured between t=86 days and t=92 days. Low flow rate values are seen randomly throughout the experiment (t=3 days, t=14 days, t=28 days, and t=171 days) and are below values of 5 mL/hour due to overflow or spillage.

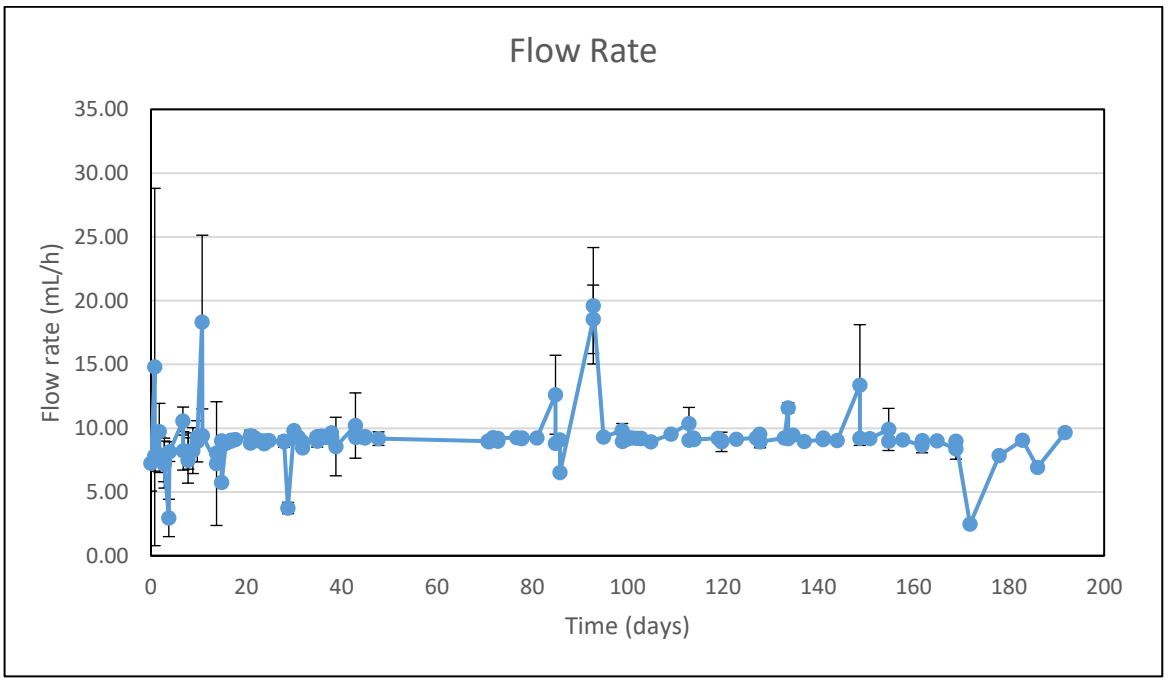


Figure 4.4: Flow rate average from the existing columns versus time. Error bars represent variation between existing columns.

4.4.2 Standard deviation as percentage of the mean

Standard deviation as percentage of the mean was calculated for the leachate of the three columns examined during column harvests. These three columns are the column being harvested, Column 1 (as it was longest running), and a column picked at random, as described in Chapter 3, Section 3.5.

The results of this calculation are given in Figure 4.5 (Appendix C). Both ferric and total iron deviations between columns were low <10% after the first 9.75 days. Cell concentration values are variable throughout the duration of the experiment but follow a downward trend after t=85 days. Copper values are also variable between columns as the ore used to form the beds was not homogenous. Overall, there is a trend downwards over time with an exception at t=113 days (harvest of Column 5) as the sample size got smaller and the columns had more time to reach steady state. No points are shown after t=176 days as after this point there was only one operational column.

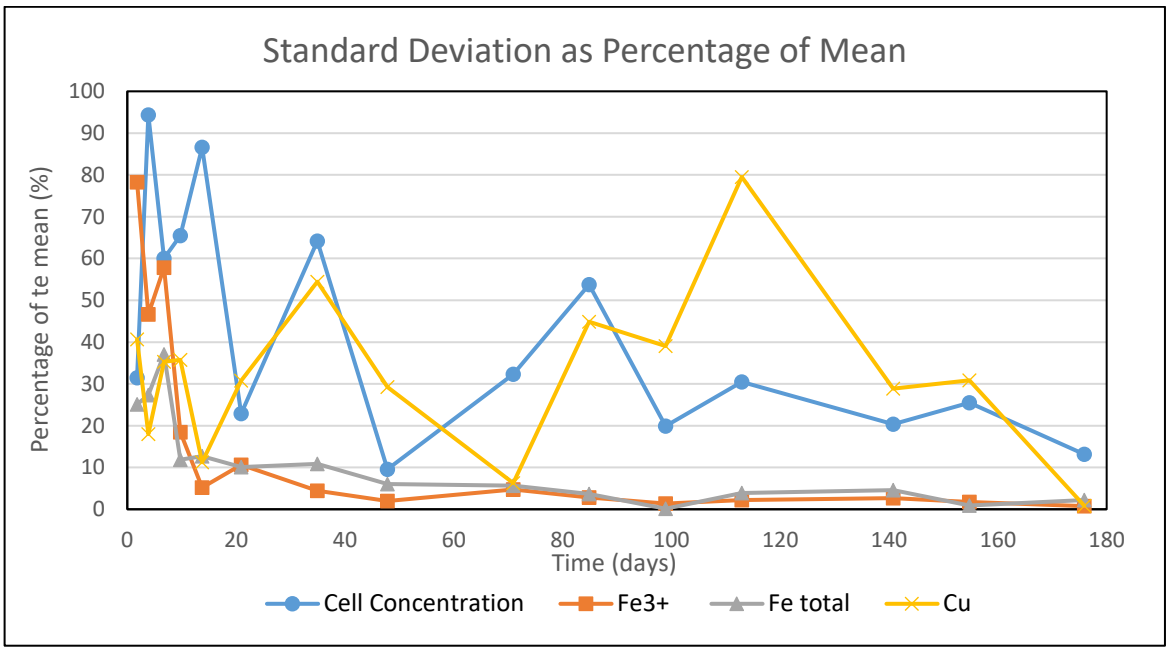


Figure 4.5: Standard deviation as percentage of the mean between the three columns during takedown experiments (leachate measurements only) in cell concentration, ferric iron, total iron and copper.

4.4.3 Cell concentrations

A difference in cell numbers between the leachate and the ore-associated phases was found (Appendix C). This persisted throughout the duration of the experiment (t=4615 hours; 192 days) and is shown in Figure 4.6. In the first 48 days of the experiment, leachate cell concentrations increased. After this point, the leachate cell concentrations were fairly constant and varying between columns from 2.50×10^{7} cells/mL to 1.17×10^{8} cells/mL (Figure 4.6).

The interstitial phase showed a notable colonisation curve, starting at 6.03×10^{8} cells/column (at t=0) and becoming more stable at t=35.9 days (5.35×10^{10} cells/column) until the end of the experiment ($5.56\text{x}10^{10}$ cells/column). Cell counts were roughly 2500x higher in the interstitial compared to the leachate.

The weakly and strongly attached curves started with high cell concentrations (7.18×10^{9} cells/column and 1.54×10^{11} cells/column, respectively). They then decreased at t=3.85 days and re-stabilised at t=35.9 days with a higher cell concentration than was evident at the outset of the experiment.

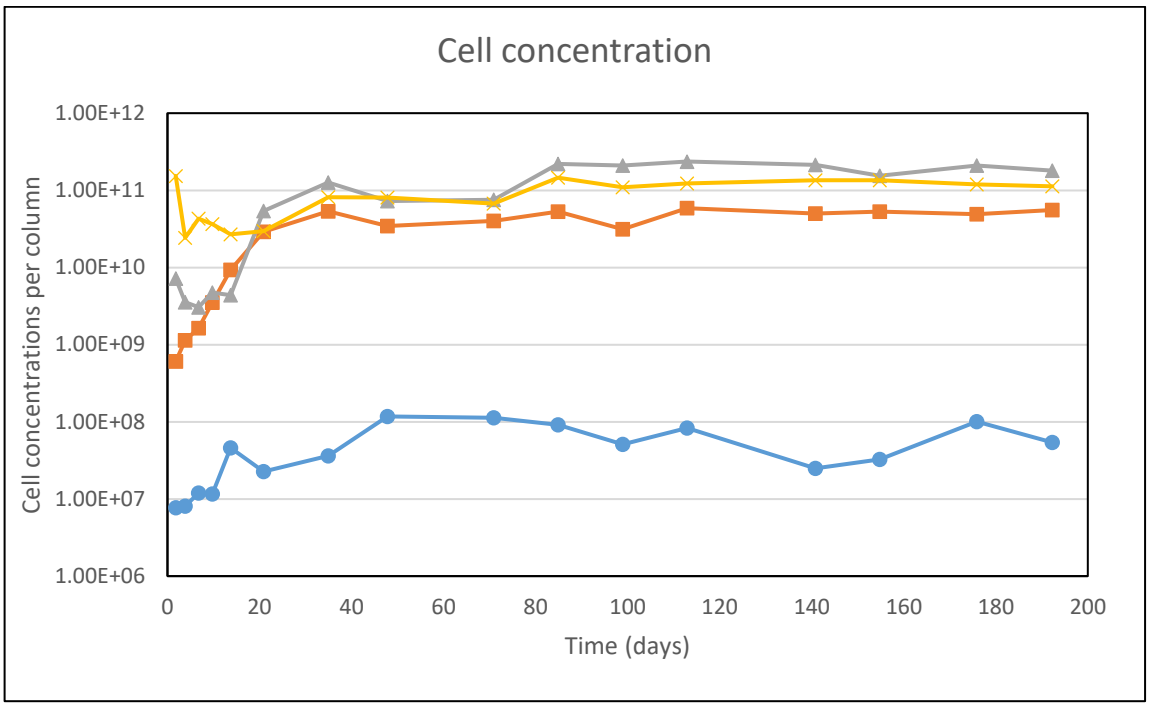


Figure 4.6: Cell concentrations per column over 192.30 days.

The strongly attached phase had the highest cell distribution at the outset of the experiment at 95.4% of the total cells per column, and overall when averaged over each column harvest at 51.0%. The weakly attached phase had the highest cell distribution at the end of the six-month period at 56.0% of the total cells per column, and the second highest overall average of the cell distribution throughout the whole experiment at 39.5%. The interstitial phase accounted for 14.5% of the average cell distribution throughout the experiment followed by the leachate at 0.03% (Figure 4.7, Table 4.2).

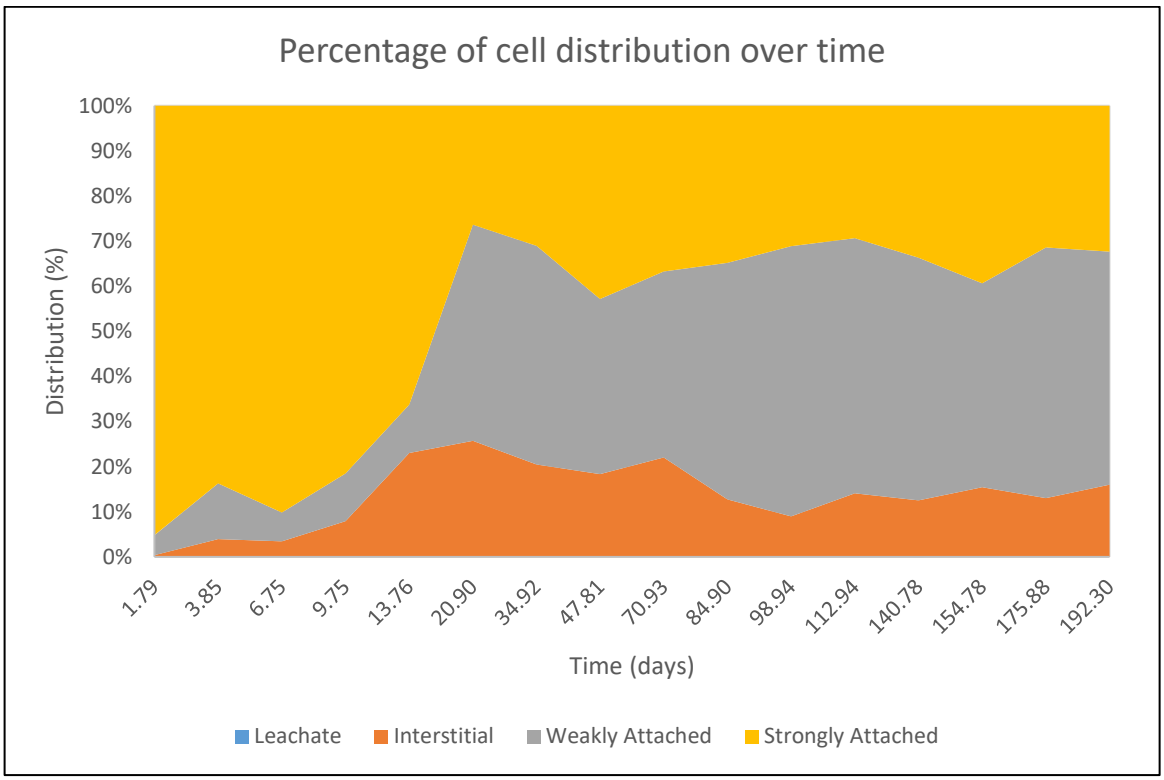


Figure 4.7: Percentage of cell distribution over 192.30 days.

Table 4.2: Percentage of cumulative cells concentrated in each phase within the column over 192.30 days.

Phase	Overall cell contribution (%)
Leachate	0.03
Interstitial	14.5
Weakly Attached	39.5
Strongly Attached	51.0

4.4.4 Maximum specific growth rate

Growth rates for the ore-associated phases were calculated using a Malthusian model and are outlined in Table 4.3 (Appendix C). The interstitial phase had the highest growth rate of the ore-associated phases, followed by the weakly attached phase (doubling time of 95.3 hours and 104.0 hours respectively). The strongly attached phase had the lowest growth rate of the ore-associated phases at 337.0 hours doubling time.

The leachate was modelled as a continuous flow system (μ=flow rate/volume) and had the highest growth rate overall at 0.54 hours doubling time.

Table 4.3: Growth rate (μ) for each phase during colonisation.

	μ (h^{-1})	t_d (h)
Leachate	1.28	0.54
Interstitial	0.0073	95.3
Weakly attached	0.0067	104.0
Strongly attached	0.0021	337.0

4.4.5 METAL DISSOLUTION

The ore associated phases were dominant in terms of metal distribution for the duration of the experiment for both iron and copper (Appendix D).

4.4.5.1 IRON

Iron concentrations in the leachate were stable at roughly 0.250 g/L, once ferric iron was added to the solution at t=9 days (Figure 4.8). The iron concentrations in the ore-associated phases were more variable than those in the leachate, from 0.19 g/L to 8.81 g/L. Iron was roughly 10x more concentrated in the ore-associated phases than in the leachate.

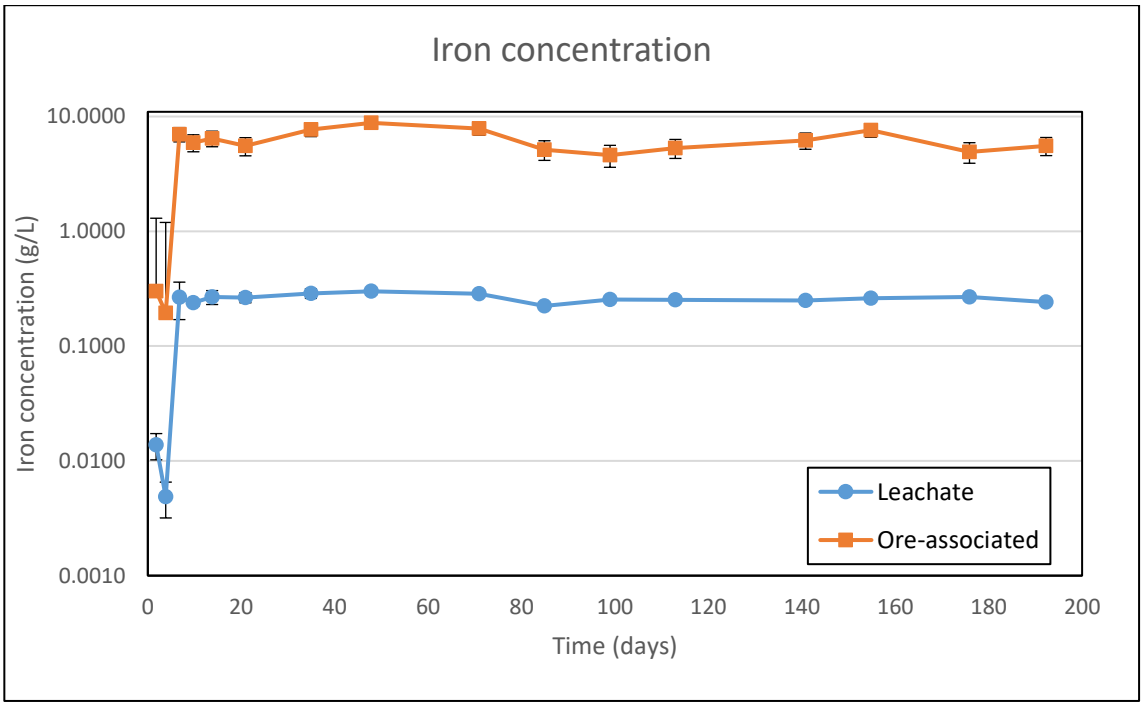


Figure 4.8: Iron concentration in the leachate and ore-associated phases. Error bars show variation in the 3 columns counted to obtain a leachate measurement.

Iron distribution across the phases as a percentage of the total iron in the column was calculated and is shown in Figure 4.9 (Appendix C). Overall, the ore-associated phases had the highest percentage of total iron throughout the experiment. The percentage of the iron distribution within the ore-associated phases and the leachate varied throughout time with no specific trend.

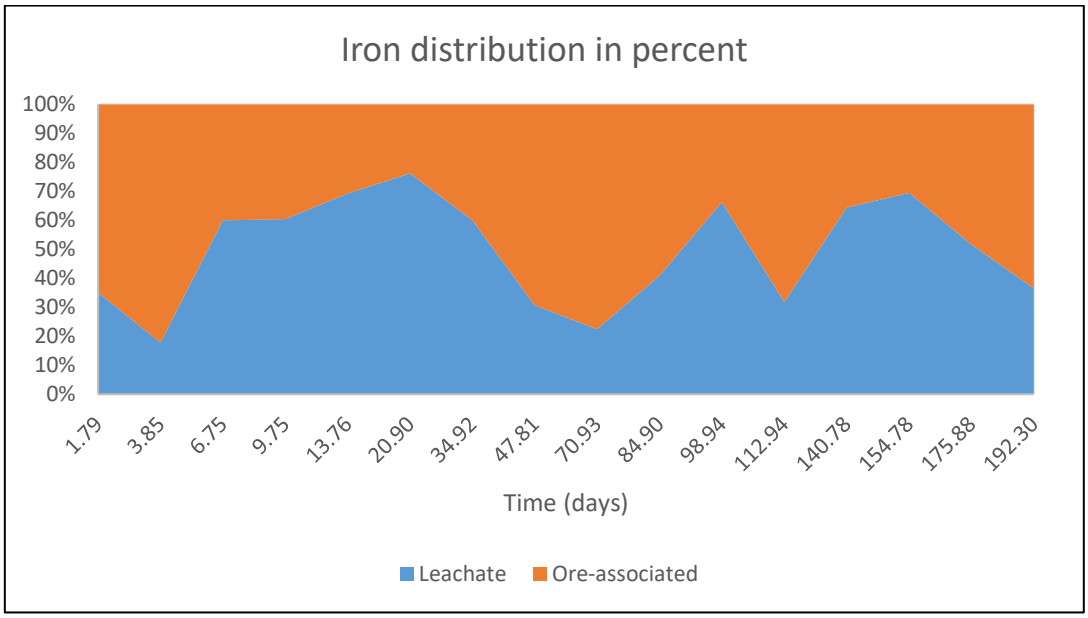


Figure 4.9: Comparison of the percentage of total in the leachate versus the ore-associated phases throughout the six-month experiment.

4.4.5.2 COPPER

The copper concentrations in the leachate ranged from 0.02 g/L to 0.0001 g/L (Figure 4.10, Appendix C), decreasing steadily with time. This decrease was also seen in the ore-associated phases and ranged from 5.21 to 0.51 g/L, however there was more variability in the ore-associated values in the different columns. There was a large peak in copper concentration at 5.21 g/L in Column 12 (t=112.9 days). Copper values were generally 100x higher in the ore-associated phases.

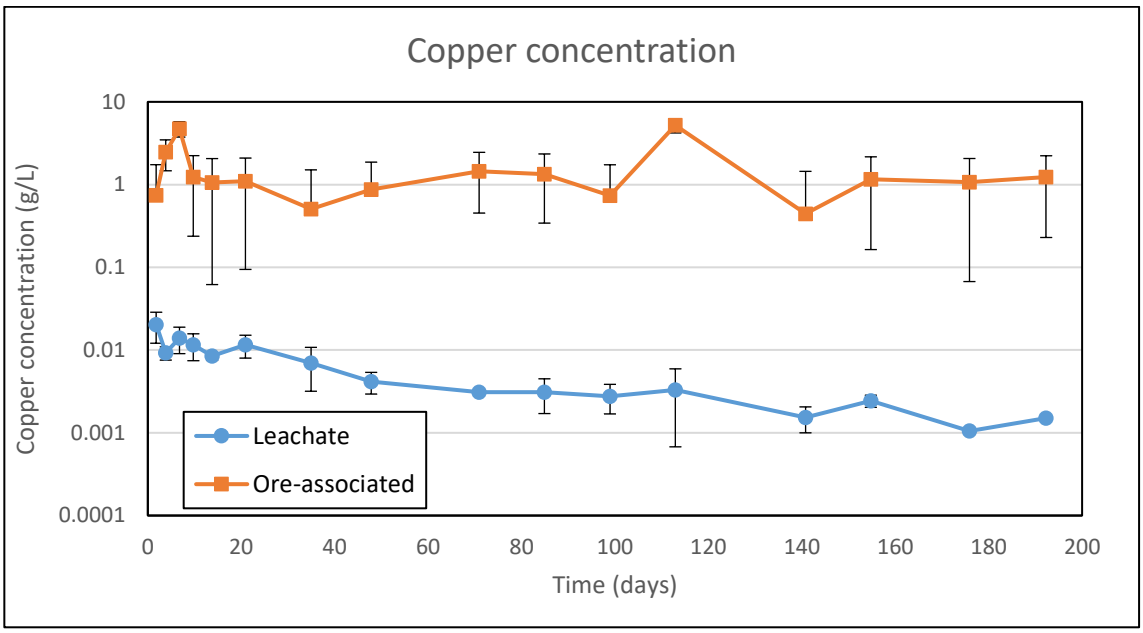


Figure 4.10: Copper concentration in the leachate and ore-associated phases. Error bars show variation in the 3 columns counted to obtain a leachate measurement.

The distribution of copper between the phases was also calculated (Figure 4.11). Overall, the ore-associated phases hosted higher percentages of copper, in comparison to the leachate. The ore-associated phases gradually hosted an increasing percentage of the copper distribution as time went on. Otherwise, no obvious trends are visible.

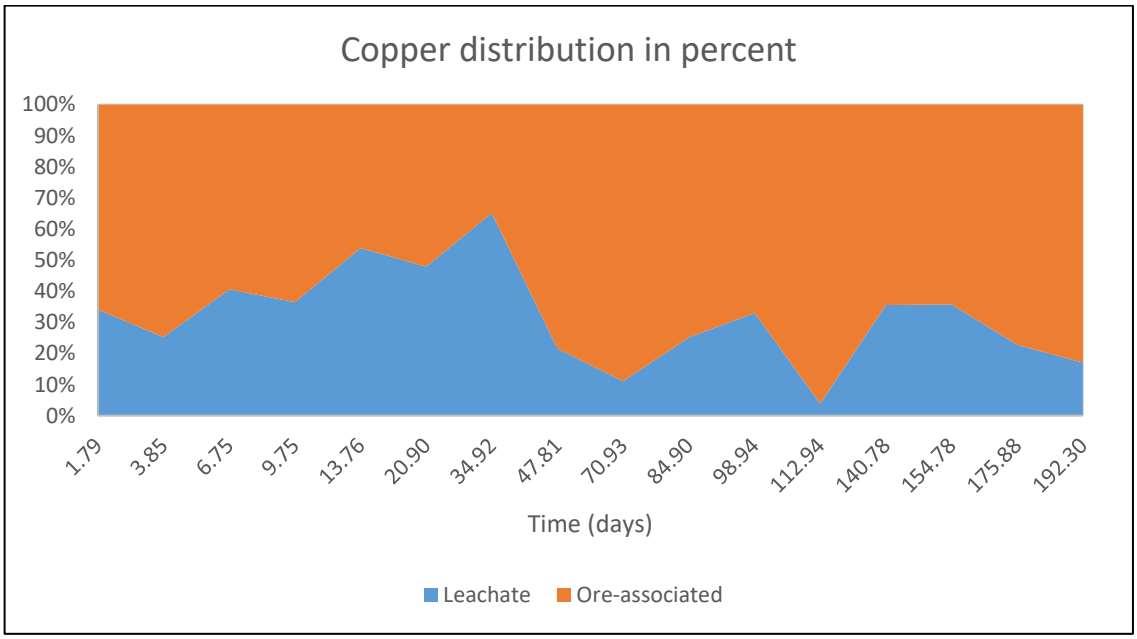


Figure 4.11: Comparison of the percentage of total iron per phase throughout the six-month experiment.

4.4.5.3 CUMULATIVE COPPER LEACHED

Figure 4.12 shows the cumulative copper leached from the ore bed, calculated by averaging the concentration of copper measured in the bulk solution and point samples (Appendix D). Error bars show the standard deviation between columns (n = 16). Total copper extracted over the six-month period was 178.2 ppm. The cumulative average includes all columns within the experiment which does not account for the mineralogical heterogeneity of the ore used to construct the columns.

Enargite is 48.4% copper, and the ore was 0.8% enargite total meaning 3.2% of the mineral would have been leached if that was the only copper mineral present in the system. This will be discussed in detail in Chapter 5.

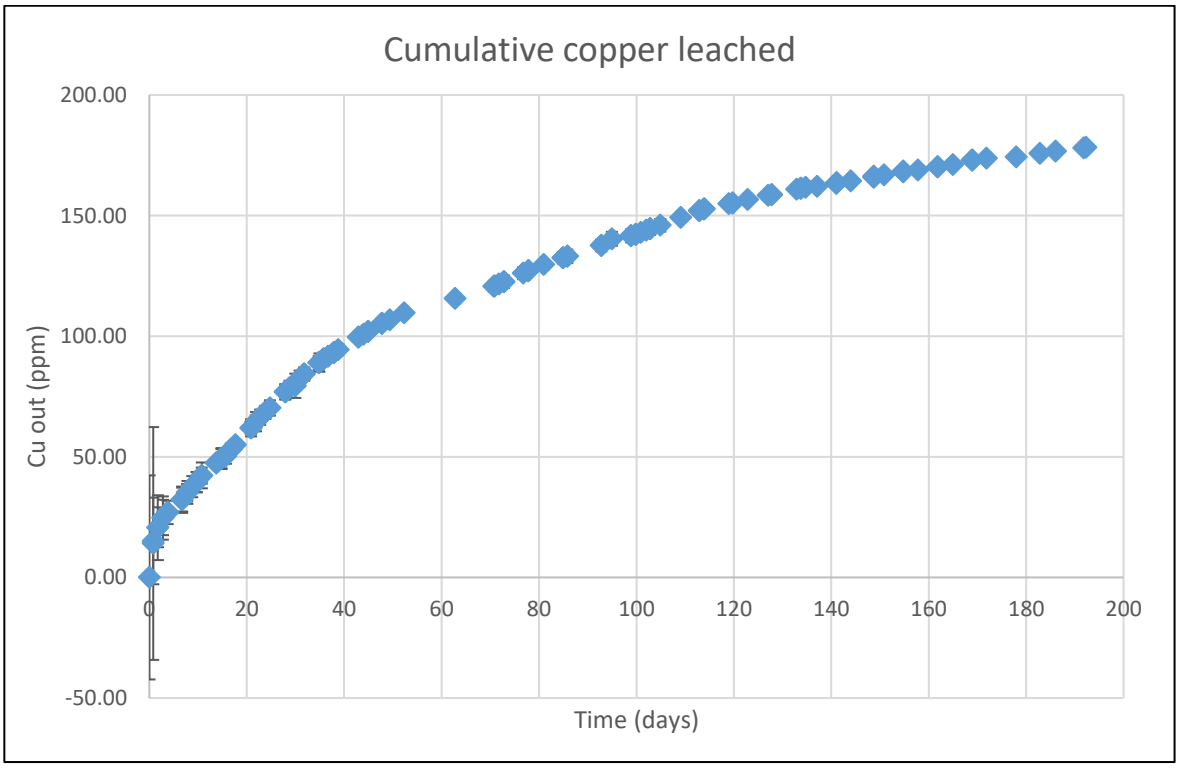


Figure 4.12: Cumulative copper leached from the ore-bed over 192.30 days. Error bars represent the standard deviation of copper values between columns.

Figure 4.13 underlines the difference in copper content between columns by showing the cumulative copper out of Column 1 and Column 2. Column 2 had a higher total cumulative copper extraction than Column 1 despite the fact that Column 1 ran for 192.3 days and Column 2 ran for only 171.9 days.

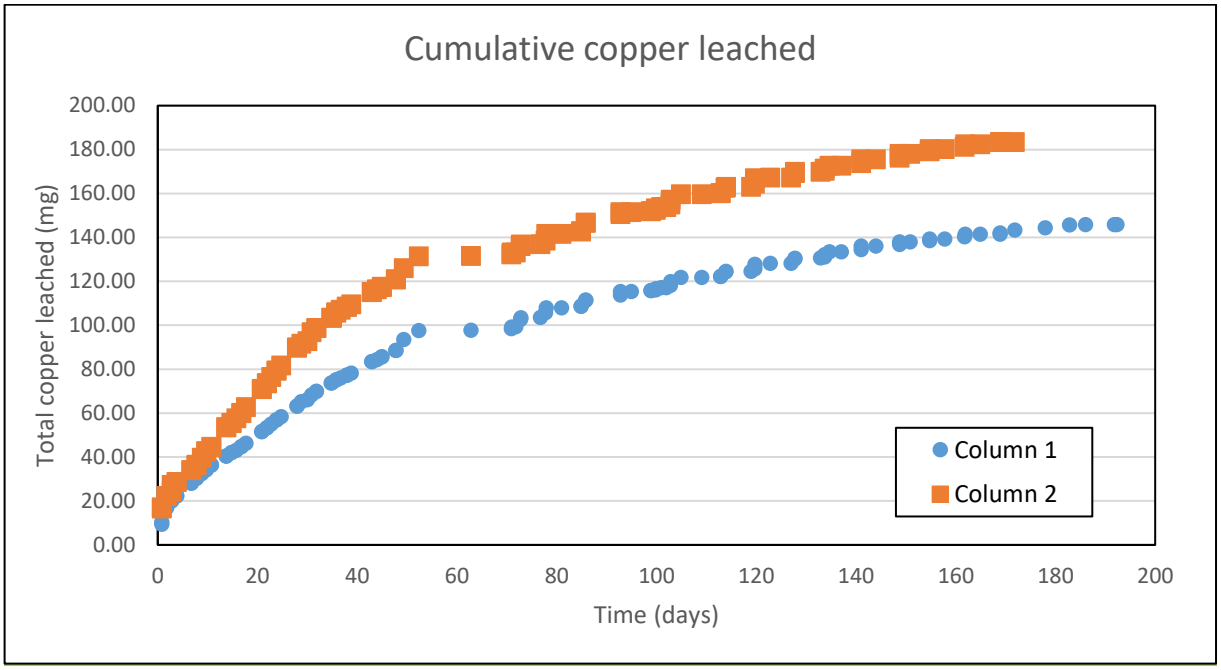


Figure 4.13: Cumulative copper out (in mg) of Column 1 and Column 2 over 192.3 and 171.9 days respectively.

4.4.6 REDOX

Redox values were calculated as explained in Section 3.10.2 and are shown in Figure 4.14 (Appendix C). The leachate had high redox values even at the outset of the experiment at 582 mV SHE (standard hydrogen electrode). This increased greatly with the addition of ferric iron at day 9, to over 800mV SHE.

Redox values for the ore-associated phases were also calculated and were fairly consistent in the 580mV SHE-range at the beginning of the experiment (first sample at t=43.05). Values entered the 700mV SHE-range once ferric iron was added to the feed.

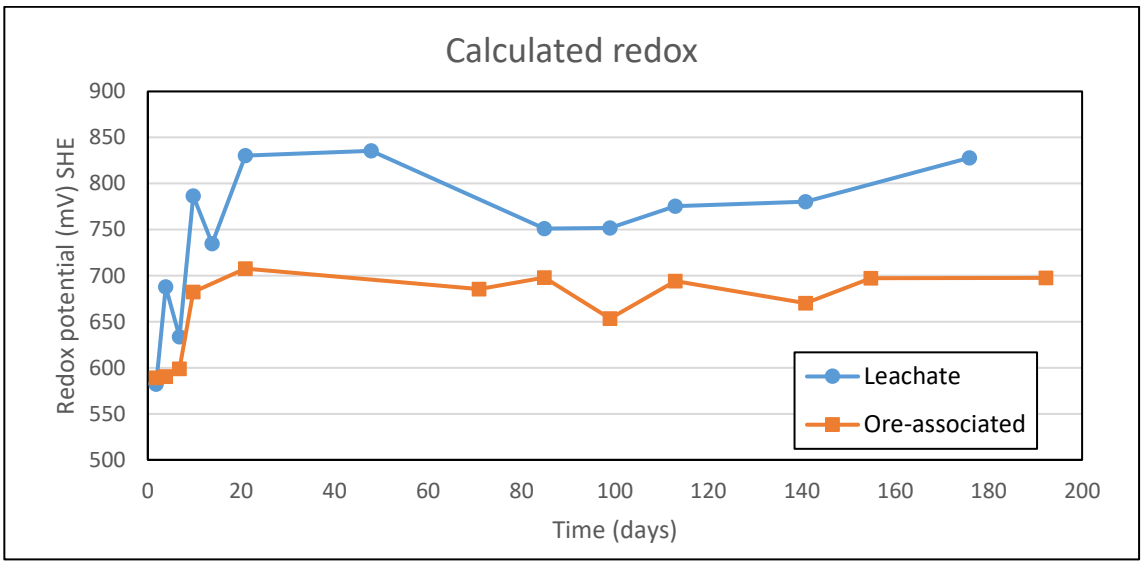


Figure 4.14: Calculated redox values for each phase versus time. Note that there is no point for 192 days for the leachate due to an experimental error.

4.4.7 Microbial ecology

This section will review the outcomes of the DNA extraction data and outline the microbial ecology between phases. The microbial ecology results are given in Appendix B. This will begin with a look at the dominant species in the system, *Leptospirillum ferriphilum*. Following this, a comparison of the ecology between the phases will be given.

The DNA samples from Columns 14-16 were not able to be analysed due to experimental error. In addition, there were some columns where there was no DNA extracted for those phases due to experimental error at the time of collection (Columns 3, 4, 12, and 13 in the leachate, Column 2 in the strongly attached phase, and Columns 1, 2, and 13 in the weakly attached phase).

4.4.7.1 *Leptospirillum ferriphilum*

Leptospirillum ferriphilum was the dominant species in this experiment in all phases. The percentage of the total cells of *Leptospirillum ferriphilum* making up the community ranged from 54.26% to 96.56% in the leachate, 73.92% to 99.28% in the interstitial phase, 54.66% to 96.66% in the weakly attached phase, and 46.11% to 91.60% in the strongly attached phase (Figure 4.15) over the 192.30-day period.

A colonisation curve is evident when examining *Leptospirillum ferriphilum* values (Figure 4.15). The colonisation of the ore-bed by *Leptospirillum ferriphilum* (in all

phases) occurs from 9.74 days to 34.25 days. Column 11 has a much lower concentration of *L. ferriphilum* than the other phases, offsetting the colonisation curve and is therefore considered an erroneous point.

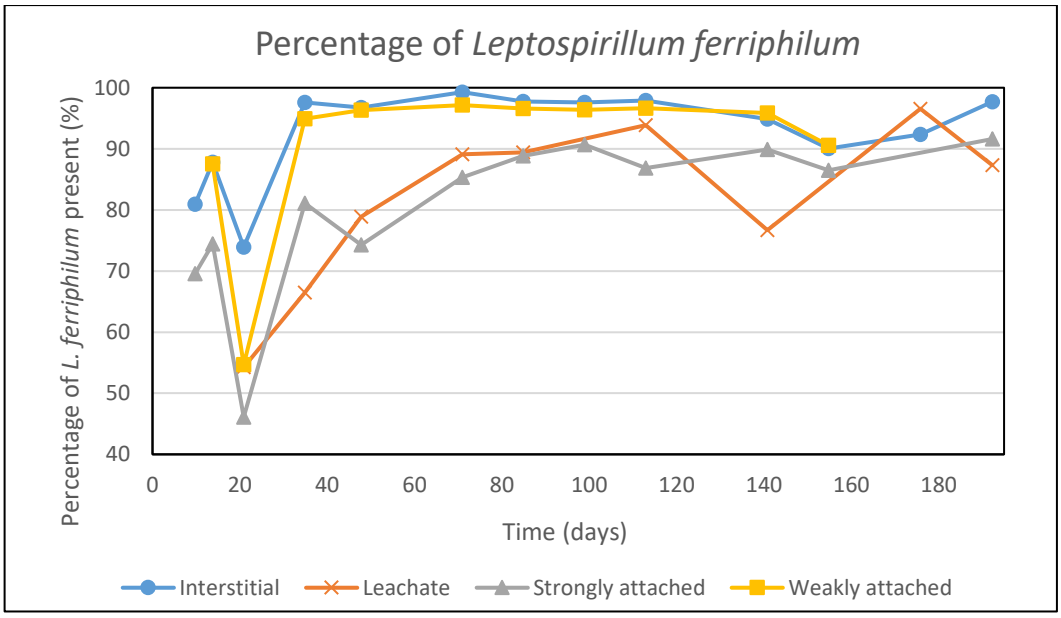


Figure 4.15: Percentage of L. ferriphilum present in each phase over time.

Maximum specific growth rates were calculated for *Leptospirillum ferriphilum* (Table 4.4). The leachate was modelled as a continuous system and the ore-associated phases as batch reactors.

The leachate had the highest growth rate with a doubling time of 0.54 hours. This was followed by the weakly attached phase, which had a doubling time of 108 hours. The interstitial phase had a doubling time of 124 hours followed by the strongly attached phase which had the longest doubling time at 426 hours.

Table 4.4: Maximum specific growth rates of Leptospirillum ferriphilum during colonisation of each phase.

	μ (h^{-1})	t_d (h)
Leachate	1.28	0.54
Interstitial	0.0056	124
Weakly attached	0.0064	108
Strongly attached	0.0016	426

4.4.7.2 Comparison of the microbial ecology between phases

Comparisons between the leachate and the ore associated phases (Figure 4.16) show that the leachate housed a wider range of microorganisms than those seen in the interstitial phase. Both phases were dominated by *Leptospirillum ferriphilum* as discussed above. The leachate had notable populations of *Acidithiobacillus albertensis*, *Alicyclobacillus disulfidooxidans*, *Acidithiobacillus ferrooxidans*, and *Acidiferrobacter sp*. compared to the interstitial phase. Figure 4.16 examines the average community structure to the end-point of the experiment in each phase (t=70.9 days to t=192 days). Colonisation of the ore-bed by *Leptospirillum ferriphilum* was apparent after t=70.9 days (Figure 4.15).

The strongly attached phase had the highest diversity in microbial ecology overall, followed by the leachate, the weakly attached phase, and lastly the interstitial phase. The interstitial phase and the weakly attached phase were very similar, and were dominated by 90% and 95% *Leptospirillum ferriphilum*, respectively. The microbial distribution in the strongly attached phase consisted of 88% *Leptospirillum ferriphilum*, 7% *Acidiferrobacter*, 1.7% *A. ferrooxidans*, 1% *Acidithiobacillus* clone, and 3.09% *Acidithiobacillus albetensis*.

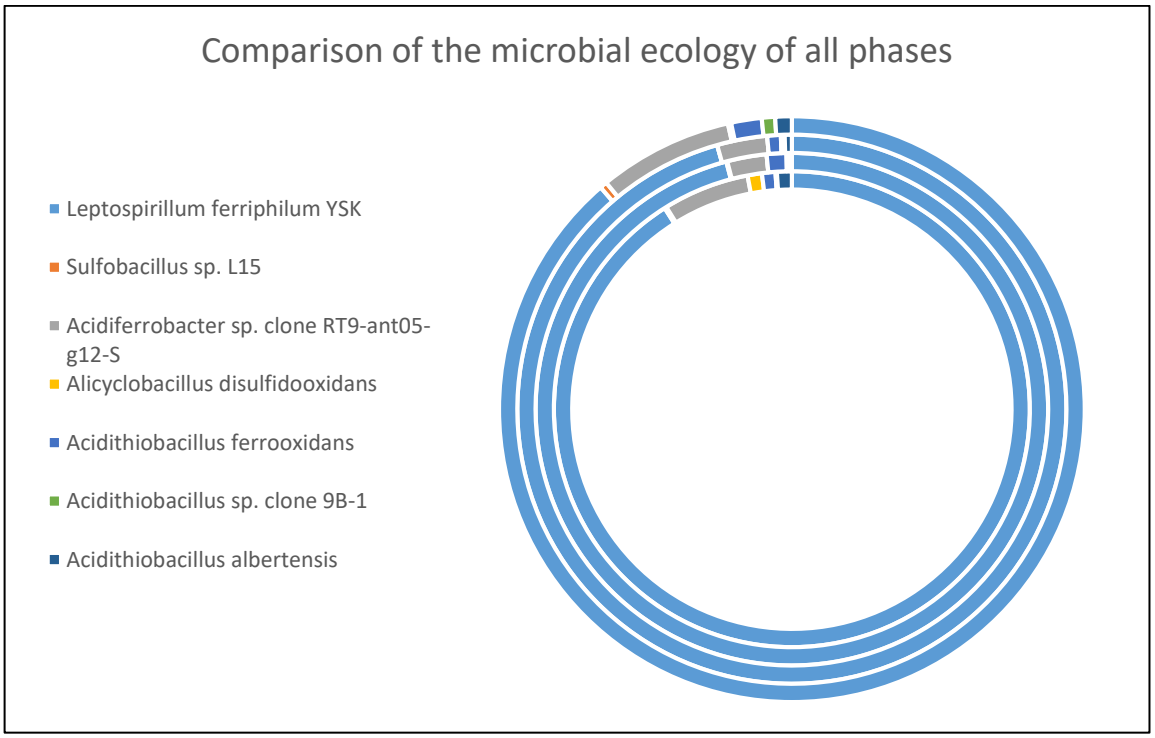


Figure 4.16: Comparison of the community structure of all phases at 70.9 days. The leachate is the closest ring to the centre, followed by the interstitial, weakly attached, and strongly attached.

As the strongly attached phase and the leachate showed the highest variation of organisms throughout the duration of the experiment, 3D bar graphs were made to demonstrate this (Figure 4.17 and 4.18). No comparisons were made for the interstitial phase and the weakly attached phase as there was very little variation between the phases in community structure (Figure 4.16).

Figure 4.17 shows the microbial ecology in the strongly attached phase over time. Blank values mean that no DNA was found for that species at that time point. The highest values of *Acidithiobacillus albertensis* were recorded in the strongly attached phase, but the percentage decreased over time.

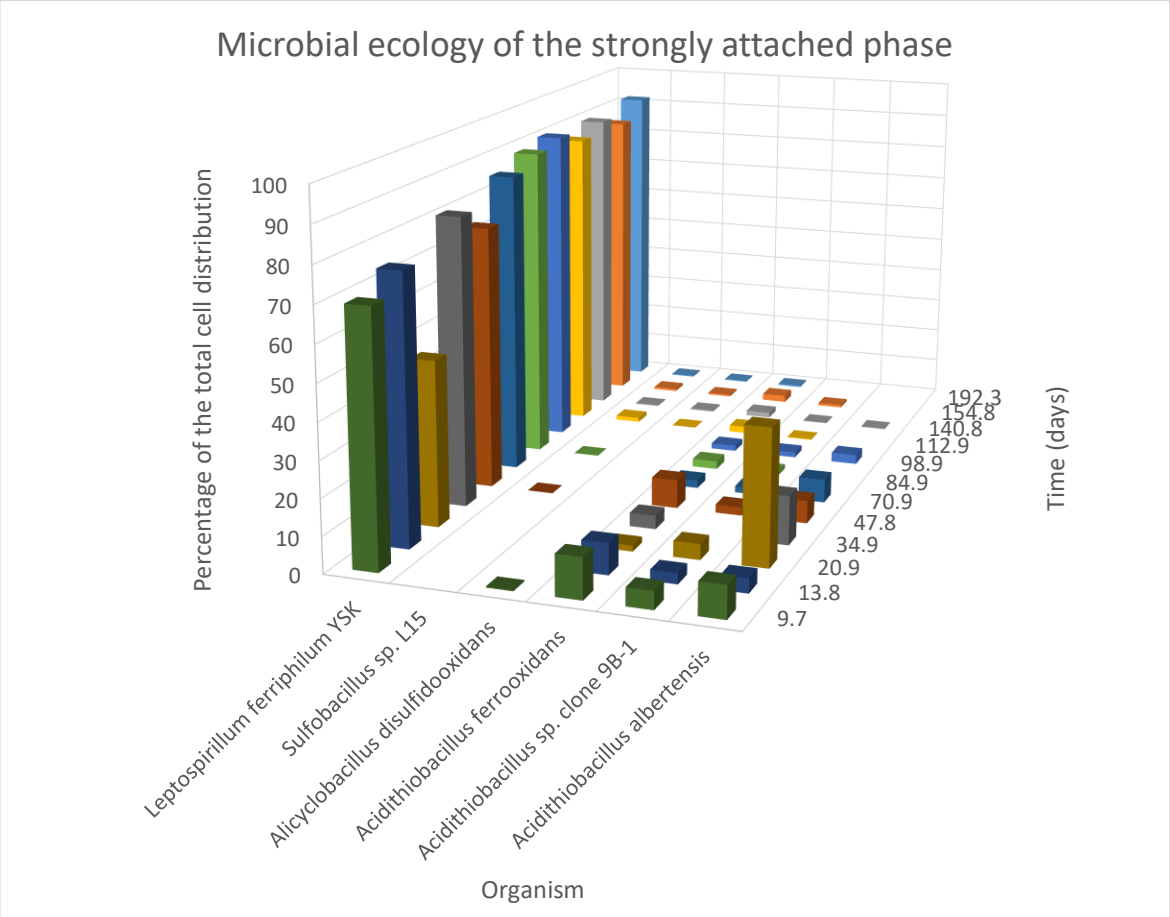


Figure 4.17: The different percentages of each organism present in the strongly attached phase at different points in time.

The leachate is represented in Figure 4.18 and also varied between the phases but had less data points due to the challenge of extracting DNA from a phase with a much lower cell concentration. *Acidiferrobacter* was present in the highest percentage in the leachate compared to the other phases. As was seen with the

Acidithiobacillus albertensis in the strongly attached phase, the percentage of *Acidiferrobacter* within the leachate decreased over time.

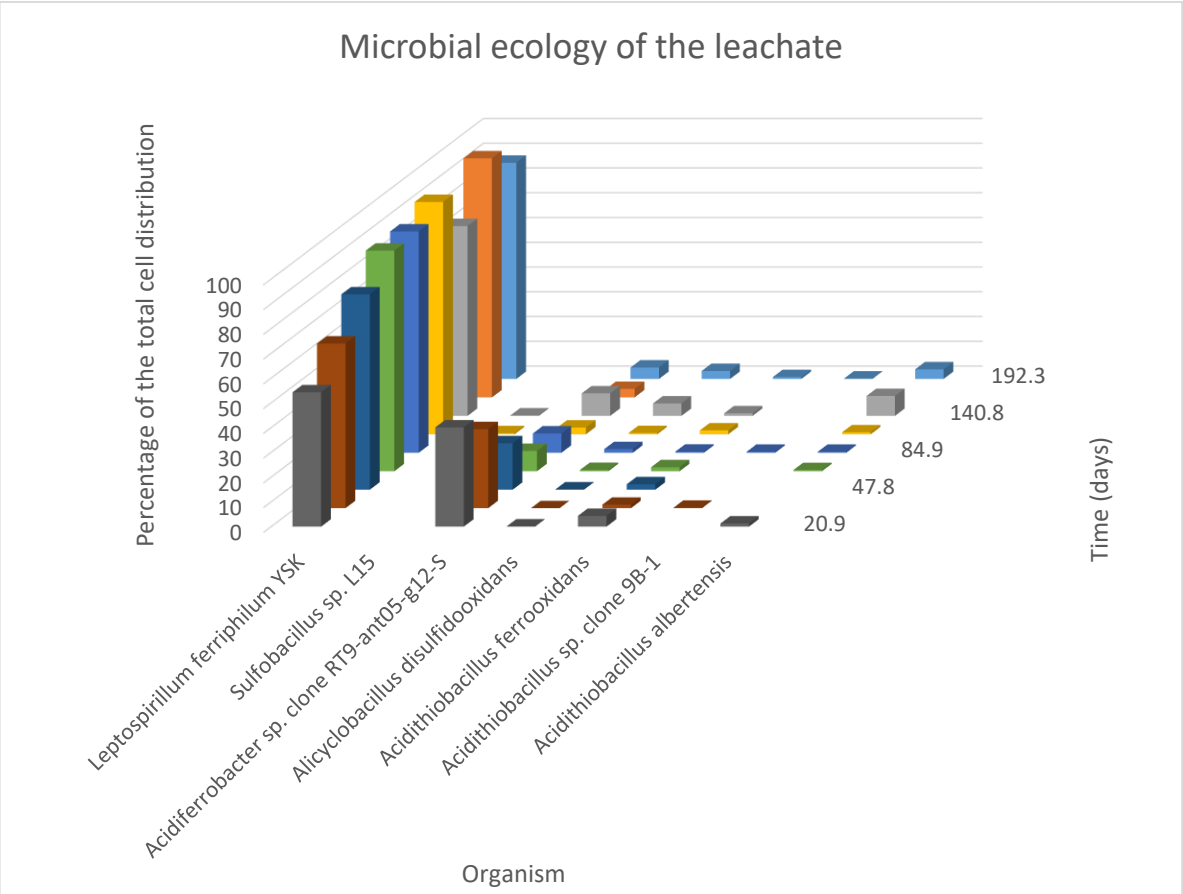


Figure 4.18: The different percentages of each organism present in the leachate at different points in time.

As the objective was to characterise the differences between the leachate and the ore-associated phases, variation between each phase and the leachate is compared using heat maps. The values can be found in Appendix B and are shown in Table 4.5, Table 4.6, and Table 4.7.

Green cells show the highest variation between the leachate and each phase it is compared against. Red cells show the lowest varations. 100% values (light green) mean that the microorganism was found in only one of the two phases. Columns were removed when there was no data for either the leachate or the phase being compared.

Table 4.5 compares the leachate with the interstitial phase, showing a high varation only in *A. ferrooxidans* in Column 7. Variation of *A. ferrooxidans* persists in every column that has data except for the final column (t=192 days).

Leptosprilum ferriphilum also has varying proportions between the leachate and the interstitial phase but as it is the dominant bacteria in both phases, the variation is lower between the two columns for the duration of the six month period (Figure 4.15).

Table 4.5: Heat map showing variation between the interstitial and the leachate, Column number is in the top row, #N/A means missing data.

	1	2	4	5	7	8	9	10	11
Leptospirillum ferriphilum YSK	11.9	4.4	23.7	4.3	9.4	11.4	22.6	46.8	36.2
Sulfobacillus sp. L15	#N/A	#N/A	100	100	#N/A	#N/A	#N/A	#N/A	#N/A
Acidiferrobacter sp. clone RT9-ant05-g12-S	50.4	49.0	57.8	50.6	88.5	100	94.3	96.0	38.2
Alicyclobacillus disulfidooxidans	100	#N/A	100	100	100	100	100	#N/A	100
Acidithiobacillus ferrooxidans	100	#N/A	24.8	53.6	144.1	55.2	26.9	52.3	90.6
Acidithiobacillus sp. clone 9B-1	100	#N/A	#N/A	#N/A	59.7	#N/A	#N/A	100	#N/A
Acidithiobacillus albertensis	100	#N/A	100	100	100	100	#N/A	#N/A	29.0

Table 4.6 compares the community structure between the leachate and the weakly attached phase. The *Leptospirillum ferriphilum* again shows variation between the phases in every column (although much lower than that seen in Table 4.5). The highest degree of variation between the distribution of *Leptospirillum ferriphilum* is again seen in Column 10. Additionally, as was seen in Table 4.5, *Alicyclobacillus disulfidooxidans* is found only in the leachate. Variations in the *A. ferrooxidans* population are much lower than those measured when comparing the interstitial phase with the leachate. The highest variation between the leachate and the weakly attached phase is in the *Acidithiobacillus albertensis* population which is more abundant in the weakly attached phase.

Table 4.6: Comparison of the community structure of the leachate to the weakly attached phase.

	4	5	7	8	9	10	11
Leptospirillum ferriphilum YSK	25.0	3.0	8.0	9.0	22.1	42.7	0.7
Sulfobacillus sp. L15	100	100	#N/A	100	#N/A	#N/A	#N/A
Acidiferrobacter sp. clone RT9-ant05-g12-S	58.8	9.4	70.5	93.7	90.7	90.9	9.7
Alicyclobacillus disulfidooxidans	100	100	100	100	100	#N/A	100
Acidithiobacillus ferrooxidans	62.2	77.8	95.2	32.9	40.9	49.0	33.9
Acidithiobacillus sp. clone 9B-1	#N/A	#N/A	58.2	#N/A	#N/A	100	#N/A
Acidithiobacillus albertensis	100	100	100	49.0	#N/A	#N/A	167.7

Finally, Table 4.7 compares the leachate and the strongly attached phase. *Acidithiobacillus albertensis* shows very high values, which can be attributed to the strongly attached phase (Figure 4.17). The *Acidithiobacillus ferrooxidans* values are much higher due to the leachate (Figure 4.18). Again, there is variation between the phases in the *Leptospirillum ferriphilum* population.

Table 4.7 Heat map showing variation between the strongly attached phase and the leachate.

	1	4	5	7	8	9	10	11
Leptospirillum ferriphilum YSK	4.9	17.2	7.5	0.6	4.2	5.9	22.0	15.0
Sulfobacillus sp. L15	#N/A	61.6	166.3	#N/A	100	#N/A	#N/A	#N/A
Acidiferrobacter sp. clone RT9-ant05-g12-S	60.0	14.4	256.5	2.7	49.9	51.9	95.0	74.2
Alicyclobacillus disulfidooxidans	92.0	93.5	90.4	100	100	100	#N/A	100
Acidithiobacillus ferrooxidans	42.0	42.8	20.5	366.7	35.9	263.2	168.9	61.8
Acidithiobacillus sp. clone 9B-1	100	#N/A	#N/A	206.3	#N/A	#N/A	100	#N/A
Acidithiobacillus albertensis	100	98.6	100	100	1239.1	#N/A	#N/A	2860.6

4.5 Reactor Study

High temperature reactors (63ºC) were used for batch testing to examine the detrimental effects of low pH, temperature, and high iron concentrations on microbial growth rates (Appendix E).

4.5.1 Maximum specific growth rate

The maximum specific growth rate (μ_{max}) decreased with decreasing pH. Values ranged from 0.01 h^{-1} to 0.016 h^{-1}. At a pH of 1.2, growth rates were faster at 0.025 h^{-1}, and at pH 1.4, the growth rate was 0.035 h^{-1}. Below pH 0.8, growth was almost completely inhibited. This is shown in Figure 4.19.

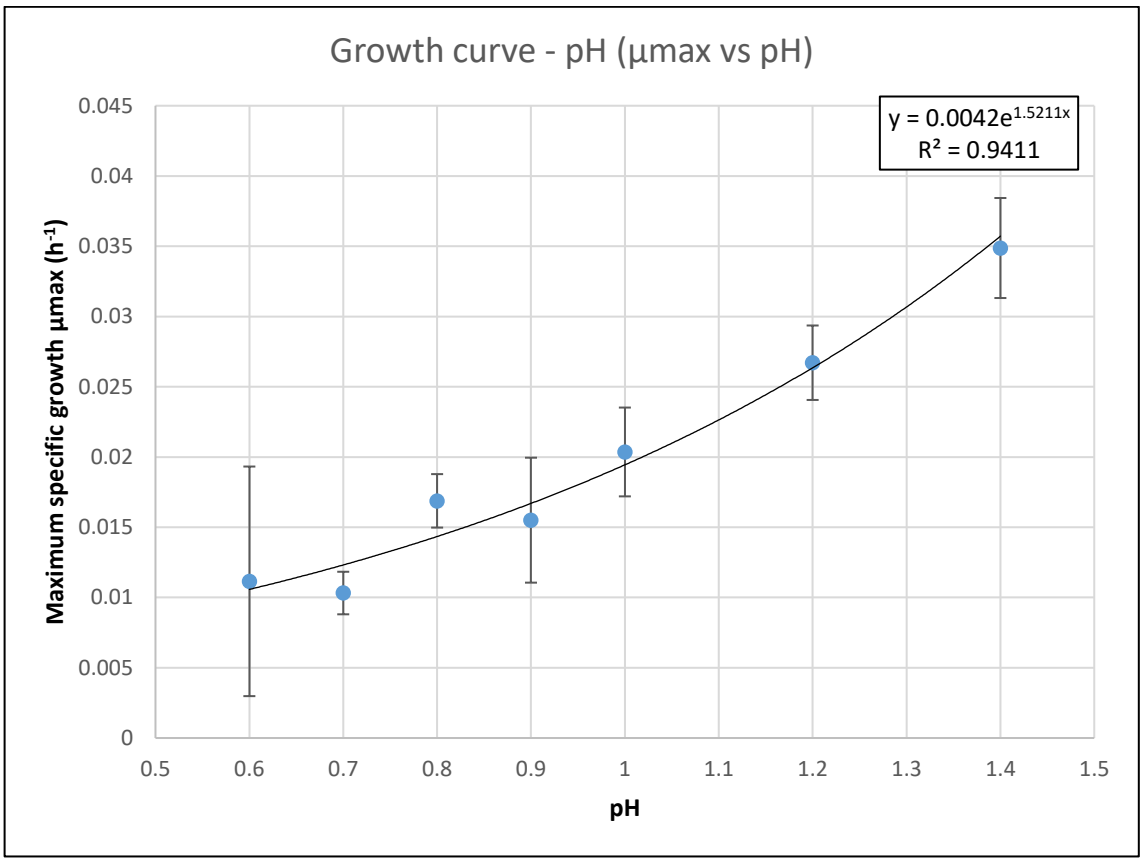


Figure 4.19: Maximum specific growth values at different pH levels, the error bars represent the standard deviation between reactors.

Maximum specific growth rates for batch reactors were calculated for the iron concentration tests as well. Iron values were in the range of 0.1 g/L to 1 g/L in the reactors with no additional iron, compared to 20 g/L, 30 g/L, and 40 g/L during the batch tests (Appendix D). At an iron concentration of 20 g/L, the maximum specific growth rate was 0.015 h^{-1}, at 30 g/L it was 0.012 h^{-1}, and at 40 g/L it was 0.003 h^{-1}. Maximum specific growth rates decreased as iron concentrations increased. At concentrations above 30 g/L, growth was almost completely inhibited. This is shown in Figure 4.20.

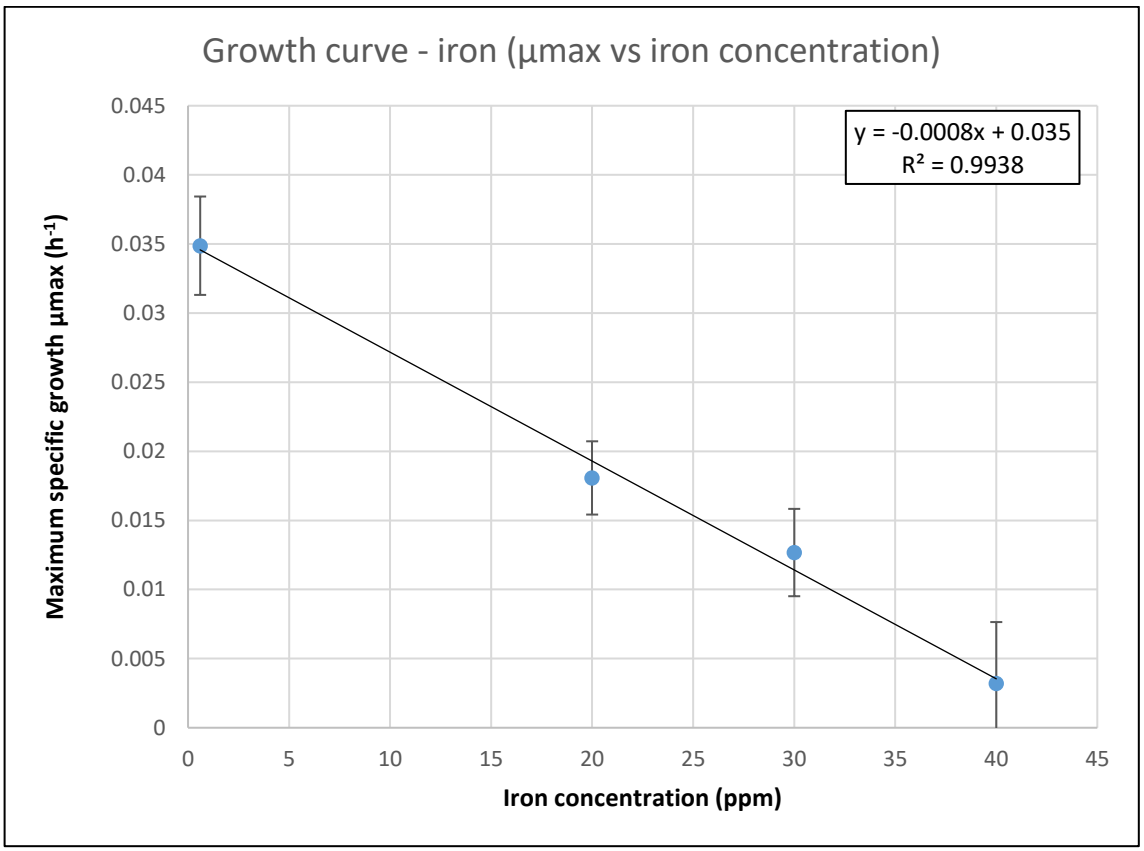


Figure 4.20 Maximum specific growth values at different iron concentrations in g/L, error bars represent the standard deviation between reactors.

4.5.2 METAL DISSOLUTION RATES

The rate of copper dissolution is linked directly to microbial activity (Chapter 2, Section 2.6). Copper dissolution was not affected until the pH dropped below 1 (0.5 - 0.6 mg/L/h; Figure 4.21, Appendix E). Below pH 1, copper dissolution was considerably slower. At pH 0.9, the dissolution rate was 0.36 mg/L/h and at pH 0.8 the dissolution rate was 0.2 mg/L/h, which was less than half the value seen at pH 1.4.

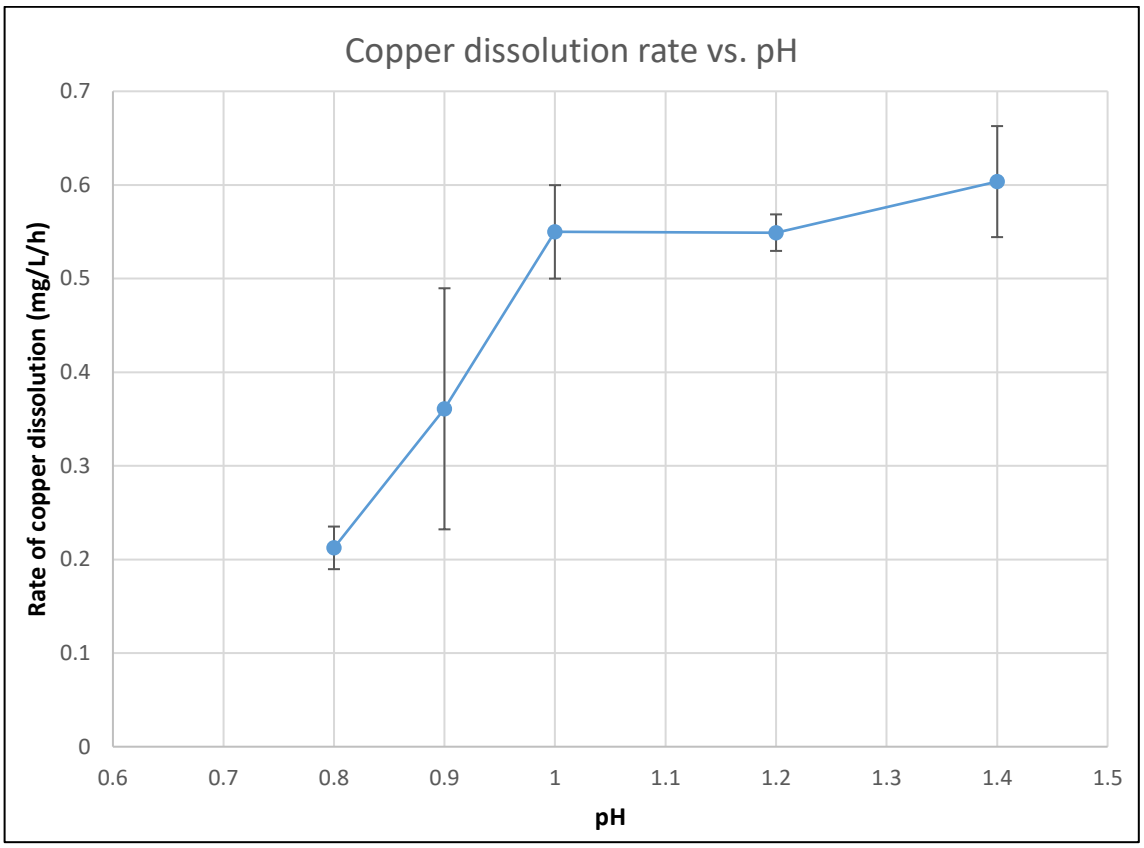


Figure 4.21: Copper dissolution versus pH value, error bars represent standard deviation between reactors.

The iron dissolution rate was also quantified at variable pH levels (Figure 4.22, Appendix E). The rates were not affected to the same extent as seen above in the copper dissolution experiments (Figure 4.21). The fastest dissolution rates were at pH 1 and pH 1.4 at 10 mg/L/h. Slower rates were seen at pH levels less than 1, with pH 0.9 showing the total iron value of 5.53 mg/L/h, at pH 0.8, total iron dissolution was at 5 mg/L/h.

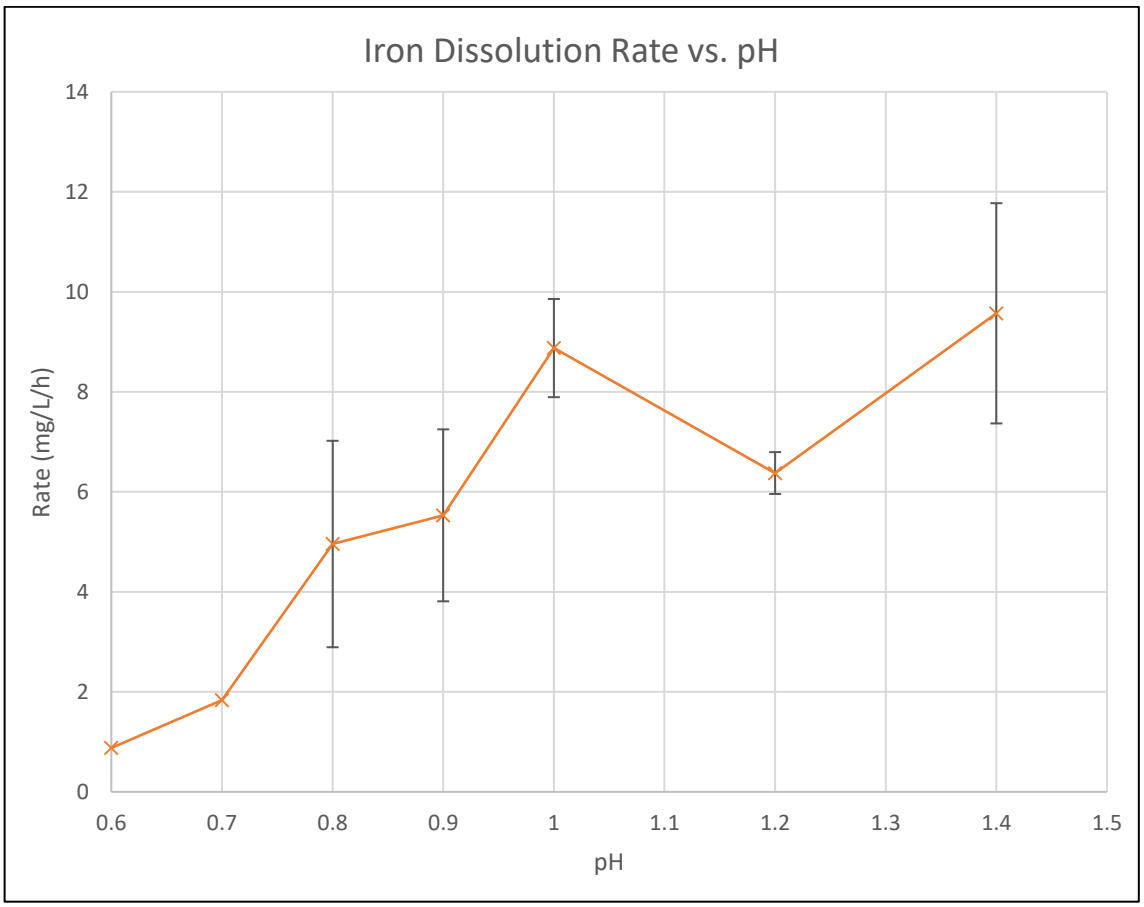


Figure 4.22: Total iron dissolution versus pH, error bars represent the standard deviation between reactors.

4.5.3 Copper extraction

Copper values were totalled and divided by the average assay value (Section 3.2.1, Appendix A) of 6260 ppm. The reactor was at 2% (w/v) ore. This means that the total copper content per reactor was 125 ppm.

The total copper leached was separated into sections based on the pH batch tests. Percentage of the total copper content extracted ranged between 75 and 69% of the total copper content at pH 1.4 and 1.2, respectively. At pH 1 and below, extraction values were lower, between 62 and 66% of the total copper content. This is shown in Figure 4.23. Data for copper extraction is missing for pH <0.8 and for the iron concentration tests.

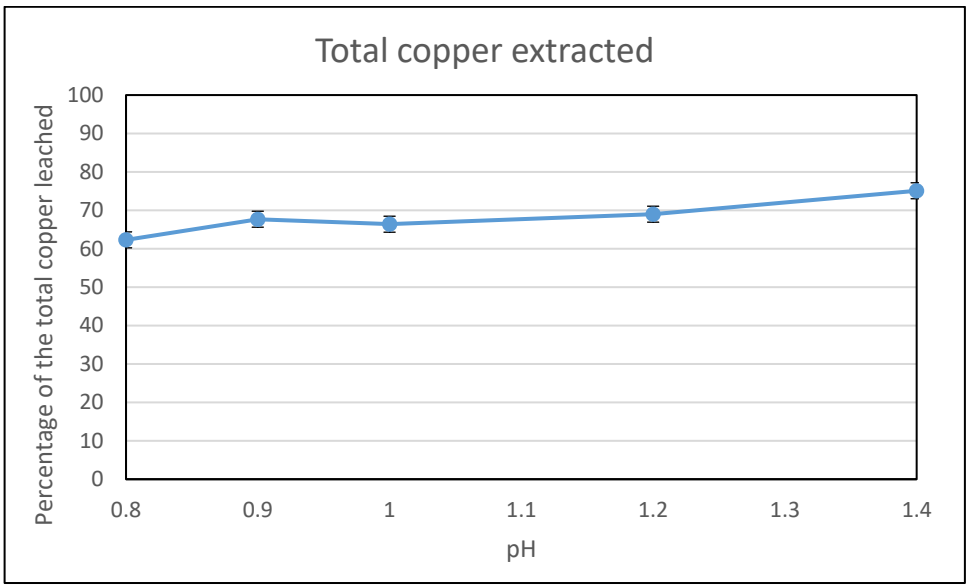


Figure 4.23: Percent of copper extracted versus pH, error bars represent the standard deviation between reactors.

CHAPTER 5. DISCUSSION

5.1 INTRODUCTION

This chapter interprets the results of the study. As in Chapter 4, the results of the microbial retention study and the un-inoculated detachment study will be discussed first. The outcomes of these tests are critical for explaining the results of the six-month agglomerate-scale leaching experiment.

The distribution of cells and metals between within the column during the agglomerate-scale leaching experiment will then be examined. This will be followed by a review of the microbial community structure based on the DNA data. The results outlined in Chapter 4 will be compared to previous agglomerate-scale leaching experiments by Chiume et al. 2012, Govender et al. 2013, and Bryan et al. 2016.

The batch reactor tests will then be discussed to provide insight into the microbial growth at low pH levels and increased iron concentrations. Finally, Chapter 5 will close with recommendations for future agglomerate-scale heap bioleaching studies.

5.2 MICROBIAL RETENTION STUDY

The microbial retention study was done to measure the retention of the inoculum within the ore bed. Microbial retention is an integral part of heap productivity, as colonisation is what allows the microbial population to contribute to metal solubilisation (Chiume et al. 2012; Watling 2016; Govender-Opitz et al. 2017).

Based on the un-inoculated detachment study, the ore would have had 6.53×10^9 cells/column already present at the outset of the experiment – this is the indigenous population (Section 5.3). This implies that the cells exiting through the leachate during the microbial retention study may include some percentage of that number. The indigenous population made up 80% of the total cells within the column at the time of inoculation. Unfortunately, no numerical estimate can be made as to the percentage of cells leaving from the inoculum vs. the number of cells leaving from the indigenous population. It's important to note that the indigenous population likely makes up a fairly small percentage of the cells leaving during the first five hours of leaching, because during the un-inoculated detachment experiment, no cells were found in the leachate (Section 5.4). Future

studies could examine the microbial communities in the inoculum and the leachate during microbial retention to estimate the amount of the indigenous population leaving.

Whatever the percentage of the indigenous population, the average cell concentrations throughout the six-month leaching experiment in the leachate were 4.78×10^7 cells/column. The microbial retention study cell counts recorded at hours four and five of the experiment showed that cells were leaving at 1.34×10^7 and 5.61×10^7 cells/column respectively. This means that three hours after inoculation, the cells leaving had already reached the same concentration, as could be expected throughout the duration of the six-month experiment. It is important to consider that the microbial retention study does not account for cell growth within the 5-hour period.

Despite this high loss of cells at the outset of the study, colonisation of the bed was apparent (Chapter 4, Figure 4.7), reaching stable values in all four phases at about 1000 hours. This will be discussed in detail in Section 5.5.

5.3 UNINOCULATED DETACHMENT EXPERIMENT

The un-inoculated detachment experiment was performed to quantify the community attached to the ore prior to the commencement of the six-month leach. The “un-inoculated” in the name refers only to the fact that these columns were not inoculated, not that they were sterile. The experiment was performed using the same detachment protocol which was used during the six-month leaching experiment (Chapter 3, Section 3.5).

It is unknown if the indigenous population was living or dead at the outset of the experiment, but it is important to consider that the ore was stored dry and transported from Peru to the UK. However, Bryan et al (2011) found that viable cells survived two years of dry storage which suggests that bacteria surviving transportation are entirely possible.

During the un-inoculated detachment experiment there were no cells present in the leachate. It is probable that the acid agglomeration and the acid leach removed any planktonic cells within the ore body if they were present at the start of the experiment. There were, however, cells found in the interstitial phase, likely

due to diffusion or convection at the start of pumping as cells would have had no stagnant liquid to reside in during dry storage. The weakly attached phase had higher cell numbers than the interstitial phase ($2.58x10^8$ cells/column) and the strongly attached phase had the highest cell numbers in total at $6.23x10^8$ cells/column. This explains the high cell counts in the weakly and strongly attached phases at the outset of the six-month leach (Chapter 4, Section 4.5.3, Figure 4.9).

The colonisation curve was not apparent in the weakly and strongly attached phases as was reported in Govender (2013). This variation from the 2013 study can therefore also be attributed to the indigenous population seen in the un-inoculated detachment experiment and will be discussed further in Section 5.4.

This test was crucial for a more complete understanding of the system because the indigenous community may have had the capacity to leach metals (Volant et al. 2014). As indigenous bacteria are best suited to potentially harmful metal concentrations in their environment (Watling 2016), it is important to document their presence and work towards a deeper understanding of the microbial ecology of these communities. This knowledge can then be applied to natural systems such as sites affected by or prone to acid mine drainage (Volant et al. 2014; Watling 2016). The inoculum used for this experiment, for example, was enriched from mine waste at Wheal Maid (Cornwall, UK) therefore it had already adapted to an acidic environment with high concentrations of dissolved metals.

5.4 Cell Distribution

5.4.1 Overview

One of the main research targets for this study was to quantify the cell distribution across all four phases past the initial heap colonisation stage. The results showed that disparity between the leachate, interstitial, weakly attached, and strongly attached phases persisted over a six-month period (Chapter 4, Figure 4.7, Appendix C). The weakly attached phase had the highest cell concentrations overall, followed by the strongly attached and interstitial phases. The leachate had much lower cell concentrations, accounting for only 0.03% of the overall cell numbers (Chapter 4, Section 4.4.3).

Most importantly, despite the fact that the cells were most concentrated in the weakly attached phase rather than the interstitial phase, the cell data still supports the notion that the leachate is not an accurate analogue for the ore-associated phases in terms of microbial concentrations over a six-month period (Chiume et al. 2012; Govender et al. 2013; Bryan et al. 2016).

5.4.2 Colonisation

Colonisation of the ore bed was ongoing during the first 1000 hours of this experiment, after which time the cell concentrations remained stable in all phases. The high cell concentrations in the weakly and strongly attached phases at the outset of the experiment were not expected based on previous studies (Govender et al. 2013; Bryan et al. 2016).

The maximum specific growth rates (outlined in Chapter 4, Table 4.3) were roughly 10x lower than those recorded in Govender et al. (2013). It is important to note that the growth rate values calculated for the leachate are likely an over-estimation (Govender et al. 2013).

The incongruence in the maximum specific growth rate values in the ore-associated phases is possibly a result of the different ore used in this experiment compared to Govender et al. (2013). The ore used in this experiment may have had less available energy for growth. It may also be due to the presence of indigenous bacteria, which slowed the colonisation of the bed by the inoculated culture as discussed above. The colonisation of the bed by the inoculated culture will be explained further in Section 5.8.

5.4.3 Cell concentrations

The higher cell concentrations in the weakly and strongly attached phases in the six-month leaching experiment may be explained by the fact that the ore used in this study was not sterile. This means that it was probably previously colonised by microorganisms and therefore accustomed to high acid and dissolved metal conditions. This would then mean that if viable cells survived storage and transport that the cell counts would be high in the phases associated with the biofilm (strongly attached phase). As previously mentioned, Bryan et al. (2011) also found that an ore which had been stored dry for a two-year period had viable cells.

It is not known whether the community found to be present on the ore was living or dead at the outset of the experiment. It is possible that the presence of dead cells may have encouraged biofilm development of the inoculum at the beginning of the six-month leaching experiment (Li & Sand 2017). This means that the strongly attached phase is composed of a mixture of dead and living cells. Microorganisms build biofilms using dead material so that the microbial community has a greater resilience to toxins within the ore bed (Li & Sand 2017). In terms of cell numbers, the strongly attached biofilm housing dead cells would have a higher cell concentration (when counted using a microscope). The strongly attached phase also shows the highest variation in community structure which may mean that the differences could be accounted for by the indigenous biofilm (Chapter 4, Figure 4.13, Section 5.8).

5.4.4 Comparison to previous studies

Govender et al. (2013), Chiume et al. (2012), and Bryan et al. (2016) all found the same result, that the leachate had much lower cell numbers than the attached phases. These studies ran for 29, 32, and 15 days, respectively. The results from the six-month leaching experiment, however, show that the weakly attached phase had the highest cell distributions followed closely by the strongly attached phase.

This difference in results could be due to the use of sterile ore in both Govender et al. (2013) and Bryan et al. (2016) and non-sterile ore in the six-month leaching experiment. In a non-sterile system, the cell numbers and biofilm development may have taken longer to establish compared to a system where there was no existing community present. The presence of arsenic in the system was not thought to cause a disparity in the results of this study compared to previous work. This is due to the low concentrations of arsenic present as there was only 6% dissolution of enargite over the duration of the experiment.

Another potential reason for disparity between cell concentrations between Govender et al. (2013) and the six-month leaching experiment is that the cell counting in Govender et al. (2013) was done by colony forming units (cfu), whereas the cell counting in the six-month leaching experiment was done using a microscope and counting chamber. Counts done by colony forming units mean that only viable cells could be counted while cell counting using a microscope

does not distinguish between dead and living cells (Govender et al. 2015). The cfu method therefore does not include dead cells in a biofilm, as can be expected from the indigenous community on the ore used in the six-month leaching experiment (Section 5.3). Ultimately, this could have resulted in lower attached cell counts overall in Govender et al. (2013) compared to the six-month leaching experiment.

This does not explain why the interstitial cell concentrations in Chiume et al. (2012) were dominant, as the ore in the 2012 study was also non-sterile, and the cell counting was also done using a counting chamber and microscope. One possible reason as to why Chiume et al. (2012) reported higher interstitial cell counts could be that the indigenous population was removed by the acid agglomeration and crushing in the 2012 study. Another possibility is that the discrepancy is due to a variation in experimental protocol, however there is no obvious reason for this based on the protocol outlined in the 2012 study. DNA samples were not taken in the 2012 study, so comparisons of the microbial communities cannot be made.

5.4.5 Cell concentrations and microbial ecology

DNA results show that the interstitial and weakly attached phases are the most similar in terms of community structure (Chapter 4, Section 4.4.7). As the cells that enter the interstitial phase do so from the weakly attached phase or vice versa this is a sensible result. The community structure in the strongly attached phase and weakly attached phase are different. The strongly attached phase has the lowest percentage of *Leptospirillum ferriphilum,* elevated concentrations of *Acidiferrobacter* and in some columns, *Acidithiobacillus albertensis*. Interestingly, whenever *Acidithiobacillus albertensis* is present, the *Leptospirillum ferriphilum* population is lower. *Acidithiobacillus albertensis* concentrations decrease over time.

As *Acidithiobacillus albertensis* is a sulphur oxidiser, it may indicate decreased sulphur levels within the system, which could be due to precipitation of compounds such as jarosite (Leahy & Schwarz 2009), a decrease in the solubilised metals, or both. The DNA results will be discussed in greater detail in Section 5.8.

5.5 METAL DISTRIBUTION

In addition to the quantification of the cells in each phase, the distribution of metals between the leachate and the ore-associated phases over time was one of the main goals of this study. Metal distribution (both Fe and Cu) were higher in the ore-associated phases than the leachate. The ore-associated phases were grouped together due to the experimental protocol, which did not make it possible to distinguish between the interstitial, weakly attached, and strongly attached phases. This is due to the 1 hour draining period in the detachment protocol (Chapter 3, Section 3.5) when metal ions would have diffused to an area of lower concentration (interstitial or leachate) once they were solubilised (Govender-Opitz et al. 2017).

5.5.1 IRON

Inconsistent iron values in the ore-associated phases throughout the six-month duration of the experiment further underline that the leachate is not an accurate window into the rest of the system. Leachate values remained stable with no evidence as to the changes in the ore-associated phases due to the addition of iron in the feed (Chapter 3, Section 3.4). Iron values in the ore-associated phases are higher than the ferric iron values in the feed (200 ppm) which can be attributed to dissolution at the mineral surface due to Fe^{3+} attack. This would then be followed by the diffusion of liberated ions into the ore-associated phases (Govender et al. 2013). The iron values are higher because the Fe^{2+} is then recycled back into the liquid and as the minerals are dissolved, additional iron is released into the system. The source of the iron is probably pyrite, which makes up 19% of the ore based on the assay results (Chapter 3, Table 3.2).

5.5.2 COPPER

It is important to remember that unlike iron, copper is only present in this system through the ore itself and that no copper was added through the feed during the six-month leach. This means that any copper present in any of the phases can be attributed solely to the dissolution of copper minerals. Enargite (Cu_3AsS_4) is the main copper bearing mineral in the ore (Chapter 3, Table 3.2), but there are also likely additional easily leachable copper minerals (Lee et al. 2011). Measurements of copper ions in the leachate are the highest at the outset of the

experiment, but then at approximately t=40 days the dissolved copper values are lower, meaning solubilisation has slowed, considerably flattening the curve. As discussed in Lee et al. (2011), Corkhill et al. (2008) and Escobar et al. (2000), this can likely be attributed to easily leachable copper metals being solubilised first as opposed to the dissolution of enargite.

If the copper leached was from enargite only, it would account for 3.2 wt.% of what was in the ore-bed at the beginning of the experiment. The mineralogical data did provide an overall copper concentration of 6196 ppm which means that 6.2% of the total copper in the system was leached over a six-month period (Appendix A). However, it is important to remember that this does not suggest which mineral is the copper source. It is very unlikely that the copper leached did come from enargite, based on previous studies (Escobar et al. 1997; Escobar et al. 2000; Corkhill et al. 2008; Lee et al. 2011; Takatsugi et al. 2011; Bryan et al. 2016). This is because reduced rates of bioleaching refractory metals such as enargite (and chalcopyrite) are not uncommon at ambient laboratory temperatures (Takatsugi et al. 2011). The six-month leaching experiment was performed at 27ºC as enargite extraction was not the goal of this study.

There is a large fluctuation in the ore-associated copper concentration at t=112.94 days, which is also likely due to variations in mineralogy. It means that this column probably contained a more copper-rich ore sample. As the ore is not a concentrate but run-of-mine, it is heterogeneous, so these fluctuations are to be expected. In industrial-scale heap bioleaching operations, microorganisms need to be able to survive spikes in concentration as seen here in the interstitial phase. The leachate at this point (Chapter 4, Figure 4.10) did show a variation (the error bar is to represent the difference between the three columns sampled that day for leachate measurements) but the leachate does not provide any idea as to the degree of heightened copper concentration residing in the ore-associated phases (Chapter 4, Figure 4.10). Throughout the rest of the experiment, the leachate does not show fluctuations in copper concentrations when they are apparent in the ore-associated phases. Again, this further proves that the leachate is not an accurate proxy for the overall heap bioleaching system.

5.5.3 SUMMARY

Metal distribution data is critical for a comprehensive understanding of heap health because high dissolved metal concentrations can be detrimental to bacterial life (Watling 2016). By understanding the extent of these challenging conditions, adjustments can be made to help bacterial communities withstand heightened metal concentrations. For example, leaching cultures can be grown in a medium that contains these toxins, including but not limited to copper, arsenic, or anions such as chloride (Escobar et al. 2000; Watling 2016). Resistance can also be built by sub-culturing or emptying and re-inoculating the heap as discussed in Lee et al. (2011).

In the six-month agglomerate-scale leaching experiment, the ore-associated phases contained higher concentrations of dissolved metals than the leachate. There was also often no detectable change in the metal concentrations in the leachate despite considerable fluctuations in the ore-associated phases. This therefore suggests that the leachate cannot be used as an accurate analogue for the metal concentrations within the whole system.

Furthermore, understanding the limits of the ore-associated bacteria in terms of dissolved metal concentration is pertinent so that they are not inhibited during heap bioleaching operations. This will be discussed further in Section 5.10.

5.6 MOVEMENT OF CELLS AND METALS WITHIN THE ORE-BED

When analysing the different microbial phases, the question arises as to how microorganisms move between them. In other words, how did the inoculum move from the flowing liquid to eventually colonise the ore bed?

Govender et al. (2013) attributes cell movement to a multitude of factors, such as chemotaxis, electrostatic and hydrophobic forces at the mineral surface, space limitation on the mineral surface and diffusion (overviewed in Chapter 2, Section 2.7). Chemotaxis is the most likely driving force for inoculated cells to swim towards the mineral surface due to its high metal concentration. Without this movement, sterile systems as seen in Govender et al. (2013) would never exhibit colonisation, as in that study there were no cells present on the mineral surface at the outset of the experiment.

There is no one clear answer for what drove cell movement between the phases throughout the duration of the six-month agglomerate scale heap leaching experiment. A potential reason is that the cells moved via diffusion. Diffusion is the movement of molecules into an area of lower concentration within a system. A well-known theory in terms of metal movement, it is not yet clear how diffusion correlates to cell concentrations in agglomerate-scale environments.

Movement of the cells from the attached phases to the interstitial phase and then the interstitial phase to the leachate may also be invoked in part due to the convective forces in any fast-moving bypassing liquid (Fogler, 2010). The results from Chiume et al. (2012) for example, show that higher irrigation rates result in higher concentrations of cells leaving.

During the column harvests in the six-month leach, the one hour draining period to separate the leachate (flowing liquid) and the ore-associated phases made it impossible to separate the phases into the interstitial, weakly, and strongly attached phases. This is due to the lack of hydrostatic pressure imposed by the feed input on the ore-bed, which forces the leachate to drain through the column (Fagan et al. 2014). This means that once there is no feed input there is no more flow within the column after the pump is switched off and the column is left to rest for one hour (Chapter 3, Section 3.5). Metals (and possibly cells) are then diffused from the ore particles into the interstitial phase. This is due to the fact that there is no driving force to pull them into the leachate, or to pull the leachate through the column. The result of this is in an increased concentration of both cells and metals trapped in the hold-up volume. Future studies should examine the effect that the one hour draining period has on the metal concentration. This could be done by following the detachment protocol immediately after the pumps are switched off and one hour after the pumps are switched off and comparing the results to examine the disparity between the measurements.

Copper concentrations are not directly linked with cell concentrations in the sense that bacteria are not processing copper ions directly. This is due to the indirect bioleaching mechanism (i.e. copper solubilisation is a product of ferric iron attack) as discussed in Chapter 2, Section 2.6.4. Although the iron ions interact directly with some of the cells in the system, it is unclear as to the percentage of which are actively oxidizing iron (living cells versus dead cells). In addition, iron input

from the feed makes it difficult to distinguish the relationship between cell and iron concentrations between phases. For example, as per the results of the residence time distribution study outlined in Govender et al. (2015), 20% of the iron rich feed would be stagnant fluid. It is also important to remember that one cell doesn't necessarily pertain to one ion and that a percentage of the microbial population is not even capable of oxidizing iron (sulphur oxidizers, etc). The intricacies of the movement of metals within the system and geomicrobiological relationship to this movement is still not well understood.

5.7 REDOX

Redox values were calculated based on the equation derived in Yue et al. (2014), discussed in Chapter 3, Section 3.9.2. The ore-associated phases show lower redox values than the leachate.

Values in the ore-associated phases at the outset of the experiment in the 500 mV-range imply that the microbial population did not oxidise all the Fe^{2+} before it was collected. This means that un-oxidised Fe^{2+} ions were likely removed by the convective force of the flowing liquid in the column before the microbial community had a chance to oxidise them (Chiume et al. 2012).

Additionally, there were some issues with iron measurements – total iron was lower than Fe^{3+} in when using the ferric chloride assay and measuring iron on the spectrophotometer (Govender et al. 2012). The Atomic Absorption Spectrometer measured total iron only, and total iron values from the AAS and the spectrophotometer were not consistent. Subtracting measured spectrophotometric Fe^{3+} values from the total iron AAS values was therefore infeasible. To avoid a situation like this in the future, redox measurements should be taken directly from the leachate using a redox probe. Redox measurements of the ore-associated phases cannot be taken directly because the phase is diluted for collection (Chapter 3, Section 3.5).

5.8 MICROBIAL ECOLOGY

Leptospirillum ferriphilum was the dominant species present in each phase, although Column 11 had much lower *Leptospirillum ferriphilum* cell numbers than the others and is considered an erroneous point.

The concentration of *Leptospirillum ferriphilum* increases over time and shows a colonisation curve. This colonisation curve implies that the species was present in the inoculum rather than in the indigenous community. Additionally, *Leptospirillum ferriphilum* is also the dominant species in the interstitial and weakly attached phases while it takes slightly longer to dominate the strongly attached phase.

The interstitial phase had the highest cell concentrations in the previous agglomerate-scale leaching experiments (Chiume et al. 2012; Govender et al. 2013; Bryan et al. 2016). Similarly, *Leptospirillum ferriphilum* cell numbers were highest in the interstitial phase in the first 96 days of the experiment. This means that the interstitial phase was dominated by the inoculated culture during colonisation of the bed, as was seen in the previous work.

The Bryan et al. (2016) study used the same inoculum as was used in the six-month agglomerate-scale leaching experiment. The results in Bryan et al. (2016) showed that *Leptospirillum ferriphilum* was present in the culture. On a much larger scale, this may mean that the interstitial phase is instrumental to the successful colonisation of the heap by an inoculated culture. It may also mean that past 96 days, the weakly attached phase may become dominant in terms of cell concentrations.

The fact that this study showed higher cell concentrations in the attached phases may therefore be attributed to dead cells or cells attached to the ore at the outset of the experiment (as was seen in the un-inoculated detachment experiment). The strongly attached phase had the highest variation of organisms followed by the leachate (Chapter 4, Section 4.5.10). The strongly attached phase also housed *Acidithiobacillus albertensis* at each time point throughout the experiment. This indicates that *Acidithiobacillus albertensis* was probably part of the indigenous culture on the ore bed rather than the inoculum. *Acidithiobacillus albertensis* was present in very low quantities (or not at all) in the other phases.

The leachate had the next highest variation of organisms, with the greatest percentage of *Acidiferrobacter* recorded compared to the other phases (Chapter 4, Section 4.5.10). It is not presently understood why at this time.

The strongly attached phase and the leachate both had increasing numbers of *Leptospirillum ferriphilum* towards the end of the experiment. This may mean that the strongly attached community is eventually dominant in terms of cell numbers when compared to the other phases, even in sterile systems. More research using a sterile ore and pure culture over a period longer than six months would be needed to prove this.

The communities in every phase were dominated by *Leptospirillum ferriphilum* in at almost every time point. Maximum specific growth values for *Leptospirillum ferriphilum* were calculated for each phase, with the highest rates in the interstitial and weakly attached phases, followed by the strongly attached phase and the leachate. The maximum specific growth rates calculated for *Leptospirillum ferriphilum* were slightly lower in the interstitial phase and about half of the rate in the strongly attached phase compared to the maximum specific growth rate calculated for the cell concentrations. The weakly attached values were very similar. The slower rate in the strongly attached phase is likely attributed to the community already present on the ore at the outset of the experiment, meaning that it took longer for *Leptospirillum ferriphilum* to colonise the biofilm. It is important to consider that the DNA data is missing from Column 14 – Column 16, which may have affected the colonisation curve for *Leptospirillum ferriphilum*.

Most importantly for this experiment, the DNA results showed that the phases were not analogous to the leachate. The microbial ecology of the phases was also different from one another with the exception of the weakly attached phase and the interstitial phase. This suggests a close relationship between the interstitial and weakly attached phases, which would be sensible as spatially, they are adjacent to one another (Li et al. 2017). As the metal concentrations are vastly different, the microbial environment between the phases is still not the same. More research is needed to further understand this relationship.

5.9 COMPARISON TO PREVIOUS WORK

5.9.1 CELL AND METAL DISTRIBUTIONS

Cell counts were highest in the weakly attached phase in this experiment. This is not what was shown in Bryan et al. (2016), Govender et al. (2013), and Chiume et al. (2012), where the interstitial phase was dominant.

Govender et al. (2013), showed that colonisation occurred before the end of the 700 hour period (both Chiume et al. (2012) and Bryan et al. (2016) ran on a much shorter time-scale), whereas in this study the results did not stabilise until 1000 hours. This can be attributed to the fact that the ore in the previous studies by Govender et al. (2013) and Bryan et al. (2016) was sterile, and therefore had no competing community or dead cells which may have inhibited microbial colonisation of the ore.

Differences in the attached numbers (especially the strongly attached) may also be due to cell counting methods used as discussed above briefly in Section 5.4. In Govender et al. (2013), cells were quantified using the Miles-Misra plating method, so only colony-forming units (cfu) were considered. This limits quantification to living cells. In this six-month experiment, the cells were counted by using a counting chamber, which doesn't distinguish between living (viable colony forming units) or dead (non-viable) cells. Therefore when counting the strongly attached phase which could very possibly incorporate dead cells (Li & Sand 2017), quantification using a counting chamber would be expected to provide a higher cell count than plating viable cells. The importance of dead cells in this environment is presently unknown.

Bryan et al. (2016) quantified the iron and copper concentrations between phases and found a similar distribution of copper and iron as seen in this dissertation. Copper was 150x greater in the ore-associated phases in that work, and 100x higher in this study. Iron was about 10x higher in both experiments. Both the 2016 study and this dissertation highlight the importance of understanding the dissolved metal concentration in the ore-associated phases, as increased dissolved metals can be detrimental to microbial communities (Watling 2016). Both studies also prove that the leachate is not an accurate analogue for the ore-associated phases within this system.

5.9.2 Comparison of community structure

The Bryan et al. (2016) study was also the first agglomerate-scale leaching experiment to examine the community structure between the phases. The culture in the 2016 study was not dominated by *Leptospirillum ferriphilum* however it was a prominent contributor to the overall community structure (Figure 5.1). The 2016 study used the same inoculum as was used in this dissertation, so it is interesting that the community structures are so different. This can likely be attributed to either the indigenous population, the flow rate, the type of ore used, or a mixture of any of these factors.

The inoculum was also used in the six-month agglomerate-scale leaching experiment roughly one year after the 2016 work. DNA data of the culture was not recorded at the outset of the six-month leaching experiment.

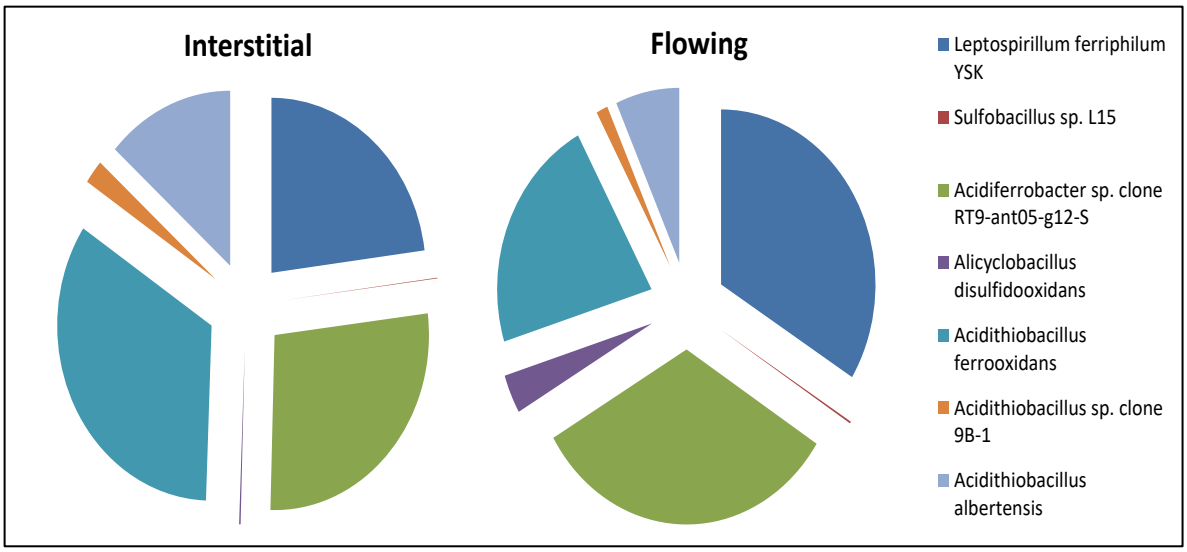


Figure 5.1: Community structure of the interstitial phase and the leachate (flowing) from Bryan et al. 2016 at 377 hours.

Figure 5.2 shows the community structure of the interstitial phase and the leachate from the six-month leaching experiment at t=510 hours. There is much less variety in the bacteria in the six-month agglomerate scale leaching experiment compared to Bryan et al. (2016). The exact cause for this is not known, although a possible reason is the mineralogy of the ore bed. *Acidiferrobacter*, for example, is sensitive to copper (Hallberg et al. 2011). This may explain why it is not present in the interstitial phase. The variation could also be impacted by the presence of the indigenous community which would not have

been a factor in the Bryan et al. (2016) study. This is especially true if the strongly attached culture was being replaced by *Leptospirillum ferriphilum* (Section 5.8).

There was also a variation in the experimental set-up as the columns in the six-month leach were acid leached with water at pH 0.6 instead of pH 1.7 due to experimental error. This may have caused additional challenges in the colonisation of the ore by the inoculated culture.

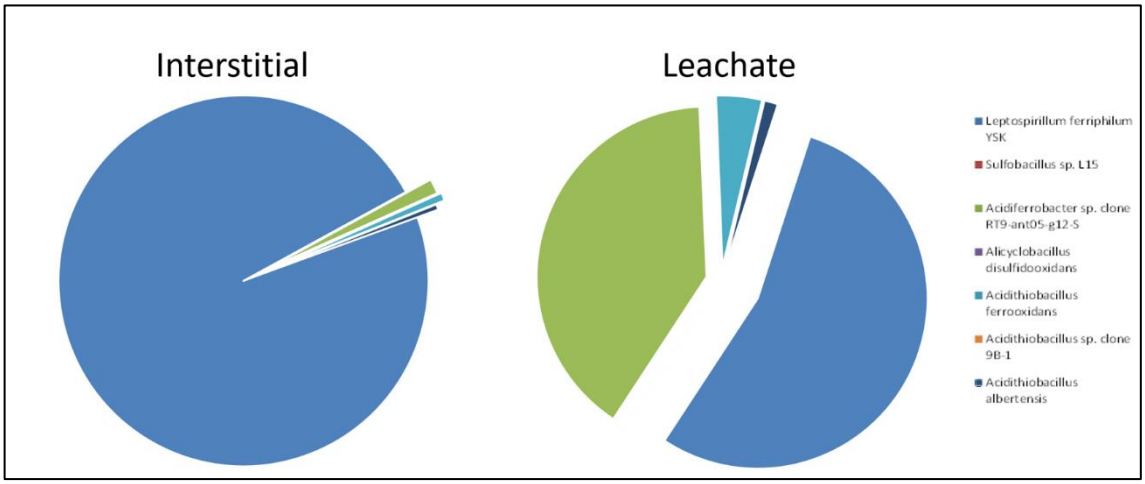


Figure 5.2: Community structure of the interstitial phase and the leachate from the six-month column leaching experiment at 20.9 days (510 hours).

5.10 Reactor batch tests

Reactor batch tests were performed to test the effects of lowered pH values and increased iron concentrations on microbial growth (Appendix E). Generally, growth rates decreased as iron concentrations increased. The iron additions may have presented additional challenges because large volumes of iron(II) sulphate were required to be added to the reactors to achieve the desired iron concentration level. The iron(II) sulphate had a 20% iron content – so roughly 80 g were required for a 20 g/L total. This therefore greatly increased the density of the slurry considering that only 14 g of mineral concentrate were added to each reactor for the required 2% solids.

The batch tests ran for 6 – 14 days. Inoculation of the following batch was done with a mixture of the previous week's culture to help build resilience to increasingly adverse conditions (Acevedo 2000). Some batches were run for longer periods to achieve the desired results. Longer duration batch tests would

probably have resulted in an overall increase in the maximum growth rate, except in the 40g/L iron batch where there were almost no cells remaining.

Calculated redox values for the reactor experiment were generally between 600mV and 700mV SHE. This implied microbial activity in the system (Watling 2006). In addition, copper dissolution rates were not affected until the pH values were below one. However, the growth rate of the archaea in the system decreased with pH. This means that the archaea were working harder at the lower pH values with each cell oxidizing more iron. This may be due to a higher fraction of cell energy being used to resist the stress of the increased acidity within the system. Alternatively, this may be due to the chemistry, as increased acidity may have had the ability to solubilise easily leachable copper minerals which would account for the rates being equal at higher pH values.

Overall, copper dissolution was between 62 and 75% of the total copper values within the reactors during the batch tests. It is important to consider that this is based on an average assay value, so it should be considered as an estimation only. There was a difference between the unadjusted reactors and the adjusted reactors in that the reactors with pH values below 1 and increased iron concentrations had less copper extraction than those which did not.

Above all, the fact that the communities in the batch tests were affected by adverse pH and iron concentrations has largescale implications with respect to the ore-associated phases. This is because the ore-associated phases are exposed to the highest metal concentrations as was shown in this dissertation and in Bryan et al. (2016). Understanding the limitations of these ore-associated communities is therefore a very useful tool when interpreting the heap bioleaching system as a whole. Especially considering that previous agglomerate-scale studies have shown that the ore-associated phases are also dominant in terms of cell numbers.

5.11 RECOMMENDATIONS FOR FUTURE WORK

First and foremost, in future experiments the residence time distribution study should be completed using the ore being studied in the experiment rather than using a value from a previous study with a slightly different set-up. Although the

disparity was thought to be minimal, it is important to ensure that the system is well represented, as heap permeability and flow play a huge role in the overall productivity of the heap. This is because the fluid within the ore-bed is a transport system for crucial ions in and out (Fagan et al. 2014). Residence time distribution values may have varied slightly between this study and Govender et al. (2015) due to differences in mineralogy and ore size. Also, if this experiment were to be replicated, the bed itself should be stacked higher to help prevent bypassing liquid. Additionally, to optimise this experiment if performed again, redox measurements should be quantified in leachate point samples using a probe. This eliminates any uncertainty in the ferric chloride assay or spectrophotometric measurements. Another RTD focused experiment that would be very valuable would be to examine the flow within the bed before and after the six-month leaching period.

Potential experiments to test different parameters in these agglomerate-scale systems are countless. One that is of interest due to its industry ties would be to perform an agglomerate-scale heap bioleach using a recycled feed (leachate) instead of fresh liquid medium. The effects of using a feed containing planktonic cells could then be examined. This is important to understand because it is presently common within industry to recycle the raffinate back through the column to minimise operating costs and promote microbial growth (Watling 2006). The flow of the system and microbial communities could also be monitored using a variety of agglomeration methods as seen in Govender-Opitz et al. (2017). This would show how microorganisms respond to different degrees of bed saturation and fluid flow.

A six-month leaching experiment with sterile columns would help to clarify the cell concentration through time. This is important as the *Leptospirillum ferriphilum* from the inoculum which colonised the bed was concentrated in the interstitial phase for the first 96 days (2300 hours). This means that it is possible that in a sterile system, over a six-month period the cell numbers in the weakly and strongly attached phase may also be dominant. Govender et al. (2013) did not see this result as this experiment ran for 700 hours.

While this study examined the community structure on a small scale, industrial scale heaps have sizeable temperature gradients (Watling 2006). The community response to different temperatures would be an interesting way to learn more about the community structure between phases. It would also be more applicable to industrial operations.

A higher temperature heap leaching experiment (70°C) would be more effective for leaching enargite in the future (Escobar et al. 1997; Escobar et al. 2000; Lee et al. 2011; Takatsugi et al. 2011). During successful enargite leaching, measurements of dissolved arsenic will help to provide insight into the geochemistry of arsenic precipitation mechanisms and the effects of arsenic on heap bioleaching cultures (Watling 2006; Corkhill et al. 2008; Lee et al. 2011; Takatsugi et al. 2011). In an experiment with a higher leaching rate, imaging of the ore bed would also be beneficial to investigate mineral dissolution and attachment sites. An abiotic column should also be run concurrently to compare mircobially induced leaching to chemical leaching. Further studies to specifically examine the effect of increased metal concentrations on community structure could also be designed using enargite ores and would help to improve the knowledge of microbial communities across phases.

A better understanding of the indigenous community could also be achieved using a very similar experiment to the one performed in this dissertation. Firstly, a microbial retention study on a non-sterile ore with a known microbial community would be beneficial, so the indigenous cells leaving could be counted over time. In addition, DNA data should be collected to examine this community and gain a deeper understanding of the role indigenous cells may play in these systems. This could be done using an un-inoculated column, run as a control. It would be valuable in any agglomerate-scale experiment with a non-sterile ore to compare leaching rates and metal concentrations of an inoculated culture to an indigenous culture. When examining the effect of an indigenous population on leaching potential, there should also be a sterile abiotic column run to compare leaching rates to a system where there is no microbial influence.

Of course, it’s important to consider large-scale sampling. Studies of the four phases at the industrial scale would be extremely enlightening. Monitoring could

be done over time at different pH and temperature gradients within the systems to quantify microbial diversity and account for community variations. As informative as laboratory scale experiments are, they cannot provide the same information that an industrial scale operation could, and so efforts must be made to move these experiments from the laboratory into the field. It is more feasible to examine abandoned sites at this time than operational ones utilising the natural communities living within those systems due to the nature of the mining industry and the restrictions on intellectual property (Brierley 2010).

CHAPTER 6. CONCLUSIONS

6.1 CONCLUSIONS

The main aim of this study was to analyse an agglomerate-scale leaching experiment over a six-month period and to quantify the cell and metal distribution between the leachate, interstitial, weakly attached, and strongly attached phases. The results showed that a disparity in cell and metal distributions persisted past the initial colonisation phase over the course of the experiment (192.3 days total). This result is congruent with previous work by Govender et al. (2013) and Bryan et al. (2016).

Colonisation of the agglomerate-scale ore beds occurred after 1000 hours (41.7 days), which was later than colonisation seen in previous work by Govender et al. (2013). Cell numbers, however, remained highest in the attached phases. This is likely due to the presence of dead cells from an indigenous community incorporated into the biofilm (Li and Sand, 2017). Overall, these results mean that the leachate cannot be used to interpret the cell distribution of the whole ore environment which should be reflected in the sampling protocols of heap leaching operations.

Iron and copper were most concentrated in the ore-associated phases throughout the duration of the experiment. The leachate had roughly 10x less iron and 100x less copper than the ore-associated phases during the six-month period. Most importantly, the metal distribution data suggests that the metal concentrations in the leachate were not an accurate portrayal of the metal concentrations in the ore-associated phases. This is also congruent with previous work (Bryan et al. 2016), and implies that the leachate does not provide insight into the variable metal distribution within the column.

Another research target was to determine the structure of the microbial communities between phases to better understand if the leachate is simply a diluted view of the communities seen in the attached phases. DNA results showed that the community structure was dominated by *Leptospirillum ferriphilum* in all four phases throughout the six-month period. However, there were still variations in the quantities of different species between the leachate and the ore-associated phases. The strongly attached phase had the most variable microbial community followed by the leachate. The microbial ecology of the interstitial and weakly attached phases was very similar, despite the drastically different

concentration of dissolved metals. This suggests that in terms of community structure, the leachate is not an accurate analogue for the rest of the system, parallel with results from Bryan et al. (2016).

Previous studies have not examined if a pre-existing indigenous community would remain intact on the ore throughout an agglomerate-scale leach, which was another main goal of this dissertation. While an indigenous community was found to be present during the uninoculated detachment experiment, it was not isolated and characterised at this time. It is therefore unknown what the contribution of the indigenous community was to the system, and if it was living or dead.

High temperature batch tests were also run in reactors to examine the potential effects of metal concentrations and pH changes within the ore-associated phases. The batch tests showed that the maximum specific growth rate decreased with elevated iron concentrations. Although the main goal of this experiment was not enargite dissolution, the total copper extracted was calculated. The best performing batch in terms of enargite dissolution was when the pH was unadjusted, with maximum dissolved copper values of 94 ppm. This is roughly 75% of the total copper content of the ore.

It often seems as though each question answered in this study raises several more. Recommendations for future work related to this topic are widespread. The best industry linked studies would be to use a recycled feed as is common practice at large heap leaching operations. It would also be interesting to use a high temperature column to optimise copper extraction from enargite, and to examine the effects of dissolved arsenic on the system. In terms of microbiology, as the contributions of the indigenous population were so poorly understood in this study, more insight on their role and potential within the system would also be very valuable.

Watling (2016) suggests that in the future there will be no need to sample the substrate in a bioleaching heap, but this work along with Chiume et al. (2012), Govender et al. (2013), and Bryan et al. (2016) proves otherwise. As the cell distribution and microbial ecology vary between the phases, sampling heap substrates is still necessary when evaluating heap productivity and microbial health. Future work that uses these protocols may be more effective in

optimisation, productivity, and maintenance of bioleaching heaps. Improvements made with regards to heap bioleaching technology encourage a widespread use of the technique in commercial operations, with countless economic and environmental benefits.

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