An investigation into the effects of preleach time period, nitrogen supplementation and iron concentration on bioleaching performance

By

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Dedicated to my parents for inspiring me to be all that I can be - who gave so much love that I could have the foundation to achieve this goal!!

Sometimes it is better not to know what can't be done, for the rest there is God...

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Abstract

Bioleaching has developed into a very important process for the recovery of valuable metals, particularly from low grade copper ores due to the ever increase in demand. A lag phase is present during the start-up phase of heap bioleach operations characterised by a low degree of microbial colonisation, a low ferrous iron oxidation rate and low microbial proliferation rates, ultimately resulting in a poor copper recovery rate. The current work investigated the effect of selected parameters on heap bioleach performance employing simulated heap leach column reactors. These parameters included preleach time period, nitrogen supplementation and different feed iron concentrations and the resultant effects on microbial activity and proliferation ultimately affecting the copper leach rate.

Higher ferrous iron oxidation and microbial growth rates were measured in the column coated with chalcopyrite concentrate compared to columns packed with low-grade ore. This phenomenon was similar to the effect of preleaching and can be attributed to the low gangue content (< 10%) of the chalcopyrite concentrate. The agglomeration process resulted in dissolution of certain gangue minerals, releasing cations and anions which were contained within the moist packed-bed. The decrease in lag phases for column reactors not preleached, preleached for a short time period and preleached for a extended time period suggest that an increase in preleach time was advantageous for microbial growth and ferrous iron oxidation activity.

Although several of the mesophilic pioneer organisms, such as *At. ferrooxidans* and *L. ferrooxidans,* are able to fix atmospheric nitrogen the process is very energy intensive. Cultures relying on nitrogen fixation grow more slowly and have a lower yield per mole of iron oxidised. The slower growth resulted in a decrease in the rate of iron oxidation as well as ore colonisation once the culture had become established, especially for the shorter preleached column experiments. The 6 day preleach was sufficient to create an environment suitable for colonisation by the inoculated culture. Copper recover was enhanced over the 60 day time period studied in the columns supplemented with nitrogen. This may be attributed to greater and more rapid colonisation as colonised mineral surfaces have been shown to leach more efficiently.

The data showed that an increased dissolved iron concentration across the range 0.2 to 5.0 $g.l^{-1}$ did enhance copper recovery initially, but not in proportion to the iron concentration. After a relatively short time copper leach rates converged to a stable value of around 0.06 $g.day^{-1}$ irrespective of feed iron concentration. This leads to the conclusion that after the initial phase copper leaching is limited by the availability of exposed mineral rather than ferric iron. Efficient copper leaching can be achieved with ferric concentrations below 0.5 $g.l^{-1}$. This could reduce the amount of iron or acid needed to keep the iron in solution and the amount of jarosite precipitation.

Declaration

I, Andries Wynand van Zyl, declare that I know the meaning of plagiarism and declare that all the work in the document, save for that which is properly acknowledged, is my own. It is submitted for the Degree of Magister Scientae (MSc) at the University of Cape Town. This thesis has not been submitted for any degree or examination at any other university.

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Andries Wynand van Zyl

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General Introduction

The current work investigated the effect of selected parameters on heap bioleach performance employing simulated heap leach column reactors. These parameters included preleach time period, nitrogen supplementation and different feed iron concentrations and the resultant effects on microbial activity and proliferation ultimately affecting the copper leach rate. These parameters were selected due to the significant impact of these parameters on the start-up phase of bioleaching.

Conditions within the heap immediately after agglomeration and stacking are likely to be unfavourable for microbial growth and colonisation of the ore surface, possibly due to the release of ions during acid agglomeration. These ions would be mobilised once irrigation of the heap was started and reach concentrations detrimental to the growth and ferrous iron oxidation activity of the bioleaching micro-organisms. This retarded activity would result in a lack of ferric iron regeneration impacting negatively on the degree of copper leaching.

In the absence of reduced nitrogen, certain organisms can employ an energy intensive process to fix atmospheric nitrogen (Madigan *et al*., 2003). The nitrogen triple bond is extremely stable, with a dissociation energy of 940 $kJ.mol^{-1}$ (Madigan *et al*., 2003). In the presence of a reduced nitrogen compound the energy otherwise expended on nitrogen fixation can be utilised for growth. Increased microbial growth would lead to a higher degree of ferrous iron oxidation as well as a higher degree of ore colonisation ultimately resulting in a reduction of the initial lag phase and a higher degree of copper leaching.

As ferric iron is the main chemical leaching agent, two main issues are of consideration. The first is to ensure a high dissolved ferric iron concentration initially in order to achieve a maximum copper leach rate. The second issue is the fact that with an increase in ferric iron concentration is an increased probability of jarosite formation which will impede leaching reactions from taking place and result in poor leaching performance. The investigation of feed iron concentrations are therefore focussed on the effect of these concentrations initially on the bioleaching process.

Chapter 1: Literature Review

1.1 Introduction to heap bioleaching

Bioleaching has developed into a very important process for the recovery of valuable metals, particularly from low grade copper ores. It has become the subject of considerable research. Currently, an important focus is the improvement of the bioleach process by elucidating the exact role of the micro-organisms in this complex, multi-step process.

During bioleaching ferric- and hydrogen ions are responsible for the chemical dissolution of metals from metal sulphides (Acevedo, 2000). The role of the micro-organisms is to regenerate the reactive leaching agents, enabling further dissolution of metal sulphides, hence the term *bio*leaching (Acevedo, 2000).

As bioleaching progresses, the pregnant leach solution (PLS) contains the metal of interest which is collected. In the case of copper ores, the soluble copper can be recovered by solvent extraction and electrowinning in order to produce cathode copper.

Traditional processes, for example pyrometallurgical methods, are widely used for the production of copper. These processes entail crushing and milling of the ore, followed by a process such as flotation to produce a concentrate. This is followed by roasting, smelting, recovery and further processing in order to obtain cathode copper. A major disadvantage of these types of processes is the immense energy input required, particularly during the milling phase. In the case of copper, 8700-11700 Kwh is required per ton of cathode copper produced. As such, these processes are restricted to relatively high grade ores. Similar processes are used for the recovery of other metals, such as zinc, nickel and cobalt. The environmentally hazardous SO_2 produced during roasting could be scrubbed off and utilised for production of sulphuric acid, but this not employed by all processes (World bank group). Heap bioleaching can be employed to recover metals from low-grade ore (< 0.5% w/w of the metal of interest), which is not economically feasible by traditional, non-biological methods. The economic advantages are associated with the lower capital costs and the reduced operating cost, largely due to the microbial regeneration of the active leach agents (Rawlings, 2002). Another important factor in considering alternatives to conventional processing of mineral ores is the fact that smelters are constrained in their ability to treat concentrates which contain high levels of deleterious impurities, particularly sulphur, resulting in difficulties with waste management (Dreisinger, 2006). Bioleaching is comparatively more environmentally benign, as sulphur dioxide and other harmful gasses, such as carbon dioxide and carbon monoxide, aren't produced in significant quantities (Rawlings, 2002). While some of the disadvantages inherent in pyrometallurgical methods are avoided in bioleach operations, there are a number of issues specifically associated with bioleaching.

Table 1.1: Advantages and disadvantages of bioleaching compared to pyrometallurgy

Advantages	Heap bioleaching	Pyrometallurgy
	Environmentally friendly due to minimal production of flue gasses (1,3).	Process is stable with predictable results (1).
	Recovery of metals from low grade ore, under mild operating conditions (1,3).	High metal quantity extraction from rich complex ores (3).
	Low capital costs and energy input (1).	Process is not affected by formation of precipitates (3).
	Compared to pyrometallurgy, relative simple operation (1).	
Disadvantages		
	Occurrence of dead zones due to nutrient limitations or inhibitor accumulation (2).	Cannot be used to recover metals from low grade ores due to high cost and low returns (3).
	Long start-up times (2).	High start-up cost (3).
	Open to the environment, temp changes, rain, snow and evaporation (2).	Produces significant levels of CO_2, CO and SO_2, detrimental to the environment (3).
	No exact process control (1).	Requires a high energy input (3).
	Heaps are heterogeneous in mineral content (1).	

References: (1) Acevedo, 2002, (2) Mazuelos *et al*., 2001, (3) Rawlings, 2002

1.2 Different types of bioleaching applications with a detailed view of heap bioleaching

Hydrometallurgical copper recovery has been described as early as the fifteenth century as follows: “For truly when the rustics in Hungary cast iron at the proper season into a certain fountain, commonly called Zifferbrunnen, it is consumed into rust and when this is liquefied with a blast-fire, it soon exists as pure Venus (copper), and nevermore returns to iron” (Davidson, 1982).

One of the earliest documented cases of the commercial application of biohydrometallurgy, was extraction of copper from mine waste (tailings from the Chino Mines) by the Kennecott Copper Corporation (Zimmerley *et al*., 1958). In 1980, leaching of copper from heaps designed to facilitate microbial activity was initiated and this technique was commercially applied. The Lo Aguirre mine in Chile processed about 16 000 tons of ore per day between 1980 and 1996, making use of bioleaching (Bustos *et al.*, 1993). From 1980 several copper

bioleaching operations have been commissioned (Brierley, 2001). Today, the largest Cu-processing plant employing heap leach technology is at the Escondida mine in Chile, processing 52 000 tons of ore per day.

Various technologies have been developed for treatment of concentrate as well as complex whole ores for example tank leaching, in-situ leaching and heap leaching. Tank leaching is generally carried out in series in order to maximize the metal recovery from concentrate. The suspension flows through a series of highly-aerated tanks which are temperature and pH controlled. Due to significant heat production continuous cooling of the tanks are necessary which significantly add to the running cost. The agitators ensure an even distribution of all the solids as well as ensuring an even suspension is carried over to the next tank in series (Rawlings, 2005). As maintenance and capital required are high this method is preferred for treatment of concentrates. Approaches for the treatment of whole ores include mainly two technologies, in-situ leaching and heap leaching. In-situ leaching entails ores are to be leached by employing bacteria without excavation of the ore prior to leaching (Rawlings, 2002). Although a large cost saving is associated with not having to do any excavation a parallel drawback is the low permeability of the ore body (Rawlings, 2002). The geological location of the ore body is also critical since the leach liquor have to be collected at the bottom where large amounts could also escape into the ground resulting in ground pollution and acid mine drainage (AMD). Heap leaching entails big dumps are setup on impermeable ground and fed with an appropriate solution resulting in metal dissolution. The heaps are constructed in a manner which allows the leaching liquor to percolate through the heap and air can also enter from the bottom and disperse through the ore (http://www.spaceship-earth.org/REM/Naeveke.htm). Micro-organisms attain energy from oxidising ferrous iron (Fe^{2+}) and/or sulphur compounds for example thiosulfate. This guarantee a constant supply of ferric iron (Fe^{3+}) which in turn results in leaching of the whole ore.

Advantages and disadvantages of different types of bioleaching

Because of the difference in setup and operational costs involved with the respective bioleaching techniques, the ore grade plays an important role in this consideration as to which process could be economically employed.

Table 1.2: Advantages and disadvantages of different types of leaching

Process	Advantages	Disadvantages
Heap leaching	Cheaper to construct and operate compared to tank leaching	Time to reach a mature population structure takes a long time
	Ability to treat low grade (< 0.5 % (w/w) desired metal ores and concentrates	Mineral solubilisation takes months
	Relative little running labour involved	Possibility of AMD formation
Tank leaching	Mineral solubilisation takes days	Keep a relatively low pulp density
	Highly robust system and adaptation from one to the next mineral is relatively simple	More expensive to construct and operate compared to heap leaching
	Mild operating conditions for example operates between 40-50ºC	Treatment of high grade ores and concentrates only
In-situ leaching	Previously mined tunnels can be hosed down, the leachate collected and used for metal extraction	Location is an important consideration as liquor can be lost (AMD)
		High costs involved for example excavating or crack introduction

Compiled from Rawlings, 2002; Rawlings, 2005; and Olson *et al.*, 2003

Different leaching techniques have their own specific advantages and disadvantages. Depending on available capital, current technology, ore grade and available time, a choice towards which bioleaching technique to be used can be made. Geographical location plays an important role when considering in-situ leaching as environmental pollution might become a problem due to the unavailability of tight process control. Where there is very specific and tight control for example over tank leaching operations and to a lesser degree over heap leaching processes geographical orientation does not play a significant role. Currently $CuFeS_2$ is the most abundant Cu-bearing sulphide ore available and as the worlds demand for Cu increases, research into obtaining Cu at faster rates and more efficiently using heap-leach technology becomes even more essential.

Heap bioleaching

A typical heap is constructed of crushed head-grade ore which is then agglomerated, with sulphuric acid addition, in rotating drums to bind the finer materials to coarser particles. This

prevents the clogging of tiny spaces between ore particles, which could impede aeration and percolation (Rawlings, 2002).

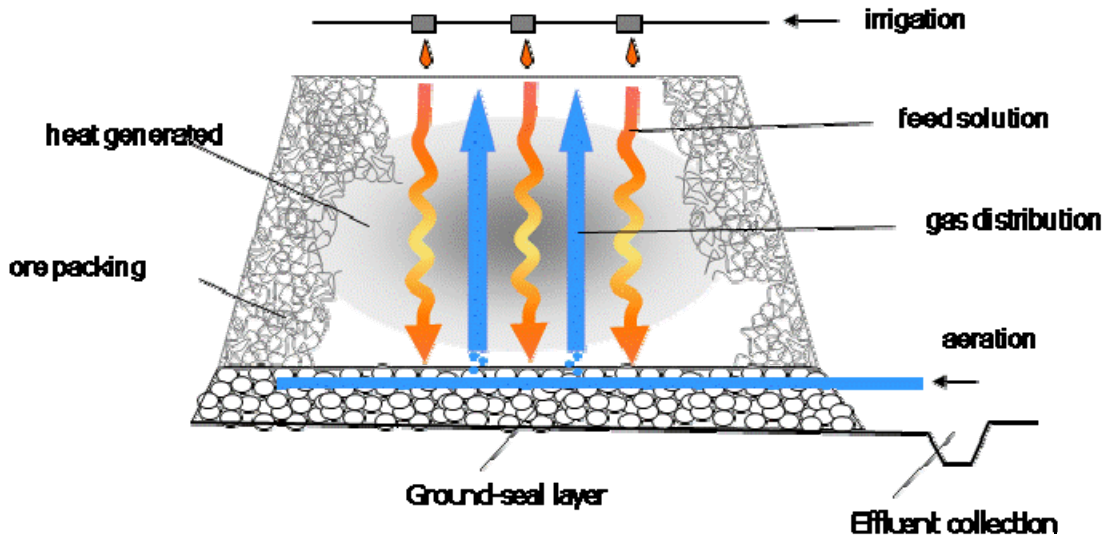


Figure 1.1: Schematic diagram of a heap leaching process (Taken from Petersen and Dixon in Rawlings and Johnson, 2007)

The agglomerated crushed ore is then piled on top of an impermeable base and supplied with an efficient leach liquor distribution and collection system, as shown in Figure 1.1. The most common liquid distribution system consists of parallel pipes, with drip points evenly spaced along the pipes, through which raffinate is pumped. The raffinate may be supplemented with nutrients or additional acid, depending on the characteristics of the ore. The microbial inoculum is generally delivered through the irrigation system, although inoculation during agglomeration may also be employed

Aeration usually occurs by blowing air into the heap through a piping system installed near the base of the heap. Compressed air is used to provide the necessary O_2 and CO_2 required for efficient growth of the micro-organisms. Micro-organisms growing on the mineral surface and the planktonic (non-attached) population in the heap are responsible for regenerating Fe^{3+} and sulphuric acid (H_2SO_4) which facilitate the dissolution of the mineral and solubilisation of the metal of interest (Rawlings, 2005). During active operation, the liquid contained within the heap can be considered to exist in two interacting phases: one phase being the stagnant solution phase consisting of fluid associated with the mineral surface as well as the pores in the mineral. The second phase is the flow-through phase, where solution moves through the heap due to gravity (Mousavi *et al*., 2006). The PLS, from which the Cu is subsequently recovered by electrowinning, is collected at the base of the heap. The stripped solution, raffinate, is modified by adjusting the pH and addition of nutrients prior to being recycled back to the heap.

In addition factors adding to the complexity of heap bioleach operations include fluid dynamics, material and gas transfer, microbial adaptation and temperature changes.
Other factors contributing to optimal Cu liberation are the necessity for maximum exposure of the mineral to the feed solution, a suitable environment for microbial growth, efficient inoculation and subsequent nutrient provision for the bioleaching micro-organisms.

1.3 Bioleaching chemistry

Metal sulphides for example zinc, copper, nickel and cobalt containing minerals are almost insoluble in water, but their sulphates are readily soluble in water (Rawlings *et al.*, 2005). This oxidation of the metal sulphide into a sulphate results in the metal being leached into solution from where it can be extracted and recovered. The oxidation of different metal sulphides proceeds via different intermediates, resulting in reactions which are not identical for all types of metal sulphides (Schippers and Sand, 1999; Cordóba *et al*., 2008b).

1.3.1 Chemistry during chalcopyrite leaching

$CuFeS_2$ is the most abundant source of Cu mineral in the world. Unlike many other ores, chalcopyrite is known to be particularly recalcitrant to hydrometallurgical processes (Qiu *et al*., 2005). During leaching attack of the sulphide moiety occurs, resulting in the solubilisation of Cu and formation of sulphate (SO_4^{2-}) via sulphur intermediates (Bharathi *et al.*, 2004).

According to equation 1.4.1 and 1.4.3 chalcopyrite is oxidised by ferric iron (Fe^{3+}) and protons (H^+), producing ferrous iron (Fe^{2+}) and an intermediate sulphur compound, for example elemental sulphur (S^0), thiosulphate ($S_2O_3^{2-}$) or tetrathionate ($S_2O_6^{2-}$) (Watling, 2006). From these equations it is clear that recycling of Fe^{2+} is vital for efficient, continuous leaching to occur.

The reduced sulphur intermediate formed is converted to sulphuric acid, according to equation 1.4, by a sulphur oxidising micro-organism. Sulphuric acid dissociates completely forming SO_4^{2-} and protons according to equation 1.5. The protons will further aid in leaching of chalcopyrite (Lizama *et al*., 2003).

$$CuFeS_2 + 4Fe^{3+} \rightarrow 2S^0 + Cu^{2+} + 5Fe^{2+} \quad \text{...Eqn 1.1}$$

$$2Fe^{2+} + \frac{1}{2}O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O \quad \text{…Eqn 1.2}$$

$$CuFeS_2 + 4H^+ \rightarrow Fe^{2+} + Cu^{2+} + 2H_2S \quad \text{…Eqn 1.3}$$

$$S^0 + 1.5O_2 + H_2O \rightarrow H_2SO_4 \quad \text{...Eqn 1.4}$$

$$H_2SO_4 \rightarrow 2H^+ + SO_4^{2-} \quad \text{...Eqn 1.5}$$

From the above discussion, the role of the bioleaching micro-organisms becomes clear. In the absence of micro-organisms the reaction described by equation 1.2 proceeds very slowly at a low pH, resulting in a reduced mineral leaching rate. The absence of sulphur oxidising organisms and a sulphur layer would likely form on the surface of the mineral (Munoz *et al*., 2007). The rate-limiting step, equation 1.1, becomes the diffusion of Fe^{3+} through this sulphur layer. The rate of mineral dissolution would decrease to a rate depending on the degree of this layer formation (passivation) (Fowler and Crundwell, 1999). The reduced sulphur compound in turn will be oxidised by sulphus oxidising micro-organisms in order to produce H_2SO_4. The H_2SO_4 will dissociate forming sulphate and protons which in turn will aid in further leaching of the metalsulphide.

1.3.2 Bioleaching mechanisms employed by bioleaching micro-organisms for oxidation of ferrous-iron and sulphur intermediate compounds

The interaction between bioleaching micro-organisms and sulphide minerals is believed to be chemical, electrochemical and biochemical. The dissolution of metal sulphides follows one of two pathways: the thiosulfate- or the polysulphide pathway depending on the ore leached. Regardless of the pathway, bioleaching micro-organisms catalyse the conversion of Fe^{2+} to Fe^{3+}.

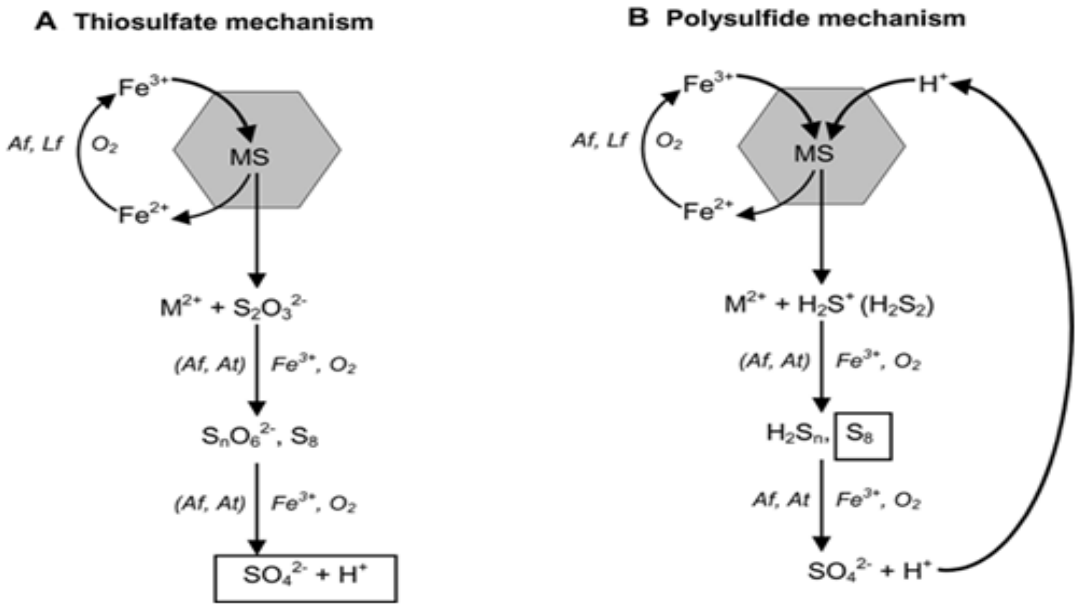


Figure 1.2: Metal dissolution pathways utilised by micro-organisms (Schippers and Sand, 1999)

Metal sulphides such as pyrite, molybdenite, and tungstenite are exclusively oxidised via electron extraction by Fe^{3+}. This pathway is named after its first free sulphur compound, thiosulphate, and is illustrated in Figure 1.2A (Schippers and Sand, 1999). Typically, free thiosulphate is oxidised via tetrathionate and other polythionates, finally to sulphate. However, significant amounts of elemental sulphur (10–20%) could be produced in the absence of sulphur-oxidizing bacteria.

Metal sulphides such as sphalerite, galena, arsenopyrite, chalcopyrite and hauerite are dissolved by the combined action of electron extraction through Fe^{3+} and H^+ attack (Crundwell, 1997). The first free sulphur compound is most likely a sulphide cation for example H_2S^+, which can spontaneously dimerise to free disulphide for example sulphur (S_8), and is further oxidised, via higher polysulphides and polysulphide radicals, to elemental sulphur which may then be transformed into sulphate by sulphur oxidising bacteria shown in figure 1.2B (Schippers *et al.*, 1996).

Bioleaching micro-organisms play a crucial role during sulphide mineral leaching operations, not only to oxidise Fe^{2+} but also to oxidise intermediate sulphur compounds. The importance of the latter is discussed in the following section (1.4.3).

1.3.2.1 Chalcopyrite passivation as researched through electrochemistry

One major influence on the rate of chalcopyrite dissolution, especially during mesophilic leaching, is solution potential (Tshilombo *et al*., 2002). Researchers have found that at 25°C and 45°C $CuFeS_2$ undergoes passivation, where dissolution is almost non-existent (Harahuc *et al*., 2000; Cordoba *et al*., 2009). Passivation was attributed to a leach product layer which eventually covered the mineral surface occurring around 450 – 650 mV, measured with a standard hydrogen electrode (SHE). As leaching progressed so did the product layer thickness (Tshilombo *et al*., 2002). This passivation was not reported to occur at 65°C under the specified conditions. The layer causing passivation at the mineral surface has been researched and due to the unstable and fragile nature of the layer the exact cause remains unsubstantiated. However, It is believed that an underlying sulphide layer(s) causes leach rates to become sluggish to an almost non-existent state (Sandström and Mattsson, 2001; Drouet and Navrotsky, 2003).

From potentiostatic experiments it was also concluded that at low solution potentials and lower, < 65°C, temperatures, only iron was preferentially dissolved and this occurred specifically in the region of severe passivation. Above the 650 mV (SHE) value preferential leaching of both copper and iron occurred (Tshilombo *et al*., 2002). Since micro-organisms oxidize Fe^{2+} it would thus be advantageous for this process to occur at a rapid rate to achieve a high potential ensuring preferential Cu liberation. Some studies have concluded also that a higher Cu liberation was achieved at lower redox potentials due to the formation of jarosite at higher potentials and the availability of higher Fe^{3+} concentrations at these high

redox potentials (Sandström and Mattsson, 2001; Drouet and Navrotsky, 2002; Córdoba *et al*., 2009).

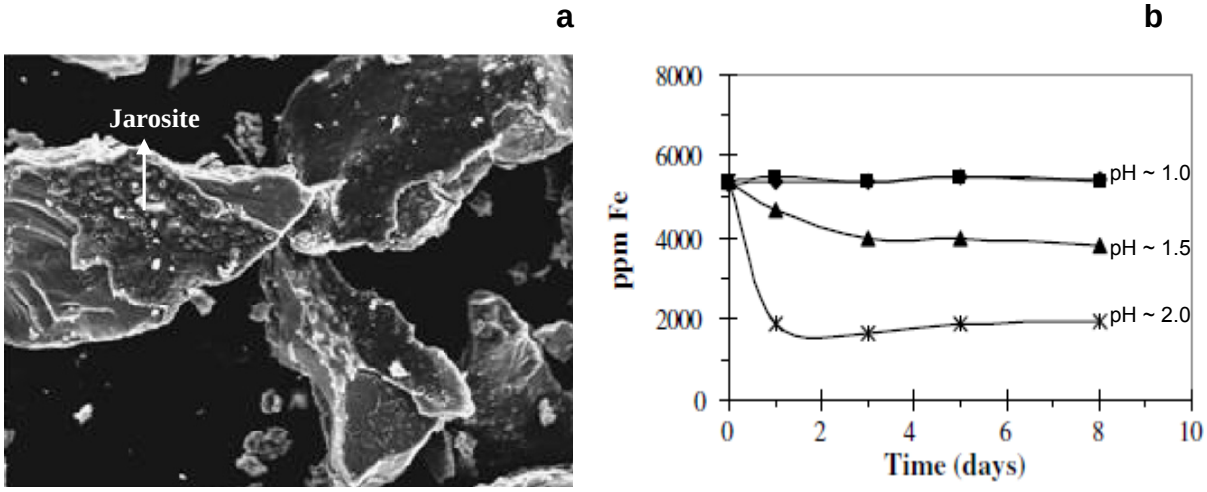


Figure 1.3: Figure a shows a scanning electron micrograph (SEM) where significant jarosite was formed on the chalcopyrite mineral surface. Figure b indicates the reduction in iron concentration with time at pH 1.0-2.0, corresponding to the jarosite formed (pH~2.0) in micrograph A (Cordoba *et al*., 2009)

Jarosites are mainly composed of Fe thus with an increase in jarosite formation occurs a reduction in the soluble iron concentration, especially of Fe^{3+}. This decrease in Fe^{3+} could potentially lead to a reduction in Cu liberation as insufficient Fe is available in order to satisfy equation 1.1, as well as creating a diffusion barrier which inhibits the leaching reactions from occurring.

The group of Cordóba further concluded that the rate of chalcopyrite dissolution cannot be improved through a decrease in pH since the function of acidity in this process was to determine the hydrolysis of Fe^{3+} (Figure 1.3). This difference in formation of ferric complexes has intrinsic differences in oxidation capabilities which in turn affect the leaching of chalcopyrite and formation of passivation layers (Ruitenberg *et* al., 1999; Cordoba *et al*., 2009).

Much debate still exists as to the passivation of chalcopyrite mostly due to the fragile nature of intermediate sulphur compounds and inconsistent Cu recoveries during leaching experiments.

1.3.2.2 Galvanic interactions within a chalcopyrite-pyrite heap system

Galvanic interactions occur between conducting and semiconducting minerals in aqueous systems. In a bioleach heap operation containing both pyrite and chalcopyrite either one or both of the minerals making up the galvanic cell would be favoured in terms of dissolution. The efficiency of the mineral leaching depends on the mineral's electrochemical characteristics and the sulphides contained within the ore (Cruz *et al*., 2005).

Direct contact of minerals with dissimilar rest potentials such as sulfides initiates the galvanic effect, where the mineral with the higher rest potential proceeds as the cathode and the mineral with the lower rest potential act as the anode. The cathode is thus galvanically protected or shielded against dissolution while the mineral acting as the anode undergoes dissolution (Cruz *et al*., 2005).

It is generally believed that progression of sulphide mineral dissolution occurs from minerals with a lower resting potential, ending with higher potential minerals (Petersen and Dixon, 2006; Qingyou *et al*., 2006).

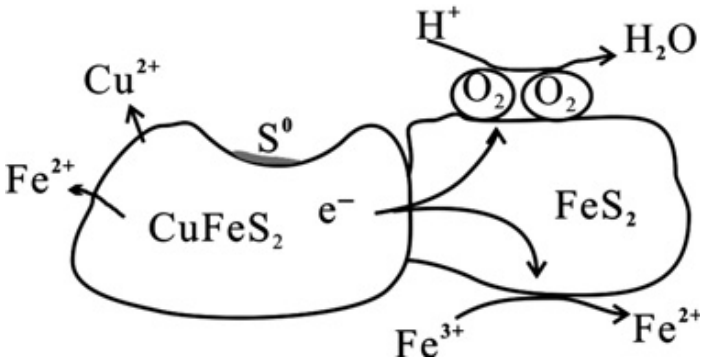


Figure 1.4: The galvanic interaction model for a pyrite-chalcopyrite system taking place in a flowing system (Qingyou *et al*., 2006)

$$CuFeS_2 \rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 4e^- \quad \text{...Eqn 1.6}$$

$$Fe^{3+} + e^- \rightarrow Fe^{2+} \quad \text{...Eqn 1.7}$$

When pyrite and chalcopyrite are in direct contact (Figure 1.4), the galvanic interactions are described by equations 1.6 and 1.7. In this case $CuFeS_2$ is the anode and is favoured for dissolution since electrons flow from mineral particles with a higher potential to mineral particles of lower potential.

The group of Qingyou observed a lag in dissolution of the anode and attributed this lag in anodic reaction to Fe^{2+} interference. As Fe^{2+} dissolved into solution, sulphur accumulated on the anode surface thereby creating a diffusion barrier at high redox potentials. As this barrier increases Fe^{2+} can accumulate reducing the concentration of H^+ near the surface and causing an increase in surface pH, whereby the formation of thiosulphate is favoured. It was shown that galvanic interactions greatly affect the dissolution of chalcopyrite.

Practically, it is possible that other galvanic interactions also occur where the dissolution of minerals with a lower rest potential is favoured for dissolution and only after these minerals are leached would chalcopyrite be favoured for dissolution. In the current study, a whole ore

containing ± 0.5% $CuFeS_2$ and ± 4.0% FeS_2 will be used where chalcopyrite will most likely be favoured for dissolution.

1.4 Microbial growth within a bioleach heap system

The bacteria and archae used in bioleaching have some physiological features in common. They are all chemolithotrophic and are able to use Fe^{2+}, reduced inorganic sulphur compounds, or both, as electron donors to generate energy for growth. Many of the important species are autotrophic, but some perform better when supplemented with some organic carbon. A number of obligate heterotrophs have also been identified. Sulphuric acid is typically the end-product of sulphur-oxidation and hence the micro-organisms involved are acidophilic and grow within the pH range of pH 1.0 - 4.0. Although a number of potential electron acceptors, such as ferric iron, are available to bioleaching micro-organisms oxygen (O_2) remains the favoured agent due to its high oxidation capacity (Rawlings, 2002).

Carbon dioxide (CO_2) is fixed by most micro-organisms involved in bioleaching, but at greatly varying efficiencies. During bioleaching the carbon source (CO_2) and preferred electron acceptor (O_2) for the growth of the micro-organisms are provided by aeration. The mineral ore supplies the electron donor (ferrous iron and/or reduced inorganic sulphur), and water is the medium for growth. Important trace elements are provided by the minerals and water (Lizama *et al*., 2003). Some bioleaching micro-organisms can also fix atmospheric nitrogen which is then used to synthesize amino acids for protein development and growth. In commercial processes, small quantities of relatively inexpensive, fertiliser-grade, ammonium sulphate and potassium phosphate may be added aid microbial growth and ensure that nutrient limitation does not occur (Lizama *et al.*, 2003).

The micro-organisms involved in bioleaching are typically resistant to a variety of metal ions, with some variation in the degree of metal tolerance at species or strain level. All these factors account for micro-organisms involved with bioleaching, being able to grow well in these extreme conditions which contain iron- and/or sulphur minerals and a liquid phase at a low pH (Rawlings, 2002).

Organisms capable of iron or sulphur oxidation have been isolated from diverse environments across a range of temperatures. The first isolates were obtained from natural acid rock drainage environments are consisted of mesophilic iron and sulphur oxidisers. The iron oxidising bacteria classified as mesophiles and moderate thermophiles are generally gram-negative (Norris, 1988). The mineral oxidation reactions which take place during bioleaching are exothermic. During commercial tank and heap leach operations, significant heating was observed (Silver, 1978). Subsequent characterisation of the microbial populations identified a number of new species capable of growth up to 55°C. These have been characterised as moderate thermophiles.

The discovery of extremely thermophilic micro-organisms in sulphidic hot springs led to the subsequent evaluation of these micro-organisms for their ability to oxidise iron and sulphur. Thermophiles are of particular interest for the leaching of recalcitrant minerals such as chalcopyrite and molybdenite, where higher metal recoveries are possible at elevated temperatures (Brierley and Brierley 1999; Rawlings, *et al*., 2002; Madigan *et al*., 2003 and Kinnunen, 2004). The metabolic requirements of a number of the most important bioleaching organisms are summarised in Table 1.3.

Table 1.3: Micro-organisms used in bioleaching and their growth parameters

Microbes	Class	Oxidation compound	Temp (ºC)	pH range	Bacterium/ Archaea
Acidianus brierleyi	T	Iron and Sulphur	45 – 75	1 – 6	Archaea
Acidithiobacillus caldus	MT	Sulphur	35 - 50	1.8 – 2.5	Bacterium
Acidithiobacillus ferrooxidans	M	Iron and Sulphur	20 – 40	1.4 – 4.0	Bacterium
Acidithiobacillus thiooxidans	M	sulphur	28 – 48	0.5 – 5.5	Bacterium
Acidmicrobium ferrooxidans	MT	Iron	45 – 50	1.5 – 2.5	Bacterium
Ferroplasma acidiphilum	M	Iron	15 – 45	1.3 – 2.2	Bacterium
Leptospirillum ferriphilum	M	Iron	30 – 37	1.4 – 1.8	Bacterium
Leptospirillum ferrooxidans	M	Iron	30 – 37	1.1 – 4.0	Bacterium
Leptospirillum thermoferrooxidans	MT	Iron	30 – 55	1.3 – 1.9	Bacterium
Metallosphaera sedula	T	Iron and Sulphur	50 – 80	1.0 – 4.5	Archaea
Sulfobacillus acidophilus	MT	Iron and Sulphur	30 – 55	2.0	Bacterium
Sulfobacillus thermosulfidooxidans	MT	Iron and Sulphur	30 – 60	1.5 – 5.5	Bacterium
Sulfolobus metallicus	T	Iron and Sulphur	50 – 75	1 – 4.5	Archaea

M – Mesophile; MT - Moderate thermophile; T - Thermophile
(Adapted from Rawlings, 2002; Rawlings, *et al*., 2002; Madigan *et al*., 2003 and Kinnunen, 2004)

1.4.1 Mesophilic and moderately thermophilic bioleaching micro-organisms

The research presented in this thesis is focussed on the initial phases of heap bioleaching. In this context the most important organisms are the mesophilic iron and sulphur oxidising bacteria belonging to the genera *Acidithiobacillus* and *Leptospirillum*. The characteristics and metabolic features of these organisms will be discussed in greater detail. Moderate and extreme thermophilic organisms are important in the latter stages of heap leaching as well as in tank leaching processes, but will not be discussed in detail in this review.

Bioleaching micro-organisms from the genera *Acidithiobacillus* and *Leptospirillum* are most efficient in obtaining energy under aerobic conditions but in the absence of oxygen a micro-organism for example *Acidithiobacillus ferrooxidans* is able to utilize Fe^{3+} as an alternate electron acceptor and using inorganic sulphur or Fe^{2+} as an energy source (Rawlings, 2002). *Acidithiobacillus ferrooxidans* is able to fix atmospheric nitrogen and utilise CO_2 as a carbon source enabling growth on very nutrient-poor solutions. Aeration provides the said CO_2 as well as nitrogen (N_2) and oxygen (O_2) where the ore supplies the energy source. Ammonium sulphate ($(NH_3)_2SO_4$)) and potassium phosphate (K_2PO_4) could also be added as supplements to ensure that sufficient nutrients are present for enhanced microbial growth (Rawlings, 1998). Because these micro-organisms are able to tolerate moderate to high levels of most metal ions for example Fe^{3+} > 20 $g.l^{-1}$ and grow at low pH values for example pH < 1.0, inter-species competition within these systems are fairly limited (Ojumu *et al*., 2006).

Acidithiobacillus ferrooxidans is classified as an acidophilic chemoautotrophic bacterium (Nagpal, 1996). The outside of the cell is usually at a very low pH of about two where the inside pH of the cell is typically at about six and a half. This vast difference in pH aids energy generation by creating a steep gradient difference in protons. The model for the oxidising system of *Acidithiobacillus ferrooxidans* is shown in Figure 1.5. This Figure suggests a short chain of three electron carriers associated with the cell envelope. Electrons flow from Fe^{2+} at the outside of the cell to a cytochrome c protein via a copper-containing protein rusticyanin. This protein is very stable at acidic pH values. Electrons are then transferred to cytochrome-a and subsequently releases its electrons to molecular oxygen. Water is produced as a result as well as ATP as shown in Figure 1.5. It is unknown specifically what helps transfer of electrons from Fe^{2+} to rusticyanin.

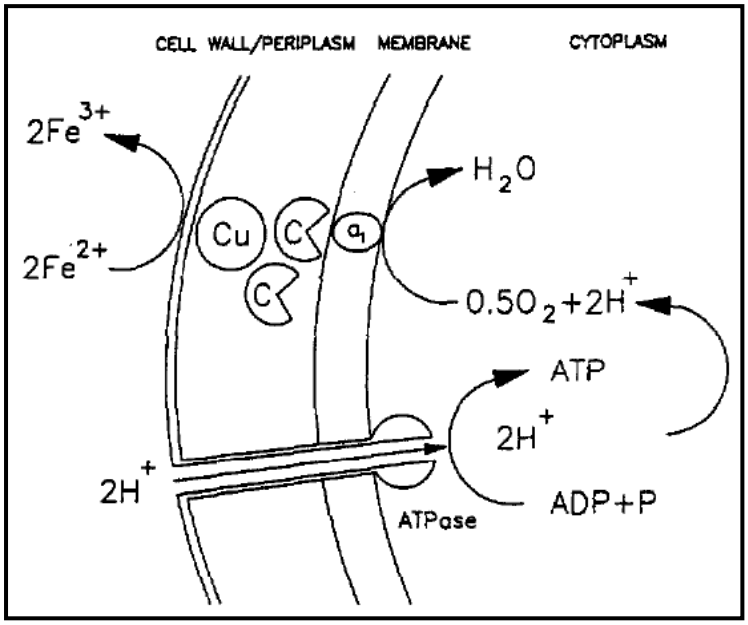


Figure 1.5: Proton circuit and ferrous oxidation for *Acidithiobacillus ferrooxidans* (Ingledew, 1982)

The Calvin-Bensen cycle is used for carbon dioxide fixation by iron-oxidizing bacteria for example *Acidithiobacillus ferrooxidans.* During this cycle three molecules ATP and two molecules NADPH are required to fix one molecule of carbon dioxide (Silver, 1978). In this process Fe^{2+} are converted to Fe^{3+} which in turn leads to further metal dissolution. Ensuring sufficient CO_2 is provided to the system could lead to a higher growth rate due to the availability of a higher carbon concentration. Understanding the bioleaching microbial metabolism plays a role in understanding microbial growth and activity, whereby a lag in growth could be better understood.

A number of bacteria are capable of utilizing iron and/or sulphur compounds as energy sources at temperatures between 45–55°C. These include the Gram-negative bacterium, *Acidithiobacillus caldus* which is capable of oxidizing sulphur between 30°C and 50°C. A significant difference between *A. caldus* and *A. ferrooxidans* is that *A. caldus* lack the genes which enable *A. ferrooxidans* to oxidise Fe^{2+} (Valdes *et al*., 2008). Another significant difference between these two species is that nitrogen fixation genes was not detected in the *A. caldus* strain (ATCC 51756) researched by the group of Valdes. This entails that this strain has to acquire nitrogen from a micro-organism able to fix nitrogen or through organic nitrogen supplied for example in a fertiliser. Due to these differences in micro-organisms it is pertinent to include different species in the inoculums in order to attain the necessary characteristics needed for a viable heap leach operation.

Bacteria from the genus *Leptospirillum* are chemolithotrophic micro-organisms oxidising Fe^{2+} as a sole source in order to gain energy and are between 0.3-0.6 µm wide and 0.9-3.5 µm long (Coram and Rawlings, 2002). It is likely that due to Fe^{2+} serving as sole energy source that these bacteria have a higher affinity (Km = 0.25) for Fe^{2+} compared to strains from the

Acidithiobacillus genus (Km = 1.34). Thus during the latter stages of heap bioleaching when high Fe^{2+} oxidation rates are reached, it is likely that species from the *Leptospirillum* genus will become dominant (Norris *et al*., 1988). The degree of metal tolerance, affinity for Fe^{2+}, growth rate as well as the ability to fix nitrogen varies amongst different microbial species. Development of the ideal microbial consortium could prove beneficial for heap bioleach operations in order to ensure optimal operation within the shortest time period.

1.4.2 Identified microbial phases within a bioleach heap system: The attached and planktonic microbial phases

The underground microbial community is divided into two related populations: planktonic and attached populations. The attached population comprise a significant proportion of cells within a system containing sulphide mineral as the attachment support base, aiding significantly in leaching (Escobar *et al*., 1996; Bouffard and Dixon, 2002). Research in this arena has also shown that the degree of attachment is dependent on the solid surface. The attachment and growth of micro-organisms can influence the flow of nutrients through small spaces, causing the formation of dead zones devoid of liquid flow (Acevedo, 2000). These zones will therefore not result in mineral dissolution.

Microbial cells are approximated as charged colloids and the ore surface as a charged surface where the interaction of cells to the ore particles are initially electrostatic and of columbic attraction (Roberts, 2004). As leaching progresses and microbial growth occurs, the attachment becomes more permanent through EPS production. Biofilm populations then develop by excretion of EPS creating a favourable environment for these bacteria to grow in as well as for the chemical reactions to occur in (Gehrke *et al*., 1998). Currently, several mathematical models are used to describe microbial attachment to mineral surfaces, for example surface complexation models (Yee *et al*., 2000).

The planktonic population also performs the function of regenerating the leaching chemicals. This occurs in solution rather than by first attaching to the ore surface, and washes out in the runoff. It is thought that there is an exchange between the attached and planktonic microbial phases where cells attach and detach as growth en development takes place.

The planktonic microbial phase would contain any species washing out of the reactor system whereby evaluation of this phase could lead to better insight regarding microbial preference to leach $CuFeS_2$ under defined conditions.

1.4.3 Microbial colonisation of an ore packed-bed under leach conditions

Bioleaching micro-organisms play a vital role in heap leaching operations. These micro-organisms need time to adapt to the heap environment if inoculated from a lab grown pre-inoculum. Due to the time needed for this adaptation and colonisation of the ore, a lag

phase is experienced where mineral dissolution is less that desired. It is thought that the rate of mineral dissolution increases as colonisation of ore occurs where the microbial population have a tight association with the ore surface.

Currently three mechanisms of leaching are thought to occur resulting in mineral dissolution. These include non-contact leaching, contact leaching and cooperative leaching (Rawlings, 2002).

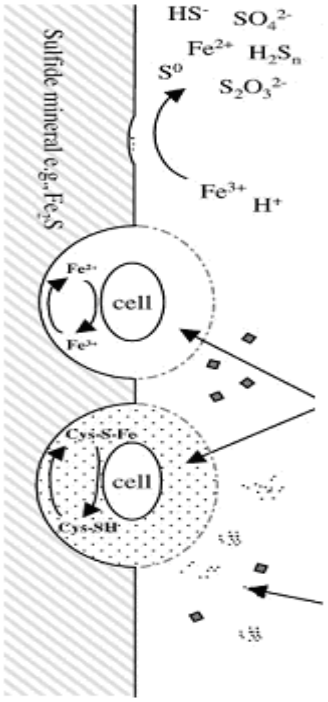


Figure 1.6: Diagram showing the means of ore leaching (Taken from Rawlings, 2002)

As microbial attachment to the ore occurs, a biofilm or extracellular polymeric substances (EPS) layer is formed, namely the direct mechanism shown in Figure 1.6, resulting in a complex and reactive chemical environment around the attached cell(s) (Sand *et al*., 2001). The EPS layer is composed mainly of lipopolysaccharides made up of fatty acids and sugar monomers (Gehrke *et al*., 1998). This formation may potentially enhance basic cell growth and metabolism. As the attached time increases the direct interaction between the cell and mineral is increased and this enhances the rate at which the mineral is recovered (Roberts, 2004).

Through the indirect mechanism or cooperative leaching some micro-organisms generate ferric ions and protons by using sulphur intermediates, thus also contributing to extraction of the metal of interest.

It is however not yet clearly understood specifically how microbial colonisation takes place, the exact population structure or how the growth thereof is governed.

1.5 Effects of environmental factors and chemical agents on the bioleaching microbial population

Many factors affect the kinetics of iron and sulphur oxidation including pH, temperature, and initial O_2 and CO_2 concentrations as well as significant concentrations dissolved cations.

1.5.1 Effect of temperature

Heat generation and retention are governed by a range of factors. These include mainly climatic conditions, heap location (altitude), heap volume, irrigation rate, air-flow rate, insulation, mineral oxidation rate and microbial activity. Most of these factors are also interdependent and affect the overall bioleaching performance (Du Plessis *et al.,* 2007 in Rawlings and Johnson, 2007).

The temperature optimum and maximum at which iron oxidizing bacteria can grow is said to be pH dependant, indicating that as pH decreases so does the optimum temperature for growth (Nemati *et al.*, 1998). Several studies have been done on optimum and maximum temperature and differences might be due to the use of different strains employed in these studies. This further indicates the necessity of required research into bioleaching micro-organisms and any involved interdependencies.

With the commencing of heap leach operations temperatures are close to ambient temperature. This requires the inoculum to contain a consortium of mesophilic bacteria performing the desired oxidation reactions. As the leaching process continuous heat is generated. When significant temperature increases occur within the heap exceeding the maximum growth temperature of the inoculated micro-organisms, it becomes vital to reinoculate the heap with a consortium of micro-organisms being able to survive the increased temperature and still carry out the desired oxidation reactions.

As the heap temperature increases it is possible to achieve a higher mineral dissolution rate especially for $CuFeS_2$ leaching. It is however also important to note that at these elevated temperatures a higher rate of jarosite formation could occur as discussed in Section1.4.2.

For the intended study the interest lies with the initial start-up phase of the bioleaching operation prior to any possible temperature increases. These conditions are therefore set for the activity of mesophilic micro-organisms, where the focus of this study will lie. The intended system would comprise of approximately 4.0 kg packing material and it is thus unlikely that significant temperature increases would occur during the study.

1.5.2 Effect of pH

Acidithiobacillus ferrooxidans is capable of growth at pH values between 1.4 and 4.0 using Fe^{2+} as the energy source. Tuovinen reported lag periods when *Acidithiobacillus ferrooxidans* was grown at pH levels of 1.2, indicating an adaptation period. Within the pH range of 1.5 – 3.5 the growth of *Acidithiobacillus ferrooxidans* was not affected but outside these ranges growth was strongly inhibited during growth in a CSTR where a concentrated $CuFeS_2$ mineral was used (Nemati *et al.*, 1998).

Due to the large pH difference between the inside and outside of the cells, they must be able to cope with the large proton gradient (Madigan *et al*., 2003). Protons are used by the cells to react with oxygen thereby forming water as well as providing a driving force for the formation of ATP.

pH is not only important for optimal microbial growth but a pH below pH 1.6 is required to reduce the formation of jarosite as discussed in Section 1.4.3.

1.5.3 Effect of dissolved ferrous- and ferric iron

Because Fe^{2+} serves as the energy source, the initial Fe^{2+} concentration significantly influences the growth of iron oxidizing bacteria. Silverman and Lundgren reported the dependency of for example *Acidithiobacillus ferrooxidans* on Fe^{2+} by showing a decline in growth of the bacteria due to the exhaustion of Fe^{2+} in the substrate (Silverman and Lundgren, 1959). Similarly, high concentrations of Fe^{2+} could also inhibit growth at reported concentrations of 2 – 3 $kg.m^{-3}$ (Barron and Lueching, 1990).

Requirements such as adapted inocula and experiments performed with inocula containing the bioleaching micro-organisms at identical growth phases have not always been met and therefore discrepancies exist for the values of maximum growth rates and activation energy (Nemati *et al.*, 1998).

Different micro-organisms possess different affinities for Fe^{2+} and as Fe^{2+} oxidation takes place a micro-organism for example *Leptospirrillum ferrooxidans* having a lower Km value (0.3-0.5) (Rawlings and Johnson, 2007) (higher Fe^{2+} affinity) compared to *Acidithiobacillus ferrooxidans* (0.7-1.3) (Norris in Rawlings and Johnson, 2007) could out compete the latter in terms of growth ability in very low Fe^{2+} concentrations. The intended study will also be geared towards investigating the changes (if any) in the microbial population during the experimental run.

An increased Fe^{3+} concentration would lead to an increased dissolution rate given the availability of $CuFeS_2$. From work done by the group of Harrison it has been shown that intertwined with precipitation of Fe^{3+} is Cu forming complexes with the precipitating phase,

resultantly prohibiting the recovery of Cu in the liquid phase (van Hille *et al*., 2009 – in press). Research has also shown Fe^{3+} concentrations below 5 $g.l^{-1}$ is not inhibitory to microbial growth (Ojumu *et al*., 2006).

One of the purposes of acid agglomeration is to dissolve oxidised sulphur compounds from the mineral surface thereby exposing the sulphide surface for microbial attachment. During this process small ore particles adhere to larger particles reducing the probability of clogging of the system. Due to the acid dissolving nature of some cations and anions could result in an environment high in ionic strength which could be detrimental the the growth and activity of the bioleaching micro-organisms. These micro-organisms respond to stress via two main strategies. The first is adaptation to a specific component, for example Si^{4+}. This takes place during initial contact with the component. This results in an extended lag phase characterised by reduced microbial growth and metabolic activity (Tuovinen *et al*., 1971). The second strategy entails microbial dormancy, where limited growth and activity occur until favourable conditions return (Madigan *et al*., 2003). The metabolic systems protecting microbial cells from specific cations and anions are molecule specific, although some genetic systems protect the micro-organisms against a variety of stressors. The induction of these systems requires time and energy, resulting in an extended lag phase. Thus, an increase in dissolved components to concentrations to which the micro-organism(s) are not adapted may result in an extended lag phase. During this phase low biological Fe^{2+} oxidation rates could result in a very low Fe^{3+} concentration leading to reduced Cu recovery.

1.5.4 Effect of nitrogen, carbon dioxide and oxygen

The main source of nitrogen in heap bioleach operations are contained within the air being provided to the heap. Nitrogen is the key component of amino acids, which are the building blocks needed for the synthesis of proteins and nucleic acids required for DNA replication. In the absence of reduced nitrogen, certain organisms can employ an energy intensive process to fix atmospheric nitrogen (Madigan *et al*., 2003). The nitrogen triple bond is extremely stable, with a dissociation energy of 940 $kJ.mol^{-1}$ (Madigan *et al*., 2003). Some micro-organisms for example *Acidithiobacillus caldus* cannot fix nitrogen from air (Valdes *et al*., 2008). All these micro-organisms would thus greatly benefit from added reduced nitrogen compounds in solution or by living in synergy with other micro-organisms providing reduced nitrogen compounds. Even for micro-organisms with the ability to assimilate nitrogen from air, it would be beneficial not having to do so as this process is highly energy intensive (Madigan *et al*., 2003). The energy saved in not having to assimilate nitrogen could be used for proliferation, maintenance and development of adaptation mechanisms.

Carbon dioxide is the main source of carbon for iron- and sulphur-oxidizing bacteria. It is thus of absolute importance to ensure adequate carbon dioxide in the air supplied to the system. CO_2 could become limited as this gas has a low solubility at low pH values. The importance of CO_2 for achieving optimal growth rates and maximum cell yields was

demonstrated by the group of Holuigue. They reported that about 5% CO_2 in air stimulated maximal growth (Holuigue *et al*., 1987). At higher levels of CO_2 the cell yields increased, although the growth rate was unaffected. A higher concentration of about 8% was found to be growth inhibitory for *Acidithiobacillus ferrooxidans.*

Oxygen is also very important for growth and serves as the final electron acceptor when ferrous iron is provided as the energy source. It has been reported by the group of Liu that oxygen becomes the limiting substrate when the concentration of dissolved oxygen is less than 0.29 $mg.l^{-1}$ and that *Acidithiobacillus ferrooxidans* is completely inhibited and does not grow at dissolved oxygen concentrations less than 0.2 $mg.l^{-1}$ (Liu and Branion (1988).

Research done by the group of Cordoba showed that in the presence of oxygen a higher dissolution rate of chalcopyrite could be obtained and attributed this to the oxidative capabilities of oxygen where Fe^{2+} is oxidised and converted to Fe^{3+} (Cordoba *et al*., 2008a,b). From SEM micrographs a surface layer was observed which appeared to be porous and non-passivating, but more research into this would be necessary.

When large heap operations are undertaken aeration could become insufficient where dead zones are created devoid of aeration. With the intended study aeration will be provided and due to the small scale insufficient aeration is not foreseen as an obstacle.

1.5.5 Microbial ecology and succession

Chemolithotrophic micro-organisms have to cope not only with elevated temperatures, high heavy metal concentrations, acidity and salinity but are also in competition with other micro-organisms for growth resources and habitat. The ability to cope with these factors makes a bacterium for example *Leptospirillum ferrooxidans* very important for industrial bioleaching processes where this micro-organisms can leach pyritic ores at redox potentials above 700 mV.

As the growth of chemolithotrophs take place, organic acids are released into the growth media. If these acids are produced at a significant rate and accumulate, they could lead to inhibition of the growth of the bioleaching micro-organisms. Heterotrophic bacteria can then metabolise these acids and grow in mutualism with the acidophiles (Remonsellez *et al.*, 2009). Synergism has been shown to occur between autotrophs and acidophiles in an industrial copper leach operation and more research could elucidate the exact link and/or dependence of either group on the other (Remonsellez *et al.*, 2009). *Acidithiobacillus ferrooxidans* has a high affinity mechanism for CO_2 uptake and production of organic carbon compounds but a relatively slow rate of iron oxidation compared to *Sulfobacillus thermosulfidooxidans* which has a high iron oxidation rate but limited ability to scavenge CO_2 from the air – these two micro-organisms can thus live in synergism serving the purpose of mineral leaching (Remonsellez *et al.*, 2009).

The group of Pizarro also reported the near absence of *Acidithiobacillus ferrooxidans* and dominance of *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans* in a Chilean copper simulated heap leaching system (column reactor experiments) (Pizarro *et al.*, 1996). *Acidithiobacillus ferrooxidans* however is usually the dominant species in systems where Fe^{2+} had been added to a high concentration and being used as an energy source. It was long believed that *Acidithiobacillus ferrooxidans* was the dominant bacterium in heap processes due to its higher growth rate on iron than that of *Leptospirillum* species. The correction for this misbelieve came about the discovery that Fe^{3+} which is produced from the oxidation of Fe^{2+} could inhibit the growth of *Acidithiobacillus ferrooxidans* in high enough concentrations where *Leptospirillum* species remain largely unaffected (Norris *et al.*, 1988). At the start of a heap leach operation a higher Fe^{2+} concentration is found within the heap. As the leach process progresses a lower Fe^{2+} concentration is maintained since more than 99% Fe^{2+} is oxidised. During the latter stage micro-organisms with a high affinity for Fe^{2+} would be favoured and likely out compete micro-organisms with a low affinity for Fe^{2+} although they possess a higher intrinsic growth rate.

It has been speculated that halotolerant micro-organisms might be able to cope with high ionic strength environments due to metal ions, since an isolate from the acidic river Tinto was closely related to a *Halomonas*-like isolate from the alkaline Lake Nakuru. Some of the other sulphur-oxidising bacteria isolated from the river grew optimally at 2% NaCl up to 10% NaCl (Lopez-Archilla *et al*., 2004). This could be highly beneficial during the start-up phases of heap leach operations since high concentrations of cations originate from the ore and could suppress microbial growth.

Due to the progression of heap leach operations and the inherent increasing temperature profile not only bacteria are found in bioleaching systems but also archaea such as *Ferroplasma acidiphilum* which is moderately thermophilic and can withstand higher temperatures being inhibitory to certain bioleaching micro-organisms. Culture-dependant studies done by the groups of Johnson and Norris demonstrated that genera such as *Acidianus, Sulfolobus* and *Metallosphaera* are some of the most efficient mineral sulfide oxidizers (Norris *et al.*, 1988).

The suggestion of using thermophiles in heap leaching has come from the exothermic biooxidation of sulfide minerals which produces significant heating in heaps as well as in continuous stirred tank reactors (CSTR's) especially in copper sulphide ore heaps and dumps (Silver, 1978). Due to the discovery of extremely thermophilic micro-organisms in sulphidic hot springs, which led to the evaluation of these micro-organisms for their ability to oxidize recalcitrant minerals such as chalcopyrite and molybdenite higher metal recoveries are possible (Brierley and Brierley 1999). Archaea have expanded bioprocessing options in terms of high temperature operation and metal tolerance especially in mineral processing.

The use of thermophiles was found to be advantageous in two ways (Brierley and Brierley, 1999). It was found firstly that increasing temperatures resulted in increased reaction rates resulting in a higher growth rate and secondly it was noted that the extent of metal extraction increased because of this, especially from chalcopyrite. It is likely that due to these higher reaction rates a higher potential could be reached in a shorter time period and well as formation of the EPS layer further enhancing the liberation of Cu^{2+}. There are also some disadvantages in using thermophiles. The increased temperature for example in a heap situation means more evaporation thus higher operation cost to provide the leaching solution and in a tank situation it means more cooling whereby running costs would increase significantly (Brierley and Brierley, 1999).

The current understanding regarding microbial inoculation in terms of species for heap leaching application is of the "the more the merrier", where it is believed that in order to obtain an optimal leach process a maximum number of microbial species should be inoculated with (Johnson, 2008).

1.6 Methods for evaluating the microbial population within a heap bioleach system

Due to the specialized growth requirements of many microbial species, these species are difficult to cultivate, or even impossible with current techniques – this fact has been known for a long time (Lorenz and Schlepe, 2002). According to "the great plate count anomaly", less than one cell in a thousand produced a colony, comparing direct microscopic cell counts and the number of colonies growing on nutrient agar (http://www.vebvzw.be/module1/uploads/24_mei_moleculaire_detectie_deel_4.pdf; http://www.cook.rutgers.edu/~dbm/lec12101204.pdf first coined by Staley and Konopka). Uncultured species represent a spectacular diversity which has been revealed by PCR-based (polymerase chain reaction) studies where DNA from samples is directly amplified (Malacinski, 2003). This approach where micro-organisms do not get cultured has been used to analyze the diversity of many different and complex environments, and to extract DNA for novel product research.

A micro-organism for example *Acidimicrobium* is more efficient at fixing carbon dioxide than *Sulfobacillus thermosulfidooxidans* and when these two micro-organisms are grown in culture, *Acidimicrobium* secretes small amounts of organic nutrients which are used by the *Sulfobacillus* enabling this bacterium to oxidize iron more rapidly. It is also possible that in nature more such symbiotic relationships exist, contributing to metal dissolution and growth of micro-organisms which are near impossible to culture under laboratory conditions.

In order to evaluate the microbial diversity within a heap leach system it is required to extract and purify the microbial DNA. PCR is used to amplify the 16s rRNA region. This region is strain specific and allows researchers to distinguish between different microbial species and strains. With the aid of techniques for example FISH (fluorescent in-situ hybridisation) and

DGGE (denaturing gradient gel electrophoresis) different 16s rRNA regions can be separated and visualised allowing the determination of the microbial strains most likely occurring in the heap leach system.

FISH is a fluorescent technique whereby a dye is used to fluoridise microbial rRNA (ribosomal ribonucleic acid) which is a highly conserved region, whereby microbial cells can be viewed under a microscope. Mahmoud and co-workers developed a fluorescent probe to detect nine isolates of the microbium *Acidithiobacillus ferrooxidans* from different AMD sites (Mahmoud *et al*., 2005). Probes are designed to be species specific where multiple species within a sample can be detected. This multistep procedure starts by fixing cells thereby maintaining the microbial population. The dye is applied and unbound dye is washed out. A microscope is employed to visualise and count the microbial cells.

Some of the drawbacks to this technique are ensuring probes have access to the rRNA (Mahmouda *et al*., 2005). Variation in the intensity of the emitted fluorescence could make distinguishing between cell colonies and background noise difficult. This variation could be due to the lower amount of cell rRNA due to the growth phase of the cells or due to different isolates having different binding affinities for the probe (Johnson and Hallberg in Rawlings and Johnson, 2007). FISH is therefore employed to verify the presence of expected microbial species within a sample.

Denaturing gradient gel electrophoresis (DGGE) is another culture independent microbial identification technique. This technique makes use of differential migration patterns of extracted and amplified DNA. The PCR amplification is done with a GC-clamp attached to the forward primer (Madigan *et al*., 2003). The DNA separates in an acrylamide gel making use of urea and formamide as chemical denaturants. These denaturants result in denaturing of the DNA up to the point where the GC-clamp is which holds the DNA together and result in different migration distances to achieve this denaturing of the DNA (Johnson and Hallberg in Rawlings and Johnson, 2007). The DNA fragments can be excised from the gel re-amplified and sequenced in order to determine the species or a standard could be run in order to determine the species contained within a sample

Care should be taken as microbial communities with an abundance of less than 1.0% of the total cell population would not be detected and therefore does not cover the total microbial richness of a given sample (Johnson and Hallberg in Rawlings and Johnson, 2007). Furthermore, this technique requires significant labour in setting up the system and finding optimal running parameters for example run time and gel concentration gradients. In order to ensure significant DNA band separation to distinguish various microbial species or strains finding the optimal gel gradient is vital where after the DNA could be excised without cross contamination. Due to the introduction of Taq polymerase errors care should be taken when interpreting sequence analysis data and the species represented (Johnson and Hallberg in Rawlings and Johnson, 2007).

Scope of Study

1.7 Problem Statement

Heap bioleaching, particularly for the recovery of copper from low-grade ores, is becoming an increasingly important alternative to conventional mineral processing technologies. While a number of heap leaching operations have been operated successfully at an industrial scale, the initial phase of process from the point of inoculation to the point where a stable redox potential is reached, remains one of the unpredictable phases.

During this start-up phase bioleaching microbes need to attach to the ore, adapt to the environment and multiply in order to colonise the entire heap. The unpredictability in start-up performance, regardless the engineering aspects may be related to conditions within the heap immediately after agglomeration and stacking, possibly due to the release of ions during agglomeration. These ions would be mobilised once irrigation of the heap was started and could result in an unfavourable environment for the microbes.

Ferric iron is the major oxidising agent responsible for sulphide mineral dissolution. It does however have limited solubility, even at acidic pH's. Jarosite, which is an iron hydroxyl-sulphate is particularly insoluble and more likely to form due to the oxidation of sulphides during leaching. It has been suggested that jarosite precipitation is responsible for chalcopyrite passivation, so the concentration of ferric iron in the PLS may have an important impact of leaching performance.

This thesis attempts to address the issues of the lag phase following inoculation and the relationship between iron concentration in solution and bioleaching performance. The current work therefore investigated the effect of selected parameters on heap bioleach performance employing simulated heap leach column reactors. These parameters included preleach time period, nitrogen supplementation and different feed iron concentrations and the resultant effects on microbial activity and proliferation ultimately affecting the copper leach rate. These parameters were selected due to the significant impact of these parameters on the start-up phase of bioleaching.

1.8 Hypotheses

The thesis is formulated to investigate three key hypotheses:

- Preleaching the ore with acidified water will flush out potentially inhibitory ions liberated by acid dissolution of gangue minerals during agglomeration resulting in an environment more favourable for attachment and subsequent microbial activity.

- Supplementing the irrigation solution with a reduced nitrogen source reduce the lag phase in microbial activity, resulting in more rapid iron oxidation and subsequent microbial growth. This supplementation will ultimately result in more efficient Cu leaching as well as a reduction in the total process time needed to achieve a maximum Cu liberation.

- Mineral leaching is a function of Fe^{3+} concentration during the initial phase only, after which the Cu leaching rate will be governed by mineral rather than Fe^{3+} availability.

These are addressed through experimental studies described in chapter 2. The results from the investigation of preleaching and its role in mitigating the lag phase are presented in Chapter 3. In Chapter 4, the results of the impact of nitrogen supplementation on minimisation of the lag phase and subsequent leaching are presented. In Chapter 5, the effect of dissolved iron concentration on leach rate is investigated. The overall conclusions and recommendations from the thesis are presented in Chapter 6.

Chapter 2: Materials and Methods

Within this chapter the materials and experimental protocols utilised during the study are presented. These include a detailed description of the ore, the microbial species employed, the bioleach column setup and configuration, experimental protocol, analytical methods employed and experimental setup.

2.1 Mineral charge

Two ore-types were used in this study. The majority of experiments were done using a low-grade whole ore. A chalcopyrite concentrate was also investigated. Both ore types were characterised by BHP Billiton prior to leaching and are described below.

2.1.1 Chalcopyrite whole-ore

The elemental composition of the whole-ore on a mass basis was 0.69% Cu, 2.95% Fe and 2.02% S. The sulphide phases and acid consuming gangue mineralogy is described in Table 2.1.

Table 2.1: Sulphide- and acid consuming mineralogy of the whole-ore

Mineral	Formula	Wt %
Sulphides		
Chalcopyrite	$CuFeS_2$	0.67
Chalcocite	Cu_2S	0.2
Covellite	CuS	0.3
Bornite	Cu_5FeS_4	0.1
Pyrite	FeS_2	4.0
Acid consumers		
Muscovite	$(KF)_2(Al_2O_3)_3(SiO_2)_6(H_2O)$	28.6
Biotite	$K(Mg, Fe)_3AlSi_3O_{10}(F, OH)_2$	0.5
Chlorite	$(Mg_5Al)(AlSi_3)O_{10}(OH)_8$	0.7
Kaolinite	$Al_2Si_2O_5(OH)_4$	7.4

2.1.2 Chalcopyrite concentrate

The major elemental composition for the chalcopyrite concentrate on a mass basis was 29.8% Cu, 25.9% Fe and 31.7% S. The major mineralogy is described in Table 2.2.

Table 2.2: Mineralogy of the chalcopyrite concentrate

Mineral	Formula	Wt %
Sulphides		
Chalcopyrite	$CuFeS_2$	79.52
Bornite	Cu_5FeS_4	4.14
Chalcocite	Cu_2S	0.01
Covellite	CuS	0.33
Pyrite	FeS_2	8.05
Other sulphides		1.23
Gypsum	$CaSO_4.2H_2O$	0.50
Silicates		
Quartz	SiO_2	1.45
Feldspar	$CaAl_2Si_2O_8$	1.83
Mica	$K_2Al_4(Si_6Al_2O_20)(OH)$	1.88
Altered silicates		0.10
Other silicates		0.29
Other		
Iron-oxides	Fe_2O_3 / Fe_3O_4	0.32
Carbonates (calcite)	$CaCO_3$	0.09
Accessories		0.17
Other		0.08
Total		100

2.2 Bacterial cultures

Mesophilic bacterial cultures were employed to facilitate the leaching process. The specific species used were obtained from the German national culture collection (DSM) *Acidithiobacillus ferrooxidans* DSM 584, *Acidithiobacillus caldus* DSM 8584 and *Leptospirillum ferriphilum* ATCC 49881.

Unless otherwise stated, all stock cultures were maintained in 1 L shake flasks with a 700 ml working volume, agitated at 120 rpm on an orbital shaker.

Acidithiobacillus ferrooxidans was grown at pH 1.6 in modified 9K media, at consisting of the following components ($g.l^{-1}$): $(NH_4)_2SO_4$ (2.0), K_2HPO_4 (0.5), $MgSO_4.7H_2O$ (0.5), KCl (0.1), $FeSO_4.7H_2O$ (20.0). *Acidithiobacillus caldus* was grown in similar media excluding $FeSO_4.7H_2O$ and with an additional 1 $g.l^{-1}$ of $(NH_4)_2SO_4$.

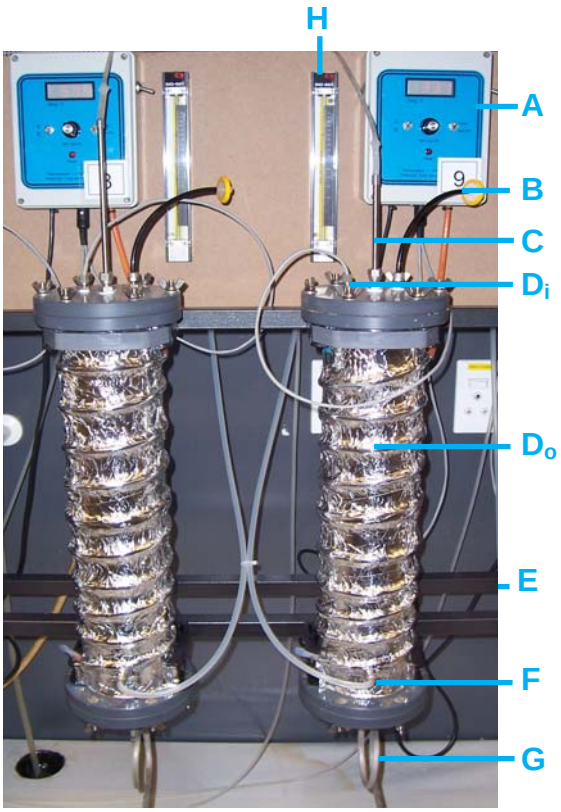
Leptospirillum ferriphilum was grown in a 1 L CSTR (continuous stirred tank reactor) fed at 0.021 $l.h^{-1}$ at pH 1.30 agitated at a rate of 400 rpm. The feed to the reactor contained the following components ($g.l^{-1}$): $(NH_4)_2HPO_4$ (0.53), $(NH_4)_2SO_4$ (1.83), $FeSO_4.7H_2O$ (5.0), K_2SO_4

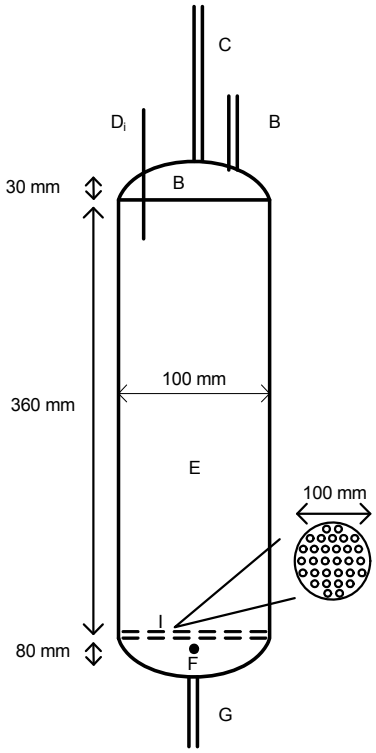
(1.11) as well as 10 ml.l^{-1} Vishniac solution. The Vishniac solution contained the following: $ZnSO_4.2H_2O$ (1.0), $CoCl_2.6H_2O$ (1.0), $MnCl_2.4H_2O$ (1.0), $CuSO_4.2H_2O$ (0.5), $FeSO_4.7H_2O$ (5.0), $Na_2MoO_4.2H_2O$ (0.5), $CaCl_2.2H_2O$ (0.5), added to 15 g.l^{-1} EDTA dissolved in 6% KOH.

The *Leptospirillum ferriphilum* strain was originally obtained from a vat-type continuous bioleaching mini-plant, treating a pyrite concentrate in Gamsberg, South Africa. The stock culture was maintained at a residence time of 72 hours.

2.3 Column configuration

A column reactor system was used to simulate the heap bioleach system. Polyvinyl chloride (PVC) columns were used as seen in Figure 2.1.





A-Temperature control unit
B-Ait outlet
C-Feed inlet
Di-Inside temperature sensor
Do-outside temperature sensor
E-packed bed
F-air inlet
G-leachate outlet
H-rotameter
I-perforated metal disc

Figure 2.1: Column reactor photograph and schematic configuration

Columns were insulated by covering the entire column with insulating aluminium tape. Temperature was controlled by a control unit and a heating coil, wrapped around each column. A controller unit was employed with two temperature sensors, one between the

aluminium insulation and the column and one inside the packed-bed, ensuring temperature control was maintained throughout the entire packed-bed.

Aeration was achieved by pumping air into the bottom of the column. The air flow rate to each column was controlled using a rotameter. The effluent pipe was bent into a coil, ensuring an airtight seal.

2.4 Analytical procedures

The bioleaching performance was determined by investigation of pH, redox potential and iron and copper liberation. The microbial response to different conditions was monitored through changes in cell morphology as well as cell number using the Canon BX40 microscope employing 1000X magnification.

2.4.1 pH and redox potential measurements

Sample pH measurements were taken using a Metrohm 704 pH meter and probe. The analytical error was calculated to be less than 1%.

Redox potential measurements were taken using a Metrohm 704 pH meter equipped with a combined Pt.Ag/AgCl electrode. The redox electrodes were tested on a daily basis by measuring a Crison standard redox solution of 468 mV at 25°C. The analytical error was found to be 0.64% against the standard.

2.4.2 Cu and total Fe measurements

Atomic absorption spectroscopy (AAS) was used to determine Cu and Fe^{T} (total iron) concentrations in the leachate. A Varian Spectra AA-200 Atomic absorption spectrophotometer incorporating Spectra AA 100/200 version 1.1 software was utilised. The analytical error was calculated to be < 1% for Fe and Cu respectively.

2.4.3 Fe^{2+} analysis

The Fe^{2+} concentration was measured colorometrically using the 1-10 phenanthroline method (Eaton *et al*., 1998). 1-10 phenanthroline complexes with Fe^{2+} to form an orange/red compound which can be quantified by measuring absorbance at 510 nm.

To measure the Fe^{2+} concentration 2 ml phenanthroline was added to 2 ml ammonium acetate solution and made up to 5 ml with appropriately diluted sample. Absorbance (510 nm) was measured using a Helios α spectrophotometer and concentration determined off a standard curve (Appendix A). Alternatively, Fe^{2+} concentration was determined from the total iron measurement (AAS) and redox potential using the Nernst equation (Appendix A).

2.4.4 Ferric iron (Fe^{3+}) determination

The Fe^{3+} concentration was calculated by subtracting the Fe^{2+} concentration obtained from either the 1-10 phenanthroline assay or from the redox potential calculations, from the total iron concentration obtained by AAS.

2.4.5 Total Kjeldahl nitrogen (TKN) analysis

The TKN method was developed and is mostly applied for the determination of nitrogen (N) in organic containing samples, especially wastewater samples. To determine the residual nitrogen concentration in the column reactor's effluent, the TKN method was employed.

All reagents used for the TKN and ammonical nitrogen analysis are described in Appendix A. The sample (5-10 ml) was pipetted into a 30 ml micro Kjeldahl flask, to which 10 ml of digestion mixture was added. The sample mixture was digested on a heating pad (380°C) until it became clear and for an additional 20 minutes thereafter. The Kjeldahl flask was transferred to the micro steam distillation unit and allowed to cool down at room temperature for approximately 30 minutes. A volume of 25 ml boric acid (H_3BO_3) solution was added to another 100 ml Erlenmeyer flask. The flask containing the boric acid was placed in position on the steam distillation apparatus with the nozzle of the condenser immersed in the boric acid solution. A 10 ml volume of distilled water was added to the contents of the Kjeldahl flask to dissolve any sediment. Sodium hydroxide (7 ml) was added to the flask containing the sample via the top opening of the distillation apparatus and the flasks were immediately sealed by putting the steam nozzle in place.

The samples were steam distilled until the H_3BO_3 solution increased to a volume of approximately 50 ml. Sulphuric acid (0.001 N) was used to titrate the samples to a change in colour from green to purple and the TKN concentration was calculated (Appendix A).

2.4.6 Ammonical nitrogen analysis

The ammonical nitrogen analysis was employed to differentiate between solution phase nitrogen and nitrogen that had been fixed in microbial biomass or contained within other organic compounds. The same procedure as for the determination of TKN was followed, with the exclusion of the digestion stage at 380°C. The nitrogen concentration for each sample was calculated as for TKN.

2.4.7 Cell concentration

Cell concentration was determined by direct counting using a phase contrast optical microscope. A Thoma counting chamber, with a well depth of 0.02 mm was used at a magnification of 1000 X (Appendix A). Direct counting by microscopy has been successfully

employed by several researchers (Konishi *et al*., 1995; Nemati and Harrison, 2000; Lamaignére, 2002; Sissing, 2002). Konishi *et al*. (1995) reported that the counting error of this method could be as high as 11% for bioleaching micro-organisms, however the reproducibility of this method was found to be acceptable. Both planktonic cell concentration and end of run detached cell numbers were measured by direct counting.

2.4.8 Molecular techniques

The monitoring of changes in microbial species composition using molecular biology techniques was an intended component of this work. However, unanticipated delays in the acquisition, commissioning and optimisation of the real-time PCR platform meant that this component was not possible within the timeframe of the project. DNA was extracted from PLS and detached cell samples and has been stored for analysis. The data will be presented in journal format at a later date. The paragraph below describes the initial work that was done and the development of the methods to be used.

Microbial DNA was extracted using the Roche High Pure Template Preparation kit according to the manufacturer's instructions. Extracted DNA concentration was measured using the Nanodrop ND-1000. Amplification of a portion of the 16s rRNA gene will be carried out using the UniF GC-clamped forward primer (5'-3': CGCCCGCCGCGCGCGGCGGGC GGGGCGGGGGCACGGGGGGTCCTACGGGAGGCAGCAG) and the rDD2 reverse primer (5'-3': CAAAGCTTCTAGACGGXTACCTTGTTACGACTT) resulting in ±1 020 bp fragments. The PCR mixture will be carried out in a total volume of 50 µl, containing ±100 ng of template DNA with the following reagents in their final concentrations: 3 µl $MgCl_2$ (1.5 mM); deoxyribonucleotide triphosphate (dATP, dCTP, dGTP, and dTTP), 2.5 µl (0.25 µM) of each primer (Uni-F and rDD2); 5 µl of 10 × PCR buffer; 33.6 µl of sterile millipore water and 0.4 µl FastStart Taq DNA Polymerase (Roche). The amplification program includes: a denaturation step for 10 min at 94°C followed by 35 amplification cycles of 30 s at 94°C, 30 s at 60°C, and 90 s at 72°C. A final step for 5 min at 72°C with the final cooling step (hold) at 4°C completing the procedure. PCR amplification will be performed in a PCR Sprint thermal cycler (Thermo Hybaid, UK). 0.8% (w/v) agarose gel and ethidium bromide (0.05 $\mu g.ml^{-1}$) staining will be used to validate amplification and size of the products. The extracted and amplified DNA will be used to perform DGGE analysis.

In order to quantify the bacterial population qRT-PCR will be employed. The amplification of a smaller portion of the 16s rRNA gene will be carried out using species specific primers designed according to the following criteria: (i) length of 17-28 bases, (ii) a base composition of 50-60% G+C, (iii) amplification of only the desired strain, (iv) melt temperature between 55-80°C, (v) self-complementarity (hairpins) should be avoided (adapted from Innis and Gelfand, 1991)

Primers have been designed and tested for specificity, using non-quantitative PCR, for
Acidithiobacillus caldus: F-primer: 5' GGGCTGACTGGAGTATGG 3'
R-primer: 5' CGAACCGCTGGCAACTAGGAA 3' and
Leptospirillum ferriphilum: F-primer: 5' ACAGAGGTGGCAAGCGTTGTT 3'
R-primer: 5' TCCGCCTTCCGGACTGAACC 3'
resulting in 550 and 790 bp fragments respectively. Primers specific for *Acidithiobacillus ferrooxidans* are currently being designed as initial designs showed cross-reactivity with other *Acidithiobacillus* species.

The PCR mixture will be carried out in a total volume of 20 µl, containing ±10 ng of template DNA with the following reagents: KAPA SYBR FAST master mix containing syber green, DNA template and the respective primers. Amplification conditions include: a denaturation step for 4 min at 94°C followed by 45 amplification cycles of 10 s at 95°C, 15 s at 58°C, and 20 s at 72°C. The melt curve will be conducted between 80-95°C concluding the PCR procedure. A 0.8% (w/v) agarose gel and ethidium bromide (0.05 µg.ml^{-1}) staining will be used in order to validate amplification and the size of the products. Analysis will include setup of standarc curves for each pure culture employed in the study where the PCR cycle number corresponds to the number of copies of the bacterial DNA amplified. The DNA from the unknown sample will be amplified where the DNA concentration will be determined. The concentration will be back calculated using the standard curve in order to calculate the number of DNA copies which correlates to cell number. The total number of micro-organisms can then be calculated as well as the proportions in which each species exist in that specific sample. This information in turn can be used to determine which species contribute significantly to Cu leaching. Universal primers will also be employed for calculation of the total number of micro-organisms present in order to ensure all species are accounted for. Furthermore, DGGE will be employed in order to detect any unknown species not inoculated for which primers will be designed and the above PCR procedure will be followed.

2.5 Experimental approach and setup

Columns were charged with agglomerated ore at the beginning of each run and operated, airtight, for approximately 60 days after inoculation. Each column reactor had a working volume of approximately 3141.6 cm^3 with a 40% void volume. Temperature was controlled at 32 ± 0.1°C and air was supplied at 200 ± 10 ml.min^{-1}. The liquid feed rate was set to 40 ml.min^{-1}. The procedure followed for each column experiment is described in the following sections.

2.5.1 Agglomeration procedure

Agglomeration was performed by mixing 4 kg of ore with 297.2 ml acidified ddH_2O (70 ml ddH_2O and 4.30 ml H_2SO_4 (98%) per kg ore) with the final pH ± 0.6. In the case of coating

chemically inert material with chalcopyrite concentrate an identical procedure and acid strength was used as was performed during agglomeration of the whole-ore. The agglomerate was packed inside the column with the temperature sensor placed inside the packed bed.

2.5.2 Leach solution

Where preleach was used, column reactors were supplied with acidified ddH_2O (H_2SO_4 pH = 1.80) at a feed rate of 40 ml.h^{-1}. The feed iron concentration, unless otherwise stated, was 2 g.l^{-1} containing ferrous- and ferric-iron at a final ratio of 1.3:0.7 . This ratio was chosen for historic comparison of leach experiments performed by this group. The pH was set to pH 1.15 by addition of H_2SO_4 (98%).

The feed was autoclaved fully made-up in all cases, to ensure a sterile feed medium. The feed solution was supplied to the column reactors through silicone tubing, using a Masterflex 8 socket peristaltic pump.

2.5.3 Daily analyses performed

Sampling was performed on a daily basis, with the total effluent collected and quantified using volumetric measuring cylinders. The pH and redox potential measurements were performed on the collected volume. A 30 ml fraction was retained for atomic adsorption spectroscopy (AAS) analysis (Cu and Fe^T) as well as for planktonic cell counts and colorimetric ferrous iron analysis.

2.5.4 Unpacking protocol upon completion of experiments

Column reactors were unpacked in three vertical sections, unless otherwise specified. A detachment regime was followed which included a series of three vortexing steps, at maximum speed, using acidified water (pH 1.3 – H_2SO_4 (98%)). Low-speed (113 x g) centrifugation was employed to remove particulate matter. This was followed by a water based detergent (Tween-20 (1%)) wash, and centrifugation (113 x g).

Cell counts were performed on the pooled acidified water supernatant fractions for each column reactor section and separately on the detergent fraction to determine the size of the attached microbial cell population. Where required, DNA extractions were performed on the relevant fractions.

2.6 Specific experimental investigations

All experiments were conducted as described in Section 2.6 with the exception of the parameter of interest as described below.

2.6.1 Effect of preleach time period

An investigation of a preleach regime was carried out to assess the impact of washing out ionic species liberated during the acid agglomeration process. The preleach regime was carried out using acidified ddH_2O (pH 1.8) at a flow rate of 40 $ml.h^{-1}$. Samples were collected at least once every 24 h for analysis as described for post inoculation.

Different preleach time periods were investigated: (i) 0 day preleach (ii) 4 day preleach (iii) 6 day preleach and (iv) 33 day preleach.

2.6.2 Effect of different feed iron concentrations

Initial observations indicated a decreased Cu liberation with a decrease in soluble Fe concentration. This relationship was not linear nor was the decrease in Cu proportional to the decrease in soluble Fe concentration. To study the effect of reduced iron concentration on leach performance, different feed Fe concentrations was investigated under more controlled conditions in order to gain insight into the relationship between soluble Fe concentration and the degree of Cu leached. The feed iron concentration was altered as follows, post preleach, maintaining a Fe^{2+}/Fe^{3+} ratio of 1.3:0.7: (i) 0.2 $g.L^{-1}$ (ii) 0.5 $g.L^{-1}$ (iii) 1.0 $g.L^{-1}$ (iv) 2.0 $g.L^{-1}$ and (v) 5.0 $g.L^{-1}$.

2.6.3 Effect of nitrogen supplementation of the feed

Due to the unpredictable lag phases experienced post inoculation, supplementation of the microbial community was investigated. Micro-organisms able to fix nitrogen from air expend high amounts of energy doing this. Micro-organisms not able to fix nitrogen requires a different source of nitrogen, for example organic nitrogen sources. Yeast extract and ammonium sulfate were chosen as the nitrogen sources for this supplementation investigation. The requirement for nitrogen was based on the nitrogen content of the maximum bacterial cell number attained in previous column experiments, and added to the feed in 100% excess prior to autoclaving. (Appendix A).

Yeast extract was utilised as organic nitrogen and $(NH_4)_2SO_4$ was used for inorganic nitrogen supplementation. A combination of $(NH_4)_2SO_4$, $NH_4H_2PO_4$, and K_2SO_4 was used as a PLS make-up (final nitrogen concentration at 50 $mg.^{l-}1$) supplementation. Experiments setup to investigate nitrogen supplementation were as follow: (i) Yeast extract (ii) Yeast extract + $(NH_4)_2SO_4$ (iii) Yeast extract + PLS (iv) PLS.

2.6.4 Geocoat® procedure

Due to high concentrations of cations and anions leached, particularly from the gangue component of the whole-ore, a coat preparation of mineral concentrate on an inert support

was used in order to investigate the effect of these cations and anions of the microbial community. Andina chalcopyrite was sieved to a size fraction range of +38 and -75 µm with a density (ρ) of 4.53 $g.cm^{-3}$, determined with an AccuPyc 1330 V1.02 pycnometer.

Inert granite pebbles were used as a support material and coated with chalcopyrite concentrate. The support material was adequately rinsed in an acidified (H_2O-H_2SO_4) solution prior to coating. A mass of 3.6 kg inert material was coated with 0.4 kg concentrate ensuring uniform coating (Harvey and Bath in Rawlings and Johnson, 2007). No preleach regime was followed and experiments were conducted as described in Section 2.6.

Chapter 3: Results and Discussion I:

The effects of preleach time period on the bioleaching performance

3.1 Preliminary experimental results employing whole ore as well as chalcopyrite concentrate

Preliminary column experiments, where no preleach was performed, yielded unexpectedly poor performance data. In order to determine whether this poor performance was related to the whole-ore used in the experiments, comparative experiments were performed using acid agglomerated whole ore as well as experiments where $CuFeS_2$ concentrate was coated onto a chemically inert support material. This was done in order to determine whether a link existed between the poor performance data obtained for the preliminary column experiments and the whole-ore used. The whole ore used in the current study was complex, comprising of many different minerals with the majority of ore being composed of gangue, as described in Section 2.1.1. The chalcopyrite concentrate coated onto the support material had a much lower ratio of gangue material to sulphide minerals compared to the whole-ore. Several of the acid-consuming minerals for example muscovite and biotite contained aluminium, magnesium, sodium and silicon which could be inhibitory to bacterial growth if liberated at sufficiently high concentrations (Nemati *et* al., 1998; Malik *et al*., 2004; Kinnunen and Puhakka, 2005; Hiroyoshi *et* al., 2007; Ozkaya *et al*., 2007).

Initial column experiments were conducted to compare system performance in columns loaded with whole ore or concentrate coated onto an inert support material. System performance was evaluated by measuring the rate of increase in redox potential, the rate of increase in planktonic cell concentration and final planktonic cell numbers.

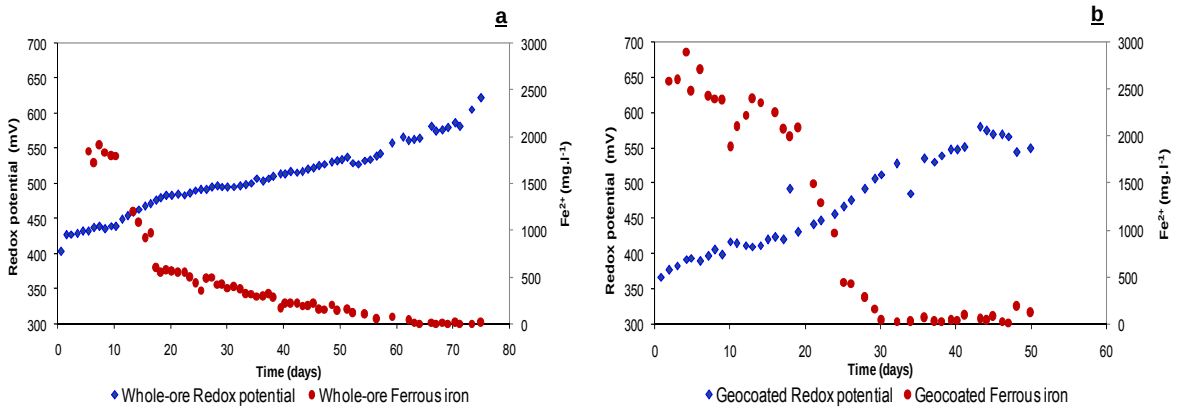


Figure 3.1: System performance profiles for (a) whole ore column reactor and (b) the geocoated® column reactor describing the change in redox potential and Fe^{2+} effluent concentrations. The material was not preleached and time zero represents the point of inoculation

The Fe^{2+} concentration measured during days 1 to 10 for the whole ore experiment (Figure 3.1.a), was significantly higher than supplied in the feed solution (1300 $mg.l^{-1}$). A similar trend was observed for the coated column reactor, although the elevated Fe^{2+} concentration lasted for 16 days (Figure 3.1b). Two factors likely contributed to these high Fe^{2+} concentrations. The total iron concentrations measured during these time periods were between three and four and three and eight ($g.l^{-1}$) for the whole ore and coated column reactors respectively, shown in Figure 3.2.a. These measurements indicate the extent of Fe dissolution which contributed to the high Fe^{2+} concentration measured initially. Furthermore, the larger proportion of sulphide minerals to gangue in the coated column reactor resulted in a Fe^{3+} leach rate greater than the rate of Fe^{2+} oxidation during days 1 to 16. Only after day 16 did the Fe^{2+} oxidation rate outcompete the ferric leaching of the mineral. Thus, the time required in order to achieve a Fe^{2+} oxidation rate significantly higher than the Fe^{3+} leach rate was reduced by ±50% where almost complete Fe^{2+} oxidation (>98%) occurred. In order to reach >98% Fe^{2+} oxidation a time period of 60 days was required for the column reactor packed with whole ore, compared to the 30 days required using concentrate as leach material.

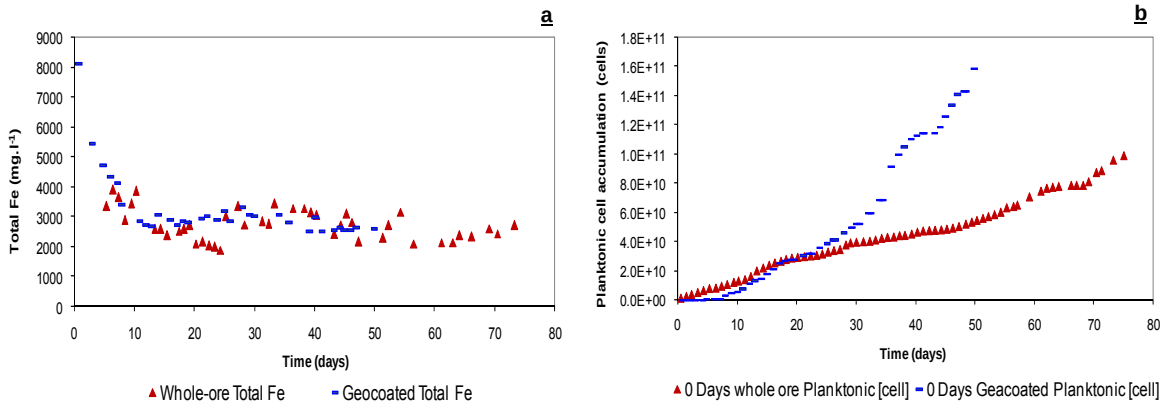


Figure 3.2: System performance profiles for (a) the total iron effluent concentration and (b) the cumulative planktonic cell number existing for both the whole ore and coated column reactors without a preleach regime employed. Time zero represents the point of inoculation

Figure 3.2.b shows a significantly higher number of cells was observed in the PLS exiting the column packed with whole ore compared to the coated column reactor during the first 10 days post inoculation. It is likely that a higher degree of microbial attachment to concentrate particles occurred compared to the degree of attachment to whole ore due to the selective attachment of micro-organisms to sulphide minerals, contributing to the different cell numbers in the PLS (Gehrke *et al*., 1998; Kinzler *et al.*, 2003; Sand and Gehrke, 2006; Africa *et al*., 2009). Furthermore, due to the dissolution of acid labile gangue components it is likely that conditions unfavourable to microbial growth were created. This resulted in a lower degree of attachment initially in the whole ore column reactor compared to the coated column reactors and thus the higher number of cells in the PLS from the whole ore column reactor. These result suggest that the poor performance observed initially was indeed linked to some property of the whole-ore used in this study.

A significantly lower planktonic cell number was observed in the PLS from the whole ore column reactor compared to the PLS from the coated column reactor following day 10 (Figure 3.2b). This indicated a lower initial degree of colonisation, followed by low microbial growth rates in the whole ore column reactor compared to the coated column reactor. The whole ore column reactor showed a planktonic cell number three times lower than that of the coated column reactor on day 50 further illustrating the long term negative effects of inhibitory components released from the low-grade ore on the microbial population.

During the agglomeration process cations and anions such as Cu^{2+}, Si^{4+} Al^{3+}, Mg^{2+}, Na^{+} and Cl^{-} ions are likely to be liberated from gangue minerals due to the concentrated acid addition. Examples of minerals containing these components are albite and sodium orthoclase. The cation concentration would be highest in the solution layer surrounding at the ore surface.

The group of Malik *et al*., (2004) reported that at an identical silicon concentration (0.096 $g.l^{-1}$) but different initial Fe^{2+} concentrations (2.4 and 5.4 $g.l^{-1}$), the rate of Fe^{2+} oxidation at 5.4 $g.l^{-1}$ Fe^{2+} was reduced by 92.5% at 40 hours run time. This work showed that dissolved solutes can adversely affect microbial growth and Fe^{2+} oxidation activity.

Micro-organisms respond to stress via two main strategies. The first is adaptation to a specific component, for example Si^{4+}. This takes place during initial contact with the component. This results in an extended lag phase characterised by reduced microbial growth and metabolic activity (Tuovinen *et al*., 1971). The second strategy entails microbial dormancy, where limited growth and activity occur until favourable conditions return (Madigan *et al*., 2003). The metabolic systems protecting microbial cells from specific cations and anions are molecule specific, although some genetic systems protect the micro-organisms against a variety of stressors. The induction of these systems requires time and energy, resulting in an extended lag phase. Thus, an increase in dissolved components to concentrations to which the micro-organism(s) are not adapted may result in an extended lag phase. During this phase low biological Fe^{2+} oxidation rates could result in a very low Fe^{3+} concentration leading to reduced Cu recovery.

From the above discussion it is clear that the microbial population in the whole ore column reactor was adversely affected, but this was not observed in the coated column reactors. This indicated that components liberated from the whole ore which were not present in the concentrate, adversely affected the performance of the microbial population as discussed above. It was therefore postulated that a preleach regime be employed in order to rid the reactor system of high concentrations of possible inhibitory components. The preleach regime was aimed at creating a more favourable environment for microbial attachment and growth, ultimately resulting in higher colonisation and Cu dissolution rates.

3.2 Evaluation of the effects of the preleach time period on bioleaching performance by redox potential and ferrous iron oxidation measurements

The preliminary experiments showed that by not employing a preleach regime, conditions for efficient microbial growth and Fe^{2+} oxidation were not achieved. Subsequent experiments evaluating different preleach time periods were performed in order to determine the effects of different preleach time periods on the performance of the microbial population as well as on bioleaching performance. The experimental programme is summarised in Table 3.1.

Table 3.1: Description of the column experiments performed where the variable of interest was preleach time period.

Column	Preleach time (days)	*Experimental run
B	0	1
E	4	2
F,R,S	6	2,4
J	33	3

Operating conditions:

Feed [iron^T]:	2 g.l^{-1}
[Inoculation^T]:	10^{11} cells.ton^{-1} of *Acidithiobacillus ferrooxidans* and *A. caldus* and *Leptospirillum ferriphilum* in equal proportions
Whole ore:	Low grade chalcopyrite ore
*Experimental run:	Describe the order in which experiments were carried out

A measured redox potential value higher than that of the irrigation solution would be indicative of significant Fe^{2+} oxidation while a lower value would indicate that the rate of mineral leaching exceeded the rate of biological oxidation.

During the 4 and 6 day preleach time periods a redox potential (387 – 430 mV) lower than the feed redox potential (470 mV) was measured (data not shown). During this time period values measured for Fe^{2+} and Fe^{3+} varied indicating incomplete Fe^{2+} oxidation and thus insignificant microbial activity. For the column reactor preleached for 33 days a net increase in redox potential from 430 - 530 mV was observed during the preleach time period determined by the Fe^{3+}/Fe^{2+} ratio. The preleach solution did not contain Fe, so any Fe in the leachate originated from the ore.

Figure 3.3 and 3.4 shows that during the first 10 days post inoculation, for the 0, 4 and 6 day preleached experiments, a lag phase was present. During this time period the ratios measured for Fe^{3+}/Fe^{2+} (mg.l^{-1}) were ≤1:1 and a constant degree of Fe^{2+} oxidation was observed for all the column reactors excluding the 33 day preleached column reactor (J). For column reactor J, the dissolved Fe^{2+} was rapidly oxidised during the first 24 hours post inoculation, suggesting a degree of microbial activity prior to inoculation. Prior to inoculation the total iron concentration was only ± 50 mg.l^{-1} implying relatively limited mineral leaching.

Following inoculation, the Fe^{2+} in the feed to column reactor J was oxidised at an increased rate, reaching almost complete oxidation (>98% of feed Fe^{2+} oxidised) within 10 days post inoculation.

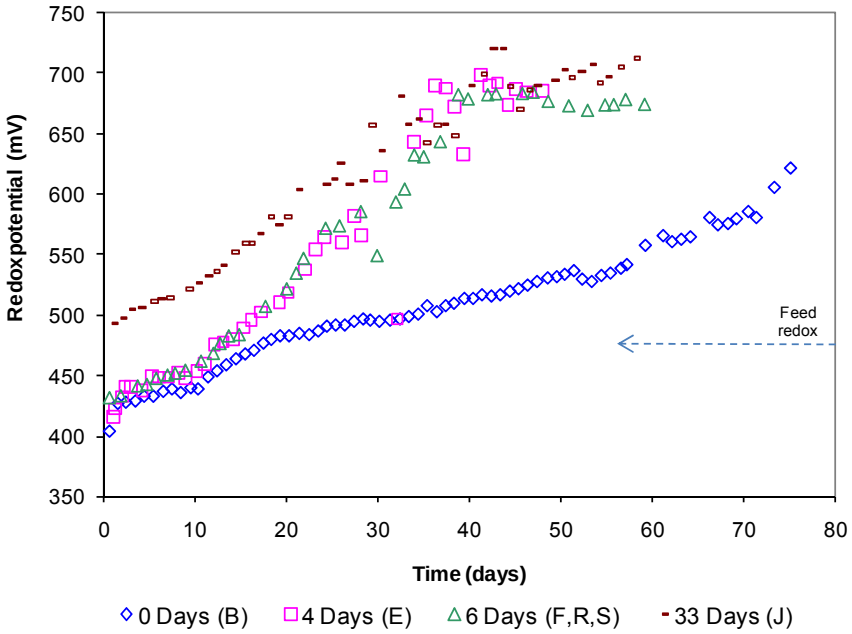


Figure 3.3: Redox potential profiles as a function of preleach time periods. Time zero represents the point of inoculation. Mean values were used for replicate columns (F,R,S)

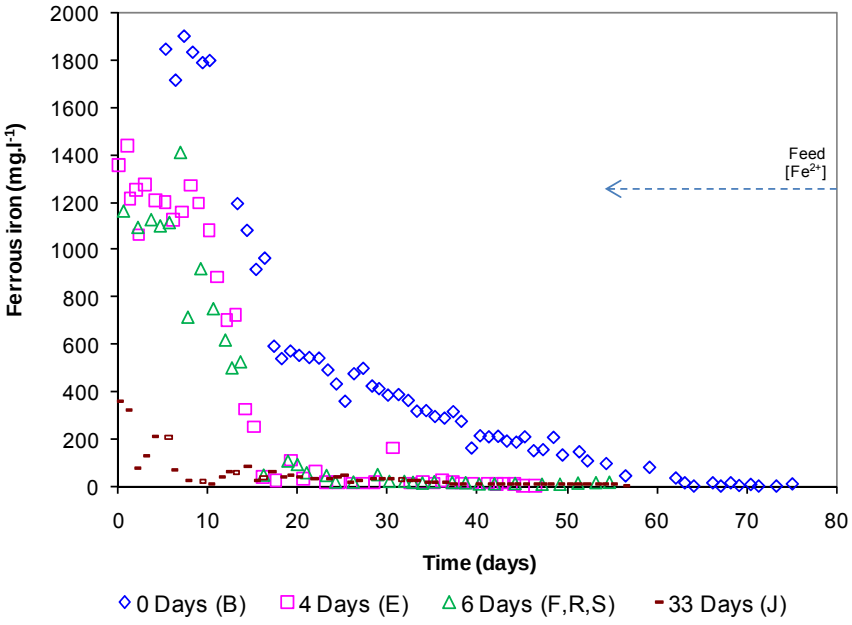


Figure 3.4: Ferrous iron concentration profile for different preleach time periods. Time zero represents the point of inoculation. Mean values were used for replicate columns (F,R,S)

From day 10, for column reactors B, E and FRS, an increase in redox potential was observed with a increase in the Fe^{3+}/Fe^{2+} ratio to >200:1, reaching a maximum redox

potential of >620 mV. The degree of Fe^{2+} oxidation for column reactor B was significantly lower than that for column reactors E and FRS. Reactor B took over three times as long (±63 days) as column reactors E and FRS (±20 days) to achieve complete oxidation of all iron in the feed.

Although the redox potential measured was only 620mV at day 75 for the non-preleached column reactor B compared to 680 mV at day 40 for the preleached column reactors, almost complete (>98%) Fe^{2+} oxidation was already achieved by day 65.

3.3 Effect of a preleach regime on the bioleaching microbial population and the degree of copper liberation

Two microbial phases exist within heap bioleach operations (Rawlings, 2002). The one phase is the planktonic or free living cell population and the other is the attached cell population. During the inoculation procedure microbial cells attach to the ore surface through an electrostatic and hydrophobic mechanism, while continuing to oxidise Fe^{2+} and using the energy for growth, maintenance and the development of adaptation mechanisms (Malik *et al*., 2004). Cells which do not attach to the ore surface through these mechanisms flow through the packed-bed, forming the planktonic population, and are washed out in the effluent. The planktonic population continues to oxidise ferrous iron dissolved in the leach solution. The cumulative number of planktonic cells exiting the columns in the PLS is shown in Figure 3.5.

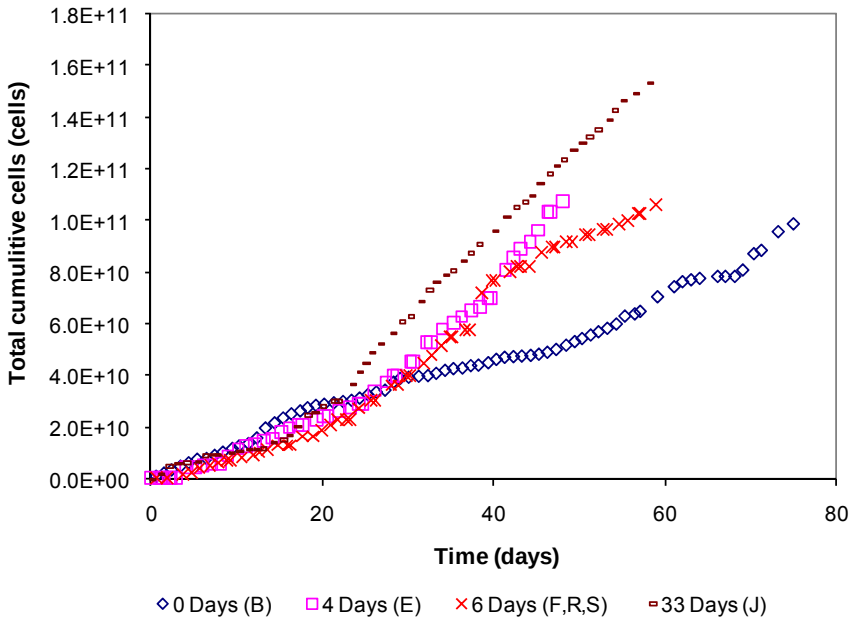


Figure 3.5: Cumulitive planktonic cell number for various preleach time periods. Time zero indicates the point of inoculation

No microbial cells were observed in the PLS for the short-term preleached column reactors during the first four days post inoculation (Figure 3.5). This was not the case for either the 0 or 33 day preleached reactors, where a significant number of cells were observed. Initially a greater proportion of the inoculated cells from column reactor B compared to column reactors E and FRS washed through the packed bed. Cells observed in the PLS after the first week represent growth and it is clear that a significantly lower growth rate was achieved in the non-preleached column reactor compared to the preleached column reactors. The data show that 20 days after inoculation the cumulative cell concentration for the non-preleached column reactor (B) increased at an average rate of 1 x 10^9 cells.day^{-1} compared to the average rate of 2 x 10^9 cells.day^{-1} for the short-term preleached column reactors E and FRS and 3 x 10^9 cells.day^{-1} for the long-term preleached column (reactor J). The data for copper recovery for the different columns is presented in Figure 3.6.

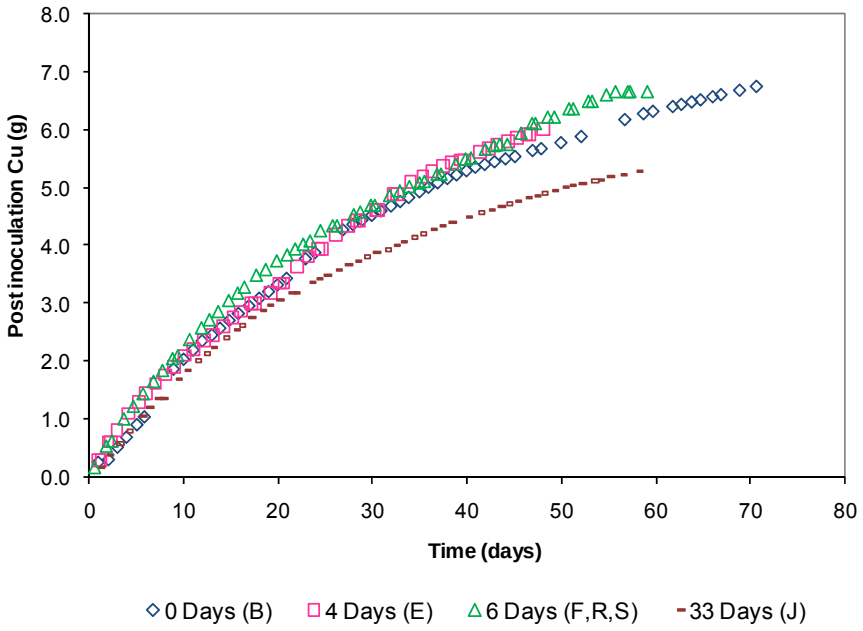


Figure 3.6: Post inoculation cumulative Cu profile for various preleach time period experiments. Liberated Cu during the first four days for the 0 days preleached column reactor was omitted from the figure

The total mass of Cu liberated from the column reactors operated at different preleach time periods did not result in significantly different Cu yields during the inoculated phases. Column reactors B, E and F,R,S (0,4 and 6 day preleach) achieved an almost identical trend where the yield for column reactor J (33 day preleach) was markedly lower, although this could be attributed to more extensive acid leaching of the ore during the preleach.

3.4 Selected elemental composition of the PLS during the preleach regime

The relatively large proportion of gangue compared to sulphide material contained within the low-grade ore, combined with the extended exposure time of the ore to acid during agglomeration can result in the release of harmful concentrations of cations and anions into the heap leach solution once irrigation is started. Microbial Fe^{2+} oxidation could be severely affected if these concentrations exceed toxicity limits, ultimately impacting negatively on the Cu leach rate (Blight and Ralph, 2004; Ojumu *et al*., 2006; Ozkaya *et al*., 2007). The leachate collected during the preleach was analysed for a number of potentially toxic cations by ICP. The data is summarised in Table 3.2.

Table 3.2: Description of selected elemental composition of the preleached PLS

Analyte	Feed	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Conductivity ($mS.cm^{-1}$)	3.23	7.06	5.39	4.20	3.72	3.57	3.68	3.70
Si	<0.02	63	63	60	63	46	50	46
Ca	3.8	242	203	160	156	95	93	95
Mn	<0.02	13	13	9.1	7.3	3.4	3.1	3.4
Ni	<0.02	4.2	2.2	1.5	0.94	0.58	0.59	0.58
Al	<0.02	>100	>100	>100	>100	78	85	78
Mg	1.4	>100	>100	>100	>100	67	65	67
As	<0.02	6.7	4.7	2.8	1.9	0.94	0.74	0.94

*All concentrations reported in $mg.l^{-1}$

Table 3.2 summarises selected elements measured by inductively coupled plasma mass spectrometry (ICP-MS) as well as conductivity. A significant conductivity difference was measured for day one compared to day four and thereafter. The increase conductivity relative to the feed, decreased by 44% by day four compared to day one, and continued to decrease by day seven. The conductivity reached a final value slightly higher than measured for the irrigation solution. Also, a significant difference was measured in the concentrations of the selected elements during day one of the preleach regime compared to day four. The highest concentrations were measured during day one and decreased over time. By day seven significant amounts of the above mentioned cations (Table 3.2) were washed out of the system. Apart from a Ca concentration of 3.8 $mg.l^{-1}$ and Mg of 1.4 $mg.l^{-1}$ measured in the feed solution, all other elements shown in Table 3.2 was measured to be <0.02 $mg.l^{-1}$.

3.5 Discussion

During the preleach phase for the short-term preleached column reactors a decrease in both Fe^{2+} and Fe^{3+} was measured indicating the absence of significant microbial Fe^{2+} oxidation and subsequent mineral leaching. The Fe^{2+} oxidation observed during the last 13 days of the preleach regime for column reactor J (33 day preleach) was likely a result of the

reactivation of the dormant indigenous microbial population. This population contributed to the degree of Fe^{2+} oxidation observed immediately after inoculation.

The change in redox potential and effluent Fe^{2+} from the time of inoculation to column unpacking can be divided into three phases, as seen in Figures 3.3 and 3.4: (i) initial ten day lag phase characterised by very slow increases in redox potential as well as very low Fe^{2+} oxidation rates for reactors B,E and FRS (not observed for reactor J), (ii) increasing redox potential and rate of Fe^{2+} oxidation (post 10 days for reactors B,E and FRS and from day 1 for reactor J) and (iii) maximum redox potential reached with minor fluctuations (reactors E, FRS and J from day 38 onwards where reactor B did not reach this high redox potential) where > 98% of the Fe^{2+} in the feed was oxidised continuously (reactor B – 60 days; reactor E, FRS – 16 days; reactor J – 10 days).

The fact that the inoculum was grown on ore-free, defined media prior to inoculation contributed to the lag phase observed initially. The micro-organisms required time (±10 days) to adapt to the new environment and limited availability of nutrients, particularly the absence of a reduced nitrogen source.

The most significant effect was seen during phase II. The performance of the non-preleached column reactor was significantly different compared to the preleached column reactors. The Fe^{2+} oxidation rate was significantly lower than that for the preleached column reactors. This was likely a result of the combined toxicity caused by components of the gangue minerals leached out during acid agglomeration. In the initial experiments where no preleach was employed the pH of the first sample collected (14h) was in excess of pH 1.4. This was higher than the pH of the feed solution (pH 1.15-1.2) and indicates that acid consumption by the gangue material was significant. This also indicated that the microbial population was not exposed to conditions where the pH was below pH 1.0.

Acute, compound-specific inhibition and toxicity has been shown by several researchers (Tuovinen *et* al., 1971; Chen *et* al., 2004; Blight and Ralph, 2004; Malik *et al*., 2004; Ojumu *et al.*, 2006; *Ozkaya et al*., 2007). Research done by Ozkaya and co-workers showed acute toxicity effects due to a combination of cations ((g/L) Fe^{2+} (20.0), Mn^{2+} (3.0), Mg^{2+} (4.0), Al^{3+} (0.1), Na^{+} (3.6), and Ca^{2+} (0.6)) simulating a heap leach solution. This group used a fluidised bed reactor (FBR) (activated carbon packing) dominated by *Leptospirillum ferriphilum* and found the addition of the above mentioned cations resulted in a 43% decrease in Fe^{2+} oxidation rate, requiring an additional 20 days to reach complete oxidation compared to an identical setup with only Fe^{2+} present (Ozkaya *et al*., 2007). A 65% decrease in Fe^{2+} oxidation rate was also observed during batch assays employing the simulated heap leach solution compared to an identical system with only Fe^{2+} present. Malik and co-workers investigated the effects of different concentrations of Ni^{2+}, Pb^{4+}, Zn^{2+}, Mn^{2+}, Al^{3+} and Si^{4+} on the Fe^{2+} oxidation rate in a batch system. Measurements were taken and compared to the control experiment (exclusion of any cations other than Fe^{2+}) at 40 h run

time. The results showed that increasing the Si^{4+} concentration from 0.096 to 0.21 $g.l^{-1}$ resulted in a four-fold extension of the lag phase as well as incomplete Fe^{2+} oxidation (65%) at the end of 110 h compared to complete oxidation by the control system after only 40 h. The micro-organisms needed to develop various protection systems in order to adapt to the different compounds (Malik *et al*., 2004).

Acid agglomeration of the ore resulted in the dissolution of cations which was measured by conductivity to be highest during day one. A significant decrease in conductivity was measured by day two due to the washout of these cations. A 44% reduction in difference between the conductivity of the feed and elluent was measured by day two and by day four an almost 90% reduction in the conductivity difference was measured. This clearly showed the washout of cations dissolved from the ore. The slight increase in conductivity measured at day seven with respect to the feed was partly caused by the leached Cu.

The concentrations of selected elements measured in the PLS during the preleach were found to be lower than the inhibitory levels reported by Ozkaya and co-workers, with the exception of Fe^{2+} (2.0 $g.l^{-1}_{\text{current study}}$ compared to 20.0 $g.l^{-1}_{\text{Ozkaya study}}$) and Na^{+} (not measured). Exposing the microbial population to these components at the concentrations reported in Table 3.2 would most likely adversely affect the Fe^{2+} oxidation activity and growth of the inoculated micro-organisms. This inhibition would be a result of the combined effect of the components released during acid agglomeration and not due to any one specific component as reported in literature. In addition, the inhospitable environment may have reduced attachment of the inoculated bacteria to the ore, resulting in slower colonisation. This is substantiated by the fact that planktonic cells were seen in the PLS from column B (0 day preleach) in the days following inoculation. The cation concentrations decreased significantly by day four as they were flushed from the interstitial spaces. These values represent the concentrations the inoculum would be exposed to after a four day preleach. The improved performance of column E (4 day preleach) provides evidence of the detrimental effects of these cations on the microbial population.

During phase III, the degree of Fe^{2+} oxidation was higher than 98%, which persisted for the remainder of the experimental run. The non-preleached column reactor required 50% longer to reach the same level of Fe^{2+} oxidation. Due to the larger proportion of the inoculum for column reactor B that washed out initially, a lower number of cells were retained in the column reactor in order to colonise the ore. This lower number of cells required more time compared to the preleach column reactors E (4 day preleach) and FRS (6 day preleach) in order to reach a similar redox potential and degree of Fe^{2+} oxidised.

Measurement of the Fe^{2+} oxidation rates and redox potential are indirect techniques giving insight into microbial activity. Cell counting is a direct technique for measurement of microbial growth. During the initial phase of bioleaching it is desirable to retain a maximum number of microbial cells within the packed-bed in order to enhancing the degree of

colonisation. For the non-preleached column reactor planktonic cells were immediately observed in the PLS following inoculation, which was indicative of a lack in cell attachment. A period of approximately six days was required in the short-term preleached column reactors before detectable microbial cells were observed in the PLS, indicating greater initial cell attachment to the ore followed by significant growth. It must be noted that the detection limit, using the counting chamber, was 3.125×10^5 cells.ml^{-1} so this result does not imply that all inoculated cells attached to the column, but rather that attachment was significantly higher than in the non-preleached column.

According to the Langmuir model an equilibrium exists between the planktonic and attached microbial populations in a mature bioleach system (Konishi *et al*., 1995). During phases II and III an increase in planktonic cell concentration was expected as significant growth took place. A plateau phase, representing constant cell growth, would occur once complete oxidation of the feed was achieved and population growth became limited by Fe^{2+}. The final, cumulative, planktonic cell number achieved by column reactor B was significantly lower than that achieved by both the short- and long term preleached column reactors. This indicated reduced attachment and slower microbial growth and colonisation where a preleach regime was not followed. This correlates with the Fe^{2+} oxidation results. A significantly lower planktonic cell concentration was measured, even after an extended run time for column reactor B. Due to the reactivation of the dormant indigenous population by the time of inoculation of column J (33 day preleach), microbial cells were observed in the PLS in the days following inoculation. This would account for the higher redox potential measured shortly after inoculation of column reactor J. Since identical inoculums and system setups were used, the differences in microbial growth were a result of the preleach regime employed.

Cu recovery was not significantly different for the different preleached column reactors, ranging between 18 and 23% of the head grade. However, a significant portion of the copper was recovered during the first four days and represented proton attack rather than ferric attack. The contribution made by bioleaching would be apparent over a longer time, so iron oxidation was used as a measure of this. For the 4, 6 and 33 day preleached column reactors the cumulative copper leached was calculated to be 15%, 19% and 22%, of the total available copper, during the preleach time periods. The majority of this (>33%) was leached during the first day.

It is clear from the above discussion that the bioleaching micro-organisms were adversely affected, most likely by an elevated concentration of ions originating from solubilisation of gangue minerals during agglomeration, when a preleach was not included. Thus by including a preleach step of at least four days most acid liberated cations and anions were washed out of the packed-bed, effectively creating a more suitable environment for microbial attachment and growth.

3.6 Conclusions

The relatively large quantity of gangue, compared to valuable materials, within a typical heap can result in the release of considerable concentrations of cations and anions into the heap leach solution. The agglomeration process resulted in dissolution of certain gangue minerals, releasing cations and anions which were contained within the moist packed-bed. These were mobilised when irrigation was started and their initial concentrations in the leach solution exceeded toxicity limits for optimal growth and Fe^{2+} oxidation activity of the bioleaching micro-organisms. Where no preleach was employed the initial attachment of the microbial cells to the ore was adversely affected. The differences in lag phases for column reactors B, E, FRS and J suggest that an increase in preleach time was advantageous for microbial growth and Fe^{2+} oxidation activity. In the current study a minimum preleach time of four days was sufficient in order to achieve significant microbial growth and Fe^{2+} oxidation rates in order to leach the available Cu.

Higher Fe^{2+} oxidation and microbial growth rates were measured in the column coated with chalcopyrite concentrate compared to column packed with low-grade ore. This phenomenon was similar to the effect of preleaching and can be attributed to the low gangue content (< 10%) of the $CuFeS_2$ concentrate.

With respect to commercial heap leach operations, water availability could be problematic and it is suggested to use water from other units/operations which can be recycled for the preleach regime.

Chapter 4: Results and discussion II:

The effect of nitrogen supplementation on the performance of chalcopyrite bioleaching

4.1 Introduction

Ensuring optimal microbial activity and growth is imperative in bioleach operations as these micro-organisms are responsible for regenerating the leaching agents (Fe^{3+} and H^{+}). Optimisation of the microbial inoculum and inoculation protocols will only result in tangible benefits if the inoculated population is provided with sufficient nutrients to rapidly colonise the heap. Tuovinen and co-authors (1979) found that the Fe^{2+} oxidation rate of *Acidithiobacillus ferrooxidans* was directly related to the available nitrogen (N) concentration. Nitrogen is assimilated by micro-organisms where it is incorporated into amino-acids and subsequently structural and functional proteins. The energy required to fix atmospheric nitrogen is substantial (Madigan *et al*., 2003). However, if these micro-organisms are provided with already fixed nitrogen, in the form of ammonium or the required amino-acids, the acquired energy could be channelled into reproduction, resulting in a higher growth rate and a shortened lag phase. The absence of nitrogen fixation genes have been reported in some bioleaching strains using bioinformatic tools (Valdes *et al*., 2008). Providing nitrogen in an organic or inorganic form would thus be essential for proliferation of these micro-organisms.

The current study investigates the effect of nitrogen supplementation, in both an inorganic and organic form, on the microbial growth rate and subsequent bioleaching performance. The experimental programme is summarised in Table 4.1.

Table 4.1: The experimental conditions examined are summarised below. The PLS supplementation consisted of inorganic nitrogen at the concentration (50 $mg.l^{-1}$) typically used to supplemented operational heaps

Column	Preleach time (days)	Experimental run	Supplementation
J	33	2	Non-supplemented
O	33	2	YE + $(NH_4)_2SO_4$
P	33	2	YE
S	6	4	Non-supplemented
W	6	4	PLS
X	6	4	PLS + YE

Feed [iron^{I}]: 2 $g.l^{-1}$
[Inoculation^{I}]: 10^{11} $cells.ton^{-1}$ of *Acidithiobacillus ferrooxidans* and *A. caldus* and *Leptospirillum ferriphilum* in equal proportions
YE and $(NH_4)_2SO_4$: 5.16 $g.l^{-1}$ and 4.58 $g.l^{-1}$

4.2 Redox potential and residual Fe^{2+} concentration as a measure of microbial growth and activity

The change in solution redox potential is shown in Figure 4.1. The data clearly shows the shortened lag phase observed for the nitrogen supplemented column reactors, compared to the non-supplemented column reactors regardless of the preleach time period. A 70% reduction in the lag phase was observed when column reactors were supplemented with nitrogen after employing a 6 day preleach regime. This effect was also observed in the column reactors employing a 33 day preleach regime. In these reactors the increase in redox potential was marginally more rapid.

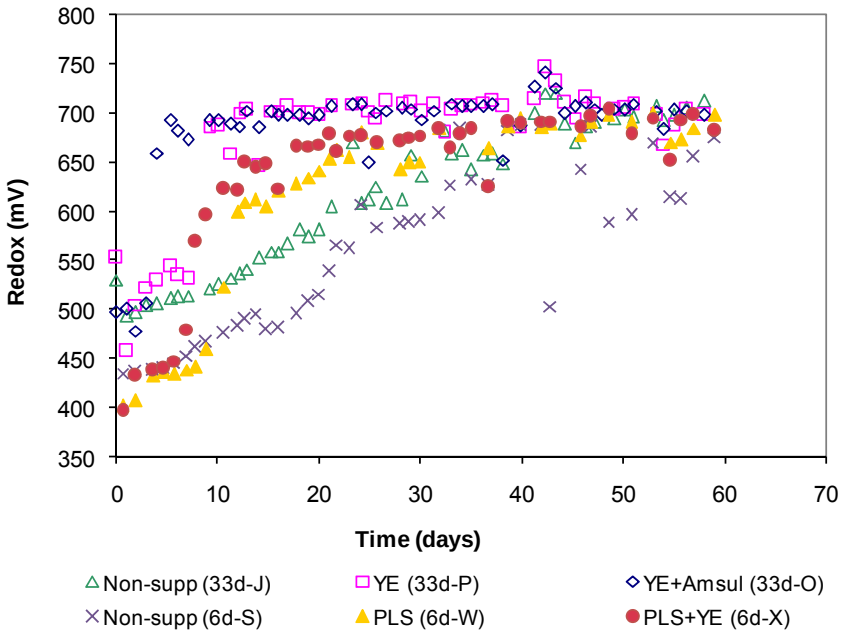


Figure 4.1: Redox profile for chalcopyrite bioleaching where time zero indicates the point of inoculation

The rapid increase in redox potential was observed for both organic and inorganic nitrogen supplementation, although the presence of yeast extract in column W resulted in a more rapid response than inorganic nitrogen alone (column X). The columns that had been preleached for 33 days appeared to show a more rapid response, but this must be interpreted in the light of the higher initial redox potential. For these columns, the response where yeast extract alone was added (P) was slower than when both yeast extract and ammonium sulphate were added. This is consistent with the results of van Hille and co-workers (2009) who showed that *At. ferrooxidans* cultures required an adaptation period before efficient growth on yeast extract as the sole nitrogen source. These sudden increases in redox potential were confirmed through calculation of the Fe^{3+}/Fe^{2+} ratio. The measured Fe^{2+} concentrations are shown in Figure 4.2.

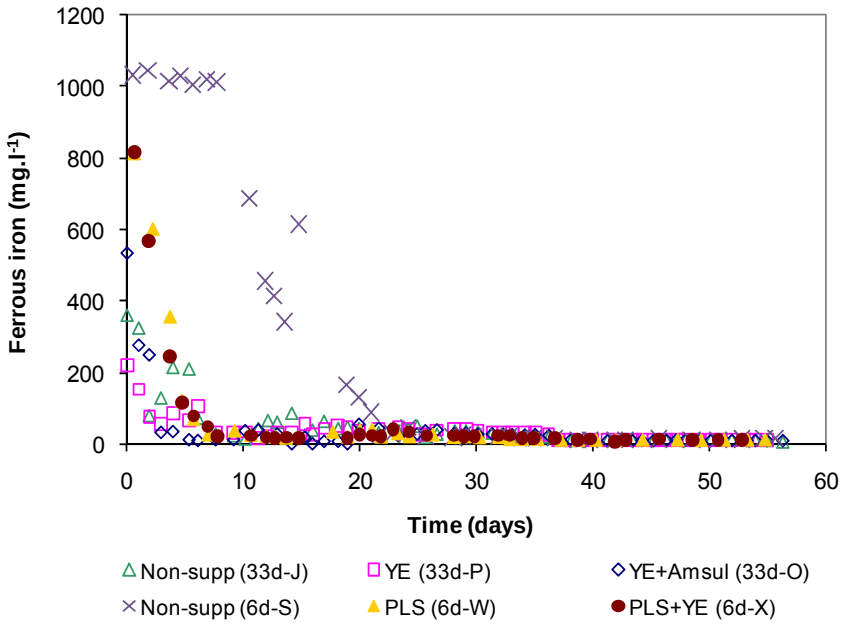


Figure 4.2: Ferrous iron profile illustrating the effect of nitrogen supplementation on the degree of Fe^{2+} oxidation. Feed ferrous concentration of 1 300 $mg.l^{-1}$

Figure 4.2 clearly shows that supplementation of the irrigation solution with organic and/or inorganic nitrogen resulted in immediate iron oxidation, with complete oxidation of the iron in the feed solution achieved by day 7. This implies uninhibited growth and rapid colonisation under these conditions. By comparison, in the absence of nitrogen supplementation a lag phase of approximately 10 days was observed. The inoculum was cultured in nitrogen containing growth media, so this lag could be attributed to the cells having to activate the nitrogen fixing pathway and the subsequent reduction in cell growth rate by nitrogen fixing organisms. This trend was not as obvious in the columns with the extended preleach period, where iron oxidation was more rapid, even in the absence of nitrogen supplementation. This may be attributed to the presence of a small adapted microbial population, as discussed in the previous chapter. These organisms were able to utilise the Fe^{2+} in the feed solution rapidly.

4.3 The effect of nitrogen supplementation on cell growth and heap colonisation

Planktonic cell concentrations in the collected PLS were determined by direct counting on a daily basis. In addition, at the end of the experiment the cells were washed off the ore using an optimised detachment protocol. The cumulative cell numbers recovered from the PLS and the cells washed off the ore are presented in Figures 4.3 and 4.4 respectively.

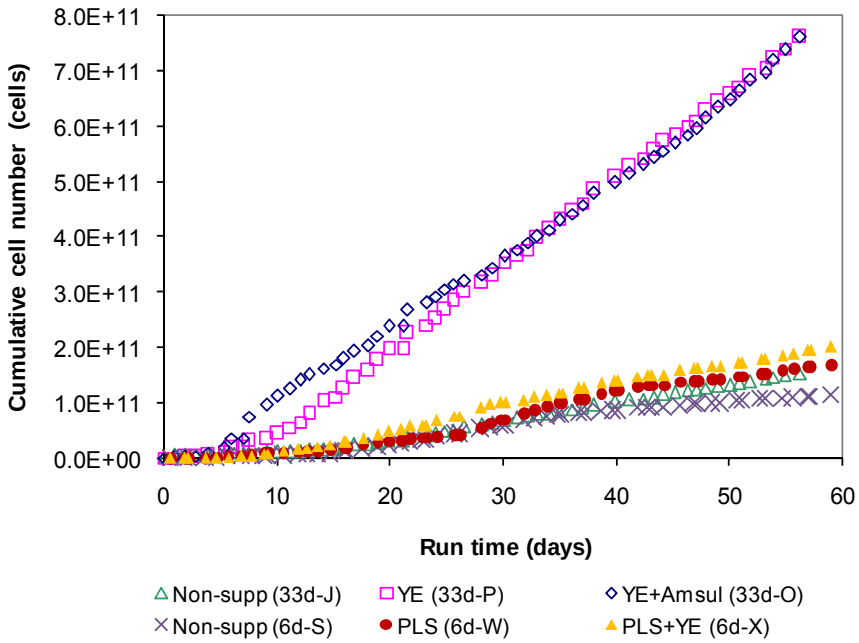


Figure 4.3: Cumulative planktonic cell number leaving the column reactor employing a nitrogen supplementation regime

Figure 4.3 shows a significant increase in planktonic cell number leaving the column in the PLS for all the column reactors supplemented with an organic and/or inorganic nitrogen source compared to the non-supplemented column reactors. The difference is far more significant in the columns preleached for 33 days, where ±7.5 x 10^{11} cells were observed for column reactors P and O, which were supplemented, compared to the 1.6 x 10^{11} cells reached by column reactor J (non-supplemented, 33 days preleached).

A similar effect was observed for the column reactors employing a 6 day preleach time period. Almost twice the number of planktonic cells in the effluent was measured cumulatively for the supplemented column reactors compared to the non-supplemented column reactor.

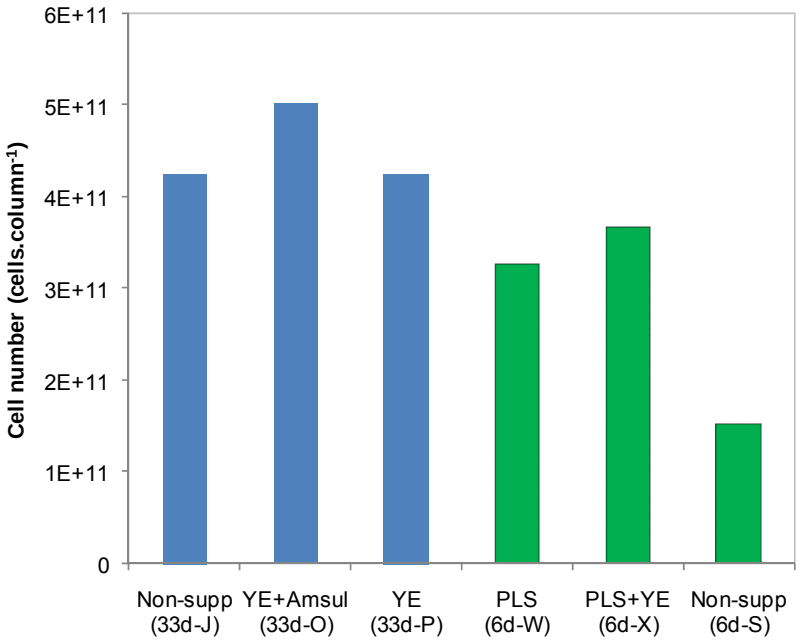


Figure 4.4: The effect of nitrogen supplementation and preleach regime on microbial colonisation in terms of microbial cell number measured in the column at day 60

A number of obvious trends are visible in Figure 4.4. The first is that column reactors supplemented with both an organic and an inorganic nitrogen source achieved a higher degree of colonisation compared to any other column reactors. Secondly, for the reactors operated with the shorter preleach time, nitrogen supplementation resulted in a significantly greater degree of colonisation relative to the non-supplemented reactor. Finally, the columns preleached for 33 days showed a greater degree of colonisation, although the difference was not that substantial relative to the supplemented 6 day preleach columns.

When the data presented in Figures 4.3 and 4.4 are combined a clearer picture emerges. The data presented in the previous chapter points to the establishment of a native population during the 33 day preleach. As the leach solution contained no iron or nitrogen during this phase the microbial growth was slow. However, there was some colonisation of the ore. Upon inoculation and subsequent addition of the iron containing feed, this population became more active. The coupled with the further attachment of cells from the inoculum, resulted in the ore being well colonised shortly after inoculation. As a result, when the nitrogen was added and the growth stimulated (columns O and J), most of the resulting cells passed through the column (Figure 4.3). By contrast, the short preleach phase did not provide sufficient time for the cells introduced with the ore to activate and colonise. Following inoculation and supplementation a greater proportion of the resulting cell growth was retained in the column to achieve a similar level of colonisation and the planktonic cell numbers were lower. As growth was not stimulated in the non-supplemented columns (J and S), the planktonic numbers were low. In the case of S the extent of colonisation was also low.

4.4 Cumulative Cu profiles post inoculation

From previous discussions it is clear that Cu liberation is dependent on a number of factors, with the microbial population playing a key role. Supplementing the microbial population with a nitrogen source resulted in higher microbial growth rates compared to the non-supplemented controls. The higher growth rate resulted in complete Fe^{2+} oxidation for a greater period of time. In addition, the higher cell concentration should enhance EPS which has been implicated in higher Cu liberation (Sand and Gehrke, 2006). The data for copper recovery after inoculation is presented in Figure 4.5.

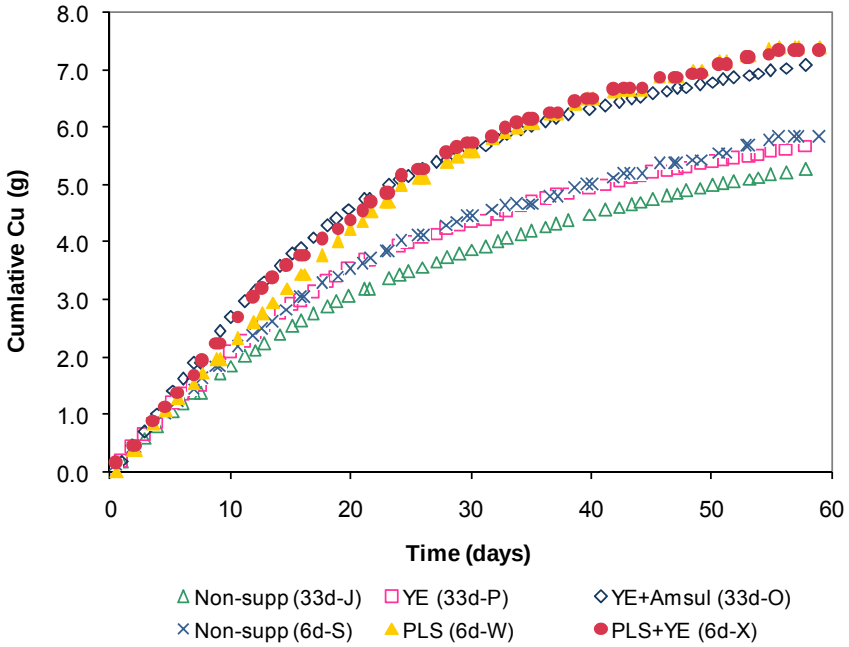


Figure 4.5: Cumulative Cu recovery from columns as a function of nitrogen supplementation regime and preleach time period. Time zero represents the point of inoculation

Figure 4.5 shows that the degree of Cu liberation was significantly enhanced by supplementing the irrigation solution with an organic and/or inorganic nitrogen source, particularly for the shorter preleach times. In most cases the copper recovery during this phase was lower for the columns with the longer preleach, which can be accounted for by the fact that more copper was recovered by acid dissolution during the extended preleach phase. Cu dissolution achieved post inoculation in column reactor O was uncharacteristic compared to similarly run column reactors, but was due to less copper being recovered during the preleach phase. When compared over the entire duration of the experiment the difference between columns P and O was only 0.7 g total Cu.

4.5 Discussion

The data presented above clearly shows the beneficial effects of nitrogen supplementation on the growth of mesophilic bioleaching organisms. Nitrogen is the second most important element, after carbon, required for micro-organisms to proliferate (Rawlings, 2005). Nitrogen is the key component of amino acids, which are the building blocks needed for the synthesis of proteins and nucleic acids required for DNA replication. In the absence of reduced nitrogen, certain organisms can employ an energy intensive process to fix atmospheric nitrogen (Madigan *et al*., 2003). The nitrogen triple bond is extremely stable, with a dissociation energy of 940 $kJ.mol^{-1}$ (Madigan *et al*., 2003). The stoichiometry of the nitrogen fixing reaction is shown below.

$$N_2 + 8e^- + 8H^+ + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$$

There is some uncertainty about the exact number of ATP's required, with numbers varying between 16 and 24 ATP's (±510 to 763.2 KJ/mole ATP) (Madigan *et al*., 2003). To put the energy requirement into perspective, the oxidation of a mole of ferrous iron to ferric by *At. ferrooxidans* yields only 33.9 kJ.

The process is catalysed by the nitrogenase enzyme complex, consisting of two active proteins, a dinitrogenase and a dinitrogenase reductase. Both proteins contain iron, while the latter also contains molybdenum. The electrons required for reduction are transferred to the dinitrogenase reductase from low potential iron-sulphur proteins like ferrodoxin or flavodoxin (Madigan *et al*., 2003). The nitrogenase complex is rapidly and irreversibly inactivated by oxygen, so aerobic organisms which fix nitrogen need to rapidly remove oxygen. In iron oxidising organisms such as *At. ferroo*xidans or *L. ferrooxidans* the reduction of oxygen during iron oxidation may be sufficient to achieve this.

Ammonia is assimilated into organic molecules via reaction leading to glutamate, glutamine and carbamoyl phosphate. The reaction between ammonia and α-ketoglutarate to form glutamate requires NADH, while the conversion of glutamate to glutamine requires energy in the form of ATP (Mathews and van Holde, 1990).

The data presented in Figures 4.1 and 4.2 shows a more rapid increase in iron oxidation and consequently redox potential when the cultures were supplied with reduced nitrogen. In the absence of reduced nitrogen a lag phase of almost 10 days was seen prior to significant iron oxidation being observed. After this time oxidation was slower than in the supplemented columns. This is consistent with a switch in the nitrogen metabolising pathway to nitrogen fixation (Valdés *et al*., 2008). Because of the high energy cost of nitrogen fixation, the genes involved are tightly regulated to prevent energy wastage when reduced nitrogen is available (Madigan *et al*., 2003). Parro and Moreno-Paz (2004) showed that a number of other genes, not directly involved in nitrogen fixation, were upregulated in *L.* ferrooxidans when the

organisms switched to nitrogen fixation. In parallel with regulation of the nif genes is coordination of energy production, development of chemotactic and pumping mechanisms, sensing and signal transduction systems as well as sulphur metabolism. This may also have influenced the lag time observed. The lower iron oxidation rate seen in the non-supplemented columns was due to the lower cell number. This is in agreement with work done using *At. ferrooxidans*, where nitrogen depleted cultures showed an iron oxidation rate almost two thirds lower than nitrogen sufficient cultures with the same starting cell concentration (van Hille *et al.,* 2009).

Figure 4.1 also shows that the column supplemented with only yeast extract (P) had a short lag phase before the fast increase in redox potential. In flask studies with *At. ferrooxidans* it was shown that there is a period of adaptation when the nitrogen source was changed from ammonium sulphate to yeast extract (van Hille *et al*., 2009). During this time limited iron oxidation was seen.

In addition to the iron data, direct measurements of the microbial population numbers were obtained. Planktonic cell counts (Figure 4.3) clearly showed higher growth rates for the supplemented column reactors compared to non-supplemented column reactors. Furthermore, there was a clear difference between supplemented and non-supplemented experiments employing different preleach time periods. Enrichment flask studies (data not shown) performed using the low grade ore confirmed the presence of an indigenous population on the ore, which was not sterilised for this work. These microbes seemed to be in a metabolically dormant state as it took over a week before iron oxidation was seen. The 33 day preleach period was sufficient time for this population to reactivate and begin to grow, although growth would have been slow due to lack of ferrous iron and nitrogen in the preleach solution. At the point of inoculation, after 33 days, the ore would have been reasonably well colonised with an active population which could immediately begin to oxidise the iron in the feed. The addition of the inoculums would have ensured almost complete colonisation of the ore, so most cells resulting from subsequent divisions would not find suitable attachment sites and pass out in the PLS (Figure 4.3). The situation in the 6 day preleach was different as the preleach time was probably too short for activation of the indigenous population before inoculation. The ore needed to be colonised and the inoculated cells would have needed some time to adapt to the new environment. As a result the planktonic cell concentration observed was lower.

The work done by van Hille and co-workers (2009) showed that the yield of *At. ferrooxidans* grown on ferrous iron under nitrogen depleted conditions was only 20-25% that of cells grown in the presence of ammonium. When the culture was adapted to grow on yeast extract as the sole nitrogen source the yield was improved further. This explains the results shown in Figures 4.3 and 4.4. *At. ferrooxidans* could not grow on yeast extract in the absence of Fe^{2+}, so growth was not heterotrophic. However, the improved yield was likely due to the fact that the cells could save the energy and NADH needed to fix ammonia into

amino acids. Microorganisms prefer to utilise pre-formed amino acids rather than synthesising their own (Mathews and van Holde, 2000), so amino acids in the yeast extract would be preferentially assimilated. An alternative explanation could be the presence of NADH in the yeast extract which is imported into the cell, reducing the energy required to reduce NAD^+ prior to carbon fixation.

Some bioleaching microorganisms, for example strains of *Acidithiobacillus caldus* and *Acidithiobacillus thiooxidans*, lack the ability to fix nitrogen from air (Valdés *et al*., 2008). In the absence of reduced nitrogen they depend on the nitrogen fixers to export fixed nitrogen. Supplying nitrogen in a reduced form would be beneficial to these micro-organisms, especially during the start-up phase of the bioleaching process, resulting in a reduced lag phase as well as higher growth rates.

The copper recovery data shows that nitrogen supplementation had a positive effect. This is most likely related to improved colonisation of the ore (Figure 4.4). Several authors have noted the benefit of microbial attachment on mineral solubilisation (Gehrke *et al*., 1998; Fowler and Crundwell, 1999; Kinzler *et al*., 2003; Harneit *et al*., 2005). The enhanced bioleaching is thought to be related to the concentration of ferric irons in the EPS layer, which keeps the microbial biofilm in place. This is thought to create an improved reaction space for the ferric attack of the mineral. The fact that the enhancement of the leaching appears greater for the columns with the 6 day preleach is likely due to greater availability of sulphide minerals. Copper can be leached from chalcopyrite by proton attack so more copper was leached from the columns during the 33 day preleach than the 6 day preleach.

4.6 Conclusions

The data presented in this chapter illustrates the benefit of nitrogen supplementation to cell growth and subsequent metal recovery. Although several of the mesophilic pioneer organisms, such as *At. ferrooxidans* and *L. ferrooxidans,* are able to fix atmospheric nitrogen, the process is very energy intensive. This was shown to result in an extended lag phase prior to efficient iron oxidation. Cultures relying on nitrogen fixation grow more slowly and have a lower yield per mole of iron oxidised. The slower growth resulted in a decrease in the rate of iron oxidation once the culture had become established. The lower growth rate also resulted in slower colonisation of the ore bed, particularly for the shorter preleach.

The study was conducted at two different preleach times and the results differed significantly. The ore was not sterile and the 33 day preleach was long enough to activate the indigenous population, although growth of this population was limited by a lack of ferrous iron and nitrogen in the preleach solution. The columns preleached for 33 days showed a more rapid response to nitrogen supplementation and a greater degree of colonisation at the end of the experiment, largely due to the activation of the indigenous population. However, in terms of operation of a real heap the extended preleach period would be less productive.

The 6 day preleach was sufficient to create an environment suitable for colonisation by the inoculated culture.

Copper recovery was enhanced in the columns supplemented with nitrogen and may be attributed to greater and more rapid colonisation as colonised mineral surfaces have been shown to leach more efficiently.

Total Kjeldahl nitrogen and ammonical nitrogen measurements confirmed the presence of residual nitrogen in the PLS throughout the experimental run where reduced nitrogen was added to the column irrigation solution, although the exact concentrations was not quantified.

Nitrogen supplementation would increase operational costs, but the data suggest that supplementation during the initial colonisation phase would have a significant benefit.

Chapter 5: Results and discussion III:

The effect of soluble iron concentrations on bioleaching performance

5.1 Introduction

Ferric iron is the primary oxidising agent in the chemical leaching of chalcopyrite, resulting in Cu dissolution and Fe^{2+} formation. Fe^{2+} is oxidised by the bioleaching micro-organisms in order to attain energy for growth, subsequently regenerating Fe^{3+}. Rawlings (2002) showed that different bacterial species possess different affinities for Fe^{2+}. This plays an important role in microbial succession and often leads to the dominance of *Leptospirillum* in efficiently operating mesophilic systems. *Leptospirillum* species have a higher affinity for the low Fe^{2+} concentrations available at high redox potentials.

The ferric ion has low solubility, even at acidic pH's, and readily precipitates as ferric hydroxide. In bioleaching, where the sulphate concentration is typically high, jarosite is the primary precipitating compound, forming at even lower pH's than ferric hydroxide. It is therefore important to ensure a liquid phase with a pH < 1.6 in order to maintain a Fe^{3+} concentration above 3-4 $g.l^{-1}$. The low pH would keep iron precipitation to a minimum. A recent study suggests jarosite precipitation is a significant cause of chalcopyrite surface passivation (Córdoba *et al*., 2009).

The dissolution of chalcopyrite in an acid ferric medium is normally described by the following general reactions (Lizama *et al*., 2003):

$$CuFeS_2 + 4Fe^{3+} \rightarrow Cu^{2+} + 5Fe^{2+} + 2S^0 \quad \text{...Eqn 5.1}$$

$$CuFeS_2 + 4Fe^{3+} + 3O_2 + 2H_2O \rightarrow Cu^{2+} + 5Fe^{2+} + 2H_2SO_4 \quad \text{...Eqn 5.2}$$

The actual mechanism is more complex and involves a number of intermediates. The equations suggest that adequate provision of ferric iron is crucial for chalcopyrite leaching and several authors have concluded that the rate is strongly affected by ferric iron concentration at lower concentrations (Kametani and Aoki, 1985). There is a lack of consensus on the threshold value with a range of 0.01-0.1 M (0.55 - 5.5 g/l) reported in literature. The effect has been related to speciation of ferric iron, with the suggestion that the Fe^{3+} and $FeHSO_4^{2+}$ species are responsible for chalcopyrite dissolution in a sulphate medium, while $FeSO_4^{+}$ is not (Konishi *et al,.* 2001). The effect of leaching of secondary

copper sulphides should also be taken into account as the ore used in this study comprised of a significant amount of secondary copper sulphides.

Previous work in a simulated heap reactor has shown a non-linear relationship between dissolved Fe^{3+} concentration and Cu liberation using a whole ore (van Hille *et al*., 2009). The relationship between soluble Fe^{3+} concentration and Cu liberation is therefore the main focus of this study.

The experimental conditions tested in this part of the work are summarised in Table 5.1. The feed solution did not contain a source of reduced nitrogen.

Table 5.1: Description of the experimental variables as discussed during the following section concentrating on the fed iron concentration. A second variable was preleach time period and is discussed accordingly

Column	Preleach time (days)	Experimental run	Feed iron concentration ($g.l^{-1}$)
U	6	4	5
FRS*	6	2,4	2
V	6	4	1
J	33	3	2
M	33	3	0.5
N	33	3	0.2

[Inoculation[1]]: 10^{11} cells.ton^{-1} of *Acidithiobacillus ferrooxidans* and *A. caldus* and *Leptospirillum ferriphilum* in equal proportions
Ore: Low grade chalcopyrite
* represents mean values of three replicates

5.2 Microbial iron oxidation

At low pH values and mesophilic temperatures, chemical oxidation of Fe^{2+} occurs at a negligible rate (Madigan *et al*., 2003). The increase in the concentration of dissolved Fe^{3+} therefore reflects the case where the rate of microbial Fe^{2+} oxidation exceeds ferric reduction during mineral leaching. The solution pH in all columns varied between pH 1.22 and pH 1.36, so no observable precipitation occurred.

The ferric iron concentration and associated redox potential measured in the PLS from the different column reactors are shown in Figure 5.1. From the data presented below it is clear that the preleach time had a significant effect on the performance of the reactors. This has been discussed in Chapter 3 and relates to the activation of a population associated with the ore during the extended preleach. Therefore, the data from the two sets of reactors (open and closed symbols) will be discussed separately. In the columns preleached for 33 days substantial iron oxidation was seen from the first day of feed introduction. The redox potential was above 450mV from the outset, indicating a Fe^{2+} to Fe^{3+} ratio of less than 1. The

ferric iron concentrations were relatively stable throughout at levels slightly higher than the total iron feed concentration indicating some iron leaching and a microbial iron oxidation rate significantly higher than the mineral leaching rate.

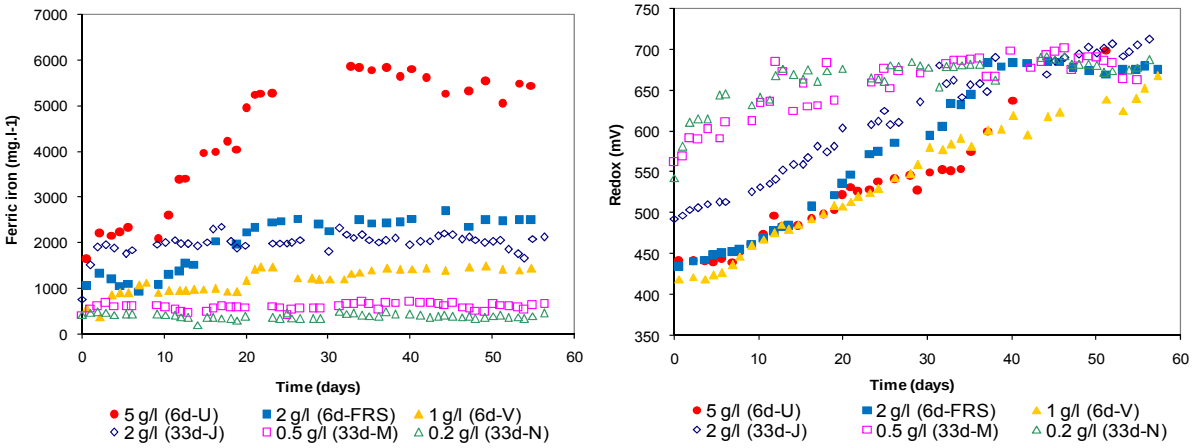


Figure 5.1: Profiles depicting (a) Fe^{3+} concentration and (b) redox potential versus time as a function of different feed iron concentrations, following preleach periods of 6 and 33 days

When the shorter preleach was used a short lag phase was seen, during which time the inoculated organisms were adapting to the environment and colonising the ore. Once microbial activity had been established, the ferric concentration increased rapidly to a stable level slightly higher than the feed concentration. The rate of increase was highest at a feed concentration of 5 $g.l^{-1}$ and lowest at 1 $g.l^{-1}$. This was unexpected, but may be due to a greater proportion of the oxidised iron being reduced during mineral leaching at the lower total iron concentration.

5.3 The effect of iron concentration on copper leaching

The copper recovery profiles for the different columns are shown in Figure 5.2. Only copper recovered after inoculation and addition of iron containing feed is showed. There was significant copper solubilisation during the preleach phase, with an average recovery of 5.7 g for the 33 day preleached columns and 4.9 g for the 6 day preleach. Almost 2 g of this Cu was recovered on the first day and most likely represents copper dissolved during agglomeration. This accounts for some of the difference seen between the 33 and 6 day preleached columns.

The results show a relationship between feed iron concentration and copper recovery, but this is not proportional. For the 33 day preleached reactors similar profiles were obtained for columns M and N (0.5 and 0.2 $g.l^{-1}$). For column J, a 10 fold increase in iron concentration over column N (0.2 – 2.0 $g.l^{-1}$) only resulted in a 23% increase in copper recovery. A similar trend can be seen initially for the columns with the shorter preleach, although the recovery

becomes similar towards the end. This trend becomes clearer when looking at the leach rate data (Figure 5.3). The copper leaching rate for the highest iron concentration in each run (columns U and J) is significantly higher initially, but converges at day 10 for the short preleach and day 16 for the longer preleach. The data points show a pattern of an exponential decay, with the leach rates for the most part converging after day 30, even though there is a 25 fold difference between the lowest and highest iron concentrations.

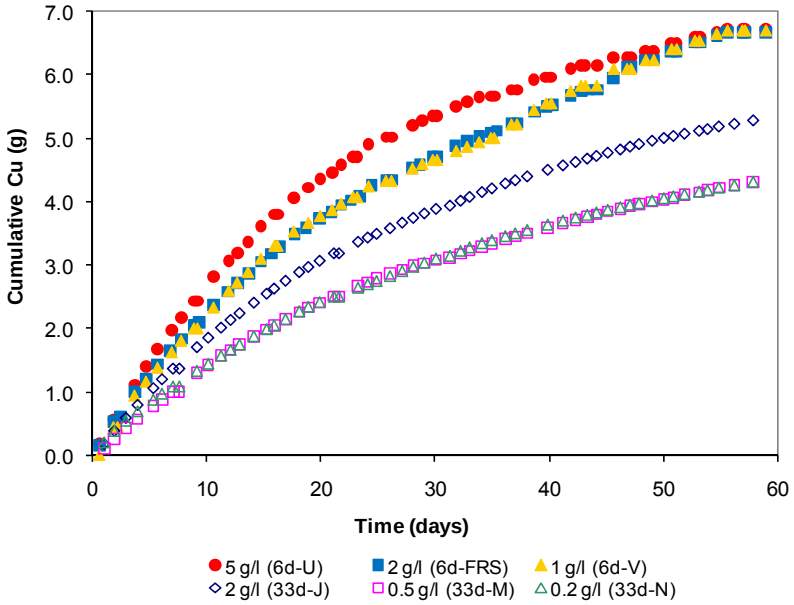


Figure 5.2: Cumulative Cu recovery as a function of feed iron concentration. Time zero indicates the point of inoculation

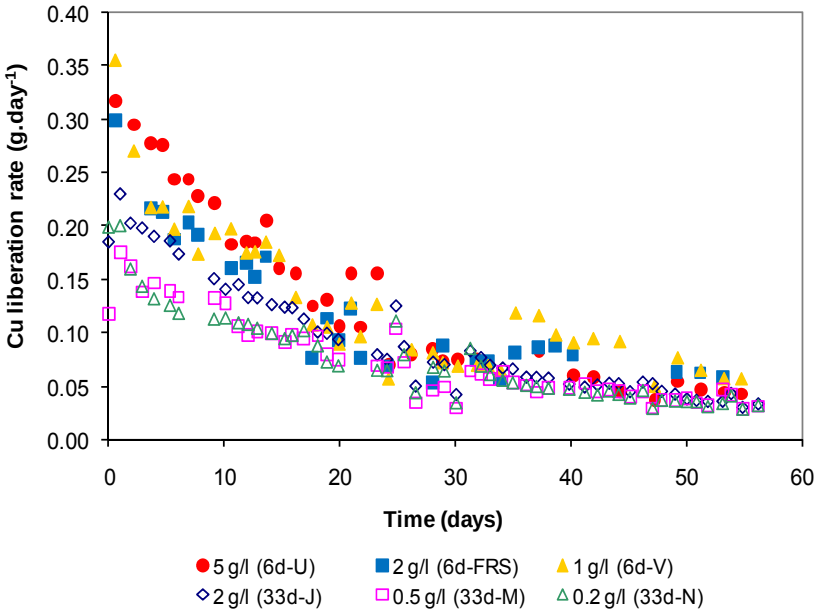


Figure 5.3: Cu leach rate profile as a function of feed iron concentration. Time zero indicates the point of inoculation. Data are presented for preleach periods at 6 and 33 days

Based on the reaction stoichiometry presented in Equations 5.1 and 5.2 and assuming both sufficient mineral surface area and rapid re-oxidation of ferrous iron, a specific copper liberation of 0.25 moles per mole of soluble ferric iron should be achieved. The specific copper liberation data for the column experiments are presented in Figure 5.4.

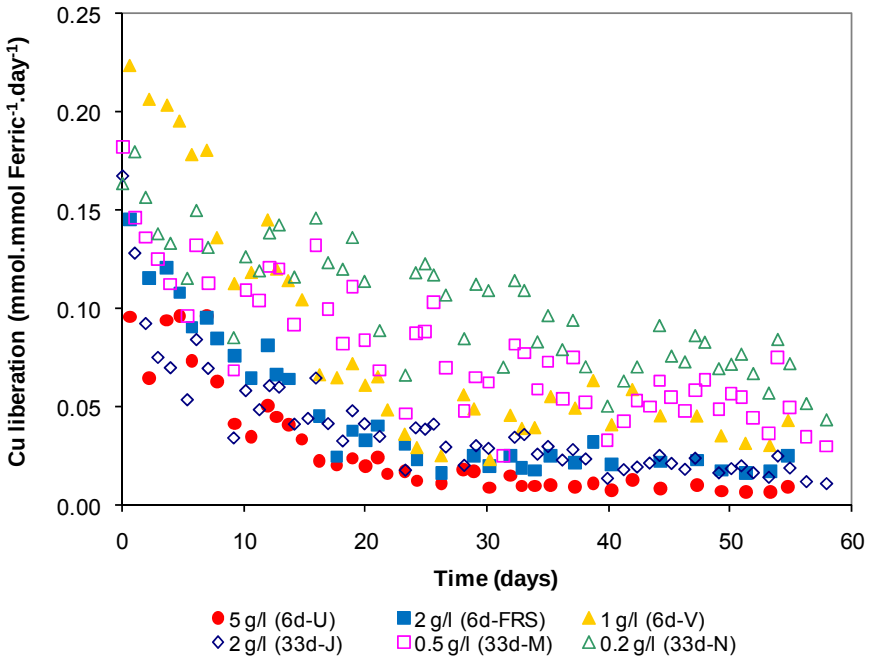


Figure 5.4: Profiles showing the specific Cu liberation rates as a function of dissolved Fe^{3+} concentration. Time zero indicates the point of inoculation. Data are presented for preleach periods of 6 and 33 days

The specific leach rate data shows a similar trend to the leach rate, with higher initial rates followed by a decrease to a stable rate. However, the trend with respect to feed iron concentration is reversed. The highest specific rates are seen for the lowest feed iron concentrations while the column receiving a feed of 5 $g.l^{-1}$ Fe^{T} showed the lowest specific rate. Because the overall leach rates converge and the specific rate is calculated by dividing the overall rate by the iron concentration the specific rates do not converge to a single value, but do reach an almost steady state.

5.4 Effect of iron concentration of microbial growth and colonisation

As Fe^{2+} is the source of energy for microbial growth, it was expected that with an increased Fe^{2+} concentration a higher microbial cell number would be achieved at the mature state.

A higher planktonic cell number was observed in the PLS for column reactors employing higher Fe feed concentrations for both the 33 and 6 day preleach times. The planktonic cell counts were higher for the column reactors employing the longer preleach time period (data not shown).

The detached cell numbers measured 60 days after inoculation for the column reactors employing a 33 day preleach time period were higher compared to the 6 day preleach time period. This is consistent with previous results and can be attributed to the activation of the indigenous population. However, no observable trends existed for the column reactors employing different feed Fe concentrations where detached cell numbers were similar for all column reactors employing a specific preleach time period.

5.5 Discussion

The ferric iron and redox potential data showed that the columns which had the longer preleach phase became active more quickly. This is due to the activation of the indigenous population, as has been discussed in Chapter 3. The shorter time required to reach a stable redox potential in the columns fed a lower total iron concentration was due to the fact that less Fe^{2+} needed to be oxidised in order to achieve a significant difference in the Fe^{3+}/Fe^{2+} ratio. During the stable state Fe^{2+} measurements were constantly below 5 $mg.l^{-1}$. The linear change in redox potential after >98% Fe^{2+} was oxidised (post day 25 for reactor FRS), was as a result of minor changes in the Fe^{3+}/Fe^{2+} ratio resulting in major redox potential changes. This occurs due to the non-linear correlation between redox potential and the Fe^{3+}/Fe^{2+} ratio. A stable redox potential state was reached at approximately 680 mV, indicating that the rate of microbial iron oxidation was significantly faster than reduction of Fe^{3+} by mineral leaching.

The stable total iron concentrations, as well as the fact that these values were slightly higher than those of the irrigation solution indicate insignificant iron precipitation. The most likely reason for this is the low pH (< pH 1.4) of the liquid in the columns.

Overall copper recovery was relatively low, but this is consistent with previous research on chalcopyrite bioleaching in the mesophilic temperature range (Córdoba *et al*., 2008b, Sasaki *et al*. 2009). The initial rate of copper leaching at 5 $g.l^{-1}$ iron is double that of 0.2 $g.l^{-1}$, but as the experiment progressed the rates for all columns converged to approximately 0.06 $g.day^{-1}$ after 30 days. This suggests that as the leach progresses, the process becomes limited by something other than ferric iron concentration in solution. Colonisation of mineral surfaces has been shown to be due to extracellular polymeric substances (EPS). In addition, the EPS layer has been shown to complex ferric ions from solution which aids attachment (Kinzler *et al*., 2003), but has also been implicated in accelerating mineral dissolution by creating a reaction space close to the mineral surface (Sand and Gehrke, 2006). This could allow the maintenance of a sufficient ferric iron concentration near the mineral surface even when solution concentrations are low, but does not account for the reduced specific rate of leaching at high ferric concentrations seen in these columns.

The iron concentration data shows that jarosite precipitation did not occur in these experiments. This suggests that if the reduced leach rate at high ferric concentrations is

caused by passivation, it is not because of jarosite precipitation. This result differs from that of Córdoba and co-workers (2008b), who showed reduced leach rates at 5 $g.l^{-1}$ Fe when compared to 0.5 $g.l^{-1}$ and attributed this to jarosite precipitation. The difference is most likely because of the higher initial pH (1.8) used in their study. In this work a more likely explanation is the reduction of available sulphide mineral surface area. This suggests that after some 25 days during which the system became colonised and initial leaching occurred, the reaction rate was controlled by mineral sulphide availability, not ferric iron concentration in the leaching of low grade ores.

The theoretical value calculated for specific Cu liberation, based on stoichiometry, was not seen during the experimental runs, regardless of the feed Fe concentration. The lowest specific rate was achieved for the highest feed Fe concentration and vice versa. Cu liberation was found to be a function of the dissolved Fe^{3+} concentration during the initial phases of bioleaching although not the extent in which the feed Fe concentrations differed. As bioleaching progressed, a convergence of the Cu liberation profiles were observed employing the 33 day preleach time period. This, together with the stable, high redox potential and the fact that the copper leach rate reached a constant value independent of feed iron concentration indicates that ferric iron was not the limiting reagent. This further supports the idea that, after an initial phase where leaching may be rapid, it becomes rate limited by the accessibility of the chalcopyrite mineral, particularly in the case of finely disseminated or brachiated chalcopyrite, such as this ore. During the initial phase of leaching the small mineral grains exposed at the surface especially that of secondary copper sulphides, are leached rapidly. After this, new mineral surfaces need to be exposed, either by dissolving gangue or penetration of the leach solution into the mineral grains, which may be limited by diffusion. During the initial phase, a higher concentration of ferric iron may enhance leaching rates after which similar rates of copper sulphide leaching could be maintained at greatly reduced iron concentrations. The results obtained correlated well with those obtained by other researchers, where additional Fe^{3+} was found only to enhance the initial leach rates but not the final leach yields (Vilcáez *et al*., 2008).

Konishi *et al.* (2001) performed bioleaching experiments employing CSTR's and found that an increase in the Fe^{3+} concentration from 0.73 to 1.46 $g.l^{-1}$ led to a negligible rate increase in copper sulphide bioleaching.

The relationship between the planktonic microbial population and the feed Fe concentration was due to the availability of a higher Fe^{2+} concentration, able to sustain a larger microbial population. As discussed in Chapter 3, the longer preleach period allowed the re-activation of the dormant indigenous population on the ore. This allowed more rapid colonisation and resulted in the planktonic cell number being higher in these columns.

5.6 Conclusions

The Fe^{2+} included in the feed solution played two important roles. The first was to provide energy for microbial growth. This was confirmed by the fact that cell concentrations in the PLS showed a positive relationship with increased feed concentration. The second role was to provide a source of Fe^{3+}, after microbial oxidation, in order to leach Cu from the ore. The data showed that an increased iron concentration did enhance copper recovery initially, but not in proportion to the iron concentration. After a relatively short time copper leach rates converged to a stable value of around 0.06 $g.day^{-1}$ irrespective of feed iron concentration. This leads to the conclusion that after the initial phase copper leaching is limited by the availability of exposed mineral rather than ferric iron. The implication for heap leaching operations is that while iron concentrations may be better for copper recovery in the short term over the longer term efficient copper leaching can be achieved with ferric concentrations below 0.5 $g.l^{-1}$. This could reduce the amount of iron or acid needed to keep the iron in solution and the amount of jarosite precipitation. Jarosite precipitation can affect solution flow in the heap and reduce copper recovery because of copper adsorption to the jarosite.

Chapter 6: General Conclusions and Recommendations

6.1 General Conclusions

Heap bioleaching, particularly for the recovery of copper from low-grade ores, is becoming an increasingly important alternative to conventional mineral processing technologies. While a number of heap leaching operations have been operated successfully at an industrial scale the phase of process, from the point of inoculation to the point where a stable redox potential is reached, is still not predictable. This study was carried out in order to gain insight into the initial bioleaching phase by investigating selected parameters significantly influencing bioleaching performance during this stage. The selected parameters included preleach time period, nitrogen supplementation and feed iron concentration.

Acid agglomeration of the ore resulted in the dissolution of gangue minerals, releasing cations and anions. This was indicated by conductivity measurements, with the highest values measured on the first day of irrigation. By day four the difference in conductivity between the leach solution and the feed had decreased by 88%. This decrease in dissolved species was confirmed by ICP analysis, where a reduction in cation concentration in the PLS was measured. The effect of the elevated dissolved solids on the inoculated microbial community was three-fold: i) higher cell washout immediately following inoculation indicated a lack in initial attachment of the micro-organisms to the ore, ii) a significant lag in microbial ferrous iron oxidation occurred, and iii) due to the reduced attachment colonisation was less extensive and occurred more slowly, ultimately impacting negatively on the leaching performance.

A four day preleach was sufficient to lead to a significant increase in colonisation rate, degree of ferrous iron oxidation and microbial growth compared to the non-preleached columns. The 33 day preleach time period was sufficiently long for the indigenous microbial community to become reactivated, although their growth was limited by the availability of ferrous iron during this time. Following irrigation of the columns with leach solution containing ferrous iron, rapid growth of the already adapted microbial community occurred contributing to the high colonisation rate as well as degree of ferrous iron oxidation.

The addition of a reduced organic and/or inorganic nitrogen source into the feed solution to the packed columns resulted in a significantly reduced lag period and was accounted for in two ways: i) supplying the micro-organisms with a reduced nitrogen source enabled these micro-organisms to channel the energy otherwise expended on nitrogen fixation to growth and ii) enabling the immediate growth of bacterial species lacking nitrogen fixation genes, for example *Acidithiobacillus caldus* and *Leptospirillum ferriphilum*. In the absence of a reduced nitrogen source a significantly lower degree of microbial colonisation, ferrous iron oxidation and microbial proliferation was observed. This suggests that short-term nitrogen

supplementation may have a practical benefit in reducing the lag phase and promoting microbial colonisation, which would ultimately improve bioleaching performance.

This study showed that a higher concentration of dissolved ferric iron was initially beneficial, resulting in a more rapid copper liberation compared to the lower iron feed concentration, although this relationship was not proportional. However, after a relatively short time (25-30 days in these tests) the copper leach rates converged and the overall degree of copper liberation was ultimately limited by accessibility of the sulphide mineral. Following this initial stage, the leach rate was little affected by Fe^{3+} in solution and the maximum specific leach rate was found at the lowest iron concentration studied (0.2 $g.l^{-1}$). Hence, after the fairly short initial stage it may be beneficial to operate at a lower (0.2 $g.l^{-1}$) soluble iron concentration in order to minimise jarosite precipitation. Jarosite coating has been implicated as a cause of chalcopyrite passivation and is also capable of adsorbing or entraining copper ions, reducing the recovery of leached mineral.

6.2 Recommendations

Based on the findings reported in this study it is recommended that the following three areas of research are to be conducted to further investigate the research questions addressed in this thesis.

1. By exposing different pure and mixed bioleaching microbial populations to a synthetic solution of similar solution chemistry to the leach solution characterised during the preleach phase, further insight into the impact of these ions on microbial activity could be determined. This research could furthermore identify species specific stress responses and provide conclusive evidence that it was the ions mobilised during agglomeration contributed to the observed inhibition of microbial activity.

2. Investigation of microbial ecology during the initial phase of bioleaching in the presence and absence of reduced nitrogen compounds in order to determine the effect on bioleaching performance. This analysis would provide information on whether nitrogen supplementation preferentially enhances the growth of particular species or the inoculum as a whole as well as indicate long term effects of supplementation on bioleaching performance. If a shift in population dominance occurs, at what point in time does this occur in the presence of reduced nitrogen?

3. Extension of the timeframe of the simulated heap reactors with different irrigation iron concentrations in order to determine the effect of feed iron concentration on copper recovery over a longer time period. Of particular interest would be if total copper recovery converges, despite the initial leaching rate being higher at higher ferric concentrations. Particularly, this should be coupled with iron precipitation studies.

References

Acevedo, F., 2000. The use of reactors in biomining processes. *Electronic Journal of Biotechnology* **8**: 184-194

Acevedo, F., 2002. Present and future of bioleaching in developing countries. *Electronic Journal of Biotechnology* **33**: 197-199

Africa, C.-J., van Hille, R.P. and Harrison, S.T.L., 2009. Investigation and visualisation of microbial attachment trends to sulphide minerals in a bioleach environment. *Advanced Materials Research* **71-73**: 345-348

Barron, J.L. and Lueching, D.R., 1990. Growth and maintenance of *Thiobacillus ferrooxidans* cells. *Applied environmental microbiology* **56**: 2801-2806

Bharathi, K., Bhagvanth, R.M., Lakshmi, N.M. and Ravindra, P., 2004. Effect of biochemical reactions in enhancement of rate of leaching. *Chemical Engineering Science* **59**: 5069-5073

Blight, K.R. and Ralph, D.E., 2004. Effect of ionic strength on iron oxidation with batch cultures of chemolithotrophic bacteria. *Hydrometallurgy* **73**: 325-334

Bouffard, S. C. and Dixon, D. G., 2002. On the rate-limiting steps of pyritic refractory gold ore heap leaching: Results from small and large column tests. *Minerals Engineering* **15**: 859-870

Brierley, C.L., 2001. Bacterial succession in bioheap leaching. *Hydrometallurgy* **59**: 249-256.

Brierley, J.A. and Brierley, C.L., 1999. Present and future commercial applications of biohydrometallurgy. In: Amils R, Ballester A (eds) Biohydrometallurgy and the environment toward the mining of the 21st century, **IBS99**. Elsevier, Amsterdam, 81–89

Bustos, S., Castro, S. and Montealegre, S., 1993. The Sociedad Mineral Pudahuel bacterial thin-layer leaching process at Lo Aguirre. *FEMS Microbology Reviews* **11**: 231–236

Chen, B.-Y., Liu, H.-L., Chen, Y.-W. and Cheng, Y.-C., 2004. Dose-response assessment of metal toxicity upon indigenous *Thiobacillus thiooxidans* BC1. *Process Biochemistry* **39**: 735-745

Coram, N.J. and Rawlings, D.E., 2002. Molecular Relationship between Two Groups of the Genus *Leptospirillum* and the Finding that *Leptospirillum ferriphilum* sp. nov. Dominates South African Commercial Biooxidation Tanks That Operate at 40°C. *Applied and Environmental Microbiology* **68**: 838–845

Córdoba, E.M., Muñoz, J.A., Blázquez, M.L., González, F. and Ballester, A., 2008a. Leaching of chalcopyrite with ferric ion. Part II: Effect of redox potential. *Hydrometallurgy* **93**: 88-96

Córdoba, E.M., Munõz, J.A., Blázquez, M.L., González, F. and Ballester, A., 2008b. Leaching of chalcopyrite with ferric iron. Part IV: The role of redox potential in the presence of mesophilic and thermophilic bacteria. *Hydrometallurgy*. **93**: 106-115

Córdoba, E.M., Muñoz, J.A., Blàzquez, M.L., Gonzàlez, F. and Ballester, A., 2009. Passivation of chalcopyrite during its chemical leaching with ferric ion at 68 °C *Minerals Engineering* **22**: 229-235

Crundwell, F.K., 1997. The kinetics of the chemiosmotic proton circuit of the iron-oxidizing bacterium *Thiobacillus ferrooxidans.* Bioelectrochemistry and Bioenergetics **43**: 115-122

Cruz, A., Luna-Sánchez, R.M., Lapidus, G.T., González, I. and Monroy, M., 2005. An experimental strategy to determine galvanic interactions affecting the reactivity of sulfide mineral concentrates. *Hydrometallurgy* **78**: 198– 208

Davidson, M.S., 1982. The effects of simulated deep solution mining conditions on the activity of iron and sulfur oxidizing bacteria. PhD Thesis, New Mexico Institute *of Mining and Technology*

Dreisinger, D., 2006. Copper leaching from primary sulfides: Options for biological and chemical extraction of copper. *Hydrometallurgy* **83**: 10-20

Drouet, C. and Navrotsky, A., 2003. Synthesis, characterization, and thermochemistry of K-Na-H3O jarosites. *Geochimica et Cosmochimica Acta* **67**:**11**: 2063-2076

Eaton, A. D., Clesceri, L. S. and Greenberg, A. E., 1998. *Standard Methods for the Examination of Water and Wastewater*; Eds.; American Public Health Association: Washington, DC.

Escobar, B., Jedlicki, E., Wiertz, J. and Vargas, T., 1996. A method for evaluating the proportion of free and attached bacteria in the bioleaching of chalcopyrite with *Thiobacillus ferrooxidans*. *Hydrometallurgy* **40**: 1-10

Fowler, T.A. and Crundwell, F.K., 1999. Leaching of zinc sulfide by Thiobacillus ferrooxidans: bacterial oxidation of the sulfur product layer increases the rate of zinc sulfide dissolution at high concentrations of ferrous ions. *Applied Environmental Microbiology* **65**: 5285– 5292

Gehrke, T., Telegdi, J., Thierry, D. and Sand, W., 1998. Importance of Extracellular Polymeric Substances from *Thiobacillus ferrooxidans* for Bioleaching. *Applied Environmental microbiology* **64(7)**: 2743-2747

Harahuc, L., Lizama, H.M. and Suzuki, I., 2000. Selective inhibition of the oxidation of ferrous iron or sulfur in *Thiobacillus ferrooxidans. Applied Environmental Microbiology* **66**: 1031–1037

Harneit, K., Goksel, A., Kock, D., Klock, J.H., Gehrke, T. And Sand, W., In: Proceeding of the 16th International Biohydrometallurgy Symposium editors Harrison, S.T.L., Rawlings, D.E. and Petersen, J., 2005. Cape Town, South Africa

Hiroyoshi, N., Kuroiwa, S., Miki, H., Tsunekawa, M. and Hirajima, T., 2007. Effects of coexisting metal ions on the redox potential dependence of chalcopyrite leaching in sulfuric acid solutions. *Hydrometallurgy* **87**: 1-10

Holuigue, L., Herrera, L., Philips, O.M., Young, M. and Allende, J.E., 1987. CO_2 fixation by mineral-leaching bacteria: characteristics of the ribulose biphosphate carboxylase-oxygenase of *Thiobacillus ferrooxidans*. *Biotechnology and applied biochemistry* **9**: 497-505

Ingledew, W.J., 1982. *Thiobacillus ferrooxidans*-The bioenergetics of an acidophilic chemolithotroph, *Biochimica et Biophysica* Acta **683**: 89-97

Kametani, H. and Aoki, A., 1985. Effect of suspension potential on the oxidation rate of copper concentration in sulphuric acid conditions. *Metallurgical Transaction* **16B**: 695–705

Kinnunen, P.H.M., 2004. High-rate ferric sulfate generation and chalcopyrite concentrate leaching by Acidophilic microorganisms. PhD Thesis June 2004 Tampere University of Technology ISBN 952-15-1190-7, p 1-55

Kinnunen, P.H.M. and Puhakka, J.A., 2005. High-rate iron oxidation at below pH 1 and at elevated iron and copper concentrations by a *Leptospirillum ferriphilum* dominated biofilm. *Process Biochemistry* **40**: 3536-3541

Kinzler, K., Gehrke, A.T., Telegdib, J. and Sanda, W., 2003. Bioleaching—A result of interfacial processes caused by extracellular polymeric substances (EPS). *Hydrometallurgy* **71**: 83-88

Konishi, Y., Asai, S. and Yoshida, N., 1995. Kinetics of *Thiobacillus thiooxidans* on the Surface of elemental sulphur. *Applied and environmental microbiology* **61**: 3617-3622

Konishi, Y., Tokushige, M., Asai, S. And Suzuki, T., 2001. Copper recovery from chalcopyrite concentrate by acidophilic thermophile *Acidianus brierleyi* in batch and continuous-flow stirred tank reactors. Hydrometallurgy **59**: 271-282

Liu, M.S., Branion, R.M.R. and Duncan, D.W., 1988. The effects of ferrous iron, dissolved oxygen, and enert solids concentrations on the growth of *Thiobacullus ferrooxidans*. *The Canadian Journal of Chemical Engineering* **66**: 445-451

Lizama, H.M., Fairweather, M.J., Dai, Z. and Allegretto, T.D., 2003. How does bioleaching start? *Hydrometallurgy* **69**: 109-116

Lopez-Archilla, A.I., Gerard, E., Moreira, D. and Lopez-Garcia, P., 2004. Macrofilamentous microbial communities in the metal rich and acidic River Tinto, Spain. *Microbiology Letters* **235**: 221-228

Lorenz, P. and Schlepe, C., 2002. Metagenome — a challenging source of enzyme discovery. *Journal of Molecular Catalysis B: Enzymatic* 19–20 and 13–19

Madigan, M.T., Martinko, J.M. and Parker, J. 2003. Brock Biology of Microorganisms (International edition). Pearson education Inc., Upper Saddle River, New Jersey, 07458, pp 87-90; 151-152; 565-590; 606-613; 669-674

Mahmouda, K.K., Leducb, L.G. and Ferroni, G.D., 2005. Detection of *Acidithiobacillus ferrooxidans* in acid mine drainage environments using fluorescent in situ hybridization (FISH). *Journal of Microbiological Methods* **61**: 33–45

Malacinski, G.M., 2002. Essentials of molecular biology 4th ed. Jones and Bartlett publishers, Sudbury, Massachusetts, **15**: 355-366

Malik, A., Dastidar, M.G. and Roychoudhury, P.K., 2004. Factors limiting bacterial iron oxidation in biodesulphurization system. *International Journal of Mineral Processing* **73**: 13-21

Mathews, C.K. and Holde, K.E., 1990. Biochemistry. The Benjamin/Cummings Publishing Company, 390 Bridge Parkway, Redwood City, CA, 94065, pp 670-682

Mazuelos, A., Palencia, I., Romero, R., Rodriquez, G. and Carranza, F., 2001. Ferric iron production in packed bed bioreactors: influence of pH, temperature, particle size, bacterial support material and type of air distributor. *Minerals Engineering* **14**: 507-514

Mousavi, S.M., Jafari, A., Yaghmaei, S., Vossoughi, M. and Sarkomaa, P., 2006. Computer simulation of fluid motion in a porous bed using a volume of fluid method: Application in heap leaching. *Minerals Engineering* **19**: 1077–1083

Muñoz, J.A., Dreisinger, D.B., Cooper, W.C. and Young, S.K., 2007.Silver-catalyzed bioleaching of low-grade copper ores. Part II: Stirred tank tests. *Hydrometallurgy* **88**: 19–34

Nagpal, S., 1996. A structured model for *Thiobacillus ferrooxidans* growh on ferrous iron. *Biotechnology and Bioengineering* **63**: 310-319

Nemati, M., Harrison, S.T.L., Hansford, G.S., and Webb, C., 1998. Biological oxidation of ferrous sulphate by *Thiobacillus ferrooxidans*: a review on the kinetic aspects. *Biochemical Engineering Journal* **I**: 171-190

Norris, P. R., Barr, D.W. and Hinson, D., 1988. Iron and mineral oxidation by acidophilic bacteria: affinities for iron and attachment to pyrite. *Proceedings of the International Symposium Science and Technology Letters.* 43–59

Ojumu, T.V., Petersen, J., Searby, G.S. and Hansford, G.S., 2006. A review of rate equations proposed for microbial ferrous-iron oxidation with a view to application to heap bioleaching. *Hydrometallurgy* **83**: 21-28

Olson, G.J., Brierley, J.A. and Brierley, C.L., 2003. Bioleaching review part B: Progress in bioleaching: applications of the microbial processes by the mineral industries. *Applied Microbiological Biotechnology* **63**: 249-257

Ozkaya, B., Sahinkaya, E., Nurmi, P., Kaksonen, A.H. and Puhakka, A., 2007. Iron oxidation and precipitation in a simulated heap leaching solution in a *Leptospirillum ferriphilum* dominated biofilm reactor. *Hydrometallurgy* **88**: 67-74

Parro, V. and Moreno-paz, M., 2004. Nitrogen fixation in acidophile iron oxidizing bacteria: The *nif* regulon of *Leptospirillum ferrooxidans*. *Research in Microbiology* **155**: 703-709

Petersen, J. and Dixon, D.G., 2006. Competitive bioleaching of pyrite and chalcopyrite. *Hydrometallurgy* **83**: 40-49

Pizarro, J., Jedlicki, E., Orellana, O., Romero, J. and Espejo, R.T., 1996. Bacterial populations in samples of bioleached copper ore as revealed by analysis of DNA obtained before and after cultivation. *Applied Environmental Microbiology* **62**: 1323-1328

Qingyou, L., Heping, L. And Li, Z., 2006. Galvanic interactions between metal sulphide minerals in a flowing system: Implications for mines environmental restoration. *Applied Geochemistry* **23**: 2316-2323

Qiu, M., Xiong, S., Zhang, W. and Wang, G., 2005. A comparison of bioleaching of chalcopyrite using pure culture or a mixed culture. *Minerals Engineering* **18**: 987-990

Rawlings, D.E., 1998. Industrial practice and the biology of leaching of metals from Ores. *Journal of Industrial Microbiology & Biotechnology* **20**: 268–274

Rawlings, D. E., 2002. Heavy metal mining using microbes. *Annual Review of Microbiology* **56**: 65-91

Rawlings, D.E. 2005. 'Characteristics and adaptability of iron- and sulphur-oxidising microorganisms used for the recovery of metals from minerals and their concentrates'. *Microbial Cell Factories* **4**: 1327.

Rawlings, D.E. and Johnson, D.B., 2007. Biomining. Springer-Verlag, Berlin, Heidelberg. pp 73-79; 97-112; 153-175; 200-213; 254-261

Remonsellez, F., Galleguillos, F., Moreno-Paz, M., Parro, V., Acosta, M. and Demergasso, C., 2009. Dynamic of active microorganisms inhabiting a bioleaching industrial heap of low-grade copper sulfide ore monitored by real-time PCR and oligonucleotide prokaryotic acidophile microarray. *Microbial Biotechnology* **2**: 613-624

Roberts, J.A., 2004. Inhibition and enhancement of microbial surface colonization: the role of silicate composition. *Chemical Geology* **212**: 313– 327

Ruitenberg, R., Hansford, G.S., Reuter, M.A. and Breed, A.W., 1999. The ferric leaching kinetics of arsenopyrite. *Hydrometallurgy* **52**: 37–55

Sand, W. and Gehrke, Tl, 2006. Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron(III) ions and acidophilic bacteria. *Research in Microbiology* **157**: 49-56

Sand, W., Gehrke, T., Jozsa, P.G. and Schippers, A., 2001. Biochemistry of bacterial leaching—direct vs. indirect bioleaching. *Hydrometallurgy* **59**: 159–175

Sandström, A. and Mattsson, E., 2001. Bacterial ferrous iron oxidation of acid mine drainage as pre-treatment for subsequent metal recovery. *International Journal of Mineral Processing* **62**: 309–320

Sasaki, K., Nakamuta, Y., Hirajima, T. and Tuovinen, O.H., 2009. Raman characterization of secondary minerals formed during chalcopyrite leaching with *Acidithiobacillus ferrooxidans*. *Hydrometallurgy* **95**: 153-158

Schippers, A and Sand, W., 1999. Bacterial Leaching of Metal Sulfides Proceeds by Two Indirect Mechanisms via Thiosulfate or via Polysulfides and Sulfur. *Applied Environmental Microbiology* **65**: 319–321

Schippers, A., Jozsa, P.G. and Sand, W., 1996. Sulfur chemistry in bacterial leaching of pyrite. *Applied Environmental Microbiology* **62**: 3424–3431

Schippers, A., Rohwerder, T., and Sand, W., 1999. Intermediary sulfur compounds in pyrite oxidation: implications for bioleaching and biodepyritization of coal. *Applied Environmental Biotechnology* **52**: 104–110

Silver, M., 1978. Metabolic mechanisms of iron oxidizing Thiobacilli, in: L.E. Murr, A.E. Torma, J.A. Brierley (Eds.), Metallurgical applications of bacterial leaching and related microbiological phenomena, *Academic press*, New York, 3-18

Silverman, M.P. and Lundgren, D.G., 1959. Studies on the chemoautotrophic iron bacterium *Ferroobacillus ferrooxidans*: II. Manometric studies. *Journal of bacteriology* **78**: 326-331

Tshilombo, A.F., Petersen, J. and Dixon, D.G., 2002. The influence of applied potentials and temperature on the electrochemical response of chalcopyrite during bacterial leaching. *Minerals Engineering* **15**: 809–813

Tuovinen, O.H., Niemelä, S.I. and Gyllenberg, H.G., 1971. Tolerance of *Thiobacillus ferrooxidans* to some metals. *Antonie van Leeuwenhoek* **37**: 489-496

Tuovinen, O.H., Panda, F.A. and Tsuchiya, H.M., 1979. Nitrogen requirement of iron-oxidising Thiobacilli for acidic ferric sulphate regeneration. *Applied Environmental Microbiology* **37**: 954-958

Valdés, J., Pedroso, I., Quatrini, R. and Holmes, D.S., 2008. Comparative genome analysis of *Acidithiobacillus ferrooxidans, A. thiooxidans* and *A. caldus*: Insights into their metabolism and ecophysiology. *Hydrometallurgy* **94**: 180–184

Van Hille, R.P., Bromfield, L.V., Botha, S.S., Jones, G., van Zyl, A.W. and Harrison, S.T.L., 2009. The effect of nutrient supplementation on growth and leaching performance of bioleaching bacteria. *Advanced Materials Research* **71-73**: 413-416

Vilcáez, J. and Suto, C., 2008. Response of thermophiles to the simultaneous addition of sulphur and ferric ion to enhance the bioleaching of chalcopyrite. *Inoue Minerals Engineering* **21**: 1063–1074

Watling, H.R., 2006. The bioleaching of sulphide minerals with emphasis on copper sulphides — A review. *Hydrometallurgy* **84**: 81–108

Yee, N., Fein, J.B. and Daughney, C.J. 2000. Experimental study of the pH, ionic strength, and reversibility behavior of bacteria-mineral adsorption. *Geochimica et Cosmochimica Acta* **64**: 609-617

Zimmerley, S.R., Wilson, D.G. and Prater, J.D., 1958. Cyclic leaching process employing iron oxidizing bacteria. *US Patent 2,829,964*

World Wide Web References

World bank group. http://www.ifc.org/ifcext/enviro.nsf/AttachmentsByTitle/gui_copperWB/$FILE/copper_PPAH.pdf (Last accessed - October 2009)

Näveke, R., 1986. Bacterial leaching of ores and other Materials. Institut für Mikrobiologie, Technische Universität Braunschweig, Republic of Germany, http://www.spaceship-earth.org/REM/Naeveke.htm (Last accessed - October 2009)

http://www.cook.rutgers.edu/~dbm/lec12101204.pdf (Last accessed - October 2009)

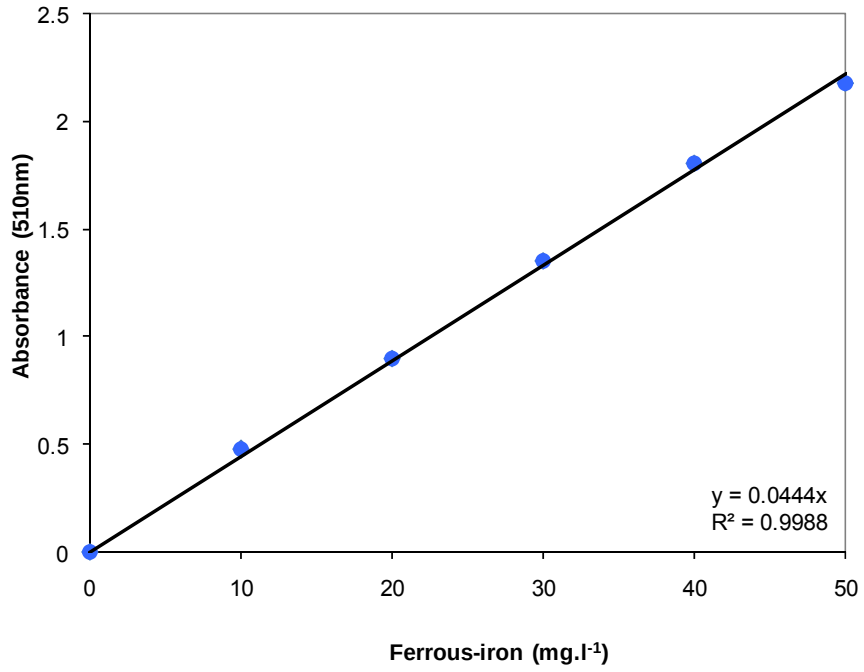
http://www.vebvzw.be/module1/uploads/24_mei_moleculaire_detectie_deel_4.pdf (Last accessed - October 2009)

Appendices

Appendix A – Analytical Methods

A.1 Colorimetric assay for ferrous-iron (Fe^{2+}) concentration measurements

Standard curves were setup using reagents in a concentration ranging between 10 – 50 $mg.l^{-1}$. In order to setup a standard curve a range of Fe^{2+} dilutions were prepared as follow: A volume of 2 ml phenanthroline and ammonium acetate solution was mixed. For the blank measurement 1 ml ddH_2O was added to the 4 ml mixture where for the individual measurements a dilution (final volume = 1 ml) of Fe^{2+} was used to achieve the desired concentration. This procedure was also followed for measuring of the column reactor effluent samples.



A.1.1 Reagents employed for Fe^{2+} standard curve setup and measurements

A.1.1.1 1-10 Phenanthroline indicator solution

A mass of 2127.708 mg of 1-10 phenanthroline was dissolved in 100 ml double distilled water (ddH_2O). The solution was diluted with ddH_2O to the 1 000 ml mark, resulting in a concentration exceeding the stoichiometric requirement.

A.1.1.2 Ammonium acetate solution

Ammonium acetate was weighed of to 250 g and dissolved in 150 ml ddH_2O. A volume of 700 ml concentrated glacial acetic acid was added to the solution allowing the ammonium acetate to fully dissolve.

A.1.1.3 Ferrous-iron stock solution

A volume of 20 ml sulphuric acid (H_2SO_4 – 98%) was slowly added to 50 ml ddH_2O. A mass of 497.629 mg ferrous-iron sulphate ($FeSO_4.7H_2O$) was added to the acid-water solution and diluted to the 1 000 ml mark with ddH_2O. This resulted in a solution with a concentration of 100 $mg.l^{-1}$.

A.4 Total Kjeldahl nitrogen (TKN) reagents required for nitrogen concentration measurement

A.4.1 Digestion mixture

Sol a Mercuric sulphate ($HgSO_4$) solution was prepared by dissolving 40 g mercuric oxide (HgO) in 250 ml dilute (1:5 – H_2SO_4:ddH_2O) sulphuric acid solution. The final solution was further diluted to a final volume of 800 ml with ddH_2O.

Sol b The solution of sulphuric acid, mercuric sulphate and potassium sulphate (K_2SO_4) was prepared by dissolving 333,75 g K_2SO_4 in 1 800 ml ddH_2O. Added to this solution was 500 ml H_2SO_4 (98%), 62,5 ml Solution a ($HgSO_4$ solution described above) and diluted to 2 500 ml.

Sol c Sulphuric acid solution (7N) was prepared by diluting 485 ml H_2SO_4 (98%) in ddH_2O and made up to 2 500 ml with ddH_2O.

For preparation of the digestion mixture: solutions b and c was mixed together in equal ratios to achieve a final volume of 5 000 ml.

A.4.2 Indicator solution

Two volumes of 0,2 % methyl red in 95 % alcohol was mixed with one volume of 0,2 %5 methylene blue in 95 % alcohol. The solution was freshly prepared every 30 days.

A.4.3 Boric acid (H_3BO_3) solution

A mass of 100 g H_3BO_3 was dissolved in 500 ml ddH_2O. A volume of 100 ml indicator solution was added to the H_3BO_3 solution and diluted to 5 000 ml with ddH_2O.

A.4.4 Sodium hydroxide (NaOH) – sodium thiosulphate ($Na_2S_2O_3.5H_2O$) solution

A mass of 500 g NaOH and 25 g $Na_2S_2O_3.5H_2O$ was dissolved in ddH_2O and made up to 1 000 ml. Care was taken as the reaction was highly exothermic.

A.4.5 Procedure followed for standard curve setup and sample analysis

A dilution range between 0 – 60 mg.l^{-1} nitrogen contents was setup.

A 5 ml sample volume was pipetted into a 30 ml micro Kjeldahl flask. A mixture of 10 ml digestion solution and 3 glass beads (3mm diameter) were added to the same Kjeldahl flask.

The flask was heated to boiling point until clear and heated for a further 20 minutes. The flask(s) was removed from the heating pad and allowed to cool at room temperature for 30 minutes.

A volume of 25 ml H_3BO_3 solution was pipette into a 100 ml Erlenmeyer flask and placed in position on the steam distillation apparatus with the nozzle of the condenser immersed in the solution. A volume of 10 ml ddH_2O was added to the flask(s) contents to dissolve any sediment.

Through the top opening of the steam distillation apparatus 7 ml NaOH solution was added and immediately sealed by placing the steam line in position.

Steam was distilled through the Kjeldahl flask contents up to the point where the volume in the Erlenmeyer flask reached 50 ml. The Erlenmeyer flask contents were titrated with 0,001 N H_2SO_4 solution in order to achieve a purple colour.

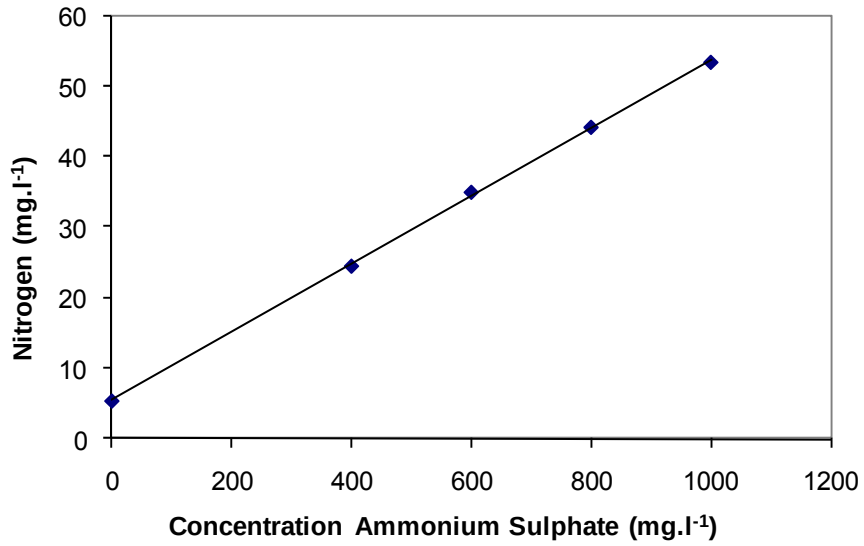
A.4.6 Final concentration calculation

The nitrogen content of the sample was calculated as follows:

$$\text{TKN}(\text{mg.l}^{-1}) = \frac{(a-b)\times N\times 14000}{A}$$

a – volume (ml) of H_2SO_4 used in titrating the sample
b – volume (ml) of H_2SO_4 used in titrating the blank
N – normality of the used H_2SO_4
A – sample volume (ml)

A.4.6 Standard curve setup for the TKN and ammonical N measurements



A.5 Planktonic cell concentration calculation

The counting grid contains four large blocks where within 400 small squares are contained. The dept of one square is 0.1 mm and the are is 1/400 mm^2. Counting the number of cells contained within four large blocks using a volume of 1.1 µl, a conversion factor of 312500 was used to calculate the cell concentration ($cells.ml^{-1}$).

A.6 Nitrogen requirements

The requirement for nitrogen was calculated through calculating the nitrogen content of a single bacterial cell. The nitrogen content of the microbial population was then calculated for a previous column reactor experiment. That calculated mass of nitrogen was doubled and added as supplementation by using either yeast extract (YE) and/or ammonium sulphate ($(NH_4)_2SO_4$) as specified.

Calculation of cell weight: A sample of a pure *Acidithiobacillus ferrooxidans* was used to perform a direct microscopic cell count on. Centrifugation at 10 000 xg was performed and a dry cell weight measurement was done after drying the sample in a oven at 80°C overnight.

Weight per cell: 5.575×10^{-13} $g.cell^{-1}$

$CH_{1.8}O_{0.5}N_{0.2}$: (12) + (1.8) + (8) + (2.8) $g.mole^{-1}$

= 24.6 $g.mole^{-1}$

5.575×10^{-13} $g.cell^{-1}$ / 24.6 $g.mole^{-1}$

= 2.27×10^{-14} $moles.cell^{-1}$

Moles N per cell: 2.27×10^{-14} moles.cell^{-1} X 0.2
$= 4.53 \times 10^{-15}$ moles nitrogen.cell^{-1}

Gram N per cell: $= 4.53 \times 10^{-15}$ moles nitrogen.cell^{-1} X 14
$= 6.35 \times 10^{-4}$ g nitrogen.cell^{-1}

Total N required: 6.35×10^{-4} g nitrogen.cell^{-1} X (7.6×10^{12} cells)
= 0.482 g N

Yeast extract required: 11% nitrogen contained within yeast extract
0.11(Yeast extract required) = 0.482 g
Yeast extract required = 4.381 g

Ammonium sulphate required: 21% nitrogen (m/m) in ammonium sulphate
0.21(Ammonium sulphate required) = 0.482 g
Ammonium sulphate required = 2.221 g

Appendix B – Raw Data Calculations

[a] Time
Experimental start date and time subtracted from date and time of when the next sample was taken.

[b, c, d] pH, Redox and volume
Measured parameters

[e] Total planktonic cell count
cells counted × volume × groups counted

$$= \text{No cells counted} \times 50\,000 \times \frac{25}{4}$$

[f] Cumulative cell number
$$(\text{cell concentration } (\text{cells.ml}^{-1}) \times \text{effluent volume (ml)}) + \text{value}_{n-1}$$

[g,h,j,] Fe^{2+}, Fe^{3+} and Cu
Measured parameters

[i] Total iron concentration
Fe^{2+}concentation + Fe^{3+}concentration

[k] Mass of Cu leached per effluent sample volume
$$\text{Cu concentration } (\text{mg.l}^{-1}) \times \text{sample volume (ml)}/1000$$

[l] Specific Cu leach rate
Calculated in order to determine the dependence of Cu leached on dissolved Fe^{3+} (mmol Cu per mmol Fe^{3+})

$$\left(\frac{\frac{Cu\ leached\ (mg)}{64}}{Time\ (days)}\right) \times \left(\frac{Fe^{3+}(mg.l^{-1})}{1000} \times Sample\ volume\ \frac{(ml)}{56}\right) + \left(\frac{Cu\ leached\ (mg)}{64} \times 4\right)$$

[m] Total cumulative Cu
$$\left(\frac{\text{Cu leached } (\text{mg.l}^{-1})}{1000} \times \text{Sample volume (ml)}\right) + \text{value}_{n-1}$$

[n] Post inoculation cumulative Cu
Point of inoculation taken as time zero

Appendix C – Raw Data

Table C1: Raw Data Column S

[a]Run time (days)	[b]pH	[c]Redox (mV)	[d]Volume (ml)	[e]Total counts (cells.ml^{-1})	[f]Cum plank cells (cells)	[g]Fe^{2+} (mg.l^{-1})	[h]Fe^{3+} (mg.l^{-1})	[i]Fe^{T} (mg.l^{-1})	[j]Cu^{2+} (mg.l^{-1})	[k]Cu leached (mg)	[l]Cu Specific leach rate (mmol Cu/day.mmol Fe^{3+})	[m]Total cum Cu (g)	[n]Post Inoc cum Cu (g)
0.00													
1.12	1.04	432	1110.00	0.00E+00	0.00E+00	458.33	365.83	824.16	1977.58	2195.11	0.24	2.20	0.14
1.58					0.00E+00							2.20	0.44
3.01	1.37	408	1935.00	0.00E+00	0.00E+00	151.35	168.82	320.17	818.80	1584.38	0.24	3.78	0.44
4.13	1.19	387	1132.00	0.00E+00	0.00E+00	52.48	213.15	265.63	516.10	584.23	0.25	4.36	0.84
5.36	1.09	397	1260.00	0.00E+00	0.00E+00	87.16	61.31	148.47	228.50	287.91	0.24	4.65	1.03
6.38	1.20	393	1018.00	0.00E+00	0.00E+00	174.77	9.05	183.82	258.60	263.25	0.25	4.91	1.23
6.98	1.29	434	600.00	0.00E+00	0.00E+00	1247.75	426.83	1674.58	225.20	135.12	0.18	5.05	1.45
8.22	1.21	439	1220.00	0.00E+00	0.00E+00	1136.26	968.58	2104.84	252.10	307.56	0.12	5.36	1.62
8.58					0.00E+00							5.36	1.85
10.06	1.23	440	1805.00	0.00E+00	0.00E+00	1115.99	964.61	2080.60	217.80	393.13	0.10	5.75	1.85
11.07	1.33	443	988.00	9.38E+05	9.26E+08	1140.77	888.32	2029.09	194.40	192.07	0.12	5.94	2.17
12.08	1.11	447	998.00	1.88E+06	2.80E+09	1109.23	1010.76	2119.99	197.70	197.30	0.11	6.14	2.37
13.32	1.08	453	1182.00	3.13E+05	3.17E+09	1110.36	1095.48	2205.84	192.30	227.30	0.08	6.37	2.49
14.15	1.11	459	858.00	7.81E+05	3.84E+09	1064.19	1128.52	2192.71	198.50	170.31	0.10	6.54	2.62
15.27	1.05	461	1107.00	1.25E+06	5.22E+09		2186.65	2186.65	204.70	226.60	0.09	6.76	2.83
15.58					5.22E+09							6.76	3.04
16.98	1.22	470	1680.00	1.25E+06	7.32E+09	780.41	1129.50	1909.91	188.80	317.18	0.07	7.08	3.04
18.32	1.30	477	1315.00	1.25E+06	8.96E+09	644.14	1272.84	1916.98	154.80	203.56	0.06	7.29	3.29

[a]Run time (days)	[b]pH	[c]Redox (mV)	[d]Volume (ml)	[e]Total counts (cells.ml^{-1})	[f]Cum plank cells (cells)	[g]Fe^{2+} (mg.l^{-1})	[h]Fe^{3+} (mg.l^{-1})	[i]Fe^{T} (mg.l^{-1})	[j]Cu^{2+} (mg.l^{-1})	[k]Cu leached (mg)	[l]Cu Specific leach rate (mmol Cu/day.mmol Fe^{3+})	[m]Total cum Cu (g)	[n]Post Inoc cum Cu (g)
19.06	1.10	485	725.00	1.09E+06	9.76E+09	547.30	1382.81	1930.11	159.50	115.64	0.08	7.40	3.42
20.01	1.12	486	932.00	9.38E+05	1.06E+10	498.87	1437.30	1936.17	139.30	129.83	0.06	7.53	3.53
21.14	1.11	485	1095.00	1.25E+06	1.20E+10	540.54	1421.89	1962.43	199.30	218.23	0.07	7.75	3.61
22.34	1.25	492	1160.00	1.56E+06	1.38E+10		1799.82	1799.82	175.20	203.23	0.06	7.95	3.71
22.58					1.38E+10							7.95	3.83
24.01	1.07	505	1660.00	1.25E+06	1.59E+10		1769.52	1769.52	152.80	253.65	0.05	8.21	3.83
25.29	1.05	511	1270.00	9.38E+05	1.71E+10	144.14	1797.08	1941.22	105.37	133.82	0.04	8.34	4.03
26.34	1.03	520	1042.00	9.38E+05	1.81E+10	90.09	2122.82	2212.91	98.79	102.94	0.03	8.44	4.12
27.38	1.07	530	862.00	1.09E+06	1.90E+10	87.84	1855.40	1943.24	96.78	83.42	0.04	8.53	4.12
28.17	1.31	539	935.00	9.38E+05	1.99E+10	83.33	2243.71	2327.04	108.50	101.45	0.04	8.63	4.31
29.23	1.11	543	1010.00	1.72E+06	2.16E+10	60.81	2286.43	2347.24	116.00	117.16	0.04	8.74	4.37
29.58					2.16E+10							8.74	4.44
30.58	1.14	556	1860.00	2.50E+06	2.63E+10	63.06	2293.27	2356.33	106.30	197.72	0.03	8.94	4.44
32.10	1.15	565	1000.00	2.50E+06	2.88E+10	21.40	2222.82	2244.22	92.57	92.57	0.02	9.03	4.58
32.58					2.88E+10							9.03	4.64
34.43	1.14	571	2280.00	2.19E+06	3.37E+10	19.37	2109.71	2129.08	81.61	186.07	0.02	9.22	4.66
35.28	1.16	579	850.00	1.88E+06	3.53E+10	34.01	2094.06	2128.07	76.00	64.60	0.03	9.29	4.68
36.20	1.17	588	920.00	2.34E+06	3.75E+10	25.45	2051.11	2076.56	75.54	69.50	0.03	9.35	4.68
36.58					3.75E+10							9.35	4.79
38.26	1.23	601	2000.00	2.58E+06	4.27E+10	24.55	2009.59	2034.14	70.68	141.36	0.02	9.50	4.79
39.21	1.11	616	960.00	2.81E+06	4.54E+10	20.50	2121.71	2142.21	61.83	59.36	0.02	9.56	4.93
40.26	1.08	649	1050.00	9.38E+05	4.63E+10	12.75	2540.53	2553.28	19.48	20.45	0.01	9.58	5.01
41.29	1.10	653	958.00	1.25E+06	4.75E+10	14.91	2706.03	2720.94	15.28	14.64	0.00	9.59	5.01

[a]Run time (days)	[b]pH	[c]Redox (mV)	[d]Volume (ml)	[e]Total counts (cells.ml^{-1})	[f]Cum plank cells (cells)	[g]Fe^{2+} (mg.l^{-1})	[h]Fe^{3+} (mg.l^{-1})	[i]Fe^{T} (mg.l^{-1})	[j]Cu^{2+} (mg.l^{-1})	[k]Cu leached (mg)	[l]Cu Specific leach rate (mmol Cu/day.mmol Fe^{3+})	[m]Total cum Cu (g)	[n]Post Inoc cum Cu (g)
41.58					4.75E+10							9.59	5.14
43.10	1.04	605	1310.00	1.56E+06	4.96E+10	13.56	2613.45	2627.01	83.53	109.42	0.02	9.70	5.19
43.58					4.96E+10							9.70	5.19
45.07	1.11	520	1630.00	1.25E+06	5.16E+10	16.60	2518.50	2535.10	88.00	143.44	0.02	9.84	5.19
46.14	1.09	553	778.00	2.03E+06	5.32E+10	17.03	2574.63	2591.66	99.76	77.61	0.03	9.92	5.36
46.58					5.32E+10							9.92	5.36
48.29	1.09	562	1550.00	2.81E+06	5.76E+10	10.74	2605.16	2615.90	87.12	135.04	0.02	10.06	5.36
49.18	1.08	476	685.00	3.13E+06	5.97E+10	1.63E+01	3887.34	3903.65	68.36	46.83	0.02	10.10	5.45
49.58					5.97E+10							10.10	5.45
50.58					5.97E+10							10.10	5.57
52.06	1.08	513	2160.00	3.44E+06	6.71E+10	17.32	2711.70	2729.02	80.73	174.38	0.02	10.28	5.57
53.24	1.10	570	880.00	2.19E+06	6.91E+10							10.28	5.68
53.58					6.91E+10							10.28	5.68
54.88	1.05	521	1260.00	1.88E+06	7.14E+10	12.03	2368.54	2380.57	69.39	87.43	0.02	10.36	5.77
55.58					7.14E+10							10.36	5.82
57.13	1.06	518	1700.00	9.38E+05	7.30E+10	11.53	2505.39	2516.92	71.04	120.77	0.02	10.49	5.82
57.58					7.30E+10							10.49	5.82
59.19	1.10	561	1559.00	1.25E+06	7.50E+10	20.90	2472.79	2493.69	68.87	107.37	0.01	10.59	5.82
59.58					7.50E+10							10.59	
61.09	1.03	567	1370.00	1.56E+06	7.71E+10	22.61	2514.51	2537.12	69.65	95.42	0.02	10.69	
62.04	1.08	585	703.00	7.81E+05	7.76E+10	24.08	2471.63	2495.71	71.91	50.55	0.02	10.74	

Table C2: ICP Analysis

Description	Mg	Cr	Si	Fe	Ca	Ti	Mn	Cu	V	Ni
PLS day 1	>100	**2.7**	63	>100	242	0.3	13	>100	<0.02	4.2
PLS day 2	>100	**1.7**	63	>100	203	0.222	13	>100	0.23	2.2
PLS day 3	>100	**1.2**	60	>100	160	<0.02	9.1	>100	0.22	1.5
PLS day 4	>100	**0.96**	63	>100	156	<0.02	7.3	>100	0.19	0.94
PLS day 5	67	**0.7**	46	>100	95	<0.02	3.4	>100	0.16	0.58
PLS day 6	65	**0.8**	50	>100	93	<0.02	3.1	>100	0.15	0.59
PLS day 7	66	**0.6**	55	>100	100	<0.02	3.0	>100	0.18	0.39
Irrigation solution	1.4	**0.1**	<0.02	3.9	3.8	<0.02	<0.02	1.9	<0.02	<0.02

Description	Co	Al	Zn	Pb	Be	Ag	Cd	Mo	SO_4	As
PLS day 1	1.5	>100	47	1.6	0.14	<0.02	1.4	2.7	>1000	6.7
PLS day 2	0.83	>100	36	1.3	0.12	<0.02	1.1	1.6	>1000	4.7
PLS day 3	<0.02	>100	21	0.90	0.08	<0.02	0.66	1.1	>1000	2.8
PLS day 4	<0.02	>100	15	0.68	0.07	<0.02	0.48	0.95	>1000	1.9
PLS day 5	<0.02	78	10	0.50	<0.02	<0.02	0.17	0.62	>1000	0.94
PLS day 6	<0.02	85	9.2	0.47	<0.02	<0.02	0.12	0.64	>1000	0.74
PLS day 7	<0.02	86	8.0	0.45	<0.02	<0.02	0.10	0.67	>1000	<0.02
Irrigation solution	<0.02	<0.02	<0.02	0.36	<0.02	<0.02	<0.02	0.14	>1000	<0.02

* All concentrations measured in $mg.l^{-1}$