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Investigation of bacterial ferrous iron oxidation kinetics in a novel packed-column reactor: pH and jarosite management

by

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DECLARATION

I, Mwema Wanjiya, declare that the contents of this thesis duly represent my original line of thinking backed by previous research studies. It has not been previously submitted for any academic examination qualification at any university.

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Signed Date

Abstract

Jarosite formation is regarded as undesirable in the bioleaching processes as it depletes ferric reagent; a critical reagent for the oxidation of most sulphide minerals, from bioleach solution. It creates kinetic barriers and clogs on mineral surfaces, thereby retarding leach rates of most minerals. However, jarosite has also been shown to serve as support for the attachment of bioleaching microbes, facilitating a high ferric-iron generation rate. In this study, a series of experiments on microbial ferrous-iron oxidation by a *mesophilic* microbe were carried out in a novel packed-column bioreactor with a view to investigating the potential use of solution pH to manage jarosite accumulation in the bioreactor. The kinetics of the oxidation was also investigated to establish base case data for the novel bioreactor.

The bioreactor was packed with glass balls 15 mm in diameter. The experiments were conducted at a constant temperature of 38.6 °C, residence time of 18 hrs, airflow rate of 20 $mL.s^{-1}$ and at desired solution pHs (1.3, 1.5 and 1.7). The results showed that the amount of jarosite accumulation is proportional to the operating solution pH and also to the duration of operation of the bioreactor. Jarosite precipitate of 4.95, 5.89 and 7.08 $g.L^{-1}$ were obtained after 10 days of continuous operation at solution pH of 1.3, 1.5 and 1.7 respectively, while after 15 days the precipitate concentration increased to 5.50, 7.90 and 9.98 $g.L^{-1}$respectively. The results also showed that a 33% and 52% reduction in jarosite accumulation could be achieved by a gradual decrease of the bioreactor solution pH after being continuously operated for 10 days from pH 1.7 to 1.5 and pH 1.7 to 1.3, respectively, for an additional five days of continuous operation. The results of the ferrous-iron biooxidation kinetics investigated at pH 1.3 show a maximum ferrous oxidation rate ($r_{Fe^{2+}}^{\max}$) of 6.85 $mmol.L^{-1}.h^{-1}$ and apparent affinity kinetics constants ($K'_{Fe^{2+}}$, $K_{Fe^{2+}}$) of 0.001 mmol $Fe^{2+}.L^{-1}$ and 0.006 (dimensionless) using Hansford and Monod equations, respectively. Although a direct relationship exists between jarosite formation and solution pH, the results of this study may be relevant in bioleach heaps, or at least in column bioreactors, to manage and control jarosite accumulation, thereby improving leach kinetics of sulphide minerals.

DEDICATION

This thesis is dedicated to my family and family members, many of who have since gone into the next life, for their moral support and for making me realise my goals thus far, which would have been difficult to pursue in my only strength and capabilities. I also dedicate this study to every one of my colleagues who contributed positively consciously or unconsciously to the success of this research study.

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LIST OF PUBLICATIONS AND PRESENTATIONS

Oral presentation

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TERMS AND CONCEPTS

Bioleaching: Bioleaching is a process that involves extraction of desired metals from low grade mineral sulphides ores, facilitated by certain microorganisms such as bacteria or archae.

Heap bioleaching: Heap bioleaching is one of the techniques used in the bioleaching process. It involves treatment of bulk and irregular shaped crushed mineral sulphide ores using the leaching solution enriched with microorganisms in order to extract the desired metals.

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Jarosite: Jarosite is a hydrous mineral with approximately 10 weight % H_2O in the form of the OH^- group, forming part of the alunite supergroup. Its chemical formula is $XFe_3(SO_4)_2(OH)_6$,where X can represent K^+(potassium jarosite), Na^+(natrojarosite),$NH4^+$(ammoniojarosite), Ag^+ (argentojarosite;), H_3O^+ (hydronium jarosite), and Pb^{2+}(plumbojarosite). Its formation is the function of the ferric-ions from the ferrous-iron biooxidation and solution pH.

Mesophilic bacteria: These are an iron and sulphur oxidising microorganism species that are mostly used commercially in the bioleaching processes. The optimum operating temperature for this group is 40 °C.

Novel packed-column reactor: This is a packed-column reactor packed with inert materials (e.g glass balls) that simulates the real solution flow dynamics of a typical heap situation.

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NOMENCLATURE

Symbol	Description	Unit
a_i	Activity of the species i	mmol.L^{-1}
C_X	Bacterial concentration	mmol C.L^{-1}
D	Dilution rate	h^{-1}
E_h	Standard redox potential	mV
Eh_o	Initial redox potential	mV
E'_h	Solution potential	mV
F	Flow rate	L.h^{-1}
F	Farady's constant in equation	Coulomb.mol.$^{-1}$
$[Fe^{2+}]$	Ferrous-iron concentration	mmol Fe^{2+}.L^{-1}
$[Fe^{3+}]$	Ferric-iron concentration	mmol Fe^{3+}.L^{-1}
$[Fe^{2+}]_{In}$	Influent ferrous-iron concentration	mmol Fe^{2+}.L^{-1}
$[Fe^{2+}]_{Out}$	Effluent ferrous-iron concentration	mmol Fe^{2+}.L^{-1}
$[Fe_T]$	Total iron	mmol Fe.L^{-1}
$K_{Fe^{2+}}$	Ferrous-iron based affinity constant	mmol Fe.L^{-1}
$K'_{Fe^{2+}}$	Affinity constant in Equation 2.16	Dimensionless
K_{O_2}	Affinity constant for oxygen utilisation rate	Dimensionless
$q_{Fe^{2+}}$	Microbial specific ferrous-iron utilisation rate	mmol Fe^{2+}.(mol C)$^{-1}$h^{-1}
$q_{Fe^{2+}}^{\max}$	Maximum microbial specific ferrous-iron utilisation rate	mmol Fe^{2+}.(mol C)$^{-1}$h^{-1}
R	Universal gas constant	J.mol^{-1} K^{-1}
R^2	Regression constant	Dimensionless
$r_{Fe^{2+}}^{\max}$	Maximum ferrous-iron utilisation rate	mmol Fe^{2+}.L^{-1}h^{-1}
$-r_{Fe^{2+}}$	Ferrous-iron utilisation rate	mmol Fe^{2+}.L^{-1}h^{-1}

T	Absolute temperature	K
t	Time	h

Greek symbols

y_i	Activity coefficient	Dimensionless
τ	Residence time	H
v_0	Volumetric flow rate	$m^3.h^{-1}$

LIST OF ABBREVIATIONS

Abbreviation	Description
AAS	Atomic Absorbance Spectrometer
ADP	Adenosine diphosphate
At.	*Acidithobacillus*
ATP	Adenosine triphosphate
BCE	Before the Common Era
BACFOX	Bacterial film oxidation
BDS	Barium DiphenyllamineSulphonate
CSTR	Continuous Stirred Tank Reactor
CycA1	Cytochrome -cA1
Cyc1	Cytochrome-c1
Cyc2	Cytochrome-c2
EDTA	Ethylenediaminetraaacetic acid Disodium salt Dihydrate
EPS	Enzopolysacharades
PLS	Pregnant Leach Solution
PVA	Poly Vinyl Alcohol
PVC	Poly Vinyl Chloride
Tf	*Thiobacillusferroxidans*
Lf	*Leptospirriliumferroxidans*
L.	*Leptospirilium*
NADH	Nicotinamide adenine dinucleotide
SHE	Standard Electrode Pontential
S.	Sulfolobus
SSE	Sum of squares error
USA	United States of America

Chapter 1

1. Introduction

1.1 Background

Bioleaching is a technique used for the extraction of valuable metals from low grade sulphide ores. This subject, well-covered by various authors, is described as a process that involves both the oxidation and dissolution of the sulphide ores. Bioleaching is applied in the pre-treatment of refractory gold ores before they are subjected to the cyanidation process. This technique is also applied in the recovery of copper from secondary copper sulphides ores (Brierley and Brierley, 2001, Watling, 2006, Morin, 1995). This process is facilitated by microbial action, mostly of bacteria and archae. Bioleaching is presently under development for the extraction of minerals such as zinc, lead, arsenic, antimony, nickel, molybdemun and cobalt from the low grade ores.

The bioleaching process proceeds in three sub-processes, one of which is driven by microorganisms, a sub-process known as the ferrous-iron oxidation process. In this process, microorganisms oxidise the ferrous-iron (Fe^{2+}) to ferric-iron (Fe^{3+}). The Fe^{3+} is the main oxidising agent in the chemical oxidative dissolution of sulphide ores. However, the chemical oxidative dissolution of the sulphide ores reduces the Fe^{3+} to Fe^{2+}. This sub-process explains the importance of the microorganisms in the generation and regeneration of solutions with relatively high redox potentials that leach sulphide ores. On the other hand, the sulphur oxidising bacteria oxidise the sulphur moieties to sulphur and the sulphuric acids which sustain the bioleaching process (Boon *et al.*, 1999c, Sand *et al.*, 1995, Schippers and Sand, 1999, Boon *et al.*, 1999b). However, the production of Fe^{3+} iron leads to the precipitation of iron (III) hydroxides and other ferric complexes such as jarosite. These precipitates have been reported to occur at high pH values (Plumb *et al.*, 2008). Jarosite formation deprives the bioleach medium of much needed Fe^{3+}iron critical to the dissolution of most sulphide ores. Furthermore, it may create diffusion barriers on mineral surfaces, thereby hindering the kinetics of metal sulphide oxidation/dissolution. This situation could lead to the entire bioleaching process being aborted,

as it may render heap beds impervious to leaching reagents and recycled pregnant leaching solution (PLS).A variety of industrial bioleaching techniques for processing of sulphide ores exist, such as heap, irrigated dump or the stirred tank. The application of these techniques depends upon the nature of the sulphide ore under consideration. If the sulphide ore is of low grade then the heap technique is the most preferable method, whereas for the marginal run of time ores, the irrigated dump leaching is preferred, and for finely milled floatation concentrates, the stirred tank is the most preferable (Brierley and Brierley, 2001, Rawlings, 2002, Rossi, 1990).

1.2 Background to the research problem

Microbial ferrous-iron oxidation is an important sub-process of the bioleaching process. The generation and the regeneration of ferric-iron (oxidising agent) are important to the success of recovery of metals from sulphide minerals. It has been shown that Fe^{2+} oxidation is 2 x 10^5 to 2 x 10^6 times faster than abiotic oxidation under the same conditions(Lacey and Lawson, 1970). Quite a number of studies have been conducted in past decades concerning the microbial ferrous-iron oxidation kinetics (Ojumu *et al.*, 2008, Breed and Hansford, 1999a, Boon *et al.*, 1999a). However, most of these studies were conducted in bioreactors that do not simulate the heap situations. The past two decades, though, have witnessed the improvement in the generation of the ferric-iron through research studies (Pogliani and Donati, 2000, Jensen and Webb, 1995, Armentia and Webb, 1992). But very few research studies have been conducted that relate jarosite/ferric-iron precipitates to solution pH in a heap bioleaching context.

Suffice it to say, much success has been recorded in the optimising of the key parameters such as temperature and pH. However, most of the studies were conducted in tank systems which are in contrast to the heap systems. This has resulted in the enhancement of the microorganism productivity in the tank system, leading to about 95 to 98% of the metal recovery achieved after five days (Brierley C.L, 2005). But due to the depletion of the rich grade ores and the high cost that would be associated with their treatment, the heap process has now become a suitable alternative technique for the treatment of low grade copper sulphide ores. The existing studies have revealed many successes pertaining to ferrous-iron biooxidation in stirred tank systems. However, the kinetics in such systems may not represent that of the attached microorganisms in heap bioleach system. It has been noted that there is little existence work directed towards the control of solution pH to minimise jarosite formation in the context of a heap bioleach or in a system that simulates the solution flow dynamics of a typical bioleach heap situation. Most of

the previous studies relating the formation of the jarosite and pH were conducted in either CSTR or fluidised/flooded packed-bed bioreactors, and thus could not be compared to the typical bioleach heap situation (Dutrizac and Hardy, 1997, Dutrizac and Jambor, 2000, Gunneriusson *et al.*, 2009, J. Daoud, 2005, Katsioti *et al.*, 2005, Liu *et al.*, 2009, Daoud and Karamanev, 2006).

Therefore, it is important to investigate the kinetics of ferrous-iron biooxidation in a bioreactor system that simulates the real solution flow dynamics of a typical bioleach heap, as it is important to investigate how solution pH can be used in the management of ferric-iron precipitates (jarosite).Ojumu and Petersen (2011) conducted a study on the effect of pH on the ferrous-iron biooxidation in a CSTR. In their study, the researchers observed that at pH 1.3, jarosite accumulation was minimised. However, the bioreactor configuration used in that study did not depict the real solution flow dynamics of a typical heap situation. This present study would provide a basis to compare ferrous-iron biooxidation kinetics in those systems.

Previous studies (Chowdhury, 2012) enlighten on the importance of the ferric-iron as the critical reagent in the metal recovery in bioleaching processes by enhancing the maximum overall rate of microbial ferrous-iron oxidation. However, excess ferric-iron readily precipitates to form hydroxides, oxyhydroxides and hydroxysulphates (jarosite complexes). Another study, according to Bevilaqua *et al.* (2002), revealed that the iron precipitates accumulating in the reactive mineral surface may contribute to the passivation effect (diffusion barrier of reactants and products). Jarosite prevents bacterial and ferric-iron from accessing the mineral sulphide surface (Nemati and Webb, 1998). This phenomenon explains the importance of jarosite management or control.

1.3 Research questions

This research intends to address the following questions:

- Will the variations in the feed pH lead to the minimisation of the jarosite formation in a packed- column reactor simulating the solution flow dynamics of a typical heap situation?
- What possible strategy can be used to minimise and control jarosite using solution pH?

1.4 General objective

The main objective of this study concerns the following:

- To investigate the kinetics of Fe^{2+}biooxidation in a novel packed-column reactor with a view to understand the kinetics in a system that closely represents a heap bioleach system.

And more specifically:

- To investigate the effects of solution pH on jarosite formation and accumulation with a view of managing its formation.
- To investigate the ferrous-iron biooxidation kinetics at a constant pH 1.3 in a novel packed-column reactor.

1.5 Delineation of the study

The present research will not investigate the influence of temperature on the formation of the jarosite, but rather restrict research to the solution pH. The intra and inter particle diffusion between the feed solution and the ore particles was assumed to not affect the kinetics of microbial ferrous-iron oxidation. Experimental trials will be conducted in a novel packed-column bioreactor that simulates the real solution flow dynamics of typical heap situation using *Acidithiobacillus ferroxidans*. Glass balls will be used as inert packing material.

1.6 Significance of this research

The findings of this research study will provide an insight into how solution pH could be used to manage the jarosite accumulation within a heap bed. The pyrite (ferrous-iron source) which is embedded within the ore body is made available through the bacteria oxidation producing ferric-iron (the main oxidising agent) to leach the desired metals (mostly copper) in a sustainable manner. Since there is no external source of ferric-iron to the heap bed, the management of the total iron is critical to the efficient metal recovery in heap bioleaching. While the ferrous-iron biooxidation kinetics investigation at the constant pH of 1.3is expected to have minimal jarosite accumulation in the bioreactor, the aspect of diffusion barriers and blockage of the heap bed may not arise. Therefore, this study will contribute a database on the management of jarosite using the solution pH and kinetics of ferrous-iron biooxidation using *At.ferroxidans* bacterium in

a heap-like bioreactor configuration. This will in turn lead to the improvement of metal recoveries within the industrial heap situation and operations of the packed-columns.

1.7 Thesis outline

This thesis is composed of six chapters outlined as follows:

Chapter 1 presents a brief summary of the background to this research problem, a summary of problems originating from previous studies, with much emphasis on the heap bioleaching context and a brief discussion of the novelty design of the bioreactor in relationship to a typical heap context. As well, it categorically points out the significance of this present study.

Chapter 2 presents a brief background of jarosite as well as historical background to the heap bioleaching. It provides a literature review on the mechanisms of the bioleaching and the microorganisms involved. This chapter reviews literature on jarosite and how the solution pH influences its formation. Intensive review of previous studies relating the solution pH and jarosite were examined, as well as the effect of the pH on the kinetics of microbial ferrous-iron oxidation through the reviewing of the previous studies conducted in various bioreactor configurations that do not simulate the flow solution dynamics of real heap situation.

Chapter 3 presents detailed experimental methods and materials used in this present study. It gives the detailed method applied in the quantification of jarosite and theoretical calculation involved in the substrate utilisation with regard to the study of ferrous-iron kinetics parameters.

Chapter 4 presents the experimental results and the discussion derived from the investigation of the solution pH on the jarosite formation and investigation of kinetics of microbial ferrous-iron oxidation at a constant pH 1.3.The experimental data obtained on the ferrous-iron microbial oxidation was correlated to appropriate rate equations derived from previous studies.

Chapter 5 presents the conclusions and recommendations generated from the experimental results obtained in Chapter 4.

Chapter 6 lists all the references cited in this thesis.

Chapter 2

2. Literature review

2.1 The history of the bioleaching process

The process of bioleaching may have been in existence since prehistory times (Ehrlich, 2001, Rawlings, 2002). This is noted by the Greeks and the Romans who extracted copper from mine water about 2000 years ago. This extraction could only be possible by the involvement of microorganisms, and at that particular time, there were no conventional methods such as pyro-hygrometallurgy established. Nevertheless, the process of bioleaching was not recognised as such until about 50 years ago (Bosecker, 1997), when it was discovered that microorganisms were mainly responsible for the enrichment of the metals in water from ore deposits and mines. This important process takes place naturally whenever the microorganisms meet suitable conditions (Colmer and Hinkle, 1947).The involvement of the microbial activities in the dissolution of the ore sulphides can be traced back to the Rio Tinto River in Spain. The river was called 'Rio Tinto' because of its red colour, obtained from the high concentration of the ferric-iron (Fe^{3+}) dissolved in it. The dissolved iron and the dissolved copper (in small quantities) were due to the presence of microorganism activities around the area. The Rio Tinto has been recognised as a river devoid of fish and drinkable water, due to this astonishing scenario (Shannon, 1976).

It is noted beyond doubt that recovery of copper metal can be dated back to the 15th or 16th centuries. However, literature has shown that the extraction of copper via leaching from the ore deposits and the precipitation of the copper from the treatment of the solution with iron (cementation) are ancient technologies that were practised by the Chinese as early as 100-200 BCE. Previous studies have reported that microbial processes are at the centre of the leaching of copper from sulphide minerals. Ehrlich (1999) noted that biooxidation of the sulphides ores, mainly copper recovery, had been in use for centuries in countries like Spain, Sweden, Germany and China.

The earliest documented commercial application of bio-hydrometallurgy in the mining industry was for copper recovery from mine wastes (Zimmerley *et al.*, 1958). It was later discovered that the design of the dump bioleaching process was not efficient in the extraction of copper. This was due to the nature of the dump design which does not promote efficient growth of the microorganisms, as well as extremely poor aeration conditions (Rossi, 1990).As a result, heap bioleaching was designed as an alternative to the dump process. Heap systems improved on aeration rates leading to the efficient growth of microorganisms, which in turn resulted in improved rates of metal extractions (Olson *et al.*, 2003, Rawlings *et al.*, 2003).After the implementation of heap bioleaching systems in the 1980s,the extraction of low grade copper and other metals such as uranium and gold became possible through bio-hydrometallurgy technology. These discoveries led to the commercial pre-treatment of arsenopyrite gold which was commissioned in the 1980s (Olson *et al.*, 2003). The Fairview mine, Barberton, South Africa, built in 1986 (Rawlings *et al.*, 2003),is such a kind. It is known to have the longest history of operation in the field of the biooxidation plants. This facility processes refractory arsenopyrites or pyrite gold bearing concentrates in large aerated stirred continuous flow bioreactors.

There have been great advances in the development of heap bioleaching systems, especially in the 1980s when the first copper mine, Lo Aguirre, located in Chile, started the industrial scale copper bioleaching process from ore containing 1 to 2% copper with a production of 14,000 tons of fine copper per year. Consequently, bioleaching technology has been used increasingly (Acevedo, 2002, Akcil, 2004, Brierley and Brierley, 2001, Montealegre *et al.*, 1993).Today, the technology of heap, dump and tank bioleaching are used for the treatment of the low grade ores, sulphide concentrates, as well as in the pre-treatment of gold bearing arsenopyrite ores. This pre-treatment, used widely around the world, is done in order to expose the gold from its arsenopyrite matrixes (Rawlings *et al.*, 2003).Significant research advances have been made in commercial applications of the bioleaching technology for the purpose of metal recovery (Brierley and Brierley, 2001).

The technology of the bioleaching has also been applied in the extraction of other valuable metals such as cobalt, zinc and uranium. It has been shown that bioleaching technology can be used in the recovery of cobalt (Olson *et al.*, 1990).The bioleaching and recovery of cobalt in the first commercial plant was established in Uganda (Briggs and Millard, 1997). It was used for

treatment of low grade cobaltiferous pyrite with a cobalt concentrate of 1.38%, and with a recovery of 92% of cobalt.

2.2 Mechanism of bioleaching

Silverman and Ehrlich (1964) proposed the first mechanisms of bioleaching. However, this phenomenon has been subjected to wide debate in recent decades (Boon, 2001, Hansford, 1997, Montealegre *et al.*, 1993, Pogliani and Donati, 1999, Sand *et al.*, 2001, Sand *et al.*, 1995, Tributsch, 2001). In spite of these debates, it is now widely accepted that mineral bioleaching processes are a combined action of two very important reactions: chemical and microbial bio-reactions. These reactions include the biooxidation of ferrous to ferric-iron, the conversion of elemental sulphur to sulphuric acid and the chemical attack of sulphide mineral ores with ferric-irons. The leaching rate is driven by ferric-iron (Fe^{3+}) availability. Therefore, the role of microorganisms in the bioleaching process can never be underestimated due to the fact that they generate and regenerate the ferric-iron and thereby maintaining a constant supply of the oxidant. The constant supply of the ferric-iron oxidant defines the degree of the extraction rate of desired metal recovery.

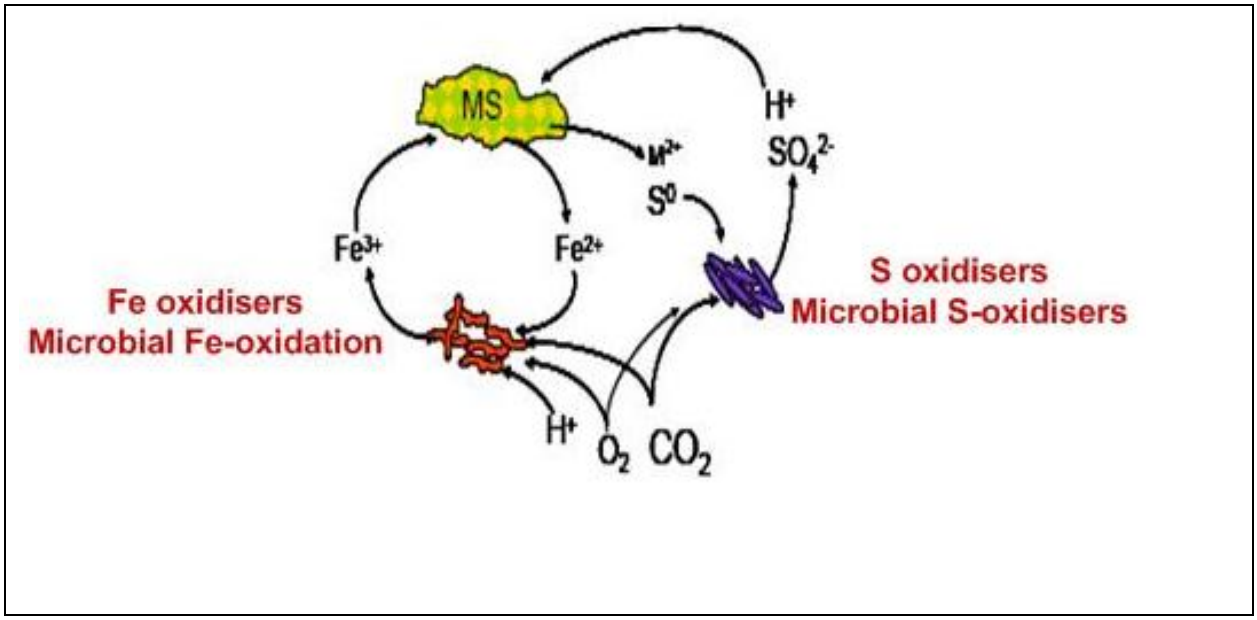

Figure 2.1: A schematic representation of the mechanism of bioleaching (Breed and Hansford, 1999c) (Hansford and Vargas, 2001)

$$MeS + 2Fe^{3+} \rightarrow Me^{2+} + 2Fe^{2+} + S^{0} \qquad 2.1$$

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{Bacteria} 4Fe^{3+} + 2H_2O \quad 2.2$$

$$2S^0 + 3O_2 + 2H_2O \xrightarrow{Bacteria} 2SO_4^{2-} + 4H^+ \quad 2.3$$

The primary attack of the sulphide mineral is a chemical ferric-iron (Fe^{3+}) leach reaction depicted by Equation 2.1, a chemical reaction which dissolves desired metal(Me) into bioleaching solution and the elemental sulphur (S^0). Ferrous-iron oxidising bacteria re-oxidise ferrous-iron back to ferric-iron form in a bio-reaction reaction, as depicted by Equation 2.2. The elemental sulphur resulting from the chemical reaction, depicted by Equation 2.1, is oxidised to sulphate ions in a bio-reaction by sulphur oxidising bacteria, as depicted by Equation 2.3.These three important reactions in the bioleaching are well-illustrated in Figure 2.1. The microbial oxidations take place within the exopolymeric substance commonly known as EPS. It is within EPS that cells divide or multiply, resulting in the formation of bio-films. The role of bio-films is to provide appropriate space for desired reactions.

Within ferrous-iron EPS, ferrous-iron is biooxidised to form ferric-iron which takes part in chemical leach of sulphide mineral ores. In return, it is reduced to ferrous iron as earlier explained. The microorganisms, therefore, re-oxidise ferrous-iron, maintaining a continuous supply of leaching agent (Fe^{3+}) in leaching liquor as mentioned previously. While in the sulphur EPS, sulphur oxidising microorganisms contain a protein carrier known as ‘cystein’ which breaks the bonds of the mineral sulphide. This forms elemental sulphur, sulphur colloids and any other sulphur intermediates. It is noted that the sulphur element is relatively stable; however, it may be oxidised to sulphate by sulphur oxidising microorganisms such as *Acidithiobacillus, Thiobacillus ferrooxidans* or *Acidithiobacillus caldus* (Rawlings, 2005).

Generally in the bioleaching process there are two main proposed mechanisms for dissolutions of sulphides ores. The first mechanism is described as the ‘thiosulphate mechanism’, and is applied to acid insoluble metals such as pyrite (FeS_2), molybdenite (MoS_2) and tungsten. In this mechanism, metal dissolution is achieved by a chemical attack of ferric-iron on the metal sulphides, thiosulphate being the main intermediate product, and the sulphate being the main end product as shown in Figure 2.2 (a). The ‘polysulphide mechanism’, represented Figure 2.2 (b), is the second mechanism and is applicable to acid soluble metals such as chalcopyrite ($CuFeS_2$), zinc sulphide (ZnS) and lead sulphide (PbS). The dissolution of desired metal is

achieved by a combination of chemical attack of ferric-iron and protons on sulphide mineral ores, with elemental sulphur as the main intermediate product and sulphate compounds being the main products, as shown in Figure 2.2 (b).

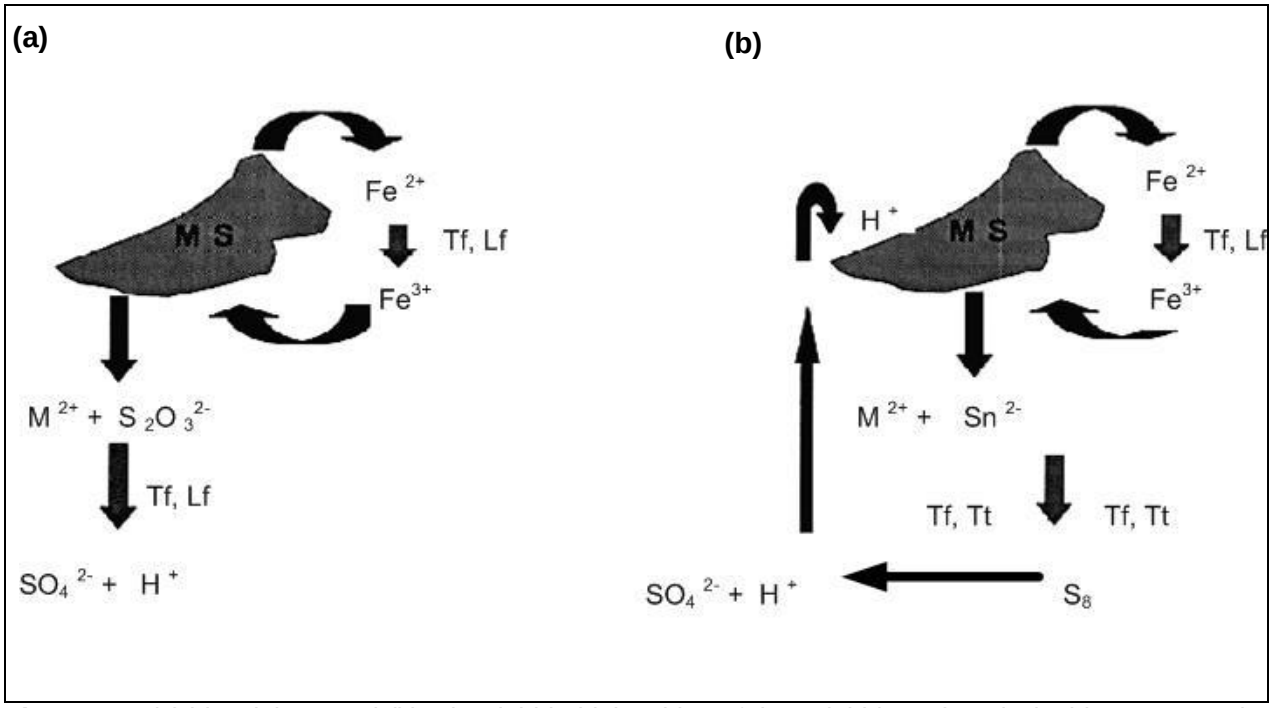

Figure 2.2: (a)thiosulphate and (b)polysulphide bioleaching of the sulphides minerals (Schippers& Sand, 1999)

2.3 The Microorganism involved in the bioleaching processes

Microorganisms are important in the bioleaching process, primarily because they control reaction kinetics within the bioleaching processes. They are acidophilic in nature, meaning that they operate under acidic conditions or under low pH conditions. This is why they are more advantageous to work within any given micro-processes. There is also no need to sterilise their environment from other competitive organisms. This phenomenon is made possible because of the nature of their acidic environment. These microorganisms perform two main functions: they oxidise ferrous-iron (Fe^{2+}) to the ferric-iron (Fe^{3+}) as well as elemental sulphur (S°) to sulphuric acid (H_2SO_4). These reactions are responsible for the control of kinetic reactions; therefore, their role in bioleaching technology can never be underestimated. The microorganisms taking part in the reactions are described according to their performance categories in Table 2.1.

Table 2.1: Acidophilic strains of bacteria used in bioleaching experiments, modified from (Chowdhury, 2012, Deveci *et al.*, 2004)

Type	Bacteria strain	Code
Mesophiles (10 °C to 45 °C)	Acidithiobacillus Ferrooxidans	DSM
	Mixed cultures	MES1 and WJM
Moderate (50 °C to 60 °C)	Sulfobacillus Thermosulfidooxidans	TH1
	Acidophilus Sulfulbacillus Yellowstonensis	YTF1
	Mixed culture	MOT6
Extreme thermophile (>70 °C)	Acidobrierley	DSM 1651

The above mentioned microorganisms possess certain unique characteristics as compared to other microorganisms, making them the best candidates within the bioleaching industry. Their physical characteristics are as follows: 1) As for the *Thiobacillus* species, these are rod-shaped, gram-negative, non-spore forming and are mesophilic except only for the same kind of species known as the *Thermophillic thiobacilli* which grows at high temperatures (Buchanan &Gibbons, 1974). 2) The *Sulfobacillus* species are gram-positive and rod-shaped with round or tapered ends. This particular species grow at high temperatures levels.3) As for *Acidianus species*, these are spherical in shape with tetrahedrons, with pyramid or saucer shaped lobes (Karavaiko and Lobyreva, 1994). 4) Finally, the *Leptospirillium ferriphelium*are spherical in shape, on-spores forming and gram-negative (Blake Ii *et al.*, 1993). For further details on microorganisms involved in the bioleaching operations refer to Ojumu *et al*. (2006).However, it must be noted that these acidophilic microorganisms are divided into three subgroups; mesophiles, moderate thermo acidophiles, and the extreme thermo acidophiles, depending on their tolerance to the temperature, as shown in Table 2.1.

2.3.1 Mesophiles

The Mesophile species grow best at prevailing room temperatures within the range of 25°C to 40°C. The most used strain within the mesophilic family is the *Thiobacillus ferrooxidans* (Das *et al.*, 1999b). The optimum pH for the growth conditions of these mesophilic strains is between 1.5 and 2.5. However, *Leptospirilium ferriphilium,* a mesophile, is also widely used in the

continuous bioleaching process. It has optimum temperature conditions of 38.6°C (Franzmann *et al.*, 2005), can survive at very low pH values, and can withstand the high concentrations of ferric-iron within the bioleaching solution (Coram and Rawlings, 2002). As such, its characteristics can be compared to the *Leptospirilium ferrooxidans*.

2.3.2 Moderate thermophiles

Moderate thermophiles, as implied by its name 'thermo', means energy or heat in engineering terms. Microbes in this category are able to grow at temperatures around 50°C to 60°C. In spite of having so many different strains of the thermophilic microorganism existing within our environment, the *Sulfobacillus thermosulfidooxidans,* which have the ability to oxidise both sulphur and iron, are widely used in the bioleaching industry (Lizama and Suzuki, 1989).It has been noted that bioleaching kinetics by the moderate thermophiles is quicker compared to mesophilic strains as a result of carrying the bioleaching technique at elevated temperature values.

2.3.3 Extreme thermophiles

Extreme thermophiles, as the name suggests, operate at elevated temperatures, as high as 80 °C. The most important among this strain is genius *Sulfolobus*. Quite a good number of *Sulfolobus* strains exists in the earth's geothermal, such as *S. acidocaldarious*, *S. sofataricus*, *S. brierley* and *S. ambioalous*. All of these strains possess the following characteristics:

- They grow in an anaerobic condition.
- They reduce the elemental sulphur.
- They grow aerobically by the oxidation of the sulphur.
- Their optimum growth is between the temperatures of 65 °C to 70 °C.
- They have the ability to oxidise the ferrous to ferric-iron as well as sulphur to sulphate.

2.4 Microbial characteristics

The commonly used microorganisms in the bioleaching technology are those that oxidise the ferrous-iron to ferric-iron, and sulphur compounds to elemental sulphur or to the sulphate compounds. These microorganisms have different optimum operating performances for different ranges of parameters such as temperature, pH, aeration rates and dissolved ions. Under these

operating conditions, they are capable of being used in different processes because of their common fundamental physiological features highlighted below:

- They are chemiolithoautotrophic and are able to use ferrous-iron or reduced sulphur inorganic compounds as the source of their substrate.
- They are acidophilic and most of them grow in the pH range of 1.5 to 2.0.
- They are able to use other electron acceptors rather than oxygen, ferric-iron for example.
- They are known to grow best in highly aerated solutions.
- They can fix carbon dioxide from the atoms, though at different efficiencies.

It is therefore imperative for effective recovery of desired metals from low grade mineral sulphide ore concentrates, that the microorganisms involved in the bioleaching process should at the very least perform quite near to their optimum performance. This can only be achieved if operating conditions for a particular microbial species involved in a particular bioleaching process are adhered to. If operating conditions are not met, the bioleaching process will be very slow or in fact, the entire process might be aborted.

2.5 Application of industrial bioleaching techniques

Many profitable industrial operations are now based on bioleaching technology. This process is used to recover metals such as copper, gold, uranium or cobalt from low grade mineral sulphide ores (Rawlings, 2005). This is with an exception of gold which bioleaching helps to be freed from the arsonpyrite matrixes prior to its extraction via the process of cyanization. Numerous applications have been designed and others are still being investigated. Examples of these designed applications are the stirred tanks, irrigated dump or heap as well as the in-situ process (Brierley C.L, 2005). Europe was the earliest continent quite active in the area of mineral bio-processing technology (Ehrlich, 2001). Recently South Africa, Australia and America have gained leadership in this particular area of mineral processing of the low grade sulphide mineral ore deposits (Brierley and Brierley, 2001). Bioleaching is widely used at industrial levels for the dissolution of the metallic sulphide ores which are the major mineral bearing ores for both precious and base metals. This process involves the bio-catalytic intervention produced by the metabolic activities of some iron and sulphur oxidising microorganisms, leading to high chemical degradation of the sulphide mineral ores.

The bioleaching process for treatment of precious and base metals is applied in two ways in industry: through the irrigated dump or through the heap system. These are controlled processes for the treatment of low grade sulphide mineral ores containing secondary minerals such as covellite, chalcopyrite and chalcocite (Palencia *et al.*, 2002),and have been in industrial operation for nearly forty years now, while the stirred tank leaching method has been in operation at industrial levels for the past twenty years.

2.5.1 The heap method

Heap bioleaching systems are operated by irrigating them from the top, either in a continuous or in an intermittent mode. This is done by allowing leaching solution to percolate through the crushed ore bed where it reacts with intended minerals. Heap bioleaching is significantly different as compared to other reactor configurations such as the tank or the in-situ systems. In the heap processes, the solids phase is stationary while the ratio of the solution to the solid phase is significantly low, usually in the range of 1:12 as compared to 8:1 in the tank systems. Mineral particles fed to heap systems are significantly larger and mostly composed of the inert matrix with only a small proportion of valuable mineral. However, particle reduction is achieved and the crushed particles of the ores are preconditioned and agglomerated through treatment with sulphuric acid or the irrigated solution. This is done to prevent separation of different particles of ore before the preparation of the pads in layers for the leaching process (Brierley and Brierley, 2001).

The other fundamental reason which can be attributed to the adoption of the heap process in industry as compared to the dump systems is the improved aeration environment whereby the air source is introduced from underneath the heaps. This situation has gotten some positive attributes in the area of microorganism activities, hence positive increase in the ferric-iron productions and the sulphuric acid (Brandl, 2008, Montealegre *et al.*, 1993, Rawlings *et al.*, 2003). This process leads to enhancement of recovery of the metals from sulphide mineral ores.

The flow of the materials within the heap is such that dissolved metals are transported with the flowing solution, with the assistance of the force of gravity, to the bottom of the heap bed. It is from this point that they are removed through the drainage system into the collection ponds as the pregnant leach solution (PLS).The desired metal is extracted from the PLS through a suitable technology by solvent extraction, cementation or adsorption, while the barred solution is

recycled to the top of the heap bed (Petersen and Dixon, 2007a, Petersen and Dixon, 2007b).The heap bioleaching technique is widely used in the bioleaching of both copper and refractory gold bearing ores (Rawlings *et al.*, 2003, Rawlings, 2002).The process of the material flow within the heap system is well-illustrated in Figure 2.3.

Heap bioleaching technique has been widely used in industry for recovery of low grade mineral sulphide ores, and incurs low operating costs. Despite these advantages, it has its shortcomings which are detrimental to the effective recovery of desired metal from low grade sulphide ores. One of these is the difficulty in obtaining a state of homogeny with regard to distribution of operating parameters, parameters which include pH, temperatures, uniform aeration rate, microorganism concentration, and dissolved metal ions. Secondly, it has not been easy to obtain a uniform gradient in terms of pH and temperature within the heap beds. These scenarios have led to low production of the ferric-iron by these microorganisms and have resulted in inefficiencies in the recovery of the desired metals.

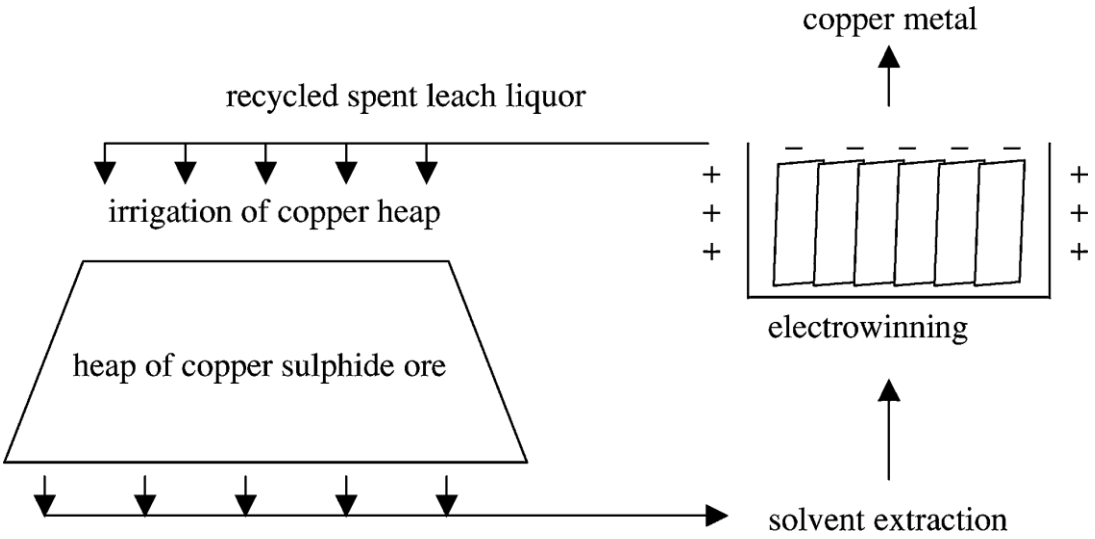

Figure 2.3: Schematic diagram showing the flow of the leaching liquor in bioleaching of low grade copper sulphide ore using the heap method (Rawlings, 2002).

2.5.2 The tank method

The tank bioleaching system is one technique in bioleaching which has been applied in industry for the past twenty years. As mentioned earlier, the operating parameters in this system can be controlled to near optimum conditions. This is in contrast to the heap system where it is much more difficult to control the operating parameters (such as the aeration rate, temperature and

pH). They operate such that feed is fed to the first tank and the overflow is channelled from tank to tank. This is done consecutively until the optimum achievement of the desired metal (Schnell, 1997), though this expensive to operate. This is shown in Figure 2.4. This issue of high expense is attributed to fact that they require long residence times, and the mineral feed needs to be finely milled concentrates. All these processes add to cost implications just like in conventional methods which use roasting and smelting. Due to this, this technique is restricted to the treatment of high grade ore concentrates. The tank method is mostly applied for treatment of the refractory gold arsenopyrite floatation concentrates. This leads to the gold being trapped in the mineral sulphide ore matrixes to be liberated by the action of biooxidation of the host mineral, through microbial actions. In the latter stage, the gold is recovered by the conventional method known as cyanidation. One example of the application of the stirred tank reactors in the bioleaching process is the Kasese Cobalt plant in Uganda. This is the first commercial bioleaching plant of the stirred tank type, even though the positive research gains were associated with the optimal operations. These were done in terms of managing the critical operating parameters within the tank bio-reactors. But they also have some drawbacks in the areas of operations, as they possess some limitations in terms of the amount of volume of mineral ores to process. As revealed in previous research studies (Acevedo, 2002, Brierley and Brierley, 2001, Van Aswegen *et al.*, 2007), above 11000 tons of gold concentrates are biologically oxidised in the reactors every year.

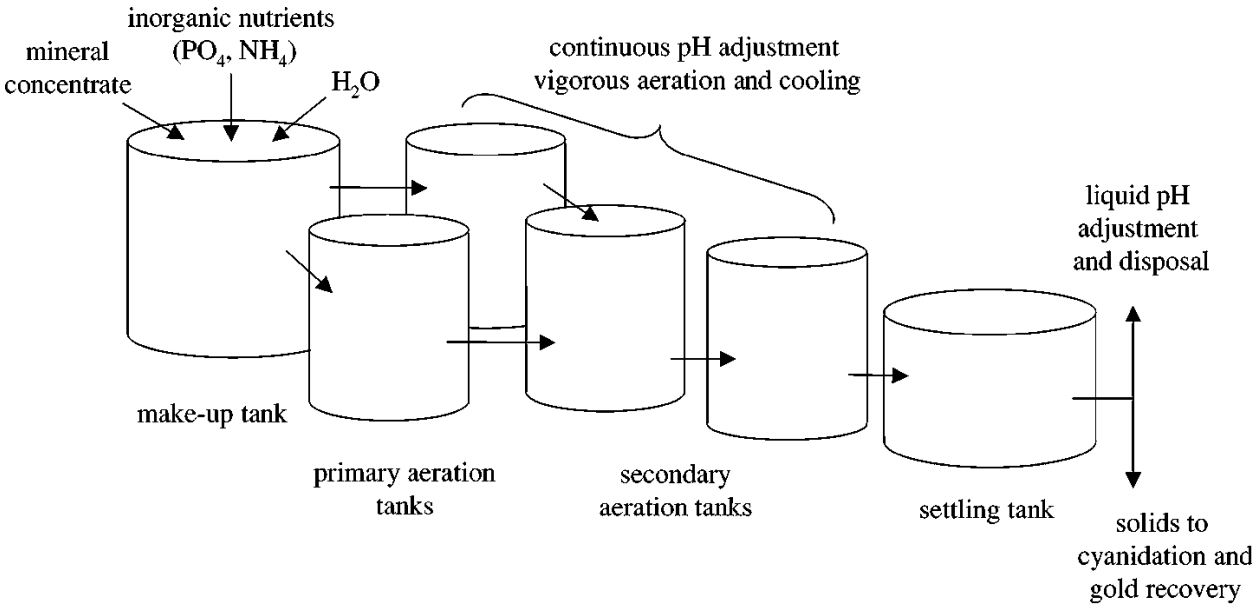

Figure 2.4: Schematic diagram showing the flow of materials between stirred tanks in the pre-treatment of gold from the arsenopyrite concentrate using the microorganisms in the stirred tank system (Rawlings, 2002)

2.5.3 The In-situ method

This is the bioleaching method that does not involve the actual physical movements of the low grade sulphide ores, as can be compared to previously mentioned techniques (see Figure 2.5). The hole containing the bioleaching solution is drilled within the mineral sulphide ore bed. It is widely used for the processing of low grade mineral sulphide ores in the areas where the use of the conventional mining process is of low economic value(Murr and Brierley, 1978, Schnell, 1997).

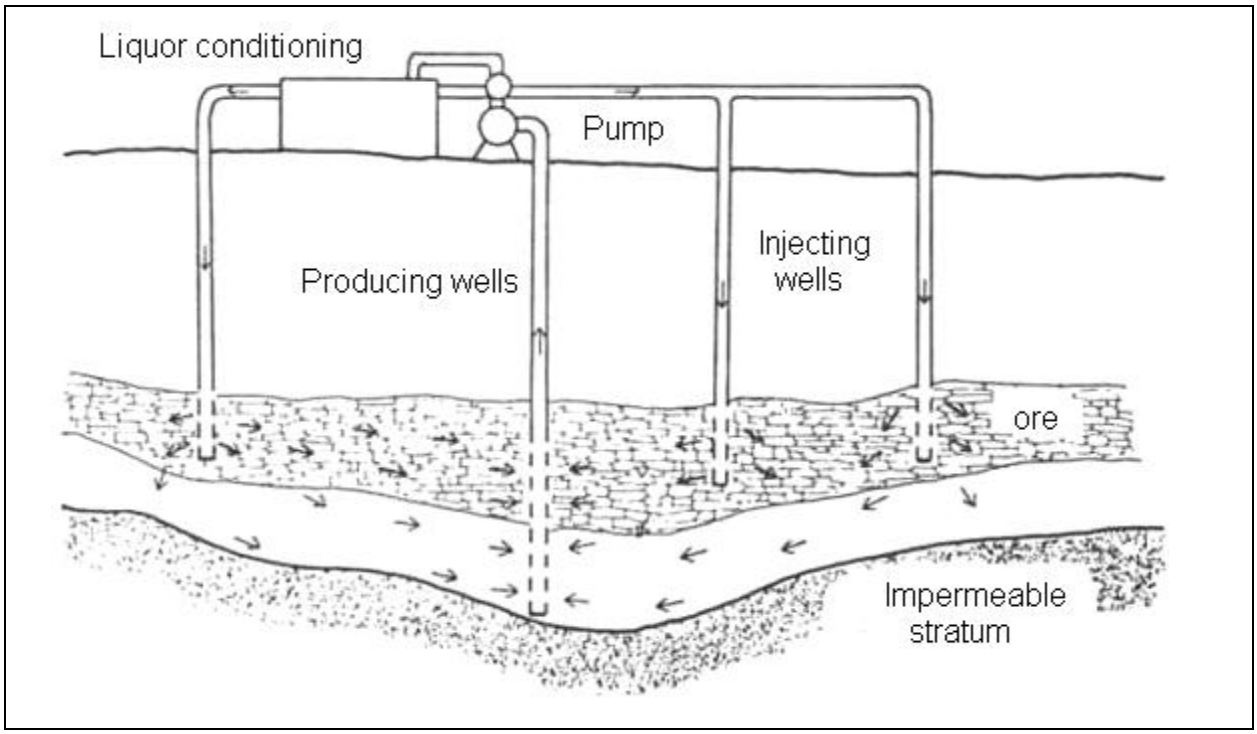

Figure 2.5: Schematic drawing showing bioleaching of the low grade mineral sulphide ore using the in-situ method

More research is being directed, even with other techniques, beyond the above mentioned techniques. This is to find other possible future ways of bioleaching other metal sulphide ores containing metals such as zinc and nickel. In this present study, much emphasis is placed on the heap system in comparison to the tank system.

2.6 The influence of pH on the ferric-iron precipitation (jarosite formation)

The relationship relating ferric-iron precipitations (jarosite) to pH rests on the basis that ferric-iron precipitations (jarosite formation) are formed at high pH values. This phenomenon occurs because at high pH values, the solubility of ferric-iron (Fe^{3+}) decreases. This statement is consistent with findings of previous studies (Sullivan *et al.*, 1988, Shum and Lavkulich, 1999) as such studies observed that iron and aluminium complexes dissolved at low pH values. Along the same lines, a previous research study by Elwood Madden *et al.* (2012) revealed that the high dissolution rate of jarosite was caused by high proton (H^+) substitution, or the dislocations within jarosite structures caused by varying the pH and the temperature. The same study also highlighted that with pH < 3.5, the dissolution rate of jarosite increases with increasing H^+ (decreasing the pH). This finding is consistent with the previous studies (Shum and Lavkulich,

1999, Sullivan *et al.*, 1988), that the solubility of ferric-iron decreases with increasing the pH. A similar study on the formation of jarosite is linked to the Eh-pH conditions. Likewise, this finding is consistent with previous studies, according to Edet *et al.* (2004). It was discovered in their research study that most of the mineral species, including Fe^{2+} at the prevailing Eh-pH conditions of pH (3.3 to 5.9) and Eh (7 to 158 mV), were immobilised through the precipitation of mineral phases. The study further revealed that iron precipitated in various iron-complex forms.

2.6.1 Chemistry of jarosite

The pH defines the structural chemistry of jarosite. The fluctuation in the pH or Eh of a particular system defines the solubility of various trace elements in growth medium solution, thereby determining the jarosite crystallographic structure. Hence, pH is very important in the determination of structural chemistry of jarosite. Figure 2.6 below shows the structural chemistry of one of the jarosite derivates which exist in the leaching systems.

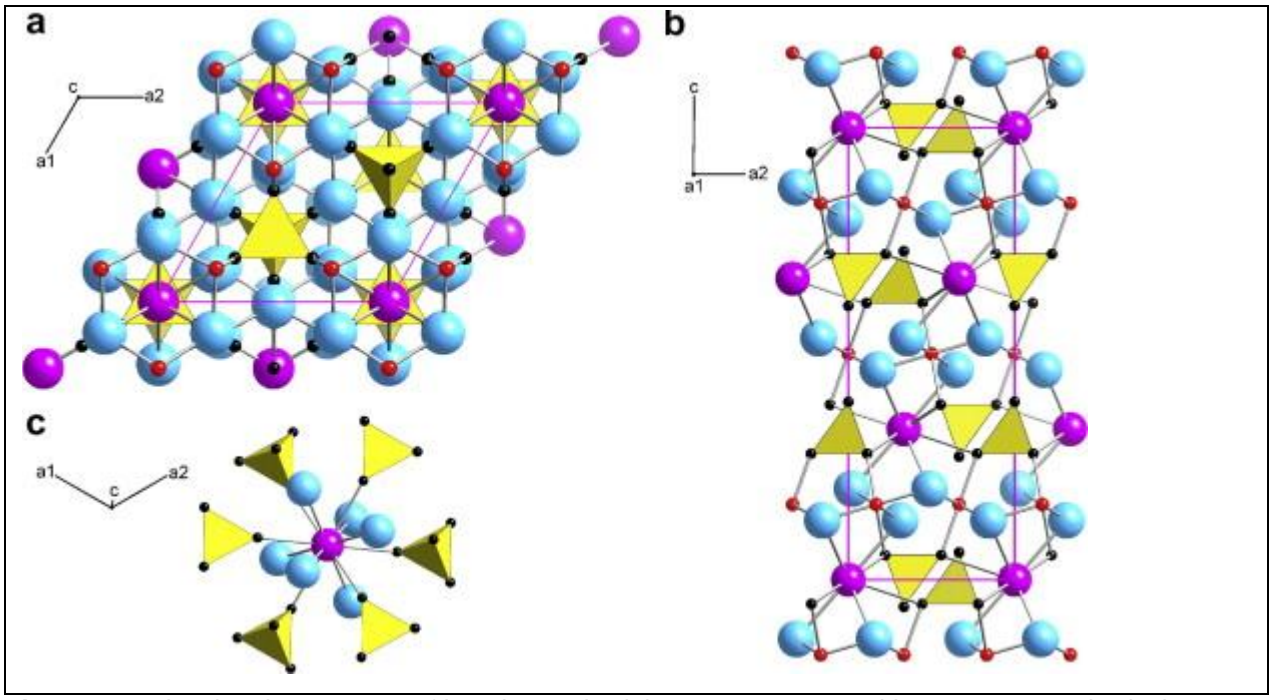

Figure 2.6: Showing the structure of jarosite; A site (K) atoms represented by magenta; B site (Fe) atoms represented by red; X site by yellow, black represents oxygen atoms and the blue spheres the hydroxyl group (Basciano and Peterson, 2007)

2.6.2 Stability of jarosite

The stability, chemistry and dissolution of jarosite depend upon the solution conditions. With much emphasis put on the pH, this is in line with the aim of this present research study. The stability of jarosite is stable under relatively oxidised conditions such that the system possesses the following conditions: that is, a redox potential of Eh > 400 mV, and having the pH range of 2 to 4 (Gasharova *et al.*, 2005). Therefore, it is very important to note that at higher pH conditions, the jarosite formed is considered metastable because of the other competitive precipitates such as goethite. Jarosite slowly hydrolyses to finely grained iron oxide (FeOOH). It is based on such findings that the solution conditions (pH) should be critically monitored to know whether indeed it is jarosite being produced or if it is other iron derivatives such as schwertmannite, ferrihydrate orgoethite. This phenomenon is well-explained according to Bigham *et al.* (1996) as listed in Table 2.2 showing different ferric-iron derivatives formed at different pH values.

Table 2.2: Chemical, physical and mineralogical properties of ferric precipitates at various pH values in the bioreactor (Adapted from Bigham*et al.,* 1996)

Sample	pH	Fe_o %	Fe_t	SO_4	Fe_o/Fe_t	Fe_t/S_t †	S.A. $m^2.g^{-1}$	Munsell	Color	Jt ‡ %	Sh‡	Gt ‡
x-13	2.3	22.2	34.5	25.1	0.64	2.4	109	0.1Y	6.9/6.6	52	48	ND
x-8a	2.5	40.8	44.2	13.6	0.92	6.1	220	9.3YR	6.7/7.4	11	89	ND
x-11	3.0	47.2	47.1	10.6	1.00	7.6	288	8.0YR	5.7/7.4	ND	100	ND
x-12	3.3	41.1	48.6	10.0	0.85	8.4	302	8.6YR	6.1/7.9	ND	88	12
x-15	3.6	26.0	50.6	8.1	0.51	10.8	256	8.4YR	5.4/7.1	ND	58	42

*Jt = Jarosite, Sh = Schwermannite, Gt = Geotheti

2.6.3 Dissolution of jarosite

Linear correlation has been established between reaction rates of the dissolution of the jarosite and the pH (Baron and Palmer, 1996). It has been observed that the reaction rate of the dissolution of the jarosite decreases with an increase in the pH values. A study conducted by Welch *et al.* (2008)reported that stoichiometry and the kinetics of the jarosite dissolution are a complex function of the solution composition. It has been observed that the dissolution of jarosite is slower in the presence of sulphuric acid as compared to other acids such as hydrochloric acid. This phenomenon is because of presence of sulphate ions (SO_4^{2-}). The SO_4^{2-} inhibits jarosite dissolution reactions because it is a reaction product. It was discovered that the rate of the dissolution of the jarosite was much faster in HCl as compared to the former. Therefore, the presence of the SO_4^{2-} hinders the dissolution of jarosite.

2.7 Review of previous studies relating pH with. jarosite formation

The pH of bioleaching solutions determines the availability of Fe^{3+}-iron within the leaching solutions, a key oxidising agent in the oxidative dissolution of the sulphide ores. Therefore, the availability of the Fe^{3+}-iron is greatly influenced by the pH of the leaching solution and is an important factor towards the formation of the jarosite and ferric-iron complexes within the bioleaching solutions, as well as influencing the rate of ferrous to ferric-iron conversion. In line with the past studies, this present study will review a number of previous research studies which are deemed to be consistent with the present study which investigate the effect of the pH on the formation of jarosite in a novel packed-column reactor. The extensive review of the previous studies on the effect of the pH on the formation of jarosite, and whether or not the accumulation of jarosite was the wanted or unwanted material, all led to the idea of linking the influence of the pH to the formation of jarosite in various bioreactor configurations used in those research studies.

A research study on the effect of pH on the formation of jarosite within the bioleaching process was conducted by Liu *et al.* (2009) who noted that at the pH of 2.3 and over, the inhibition of the microbial oxidation activity was profound. This phenomenon was attributed to formation of a layer of precipitation on the microorganisms which hindered the diffusion of protons (H^+). While investigating the formation of jarosite at the pH values of 1.58, 1.63, 1.69, 1.73, 1.79 and 1.99, the researchers discovered that as the pH of the system increased so did the jarosite formation. It was noted, that particular research study was based on the investigation of the effect of the initial pH, the ferrous-iron concentration and the applied potential using cultivated culture of the 9K medium using the *Thiobacillus ferrooxidan*. However, for the sake of this present study in comparison to this previous study, emphasis was directed only on the effect of the initial pH. Their results have further substantiated just how crucial a role the pH of a particular bioleaching system plays with respect to the formation of jarosite.

Several previous research studies (Asta *et al.*, 2009, Daoud and Karamanev, 2006, Jönsson *et al.*, 2006, Webster *et al.*, 1998) also reported on how jarosite production was used to enhance the removal of the hazardous elements from various systems. And the results obtained have shown that the quantities removed were directly proportional to the pH of the operating system. It is also noted that pH defines the amount of jarosite formed in the due process determining the amount of the hazardous element to be removed. For example the removal of fluoride during the leaching of the pyrite is now possible because of the formation of the jarosite. This was

made possible because the ionic radii of the hydroxide and the fluoride ions are closely related structure wise, with each having radii of size of r (OH^-) = 0.121 nm and 0.117 nm, respectively, for the tetrahedral coordination (Shannon, 1976). It is for this reason that the exchange between these ions was possible. It was based on this factor that the researchers in that particular study discovered that pH played a significant role in the successful mixture of the jarosite and fluoride ion concentrations within the bioleaching system. It was discovered that the amount of fluoride ions absorbed by jarosite increased with an increase in the pH and it was further observed that the concentration of the fluoride within the jarosite structure decreased at low pH values, resulting in low fluoride concentration. Essentially, they discovered that pH played an important role in the generation of jarosite as well as in the reduction of the fluoride concentration within the system. For further information, refer to Gunneriusson *et al.* (2009).

Research conducted to investigate the recovery of the sulphate ions ($S0_4^{2-}$)from the acid mine drainage by Rose and Elliot (2000), it was discovered that $S0_4^{2-}$recovery from the Fe^{3+} precipitates increased with an increase in the pH values. Therefore, this result was further justification demonstrating the role of pH in the recovery of sulphate ions from acid mine drainage. That research study also revealed the control of thalium from the zinc residue, a very dangerous material. This was possible because the pH and the jarosite formation were used to remove thalium. It was earlier mentioned that the formation of jarosite has been used as a mechanism to remove dangerous materials from the metals or just to eliminate unwanted materials from the process system.

A research study conducted by Dutrizac and Hardy (1997) with regard to the effect of pH on the jarosite formation further demonstrated the importance of pH on this particular scenario. The objective of their study was to remove thiocynatate which was embedded within the jarosite structure. Therefore, its removal depended on the quantity of jarosite formed. This particular study revealed the impact of varying the pH of the solution from 2.0 to 0.6. It was noted that from the pH of 1.6, the amount of jarosite declined to zero at the pH of 0.6. Their outcome was also in line with what was discussed in section 2.6 on the role of pH with respect to the jarosite formation.

To substantiate the role of the pH in relationship to the formation of jarosite, a study conducted by Arslan and Arslan (2003) also revealed that the precipitation of the iron was controlled by both the thermodynamics and the kinetics factors taking place within the iron solutions as well

as in the precipitates. It was observed in that study that pH was one of the key factors governing the formation of jarosite.

In order to fully comprehend the scope of this present research study, more reviews pertaining to the effect of pH on the formation of jarosite were evaluated so as to fully understand the main objective of this present research study. Somewhat recently, a study was conducted by Changqiu *et al*. (2006) on the solution conditions for the formation of the ammoniojarosite with the aim of the removal of the iron in the wet metallurgical industry. That particular study was conducted at a constant temperature of 90°C and within a pH range of 1.2 to 3.1. It was observed that the formation of the ammoniojarosite was enhanced as the pH of the system increased within the range stated above. Therefore, it was concluded that the pH played a central role with regard to the increased formation of ammoniojarosite. These results further substantiate the role of pH with respect to the formation of jarosite. In the same vein, a study was conducted by Pina *et al* .(2005) on the effect of the ferrous and ferric-iron on the sphalerite (ZnS) bioleaching using the *Acidibacillus* species. Their outcome showed that the pH range of 1.75 to 2.00 gave maximum zinc dissolution attributed to the higher presence of the Fe^{3+}-iron within this pH range. However, at the higher pH of 2.25 and above, there was a reduction in the ZnS dissolution due to the formation of jarosite at this elevated pH range. This situation led to the depletion of the main oxidant, $Fe^{3+,}$, as well as the microbial population which were adhered onto the jarosite matrixes within the leaching solution. That particular research study revealed the negative repercussions of the formation of jarosite on the chemical oxidative dissolution of the sulphide mineral ores, through depriving that particular system of the much needed Fe^{3+}-iron, the main oxidising agent in chemical sulphide mineral ore dissolution.

A research study was conducted by Wang and Zhou (2002) on the recovery of cobalt from zinc residues. The jarosite method was applied, involving the entrapment of cobalt on jarosite. This was done to free the cobalt from the zinc residues. Hence, it was discovered that at the pH <1, there was no jarosite formation, while at the pH of 1 the formation of jarosite had commenced, though slowly. But at the pH of 2.25, the jarosite precipitation had increased and had proven difficult to filter. This phenomenon led to the loss of the cobalt through co-precipitation and the problem escalated at the pH of 3.0 resulting in even greater cobalt losses. The obtained result is consistent with previous studies presented in this literature review on the effects of pH on the formation of jarosite.

It is important to note that pH was successfully used in the previous studies, as revealed above, to control the amount of jarosite formed. This is to enhance the microbial populations and thereby increase their microbial activities or to remove unwanted materials from desired processes. However, suffice it to say, most of these reviewed research studies were either conducted in a batch or continuous mode. These research studies were conducted in different bioreactor configurations and using different bacterial strains in contrast to the present study which is conducted in a novel packed-column simulating the real solution flow dynamics of a typical heap bioleaching system. The fundamental objective of the present study, then, is not to use jarosite to eliminate the unwanted materials but to control its formation through the manipulation of the feed pH. This is to improve the ferrous-iron biooxidation kinetics in the packed-column bioreactors, as elaborated earlier in this literature review.

2.8 The synthesis of jarosite

It is very important firstly to understand how jarosite is produced from a chemical reaction occurring simultaneously alongside other reactions, expressed by the Equations 2.4, 2.5 and 2.6 respectively. The jarosite is just like the alunite as both are isostructural hydrous sulphate minerals belonging to the alunite group having a general formula of $AB_3(SO_4)_2(OH)_6$ (Burger *et al.*, 2009). The B (cation) hasan octahedral coordination and normally is Fe^{3+};in this particular case it is the jarosite subgroup, while if it is Al^{3+}then it is alunite group. The A (cation) poses a coordination of less than or equal to (≤) 9. It is usually equated to K^+ (potassium), NH_4^+ (ammonium), Ag^+ (silver), Na^+ (sodium) or H_3O^+ (hydronium) (Jensen and Webb, 1995). For the purpose of this study, emphasis is placed on the jarosite subgroup as compared to the other. However, it must be noted that different kinds of jarosites do exist from various reactions as by-products, as shown in Table 2.3. The main parameters which affect the jarosite formation are the pH of the operating solution, the ionic composition and the concentration of the medium. During the biooxidation of the ferrous-iron, the hydrogen (H^+) ions are constantly consumed, increasing the pH of the system. However, this effect is counteracted by the hydrolysis of the Fe^{3+}-iron as shown below:

$$Fe^{3+} + H_2O \rightarrow FeOH_2 + H^+ \quad 2.4$$

$$Fe^{3+} + 2H_2O \rightarrow Fe(OH)_2 + 2H^+ \quad 2.5$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \quad 2.6$$

There is a reaction competitive to the hydrolysis reaction shown by the equations above giving products of ferric hydroxylsulphates, commonly known as jarosite, which has a chemical formula as shown by Equation 2.7:

$$AFe_3(SO_4)_2(OH)_6 + 8H^+ \qquad 2.7$$

Whereby the unknown (A) in the equation represents a good number of different metals such as K^+, NH_4^+, Ag^+, Na^+ or H_3O^+ depending on their individual concentrations within the growth medium. This product compound is commonly known as jarosite, as earlier mentioned in this literature. Jarosite is chemically formed in the reaction shown by Equation 2.8:

$$3Fe^{3+} + X^+ + 2HSO_4 + 6H_2O \rightarrow XFe_3(SO_4)_2(OH)_6 + 8H^+ \qquad 2.8$$

Since the predominant metal ions in the growth medium are the ammonium ions or potassium. Therefore, the jarosite formed constitute the ammonium or the potassium ions, thereby forming the NH_4-jarosite or the K-jarosite. The crystal structure of jarosite is shown by Figure 2.6 while Figure 2.7 shows the actual jarosite from smelter plant.

Table 2.3: Mineral of the jarosite subgroup and the sythenticanalogs (Dutrizac and Jambor, 2000).

Formula	Mineral name	Synthetic equivalent
$KFe_3(SO4)_2(OH)_6$	Jarosite	potassium jarosite
$NaFe_3(SO4)_2(OH)_6$	Natrojarosite	sodium jarosite
$RbFe_3(SO4)_2(OH)_6$	no mineral equivalent	rubidium jarosite
$AgFe_3(SO4)_2(OH)_6$	Argentojarosite	silver jarosite
$(NH_4)Fe_3(SO4)_2(OH)_6$	Ammoniojarosite	ammonium jarosite
$TlFe_3(SO4)_2(OH)_6$	Dorallcharite	thallium jarosite
$PbFe_6(SO4)_4(OH)_{12}$	Plumbojarosite	lead jarosite
$HgFe_6(SO4)_4(OH)_{12}$	No mineral equivalent	mercury jarosite
Pb $(Fe,CU)_3(SO4)_2(OH,H2O)_6$	Beavirite	lead copper jarosite
$(H_3O)Fe_3(SO4)_2(OH)_6$	hydronium jarosite	hydronium jarosite (Synth)

Figure 2.7: Jarosite produced from the Debari Zinc Smelter plant, Ranjasthan, India (Pappu *et al.*, 2006).

Various supporting mediums have been used with the intention of enhancing the bio-film formation so as to promote microbial growth as well as to protect the organisms from inhibitory compounds. The selection of the packing media as carriers, however, must be done carefully to minimise the adverse effects that can result from these materials and at the same time maximise the positive outcome of the bioreactors.

The packing materials play an integral role within the bioleaching systems; they retain the microorganisms on them, improving on the ferrous-iron oxidation rate. Extensive research studies have been conducted on different types of carrier materials with the goal of improving their performances. For details on the role of the different kinds of the carrier materials within the bioleaching industry refer to this literature by various authors: on poly(vinyl alcohol) (PVA), cropgel carries, ion-exchange resin, activated carbon (Grishin and Tuovinen, 1988); low grade ore (Carranza and Garcia, 1990); nickel alloy fiber (Gómez *et al.*, 2000); and siliceous stone particles (Mazuelos *et al.*, 1999). These studies revealed that Fe^{3+} precipitates were entrapped within the carrier material matrixes and enhanced the microbial populations within the bioleaching system. For example, Jensen and Webb (1995) observed that the reduction in the iron oxidation rate with the activated carbon as a carrier material was caused because of the

ferric-iron precipitation at the pH value of 2.0. This finding was further substantiated by Ozkaya *et al.* (2007b) using the same carrier material but in a different bioreactor, a fluidised bed reactor. At the pH of 2.5, ferric-iron precipitation was recorded.

The previous studies (Cavazza *et al.*, 1995, Gómez *et al.*, 2000, Grishin and Tuovinen, 1988, Jensen and Webb, 1995, Ozkaya *et al.*, 2007a), thus far have indicated that the carrier materials in those particular studies could not prevent the formation of jarosite. Based on this conclusion, glass balls have been chosen for use in this present study because of their smooth surface, as the smooth surface may limit the formation of the jarosite.

2.8.1 The importance of the jarosite formation

The formation of jarosite is considered an important aspect with regard to different applications. However, in the present research study, it is considered an undesirable material due to its negative effects with respect to depletion of the availability of the ferric-iron (the main oxidising agent). However, it has to be noted that to some extent, the presence of jarosite within the bioleaching industry can also be beneficial (Armentia and Webb, 1992). It has been observed that the jarosite precipitate could directly participate in the bio-film formation in the production of ferric-iron which is of great industrial importance. This discovery was also echoed by other authors (Grishin and Tuovinen, 1988, Kinnunen and Puhakka, 2004, Toro *et al.*, 1988) who reviewed that jarosite plays a very important role in the formation and the stability of the bio-film. A study by Karamanev (1991) reported that the bio-film is principally comprised of jarosite attached to the solid support and cells adsorbed on the pores; this Karamanev model is referred to up to date. In a more recent study (Van der Meer *et al.*, 2007), after analysing different types of carrier materials such as activated carbon, diamaceous earth and aluminium oxide in the fluidised bed bioreactors, using electron microscopy coupled with energy dispersive spectroscopy, found that all of the materials covered with the jarosite precipitates had the bacteria covered on the jarosite areas.

Basically, a bio-film consists of an aggregate of the immobilised cell attached to the inert support material. The inert support material can be composed of glass balls, activated carbon, ion exchanger resin, sand, polysthyrene or polyurethane, as mentioned under Section 2.8 However, some studies have indicated that inert solid particles which are used as supporting materials in some reactors inhibit ferrous-iron oxidation. Other studies attested to the

importance of formation of jarosite to some extent. For example, Pogliani and Donati (2000) reported that the number of attached cells to the supporting materials was enhanced due to the presence of jarosite.

2.8.2 The negative effects of jarosite within bioleaching processes

A previous study by Du Plessis *et al*. (2007) found that pH greater than 2.0 affects the population of microorganisms. This phenomenon could be attributed to the scenario whereby new Fe^{3+}-iron deposits cover the previously immobilised bacteria and hence prevent them from accessing the important nutrients. On the other hand, a gradual increase of bacteria seriously affects the nutrients availability, especially oxygen and carbon dioxide, thereby retarding their growth. Van Aswegen *et al.* (2007) observed that within the pH range of 2 to 3, the formation of the ferric-iron precipitation (jarosite) is enhanced, decreasing the concentration of the Fe^{3}-iron in solution. This negatively affects chemical oxidative dissolution of the low grade sulphide mineral ores. Therefore, a high pH atmosphere within the bioleaching environment not only negatively affects the microbial activities but also reduces the smooth flow of the leaching reagents such as the ferric-iron, oxygen and carbon dioxide supply within the bioleaching process; this scenario is caused by the presence of ferric-iron precipitates (jarosite).

Several of the previous research studies on the effect of the pH on the formation of the jarosite and its negative impacts on the kinetics of ferrous-iron biooxidation have been intensively investigated in various bioreactor configurations such as the tank, CSTR, packed-bed reactor and flooded bed column bioreactors. However, this present study uses a novel packed-bed column bioreactor with its operation in such a way that simulates the real solution flow dynamics of heap bioleaching system. The variations of the pH in any of the bioreactor configurations, either operating in a batch or continuous mode, play a critical role in the management and the control of the jarosite. The changes in the pH values result from the simultaneous consumption of the (H^{+}) in the oxidation of the Fe^{2+} and the release of (H^{+}) related to the Fe^{3+} precipitates. According to Lui *et* (2009) their research study demonstrated that the formation of the jarosite was largely detrimental to the effectiveness of the microbial oxidation activities at the pH of 2.3. That research outcome demonstrated that pH led to the formation of the layer of the precipitation on the microbial surface which created diffusion barriers in terms of the flow of critical nutrients as well as the flow of the protons (H^{+}) to the active site on both the microorganisms and the mineral ore sulphides. The following are the negative implications of

jarosite formation in the context of the bioleach heap:

- It deprives the system of the much needed ferric-iron which is the engine of chemical oxidative reactions in the dissolution process of the mineral sulphides (Rawlings, 2002).
- It leads to kinetics barriers by hindering the diffusion of the reactants and the products through the precipitations zones (Jensen and Webb, 1995).
- It leads to the blockage of the pumps, valves, pipes and any other periphery equipment in the processing set-up.
- Its attaches its precipitations, which are crystal-like structures, to the surface of the sulphide ores resulting in a compact thin layer of membrane which hinders the transfer of the substrate and the metabolites.
- It affects the respiration process of the bacteria by denying them full access to the available nutrients due to hindrance caused by the jarosite precipitates.
- It occupies the available sites on mineral surfaces thereby hindering the bacteria from adsorption on the mineral deposits and in this process hindering the effective biooxidation process.
- It limits, due to Jarosite formation in the immobilisation matrices, the amount of biomass retention because ferric-iron deposits occupy most of the available space (Jensen and Webb, 1995).
- It clogs up the heap bed systems causing partial or in some cases total obstruction of the channels of passage of the liquid and gas flows within the heap bed systems (J. Daoud, 2005, Jensen and Webb, 1995).

2.8.3 The solution chemistry of iron with respect to iron complexes (jarosite) in bioleaching solution

Iron is the fourth most dominating metal in the earth's crust and the second most abundantly occurring metal after aluminium. This explains why most jarosite formed is either aluminium or iron based. Iron is distinct in colour and has a complex nature. It can exist under three different oxidation states, so it forms three strong complexes with different unique properties, hence its influential role within the bio-hydrometallurgical industry.

It is noted that iron forms different kinds of complexes with both sulphate and hydrogen ions, with the formation of the jarosite being non-exceptional. Among the notable complexes are Fe

SO_4^{+}, $FeHSO_4^{+}$, $FeOH^{2+}$, $Fe_2(OH)_2^{4+}$, and $Fe(OH)_2^{2+}$. However, the iron ions will not form complexes whenever the solution is infinitely diluted. This statement is in agreement with the research study conducted by Huberts (1994) from which he reported that the percentage of the free ferrous and ferric-iron in the solution containing 9 $g.L^{-1}$ total iron concentrations was estimated to be 50 and 1%, respectively, while the rest was in the complex form. It has to be noted that these proportions depend upon key important factors such as the pH, sulphate concentration, ionic strength as well as the temperature of the solution. The stability of the iron species depends upon their corresponding equilibrium constant. Since the bioleaching solutions are bound to have high concentrations of dissolved ions, as observed by Petersen and Dixon (2004),the interactions of these ions (chemical species at certain bioleaching conditions) result in the high possibility of ion paring. This situation explains why both ferrous-iron and the product ferric-iron not only exist as hexa-hydrated complexes but also in different forms of complex ions, bonding with different ions such as the sulphate ions (SO_4^{2-}) in the bioleaching solution.

2.8.4 The estimation of the ionic species (iron in the solution)

In recent years, a number of industrial software programmes such as the Visual Minteq and HSC chemistry have been developed in the quest of analysing different species existing in different processes such as in agricultural soils and in the bioleaching process in the bio-hydrometallurgy. In bio-hydrometallurgy, these programmes have helped in the estimation of ionic species and their compositions in a simple simulated typical bioleaching solution as well as the theoretical details involved. This computation gives the distribution of the ionic species at certain operating conditions of a particular bioleaching system. Take, for example, a solution containing 5 $g{\cdot}L^{-1}$ of the total iron with a ferric to ferrous-iron ratio of 650mV at a pH 1.3, using the Visual Minteq programme, with results shown in Table 2.4. The data shows that different types of ionic species exist within the bioleaching solutions, and that iron exists as complex species within the typical bioleaching solution. It is noted that most is associated with sulphate ions (SO_4^{2-}) (Barrett *et al.*, 1993).

Table 2.4: Concentrations of iron-ions speciation in the bioleaching leaching solution, of 5 g.L^{-1} of total Fe^{2+} at ferric to ferrous iron ratio of 650 mV at a pH of 1.3. Calculated using Visual Minteq

Ferric iron complex	Concentration	Activity	Log activity
$Fe(OH)_2$ (aq)	6.563 X 10^{-19}	6.560 X 10^{-19}	-18.183
$Fe(OH)^{2+}$	5.470 X 10^{-07}	5.470 X 10^{-07}	-6.262
$Fe(OH)^{3-}$	4.701 X 10^{-28}	4.710 X 10^{-28}	-27.328
$Fe(OH)_3$ (aq)	1.189 X 10^{-14}	1.190 X 10^{-14}	-13.925
$Fe(OH)^{4-}$	1.918 X 10^{-20}	1.920 X 10^{-20}	-19.717
$Fe(SO4)^{2-}$	4.816 X 10^{-06}	4.820 X 10^{-06}	-5.317
Fe^{2+}	0.0627	0.0627	-1.203
Fe^{3+}	0.000398	0.000398	-3.400
$Fe_2(OH)_2^{4+}$	2.172 X 10^{-07}	2.170 X 10^{-07}	-6.663
$Fe_3(OH)_4^{5+}$	1.622 X10^{-11}	1.620 X 10^{-11}	-10.790
$Fe\ OH^{+}$	1.338 X 10^{-09}	1.340 X 10^{-09}	-8.874
$Fe\ OH^{2+}$	0.000118	0.000118	-3.928
Fe SO4 (aq)	0.00336	0.00337	-2.474
$Fe\ SO4^{+}$	0.00208	0.00208	-2.681
$SO4^{2-}$	0.000190	0.000190	-3.722

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The determination of the equilibrium constants for the above iron species involves mathematical expressions of some complex algebra, somewhat simplified by the use of simplified notations as revealed by Barret *et al.* (1993). These notations use such expressions as Fe (x,y) whereby x represents the number of the Fe^{3+} centres, and the y the number of the (OH) groups. While Fe (1,S), Fe (1,HS) and Fe (1, 2S) represent the complexes $[FeSO4]^{+}$, $[FeHSO_4]^{2+}$, and $[Fe(SO_4)_2]$, respectively. Therefore, the equilibrium constants of the following reactions are derived, as shown below by Equations 2.12, 2.13 and 2.14:

$Fe\ (1, 0) + SO_4^{2-} \Leftrightarrow [Fe\ SO_4]^{+}$ 2.9

$Fe\ (1, 0) + HSO_4^{-} \Leftrightarrow [FeHSO_4]^{+}$ 2.10

$Fe\ (1, 0) + SO_4^{2-} \Leftrightarrow [Fe\ (SO_4)]^{2-}$ 2.11

$K1s = [Fe\ (1,s)] / [Fe\ (1,0)][SO_4^{2-}]$ 2.12

$KHS = [Fe\ (1,HS)] / [Fe(1,0)][HSO_4^{-}]$ 2.13

$K2s = [Fe\ (1,2S)] / [Fe\ (1,S)][SO_4^{2-}]$ 2.14

2.9 The microbial ferrous-iron oxidation

The oxidation of ferrous iron is cardinal to the respiratory mechanisms of the iron oxidising microorganisms. The high yield in the respiratory mechanism determines the efficiency of the bioleaching process. It is for this reason that it is the main driving force in metal recovery from the sulphide minerals in the bio-hydrometallurgy industry. This phenomenon was first proposed by Mitchell (1996) who investigated this mechanism and recorded that it is a chemiosmotic mechanism which involves the transfer of electrons across an energy transducing membrane to the production of energy rich molecules. Only one mole of electrons is released during the conversion of ferrous-iron to ferric-iron. Therefore, a large amount of ferrous-iron is needed for the adequate amount of energy to be released. The research (Mitchell, 1966)was also applied by Ingledew (1982) to determine the bio-energies of the growth of *Acidothiobacillus ferrooxidans* on the ferrous-iron. It is assumed that this mechanism holds for both bacteria and archea, even though the two microbes have different cell structures which could mean different metabolic pathways.

The microorganisms obtain their energies for the metabolic activities from the conversion of ferrous to ferric-iron and elemental sulphur and the sulphur compounds to sulphate compounds. It should be noted that cell pH of these microorganisms, even for *Acidothiobacillus ferrooxidans,* is maintained around the neutral point of 6.5 to 7.0, even in their highly acidic environment. The metabolic activities of these microorganisms involve half-cell reactions shown by Equations 2.15 and 2.16 and oxidation of ferrous to ferric-iron and the acceptance of the electrons by the oxygen, as shown by Equation 2.17 below. These microorganisms conserve some of the energy obtained from the redox potential reactions and use it to convert inorganic carbon from carbon dioxide into biomass.

$$2Fe^{2+} \rightarrow 2Fe^{3+} + 2e^{-} \; E_h = +770mV \qquad 2.15$$

$$2e^{-} + 0.5O_2 + 2H^{+} \rightarrow H_2O \; E_h = -1120mV \qquad 2.16$$

The overall reaction being:

$$2Fe^{2+} + 0.5O_2 + 2H^{+} \rightarrow 2Fe^{3+} + H_2O + Energy, \quad -350mV \qquad 2.17$$

The overall reaction results in water (H_2O), ferric-iron (Fe^{3+}) and energy for the carbon dioxide (CO_2) fixation and cell growth, as reported by Ingledew (1982). The process of oxidation of

ferrous-iron takes place in the periplasmic space of the cells and is well-catalysed by the enzyme complex within that system. These enzymes are composed of rusticyanin, cytochrome c and a c4 type cytochrome (Cavazza *et al.*, 1995). This process involves quite a number of mechanism steps, such as the electron transfer across the cell membrane which promotes the reduction of oxygen. This process requires the availability of oxygen. The required oxygen is transported inside the cell from an outside environment, as shown in the below diagram in Figure 2.8.

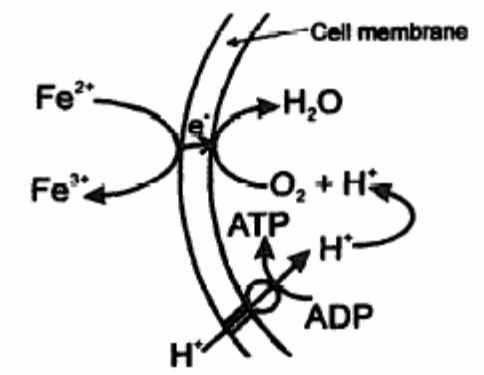


Figure 2.8: Schematic representation of the proton circuit and ferrous iron oxidation by *At. ferrooxidans*. (Extracted from Crundwell, 1997)

The particular catalytic conversion of ADP to ATP, which is an energy carrier within the cell physiology, is achieved by the difference in the cytomplasmic pH and the bulk solution. This ATP is utilised for the oxygen reduction to form water. This phenomenon, which has been discussed elsewhere (Ingledew, 1982, Rossi, 1990) is based on such revelations that conclusions have been deduced that ferrous-iron oxidation is an energy generation process for the bacteria and forms the basis for their survival.

The mechanism of iron oxidation has been studied extensively for the bacterium *At. ferrooxidans,* as illustrated by the diagram in Figure 2.9. This particular bacterium contains a rus operon responsible for the electron transport chain which is responsible for the oxidation of ferrous-iron. The operon is composed of genes of aa3-type cytochrome oxidase, a high molecular weight outer membrane located cytochrome-c (Cyc2), a C4- type cytochrome and a low molecular weight copper containing protein rusticyanin. Rusticyanin is accredited to the task of functioning like an electron reservoir by readily channelling the available electrons on the outer membrane down the respiratory pathway. It also serves the task of being a redox buffer in

the sense that it ensures that the outer membrane CyC2, which is an electron acceptor, remains in a fully oxidised state. In this way, it is ready to accommodate electrons from ferrous-iron even amidst short-term fluctuation of oxygen. For further details refer to Rawlings (2005).

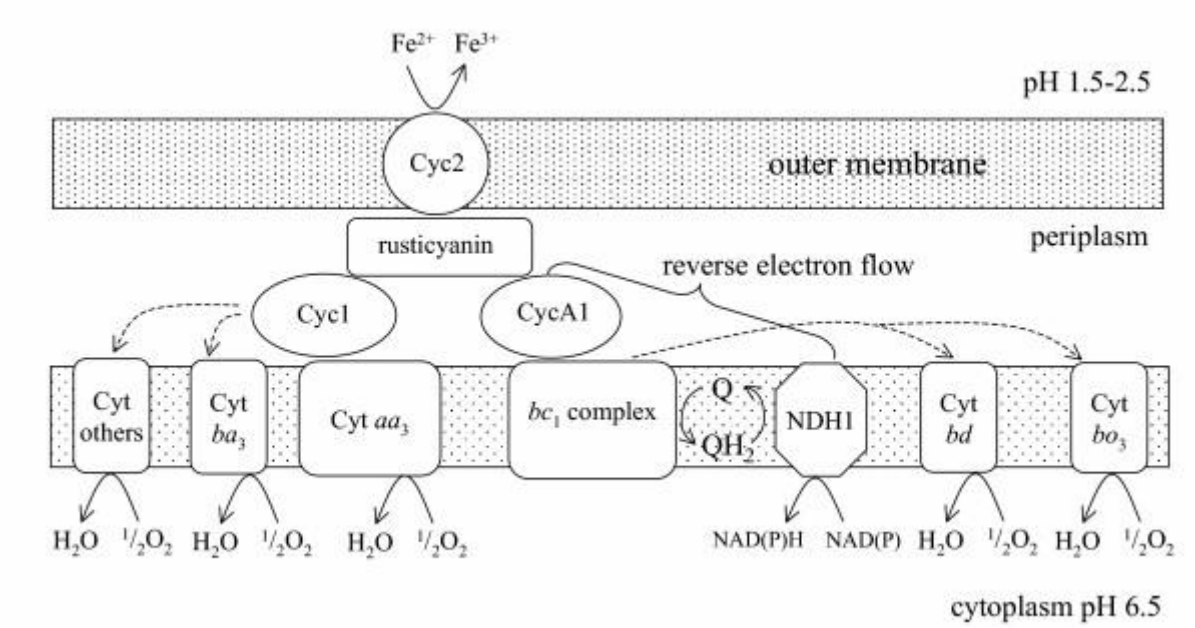


Figure2.9: A model of iron oxidation electron transport pathway of *At.ferroxidans* showing electron transport generating the proton gradient and reverse electron transport for NADH formation (Adapted from Rawlings, 2005).

It has to be noted that the mechanisms involving *L. ferrooxidan* (presumably *L. ferriphilum)* could be substantially different in comparison to *At. ferrooxidan,* which is best studied for the iron oxidation. *At. ferrooxidan* was capable of growth in ferrous-iron at redox potential of up to +800 mV while *L. ferrooxidans* was closer to 950 mV. However, *At. ferroxidan* outgrows *L. ferrooxidan* at high ratios of ferrous to ferric, but *L. ferrooxidan* out-competes *At. ferroxidan*s because of high ferric-iron concentrations which inhibit its performance. Ferrous-iron oxidation is also needed to produce reduced NAD (P) + responsible for carbon dioxide (CO_2) fixation and other anabolic processes by the transfer of the electrons from Fe^{2+}.

2.10 The kinetics of microbial ferrous iron bio-oxidation

The kinetics of ferrous-iron, as shown by Equation 2.1, is a crucial aspect of the bioleaching process, defining the efficiency of the metal sulphides dissolution rates. The Fe^{3+} from that reaction is the main oxidising agent in the chemical oxidative dissolution of the sulphide mineral ores. Therefore, studies investigating different ways by which to improve kinetics of bioreactors

cannot be under-estimated. Extensive studies on the ferrous-iron biooxidation kinetics in different bioreactor configurations, using different microbial strains, have been conducted previously. This experimental research was conducted in either batch or continuous operational modes, for example, in a continuous system using different kinds of microbial strains such as *Acidithiobacillus ferooxidan* (Boon, 1996) or *Leptospirilium ferooxidans* (Breed *et al.*, 1999). More recently, oxidation kinetics has been studied in a novel packed-column bioreactor (Chowdhury, 2012).

Much success has been recorded with regard to achieving at least near optimum kinetics conditions in the CSTR bioreactors, as mentioned earlier in Section 2.5.1. However, much work still remains to be done on kinetics improvements within the heap bioleaching systems, especially as a result of its high demand as an alternative and less expensive way of exploiting low grade mineral sulphides.

It is very difficult to obtain a homogeneous pH gradient within the heap set up as mentioned earlier in this literature review (Pradhan *et al.*, 2008). Consequently, the variation of the pH causes operational problems within the heap system. The pH is one of the key factors which affect the kinetics (Ojumu *et al.*, 2006). High pH values, the generation of the ferric precipitates, is very prominent and thereby causes the kinetics barriers. It is paramount to note that within the bioleaching process, there are a good number of different sub-processes which take place in a sequential or parallel manner. The overall kinetics of these sub-processes determines the overall performance of the bioreactors for a wide range of different categories of minerals, microorganisms and different operating conditions .It is important to note that kinetics reactions derived from the microbial activities are the driving forces in the efficiency dissolution of the mineral sulphides and in the pre-treatment of the gold bearing arsenopyrite (Ubaldini *et al.*, 1997).

2.10.1 Parameters affecting the kinetics of the bioleaching process

There are numerous factors which determine the overall efficiency of the kinetics of bioleaching processes. The effective metal recovery from mineral sulphide ores depends on such factors. Beyond the required limits, these factors can potentially have a negative impact on the microorganisms chosen for a particular research process. Therefore, measures should always be taken to ensure that the appropriate operating conditions for specific microbial strains are

available for effective biooxidation reaction to take place. This scenario leads to successful metal recovery. Below are some of the main operating parameters which play an important role in the kinetics of the bioleaching process:

- Supply of oxygen
- Activity of microorganisms
- pH and Fe^{3+}-iron concentration
- Product layer.
- Bacterial concentration

2.10.2 Supply of oxygen

The microorganisms that carry out their metabolic activities, such as during the oxidation of Fe^{2+} and S°, and other sulphur compounds need a constant supply of oxygen. Karavaiko and Lobyreva (1994) stated that the solubility of O_2 in the water at 35°C is 8 $g.m^{-3}$ and it decreases with an increase in the ionic concentration in the solution. According to the stoichiometry proportions, ferrous-iron oxidation reactions by bacterium requires 0.07 g of O_2 per gram of Fe^{2+}-iron oxidised, and this amount cannot be readily available in solution because the solubility of O_2 in the water is very low. Consequently, extra O_2 has to be provided from external sources. These acidophilic microorganisms are obligate aerobes and low concentration of O_2 would impose constraints on the rate of oxidation by these microorganisms. The mineral sulphide dissolution rate, especially for copper solubilisation in the heap or dumps leaching systems, greatly depends on the availability of the O_2 gas. In an ideal situation, the heap systems are constructed in such a way that the air supply is pumped through the bottom of the heap bed, percolates up through the heap bed, and transcends horizontally before the same ascends vertically. The main driving force of the air inside the heaps is the difference in the air density. The difference in the air density is directly associated with the temperature distribution within the heap bioleaching system because of the exothermic nature of the sulphide minerals dissolution. Temperature gradient is always observed inside the heap. It is observed that the air convection inside heaps depends upon the following three factors: 1) heap heights 2) the irrigation rate and 3) heap permeability. In a large heap, the high solution flow rate favours a good air distribution inside the heap as the core temperature of the heap is high and the high flow rate would bring down the temperature as compared to the smaller heap system which will require a lower solution flow rate to retain good convection. A good number of mathematical models have been

developed with regard to the air distribution inside the heap, with much emphasis on the various leaching parameters including flow rate, temperature, permeability and ore minerals (Pantelis and Ritchie, 1991).

The biological dissolution of sulphide ore is specific to the particular mineral or the microorganism system. Consequently, after evaluating the overall kinetics with reference to the metal dissolution of iron as well as for sulphur oxidation, the total demand of O_2 in the system can be evaluated. In order to utilise O_2 optimally, there is need for proper design to be followed: that is the reactor, impeller, oxygen distribution and the bubble (Boogerd *et al.*, 1990, Hoffmann *et al.*, 1993).

2.10.3 The activities of microorganisms

The activities of the microorganisms such as *Thiobacillus* and any other acidophiles were indirectly measured in terms of Fe^{2+} and the S° oxidation rate, and this phenomenon differed from one particular strain to another (Bhattacharyya *et al.*, 1992, Mason *et al.*, 1987). The difference in the oxidation rate is due to many factors such as change of morphology (Silverman, 1967), the difference in the chemical composition of lipopolysacharides which is the main component of the cell-envelop (Hirt and Vestal, 1975, Vestal *et al.*, 1973, Petersen and Dixon, 2007b), nutrient metabolism (Ralph, 1985), and tolerance towards organic materials (Wichlacz *et al.*, 1986). Many ways of improving microorganism activities exist, with the adaptation technique being one of them. It is observed that most of the metal ions are toxic to the microorganisms. Due to this fact, the adaptation technique helps these microorganisms to survive and grow in these alien conditions. For example *Leptosphilium ferriphelium* have the ability to grow in solutions of high Fe^{3+}-iron as compared to the acidophiles, such as *Thiobacillus,* which have the capability of growing in the presence of various kinds of metal ions after adaptation.

2.10.4 Galvanic interaction

In the galvanic interaction, the dissolution reaction of the sulphide minerals acts like cathode and this could be enhanced, whereas the dissolution of anodic sulphide minerals would be minimised because sulphide mineral dissolution can be considered as a half cell reaction, such as the cathode and the anode. It was observed that each sulphide mineral has its own rest potential depending on factors such as solution composition, crystallographic structures, ionic

concentration and the nature of the microorganism used for that particular study (Natarajan, 1992). Consequently, the galvanic interactions not only increase the dissolution reaction but preferentially leach a particular desired metal. The galvanic effect depends on many factors: its rest potential, nature and the duration of the contact, the presence of O_2 and the nature of the electrolyte such as pH, conductivity and the presence of other redox species (Jyothi *et al.*, 1989, Natarajan, 1988, Rao and Finch, 1988).

2.10.5 The influence of the pH

The pH of the growth medium is very important because it affects the growth and the activity of acidophilic microorganisms. *Thiobacillus ferrooxidan* responds to external pH changes by regulating the synthesis of several of its cellular components (Amaro *et al.*, 1991). Many researchers reported that microorganism activities are improved significantly by the process of adaptation of the microbial strain to the growth medium at a particular pH level (Barr *et al.*, 1992, Elzeky and Attia, 1995, Menon and Dave, 1995). The pH influences the kinetics of a particular bioleaching system. For more details, refer to Section 2.8 in the literature review concerning the effect of the pH on jarosite and its effects on the kinetics.

2.10.6 The product layer

In the dissolution of mineral sulphides, it is assumed that the substrate volume goes on decreasing with leaching. In the bioleaching process, there are three main product layers: gypsum, precipitated iron compounds (jarosite) and sulphur. The mineral concentrates contain different kinds of acid consuming gangue minerals such as carbonates and silicates and during the leaching operations, these gangue minerals are neutralised by the acid, forming gypsum. One of the major contributors to the deposits on the mineral ores is the iron. The iron chemistry is rather complicated because it precipitates in various forms such as hydroxide, goethite, jarosite or hearmite (Bigham *et al.*, 1996). This iron compound precipitates, and their formation depends on the following factors such as electrode potential, the pH and the concentration of the Fe^{3+}-iron. The elemental sulphur (S°) is formed during the dissolution of metal sulphides, resulting from incomplete biooxidation using sulphur oxidising microorganisms as mentioned earlier in the literature review. It forms a dense, tenacious insulating protective layer over the sulphide matrix. This layer limits the transport of the species to and from the reaction site, causing limitations to the dissolution rate of the sulphide minerals in the bioleaching processes (Watling *et al.*, 2009). The negative impact of the product layer on the overall bioleaching

kinetics, as well as on the entire metal recovery from the mineral sulphide, is discussed in detail in Section 2.8.3.

2.10.7 The bacteria concentration

The microbial concentration in the bioleaching process forms one of the most important parameters among the operating parameters, one which drives and determines the rate of the biooxidation reaction of the sulphide minerals both in the direct or indirect mechanism. Consequently, to increase the kinetics, the population of the microorganisms has to be increased. There are many ways of increasing the microbial population, such as in the bio-film type of reactor known as bacterial film oxidation (BACFOX) used for the processing of the low grade sulphide ores (Murayama *et al.*, 1987, Grishin *et al.*, 1988, Das *et al.*, 1999a). Jarosite plays a very important role in the retaining of the acidophilic microorganisms due to their high special affinity toward them. A thin film of jarosite can be developed in a reactor under suitable conditions, thereby allowing the acidophilic microorganisms to get a necessary site for surplus growth. The mechanism for jarosite formation and the growth of bacterial population with regards to this phenomenon was proposed by Pesic *et al.* (1993). The other method which exists toward the increase of the microbial population in the solution is to apply an electrical potential.

It is a known fact that microbial population depends on the amount of ferrous-iron (Fe^{2+}) oxidised. In a rough estimate, to produce 1 g of biomass, 100 g Fe^{2+}-iron is required; that is, a high amount of ferrous sulphate ($FeSO_4$) is required (Das *et al.*, 1999b). In order to reduce the amount of $FeSO_4$ required, the microbial oxidation reaction can be carried out under the applied potential. In this scenario, the anode and the cathode chambers are separated by a membrane which allows the movements of the electrons. In the cathode chamber, the Fe^{2+} is oxidised by the microorganism and in the process it is reduced back to Fe^{2+} by the application of applied potential. Consequently, in the cathode cell there would be a perennial source of Fe^{2+} without the external addition. In an ideal situation, the scenario is that if the microbial iron oxidation rate is equal to the iron reduction rate by the application of the applied potential, then it will result in leaching solution containing a high microbial concentration as well as the high oxidation potential, both of which are favourable for the sulphide dissolution kinetics (Natarajan, 1992).

2.11 Bioleaching kinetics in the heaps system

The bioleaching kinetics of heap systems are very complex in nature, so while various research studies have been conducted, some others are yet to be conducted on ways of improving efficiencies of the kinetics in this particular system in the quest of extraction of desired metals from the mineral sulphides. Various sub-processes occur within the heap bed, such as the percolation of the leaching solution, which predict the overall kinetics of this particular system.

In the quest to understand this complex system, the fundamentals of both the mass and energy flows should be well understood. The kinetics within the heap system are controlled by the interplay of the mass and the energy flow in the form of solution; heat gas flows through the heap bed via the core distinct categories such as heap bed, macro, aggregate, particle and the grain scale (Petersen and Dixon, 2003). Figure 2.10 below provides a summary of the core distinct categories.

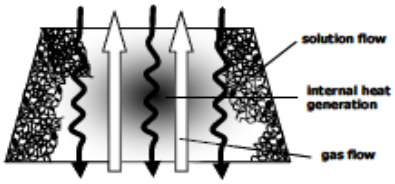
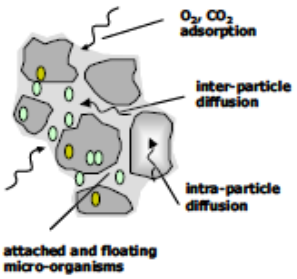
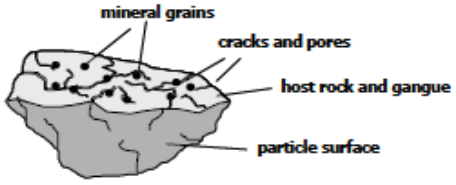
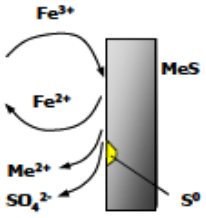
Level	Sub-processes	Illustration
Heap Scale	Solution flow through packed bed Gas advection Water vapour transport Heat balance	
Aggregate Scale	Gas adsorption Particle diffusion Microbial growth Microbial attachment Microbial oxidation	
Particle Scale	Topological effects Intra-particle diffusion Particle and grain size distribution	
Grain Scale	Ferric/ferrous reduction Mineral oxidation Sulphur oxidation Surface processes	

Figure2.10: Schematic illustration of the scales and the sub-processes taking place within the heap bioleaching systems (Petersen and Dixon, 2007a)

These categories and their particular activities are explained as follows:

- *Heap scale:* This stage involves the interactions of important elements such as the flow of liquid solution through the heap bed, the gas advection, the water vapour transport and the heat balances derived from internal heat generations. These are very important processes aiding the bioleaching mechanisms as earlier explained under Section 2.7.
- *The aggregate scale*: This particular stage compared to the previous stage is cardinal to the overall kinetics of the heap bioleaching process. Important processes take place within this category such as the adsorption of the gas into the liquid phase, and the intra and the inter particle diffusion within the stagnant zones occurs. Microbial growth and the oxidation take

place, crucial to the kinetics of this particular system. However, it has to be noted that the microbial activities are governed by the overall interactions of different microbial strains to the system parameters such as the temperature and the concentrations of the dissolved constituents like the ferrous, ferric-iron as well as the dissolved gases O_2 and CO_2 and other important elements.

- *The particle scales:* This stage can also be referred to as the 'topological effects zone'. It gives details on how minerals are distributed on a single particle. They have no specific places within the single particle but are normally spaced in an uneven manner. It is for this reason that the distribution and the accessibility of the mineral grains by the microorganisms define the leaching ability of different mineral sulphides.
- *The grain scale:* This is the final stage in this scenario because the actual metal extraction takes place in this phase. The important process of both chemical and electrochemical interactions occurs. This process, therefore, determines the heap kinetics. However, other factors should be taken into consideration in the sense that they may influence heap kinetics in one way or the other, such as the preference of a particular metal to another (galvanic interactions), the ratio of ferric to ferrous and the condition of the solution with respect to the operating temperature (reaction activation energy), and direct microbial interaction with the exposed mineral. For further details refer to (Sand *et al.*, 2001).

2.12 The developed kinetics models

Several kinetics models have been developed and proposed up to date, using different kinds of living microorganisms (Ojumu *et al.*, 2006) in the quest to improve the efficiency of metal recovery in the bio-hydrometallurgical industry. These models have been classified either as empirical, Michealis-Menten or Monod based. What distinguishes these is the fact that the empirical model uses instruments such as the logistic equation to model the kinetics as compared to the Michealis-Menten which uses traditional enzyme kinetics to evaluate the rate limiting reactions.

These kinetics models were further subjected to modifications. Boon *et al.* (1995a) proposed modified Michealis-Menten type model in regards to the microbial specific oxygen utilisation rate. This modified model also incorporated within itself two factors: ferric-iron inhibition and as a threshold ferrous-iron concentration. Investigating further, Boon *et al*. (1995b) found that the kinetics patterns of the conversion of ferrous-iron to ferric-iron could be related to the

Ferric/Ferrous ratio or the reduction or oxidation potential. This phenomenon is in agreement with the chemiosmotic theory suggested by Ingedew (1982).

It was also observed that apart from the above mentioned kinetics models, many of the kinetics models had been developed in the past few decades using different kinds of microbial strains. For example, many researchers (Jones and Kelly, 1983, Lacey and Lawson, 1970, MacDonald and Clark, 1970, Nakamura *et al.*, 1986) described the kinetics of *At .thiobacillus* with reference to the Monod-type. Others (Boon, 1996, Boon *et al.*, 1999c, Hansford, 1997, Van Scherpenzeel *et al.*, 1998) simplified the inhibition proposed to include the dependence on the ferric to ferrous-iron ratio. They revealed that the redox potential, that is the ferric to ferrous-iron ratio concentration, is the dominant factor in determining the microbial ferrous oxidation. This phenomenon is expressed within Boon/Monod and Hansford models as expressed by Equations 2.18 and 2.19. Further details on the development of these of kinetics models have been reported elsewhere (Chowdhury, 2012, Ojumu *et al.*, 2006, Ojumu *et al.*, 2008).

$$-r_{Fe^{2+}} = \frac{r^{\max}_{Fe^{2+}}}{1 + K'_{Fe^{2+}} \dfrac{[Fe^{3+}]}{[Fe^{2+}]}} \qquad 2.18$$

$$-r_{Fe^{2+}} = \frac{r^{\max}_{Fe^{2+}} [Fe^{2+}]}{K_{Fe^{2+}} + [Fe^{2+}]} \qquad 2.19$$

$r^{\max}_{Fe^{2+}}$ (mmol Fe^{2+}. L^{-1} h^{-1}): Maximum ferrous iron oxidation rate

$[Fe^{2+}]$ (mmol $Fe^{2+}.L^{-1}$) : Ferrous-iron

$[Fe^{3+}]$ (mmol $Fe^{2+}.L^{-1}$) : Ferric-iron

$K_{Fe^{2+}}$ (mmol $Fe^{2+}.L^{-1}$): Monod affinity constant

$K'_{Fe^{2+}}$ (Is dimensionless): Hansford affinity constant

In spite of the development of these kinetics models, the study of the kinetics of the Fe^{2+}-iron biooxidation still remains as 'research in progress' as well as an enormous challenge in fitting into these models convincingly the influence of the key operating parameters; temperature, concentration of the dissolved oxygen and carbon dioxide, pH and extending the application of these models into heap operation conditions (Nemati and Webb, 1998, Ojumu *et al.*, 2006).

2.13 A review on studies on the effect of pH on kinetics of the ferrous iron biooxidation

In the bioleaching industry, pH plays a significant role in regard to the kinetics of the ferrous-iron biooxidation phenomena, as well as in the chemical oxidative dissolution of the mineral sulphides (MS). This phenomenon is well-covered in different areas of this literature review. The physiological stability of the microbial strains, and hence their activities, significantly depend upon acidity of the environment in which they operate. Lower pH values result in a decline of oxidation rates; therefore the microorganisms are more involved in channelling most of their energies into maintaining the neutrality of their cytoplasmic pH at the expense of iron oxidation. This phenomenon results in the reduction in the oxidation rates. Therefore, the role of pH within the bioleaching system cannot be underestimated. It is very important to note the increase in the pH of the bioleaching system is a result of consumption of the protons (H^+). The lack of availability of the protons in the bioleaching system is detrimental to the rates of ferrous-iron oxidation. This is attributed to the fact that a high presence of protons within the bioleaching systems assist in the reverse electron transport within the chemolithotrophic autotrophic bacteria for the purpose of the cell nutrition (Ingledew, 1982). Section 2.9 revealed the effect of the pH on the formation of jarosite and its negative effects on ferrous-iron biooxidation kinetics. Therefore, the ferrous-iron biooxidation kinetics is affected with a variation in pH values.

Section 2.13 reviews on some of previous research studies investigating the effects of pH on the ferrous-iron biooxidation kinetics using different bioreactor designs as well as modes of operation, either in batch or continuous operating mode. A research study conducted by Kinnunen and Puhakka (2005) revealed that the ferrous-iron oxidation rate did not differ significantly within the pH range of 0.9 to 1.6. This particular research study was conducted in a fluidised bed packed bioreactor, packed at the bottom with glass balls and the bed with activated carbon as carrier material. The pH was gradually decreased and it is imperative to note in that particular research study, that even at a lower pH of 0.9, jarosite had accumulated on the activated carbon with bacteria attached on them.

Breed and Hansford (1999b)worked on the kinetics of the ferrous-iron using *Leptospirilium ferrooxidan,* within a dilution rate range of 0.01 to 0.10 h^{-1} and a pH range of 1.10 to 1.70. It was observed in those studies that within that pH range, the kinetic constants of ferrous-iron and oxygen utilisation, $K_{Fe^{2+}}$ and K_{O_2} increased linearly corresponding with increase in the pH. The pH of 1.3 conducted within the dilution range of 0.01 to 0.10 h^{-1} recorded bacterium 'wash out'

indicated by lower redox potentials at dilution rate 0.10 h^{-1}, the highest dilution rate. A more recent study on the effects of the pH on the ferrous-iron biooxidation kinetics by Ojumu and Petersen (2011) was conducted within the pH range of 0.8 to 2.0. It was reported that the pH of 1.3 gave the maximum oxidation rate ($q_{Fe^{2+}}^{max}$) of 14.54 mmol Fe^{2+} (mmol Ch)$^{-1}$ and also revealed minimal jarosite accumulation at pH 1.3.

However, most of these studies were conducted in different kinds of bioreactor configurations in comparison to the one used in the present study, configurations which could not simulate the real solution flow dynamics of a heap bioleaching process. Much emphasis is placed on the findings of Ojumu and Petersen (2011) who discovered very interesting results at the pH of 1.3 in terms of high maximum ferrous-iron biooxidation rates and minimal jarosite accumulation as mentioned above. However, these positive results (high kinetic parameters) may not be applicable to a typical heap set up due to the difference in the solution flow dynamics. Based on this fact, the present research study will investigate the ferrous-iron biooxidation kinetics in a packed-column bioreactor that will at least simulate the solution flow the dynamics of a typical heap system. The results obtained from this study could be used to improve ferrous-iron biooxidation kinetics in packed-column and ultimately the effective recovery of metals in the heap systems.

2.14 Summary and problem statement

Bio-hydrometallurgy has offered an alternative vehicle of processing the low grade sulphide ores, an alternative which is both environmentally friendly and economically viable in terms of processing of bulk materials of low grade concentrates compared to the conventional methods (pyro-hydrometallurgy). Bioleaching at an industrial scale is applied through two techniques: tank and heap systems. However, much success has been recorded in optimising the key operating parameters such as temperature, pH, aeration rate, and dissolved ions in tank systems as compared to the heap system. The improvement of the kinetics of heap systems is still under review due to the complexity of the system. This phenomenon is well-explained in Section 2.11. This is the motivation behind this study: using bioreactor configuration simulating the real solution flow dynamics of a heap set up with the intention of improving the ferrous-iron oxidation kinetics in them.

The minimisation of the jarosite is achieved through the manipulation of the feed pH. Jarosite is generated from Fe^{3+} which is the main oxidising agent and forms at pH > 3. In order to fully comprehend the concepts of jarosite, the literature review has extensively revealed important aspects on jarosite such as how pH influences its production, its general importance, and negative effects on the bioleaching process. These are well-explained in Section 2.8. The deprivation of this important oxidising agent within the leaching solution creates kinetic barriers which are detrimental to the entire bioleaching process. It is on this ground that microbial ferrous-iron kinetics has been extensively covered in this chapter, and conditions of how pH plays an important role toward the formation of the jarosite, as well as the ferrous-iron kinetics. The brief review on the development of the kinetics models has been also covered in this literature review. Extensive reviews of previous research studies on the effect of the pH on both formation of jarosite and the ferrous-iron biooxidation kinetics have been done to comprehend the context of the present research study.

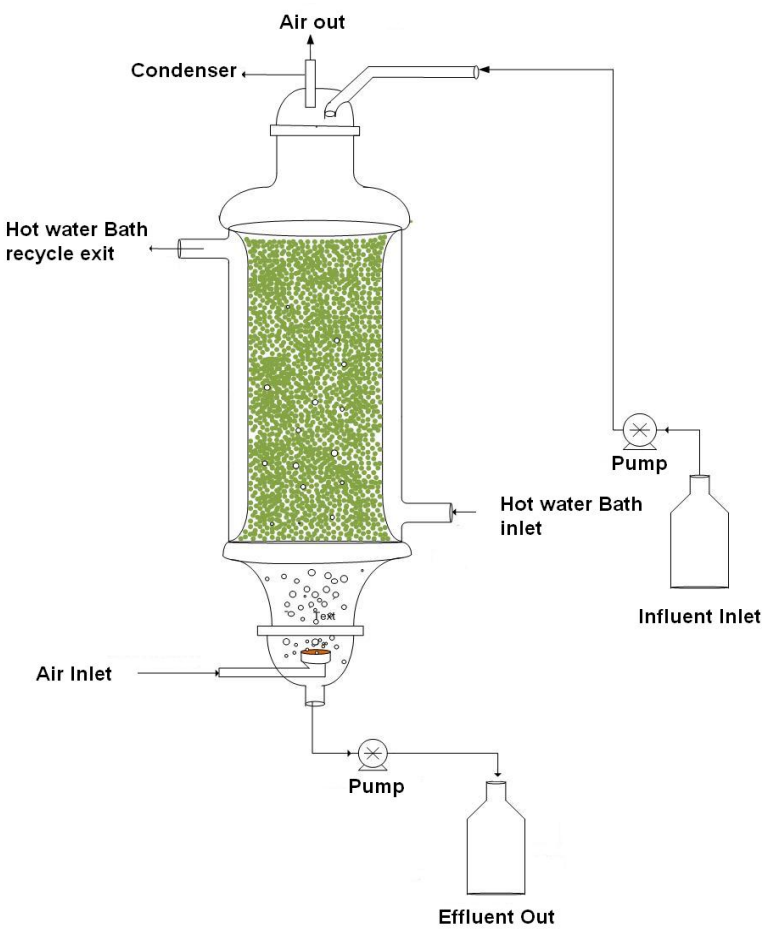
Chapter 3

3. Materials and Methods

3.1 Materials

3.1.1 Experimental rig

Figure 3.1 illustrates the experimental rig utilised in this present study. It is composed of two jacketed column bioreactors made of borosilicate glass with the total volume of 750 mL. The bioreactors have a height to diameter ratio (H/D) of approximately 12.5. The inert glass balls of 15 mm in diameter were used as the inside packing material occupying a volume of 700 mL. A working liquid volume of 500 mL was applied inside the bioreactor. The water bath supplied hot water at a constant temperature of 38 °C to the column jacket by circulating the hot water in the water jacket of the bioreactor. The feed was pumped to the top of the column bioreactor using a low-flow multi-channel Master Flex pump (model: Watson Marlow 205S) with a variable speed drive and using 0.5 mm PVC tubing of orange/yellow colour code. The liquid percolates through the packing materials by the help of natural force of gravity. A low-flow rate single channel pump (mode: Watson Marlow 101U/R) using a silicone tubing of 5 mm inner diameter was used to pump the effluents out, and to maintain a constant working volume within the bioreactor at the steady state. Inlet air to the bottom of the bioreactor column was supplied by means of an air compressor. The air supply rate to the column bioreactors was controlled and maintained at constant air-flow rate of 20 $mL.s^{-1}$ by means of bubble air flow meter. This was monitored constantly for any fluctuation in the set-point air flow rate. The off-gas was allowed to pass through the condensers to minimise the loss of the liquor in the bioreactor via the evaporation process.



(a) **(b)**

Figure 3.1: (a) Schematic diagram of the experimental rig; and (b) the actual laboratory experimental rig

3.1.2 Growth Medium

All the reagents used in this experimental research study were of analytical grade. The ferrous-iron media consisted of the desired quantity in ($g.L^{-1}$) of Fe^{2+} added as ($FeSO_4.7H_2O$), 1.11 K_2SO_4, 1.83 $(NH_4)_2SO_4$, 0.53 $(NH_4)_2\ HPO_4$ and 10 mL of vishniac solution, a trace element solution(Vishniac and Santer, 1957) adjusted to the desired pH ($0.7 < pH < 1.30$) using concentrated H_2SO_4. It must be noted that there was no attempt to keep sterile conditions due to the acidic environment prevailing. The vishniac solution was prepared thus --15 $g.L^{-1}$ of EDTA $C_{10}H_{12}FeN_2NaO_8.3H_2O$ ($Mw = 421.10 g.mol^{-1}$) was dissolved in demineralised water, then 1 $g.L^{-1}$ of $ZnSO_4$ was added and the pH adjusted to 6.0 using concentrated H_2SO_4. These compounds were added to each other successively: 1.0 $g.L^{-1}$ $CoCl_2.6H_2O$, 1.0 $g.L^{-1}$ $MnCl_2.4H_20$, 0.5 $g.L^{-1}CuSO_4.5H_2O$, 5.0 $FeSO_4.7H_2O$, 0.5 $g.L^{-1}Na_2MoO_4.2H_2O$ and 0.5 $g.L^{-1}$ $CaCl_2.2H_2O$. Having had the complete dissolution of all the compounds, the pH was set to 4.0 with 1 M of H_2SO_4 solution.

3.1.3 Bacteria Culture

The bacteria strain was obtained from a continuous two stage mini-plant (2 X 20 L) treating pyrite-arsenopyrite concentrate in Gamsberg, South Africa. The pre-dominant bacteria strain used was *Acidithiobacillus ferroxidans* sp. Nov., which was recently found to be ferrous-iron oxidising microbial species (Coram and Rawlings, 2002). The stock bacteria culture was maintained in batch continuous stirred tank bioreactors at a constant temperature of 38.6 °C, and at a residence time of 24 hours. This was fed on ferrous-iron substrate, supplied as $FeSO_4.7H_2O$ containing 5 $g.L^{-1}$of total iron.

3.2 Methods

3.2.1 Microbial ferrous-iron oxidation under continuous operation: Jarosite accumulation and control

A series of continuous culture experiments were conducted and repeated in identical double-walled packed-column reactors packed with 15 mm glass balls as the packing material. The working volume in the bioreactor was 500 mL. The diagrammatic representation is shown in Figure 3.1a. The detailed description of the bioreactor can be found elsewhere (Chowdhury, 2012). The experiments were conducted at a constant temperature, aeration rate and the dilution rate of 38.6°C, 20 $mL.s^{-1}$ and 0.05 h^{-1}, respectively. Each experiment was carried out in triplicate and allowed to run for 10 and 15 days at a desired bioreactor solution pH of 1.3, 1.5

and 1.7 with a pH deviation of $\pm$ 0.05. It must be noted that feed solution pH was used as a proxy measure to control the bioreactor pH to the desired value. The pH of the feed was controlled with the concentrated sulphuric acid (98%) only. The solution redox potentials and pH were monitored using the CRISON GLP 21 meter.

Another set of experiments were carried out such that the bioreactor solution pH of 1.7 was maintained for the first 10 days, and at the end of the 10th day, the bioreactor solution pH was controlled to pH 1.5 by adjusting the feed pH. The experiment was allowed to continue for another five days. The experiment was repeated in the same fashion for a change in pH from 1.7 to 1.3. At the end of each experiment, jarosite precipitate that had accumulated was determined using a combination of methods such as filtration and dissolution in hydrochloric acid and was analysed using AAS (Atomic Absorbance Spectrometer). Each experiment was conducted in a pre-cleaned identical bioreactor, and in triplicate.

3.2.2 Kinetics of microbial ferrous-iron oxidation under continuous operation

A series of continuous culture experiments were conducted in the same bioreactor configuration identified in Section 3.2.1. The same operating conditions were maintained except for the bioreactor solution pH that was kept constant at pH 1.3 $\pm$ 0.05. The microbial oxidation kinetics was investigated at five different dilution rates ranging from 0.05 to 0.10 h^{-1}. The bioreactor was operated at each dilution rate for at least three residence times before steady state was assumed. Steady state was assumed only when the pH and redox potential in the culture liquor were constant. Steady state was maintained for at least one residence time in order to allow for the chemical analysis of the influent and effluent samples. Each experiment was start-up in a batch mode of operation by adding 50% of stock solution with ferrous-iron feed in the packed column bioreactor; the solution potential was allowed to get to about 600 mV (on Ag/AgCl electrode) before switching into continuous mode. The jarosite precipitate accumulation was minimised in the bioreactor by cleaning both the bioreactor and the packing after every dilution rate with concentrated HCl (32%) and distilled water.

Ferrous-iron and total iron concentrations were determined by titration with potassium dichromate solution using the BDS indicator. The measurement of redox potential using CRISON redox electrodes (Ag/AgCl) allowed the determination of ferric-to-ferrous iron ratio from a calibration curve for the electrode derived using a Nernst equation, as described previously (Ojumu and Petersen, 2011).

3.3 The Analytical procedure

3.3.1 The iron analysis

In order to calculate the ferric to ferrous iron ratio within the bioreactor and in the feed solution, the solution potential had to be determined periodically by the use of the redox electrode probe (Ag/AgCl). The redox probe was calibrated regularly (refer to Section 3.6.2) under the same conditions as the bioreactor operation prior to use. The total iron concentration in both the feed and bioreactor effluents was determined by a titration technique with potassium dichromate using the BDS indicator (Vogel *et al.*, 1989). The difference between the total iron concentration in the feed entering the packed bioreactor and the total iron concentration in the effluents leaving the bioreactor is used to the determination of the ferric-iron precipitation in the effluent. The ferrous-iron concentration in both solutions were determined by titration with potassium dichromate (Vogel *et al.*, 1989). The ferric-iron concentrations were determined by subtracting the ferrous-iron concentration from the total iron concentration. The solution reduction-oxidation (redox) potential and the pH were determined using a CRISON GLP 21 meter. This process leads to the careful determination of ferric-iron concentration and the ferrous-iron utilisation rate ($r_{Fe^{2+}}^{\max}$) calculation at every dilution rate.

3.3.2 Redox probe calibration

The initial redox potential (Eh_o) shown in the equation 3.21 below, was calculated at the same operating conditions to that of experimental procedures. The calibration of redox electrode (i.e. Ag/AgCl electrode) was calibrated against the half reaction of ferrous to ferric oxidation, as depicted by the reaction shown by Equation 3.20 which is the only redox couple available in the bioreactor. Based on this calibration, the iron species can be estimated using the Nernst equation shown by Equation 3.21.This procedure facilitated the determination of the ferrous to ferric conversion rate within the bioreactors.

$$Fe^{2+} \rightarrow Fe^{3+} + e \qquad 3.20$$

$$Eh = Eh_o + \frac{RT}{nF} \ln \frac{[Fe^{3+}]}{[Fe^{2+}]} \qquad 3.21$$

Whereby the Eh_o is defined as the solution potential measured at equal total ferric and ferrous iron concentrations, which accounts for activity coefficient, formation of complexes, electrode type and the fouling on the electrode.

3.4 Analysis of the kinetic data

3.4.1 The determination of the maximum ferrous-iron oxidation rate

The experimental data were fitted into the Hansford (1997) and the Monod models expressed by Equations 2.18 and 2.19. These equations were used to determine the maximum ferrous-iron oxidation rate and apparent affinity constants. These models are capable of interpreting the kinetics of the specific microbial ferrous-iron oxidation rate using the ferric to ferrous ratio [Fe^{3+}/Fe^{2+}] measured in the bioreactor (Ojumu *et al.*, 2006).

3.5 Conclusion

This chapter revealed all raw materials, equipment and all methodological approaches applied in this study. The methods applied are for both quantification of the jarosite and the analytical techniques applied for the calculations of the ferrous-iron biooxidation kinetic studies. These are presented below:

- The experimental rig
- The growth medium
- Bacteria culture
- Methods: the preparation of the samples for the quantifications of the jarosite
- Analytical procedure for the iron analysis
- Calibration of redox probe
- Analysis of the kinetic data

Chapter 4

The effect of pH on jarosite management and investigation of bacterial ferrous-iron oxidation at pH 1.3 in a novel packed-bed reactor

4. Introduction

This chapter presents the results and discussions obtained from the series of experimental studies on the effect of pH on the management of the jarosite accumulation and investigation of bacterial ferrous-iron oxidation at pH 1.3 within a novel packed-column reactor. These results simulated the real solution flow dynamics of a typical bioleach heap situation. The operating parameters used in the jarosite accumulation and the control study were fed to computer code tool-the Visual Minteq to give anticipated outcome on the formation of the ferric-iron complexes, equilibrium concentrations and saturation indices at varying pH levels. This was done in order to assess the general trend of the expected outcome on the formation of the jarosite in relation to the solution pH at the same operating conditions.

Therefore, chemical modelling was performed using the Visual Minteq computer aided codes as mentioned above. This model was used to calculate ionic activities and mass distribution. Table 2.4 in Chapter 2 has shown the appropriate data obtained at a specified pH and redox potential (the ratio of the ferric to ferrous-iron) at a constant temperature of 38.6°C. The data in Table 4.1 revealed that the saturation index for the various iron ionic species either increased or decreased with increasing pH (that is 1.3, 1.5 and 1.7) while for the K-jarosite remained at 0.000 (the equilibrium value) at all the pH used in chemical model.

Table 4.1: The relationship between the pH and the saturation indices of iron complex species in the bioleaching leaching solution, calculated using Visual Minteq

	pH		
Ferric-iron (Fe^{3+}) species	**1.3**	**1.4**	**1.5**
$Fe(OH)_2Cl_{3(s)}$	2.284	2.795	3.326
$Fe(SO_4)_{3(s)}$	-12.382	-13.948	-15.670
$Fe_3(SO_4)_{8(s)}$	-17.825	-16.313	-14.741
Ferrihydrite	-1.933	-1.043	0.771
$Ferrihydrite_{(aged)}$	-1.472	-0.852	-0.261
Geothite	0.472	1.043	1.633
Hematite	3.404	4.545	5.727
H-Jarosite	-4.048	-4.140	-4.304
K-Jarosite	0.000	0.000	0.000

*Oversaturation SI = (+),*Under-saturation SI = (-), * Apparent equilibrium SI = (0.000).

Chemical modelling has further illustrated that within the bioleaching solution, the Fe^{3+} do not only form the jarosite derivates such as NH_4^+, K^+, H_3O^+ or H^+ jarosite, but also the other forms of the ferric-iron complexes such as hematite, stregite and goethite. However, the K-jarosite tends to dominate in most of the bioleaching solutions; this may be attributed to the strong possibility that the potassium concentration could be high in such medium. The other factor is the role of the pH because at certain pH values, the formation of certain ferric-iron complexes, for example at certain pH > 4, favours the formation of the other ferric complexes such as goethite, schwertmannite and ferrihydrate (see Section 2.6.2 for further details).

Table 4.2: Effect of pH on the formation of ferric- iron complexes within the bioleaching solution with a ferric to ferrous-iron ratio of 650 redox potential (mV), calculated using the Visual Minteq

pH	Component	Total dissolved	Dissolved %	Total sorbed	Sorbed %	Total precipitated	Precipitated %
1.3	Fe^{+2}	0.069	100	0.000	0.000	0.000	0.00
	Fe^{+3}	0.003	15.16	0.000	0.000	0.019	84.84
	K^{+1}	0.012	64.41	0.000	0.000	0.006	35.59
	SO_4^{-2}	0.007	35.56	0.000	0.000	0.013	64.44
1.5	Fe^{+2}	0.062	100	0.000	0.000	0.000	0.00
	Fe^{+3}	0.002	7.32	0.000	0.000	0.027	92.68
	K^{+1}	0.009	50.19	0.000	0.000	0.009	49.81
	SO_4^{-2}	0.002	9.82	0.000	0.000	0.018	90.18
1.7	Fe^{+2}	0.060	100	0.000	0.000	0	0.00
	Fe^{+3}	0.002	6.23	0.000	0.000	0.0293	93.77
	K^{+1}	0.008	46.14	0.000	0.000	0.0097666	53.86
	SO_4^{-2}	0.0005	2.47	0.000	0.000	0.019533	97.53

*Units: $mg.L^{-1}$

This phenomenon (chemical modelling) revealed certain important aspects attached to the pH and the formation of the ferric complexes, of which the jarosite is non-exceptional. The data presented in Table 4.2, with special emphasis on the ferric-iron (Fe^{3+}) precipitates highlighted in red colour, revealed that at a constant temperature (38.6°C), varying pH levels (1.3, 1.5 and 1.7) and performing the Visual Minteq on all the elements fed to the bioleaching solution, outcome showed that the percentage of ferric complexes with other ionic species in the bioleaching solution increased with the increase in the pH. The data obtained by performing the Visual Minteq programme at the above stated conditions revealed that the potassium jarosite equilibrium concentration ($mol{\cdot}L^{-1}$) increased subsequently with pH increase, following the same trend as the ferric complexes at the same pH values as revealed in Table 4.3.

Table 4.3: Equilibrium concentration ($mol.L^{-1}$) of K-jarosite at different pH values calculated using Visual Minteq

pH	1.3	1.5	1.7
K-Jarosite ($mol.L^{-1}$)	6.4523×10^{-3}	9.031×10^{-3}	9.767×10^{-3}

The chemical modelling done using the Visual Minteq as a tool for analysing the possible trend

of the reactions resulting from the iron ionic species chemical interactions in the bioleaching solution at different pH, could be beneficial in analyses of the laboratory experimental data.

4.1 Results and Discussions

4.2 Jarosite accumulation and control – Jarosite management

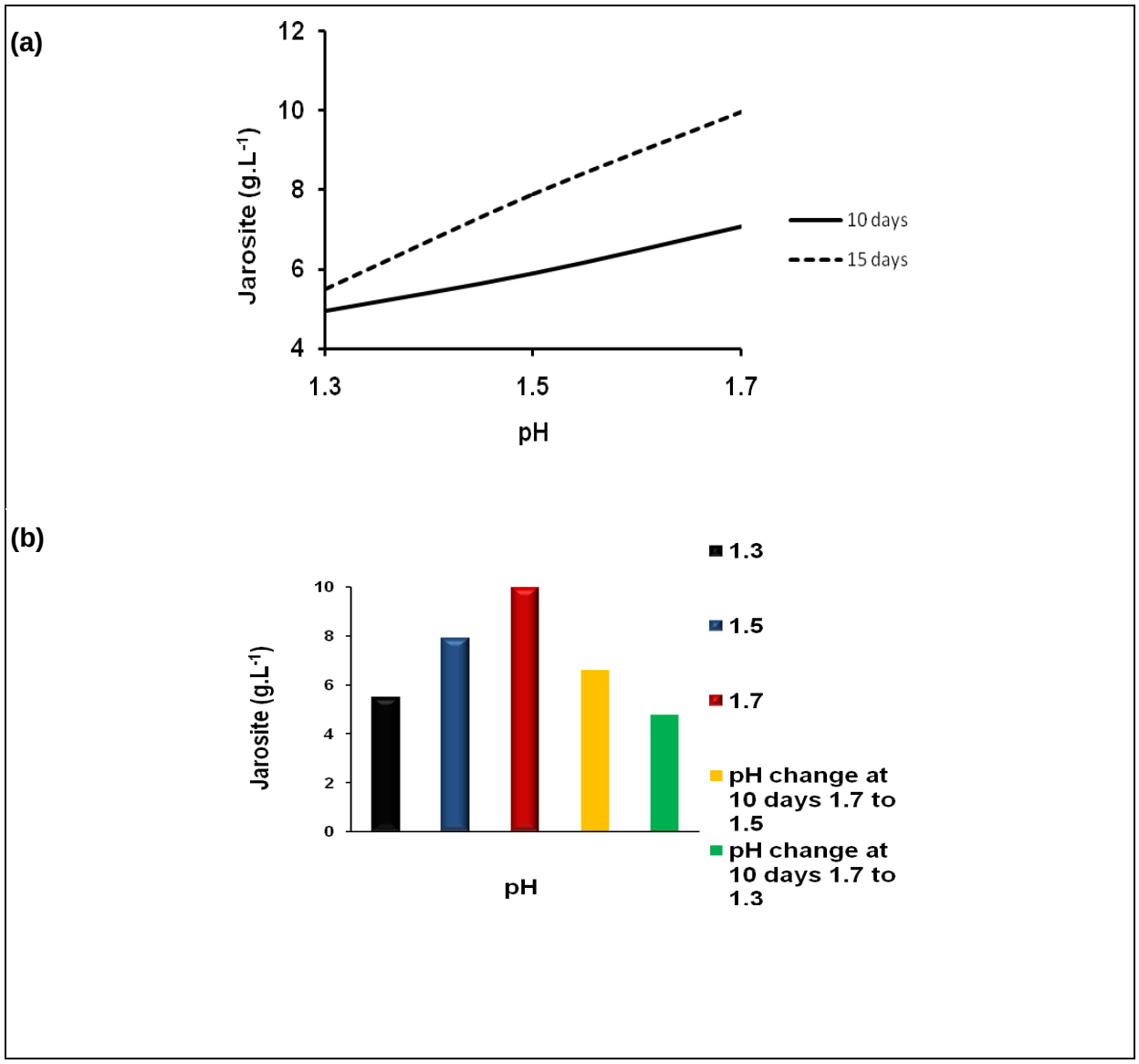


Figure 4.1: Variation of jarosite precipitate accumulation versus bioreactor solution pH after (a) 10 and 15 days of continuous operation. (b) 15 days of operation at constant pH compared to the variation when pH was lowered to 1.5 and 1.3 after 10 days for additional five days of operation.

The experimental results revealed that jarosite precipitate accumulation in the bioreactor shows an increasing trend (Figure 4.1a) with an increase in operating bioreactor solution pH. Figure

4.1a also shows significant increase in jarosite accumulation with an increase in the period of operation of the bioreactor: an increase of 11, 34 and 40.9% can be obtained from Table 4.4 at pH 1.3, 1.5 and 1.7, respectively, when the bioreactor was left to run at these conditions for an additional five days. This trend is consistent with previous reports (Grishin *et al.*, 1988, Karamanev, 1991, Kinnunen and Puhakka, 2004, Mazuelos *et al.*, 2010, Nikolov and Karamanev, 1992, Pogliani and Donati, 2000). The pH-jarosite relationship is also consistent with the trend of data obtained using a geochemical simulation package, Visual Minteq, showing that the percentage of ferric ion precipitate complexes increased with the solution pH (see Table 4.3). It can also be seen that increase in jarosite precipitate increases redox potential (oxidation rate), which might be attributed to the increase of immobilised microbial population on the jarosite matrixes as this enhances the rate of ferrous-iron oxidation (see Table 4.4).Therefore, manipulation of the feed pH leads to jarosite minimisation and control. This phenomenon answers one of the research questions asked under Section 1.3 concerning the possible strategy that could be used to minimise and control jarosite using solution pH.

Table 4.4: Jarosite accumulation obtained from the experimental trials at different pH levels

Days	**pH**									
	1.3		**1.5**		**1.7**		**Change of pH after 10 days from 1.7 to 1.3**		**Change in the pH after 10 days from 1.7 to 1.5**	
	Mass of Jarosite	**mV**	**Mass of Jarosite**	**mV**	**Mass of Jarosite**	**mV**	**Mass of Jarosite**	**mV**	**Mass of Jarosite**	**mV**
10	4.95	678	5.89	630	7.08	610	-	-	-	-
15	5.50	711	7.90	645	9.98	634	4.78	635	6.60	625

*mV= redox potential, $g.L^{-1}$ = mass of jarosite

A decrease in jarosite accumulation of 33 and 52% can also be seen (see Figure 4.1 and Table 4.4) by dropping the solution pH of the bioreactor after 10 days of continuous operation from pH 1.7 ± 0.05 to 1.5 ± 0.05 and pH 1.7 ± 0.05 to 1.3 ± 0.05, respectively (through the manipulation of the feed pH using concentrated sulphuric acid), allowing the bioreaction to continue for an additional five days of continuous operation. It should be noted that these values were obtained by comparing the amounts of jarosite obtained during the decrease in pH (i.e. pH of 1.7 ± 0.05 to 15 ± 0.05 and 1.7 ± 0.05 to 1.3 ± 0.05) to the individual amount obtained when the bioreactor was allowed to run at pH of 1.7 ± 0.05 for 15 days of continuous operation. The reduction in

jarosite precipitate accumulation in the bioreactor by lowering of operational pH appears to be linked to the chemistry of dissolution of jarosite precipitation at low pHs as reported by Mazuelos *et al.* (2010). These researchers reported dissolution of the precipitate when the solution pH was reduced from 1.0 to 0.82. The results of this study suggest that jarosite precipitate would be better managed and controlled in the packed-bed system by running the bioreactor system initially at pH 1.7 ± 0.05 before reducing to pH 1.3 ± 0.05. Doing this would ensure much reduction in the precipitate while retaining considerable biomass in the bioreactor, as it can be seen that a 13 and 17% decrease in jarosite precipitate would be achieved (see Figure 4.1b and Table 4.4) by the stepwise pH reduction from 1.7 ± 0.05 to 1.5 ± 0.05 and 1.3 ± 0.05 respectively compared to the individual amounts obtained at a pH 1.3 ± 0.05 and 1.5 ± 0.05 for 15 days of continuous operation. These results show that reduction in the feed solution pH lead to minimisation of the jarosite formations, providing answers to a research question under Section 1.3.

4.3 Experimental results (Ferrous-iron oxidation)

4.3.1 Variation of ferrous iron oxidation rate, conversion, redox potential and iron species with the dilution rate

Figure 4.2 shows the variation of the overall microbial ferrous-iron oxidation rate, conversion, redox potential and iron species in the bioreactor with dilution rate. The oxidation rate, $r_{Fe^{2+}}$, increases with dilution rate in a somewhat a linear fashion that is consistent with the substrate balance equation over the packed-column reactor system (Chowdhury, 2012). It should be noted that the trend of conversion, redox potential and the iron speciation with dilution rate should be the same; as dilution rate increases the redox potential decreases from high potential to lower values, indicative of a decreasing ferric-iron concentration in the bioreactor. Therefore the decreasing trend of conversion with dilution rate (see Figure 4.2b) was expected. At low dilution rate the cells were in contact with the feedstock for a much longer period to achieve higher conversion while the cells progressively washout at high dilution rates. This also is consistent with previous studies (Boon *et al.*, 1995a, Breed and Hansford, 1999b, Ojumu *et al.*, 2009, Ojumu and Petersen, 2011, Chowdhury, 2012).

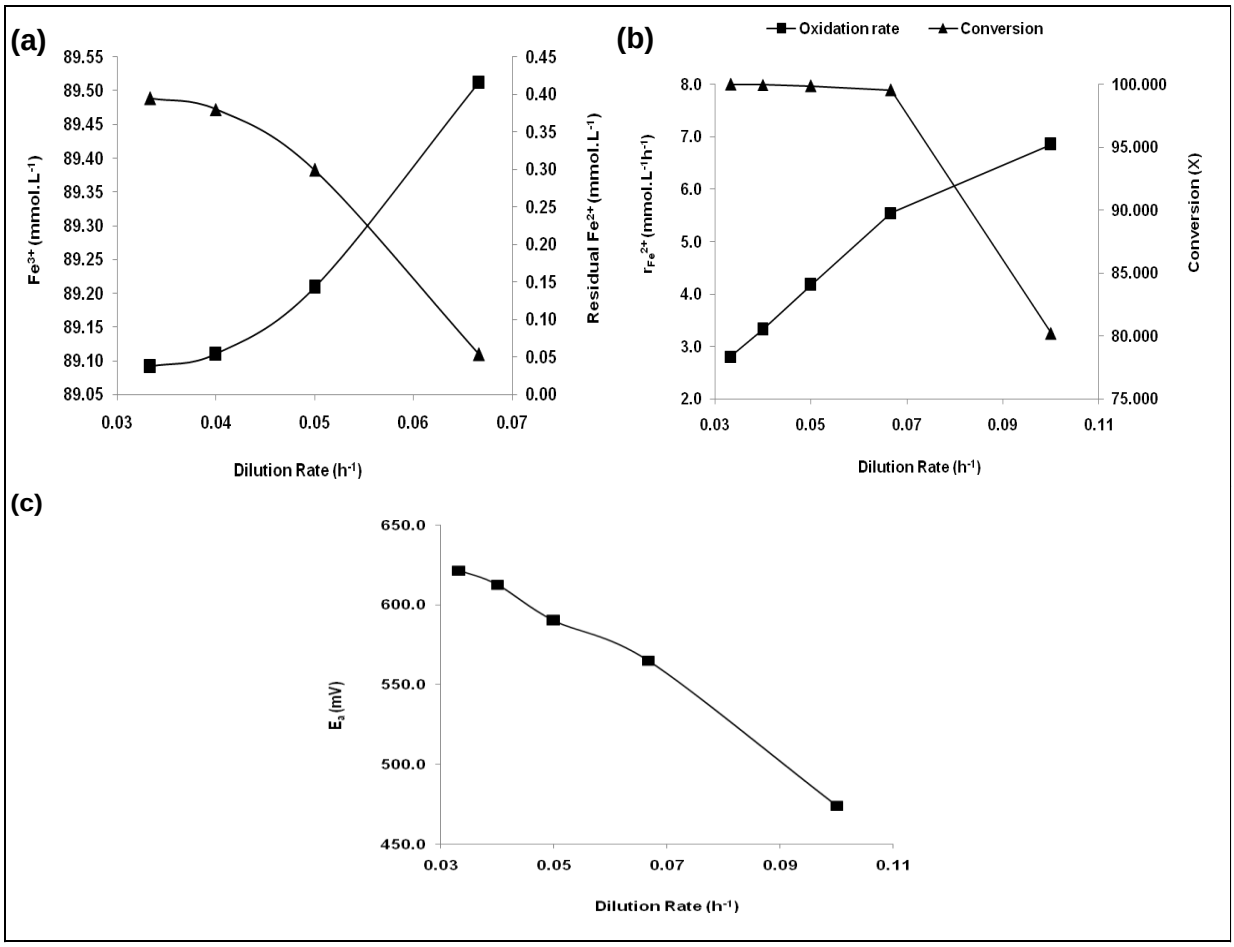


Figure 4.2: Dilution rate versus (a) iron species (ferric-iron and residual ferrous-iron); (b) ferrous-iron oxidation and conversion; (c) solution redox potential (Ag/Agcl)

4.3.2 Kinetic parameters

The simplified competitive inhibition model, Hansford model and the Monod's (Ojumu *et al.*, 2006), represented by Equations 2.18 and 2.19 shown below, were used to analyze the experimental data (Table F1.1, Appendix F). It has been shown previously that these two equations can be used to fit the same data fairly accurately (Ojumu *et al.,* 2006). This has been confirmed in the literature (Chowdhury, 2012, Ojumu *et al.*, 2006, Ojumu *et al.*, 2009, Ojumu *et al.*, 2008). The kinetics parameters, the maximum ferrous-iron oxidation rate $r_{Fe^{2+}}^{\max}$ and the affinity constants ($K'_{Fe^{2+}}$, $K_{Fe^{2+}}$) were determined using a Solver routine in Excel™ to minimise the sum of the squared errors (SSE) between the measured and predicted values of $r_{Fe^{2+}}$ as shown in Figure 4.3, described previously (Ojumu *et al.*, 2009, Ojumu *et al.*, 2008). It was shown that similar results can be obtained using Lineweaver-Burk plots (data not shown).

$$-r_{Fe^{2+}} = \frac{r^{max}_{Fe^{2+}}}{1 + K'_{Fe^{2+}} \frac{[Fe^{3+}]}{[Fe^{2+}]}} \quad 2.18$$

$$-r_{Fe^{2+}} = \frac{r^{max}_{Fe^{2+}}[Fe^{2+}]}{K_{Fe^{2+}} + [Fe^{2+}]} \quad 2.19$$

There is a solid correlation between the experimentally determined and predicted values for $r_{Fe^{2+}}$. However, the maximum overall microbial ferrous-iron oxidation rate obtained in this study is much lower than that obtained by Chowdhury (2012) in a similar study (see Table 4.5) with the main differences between these studies being the type of microbe used, the temperature and pH conditions of both experiments (see Table 4.5); *Acidithiobacillus ferrooxidans* was used in this study while Chowdhury (2012) used *Leptospirillum ferriphilum*. The lower values of the affinity constants in this study suggest that *At. ferrooxidans* has higher affinity for the substrate in the bioreactor than *L. ferriphilum* which is consistent with the studies of Rawlings (2005).

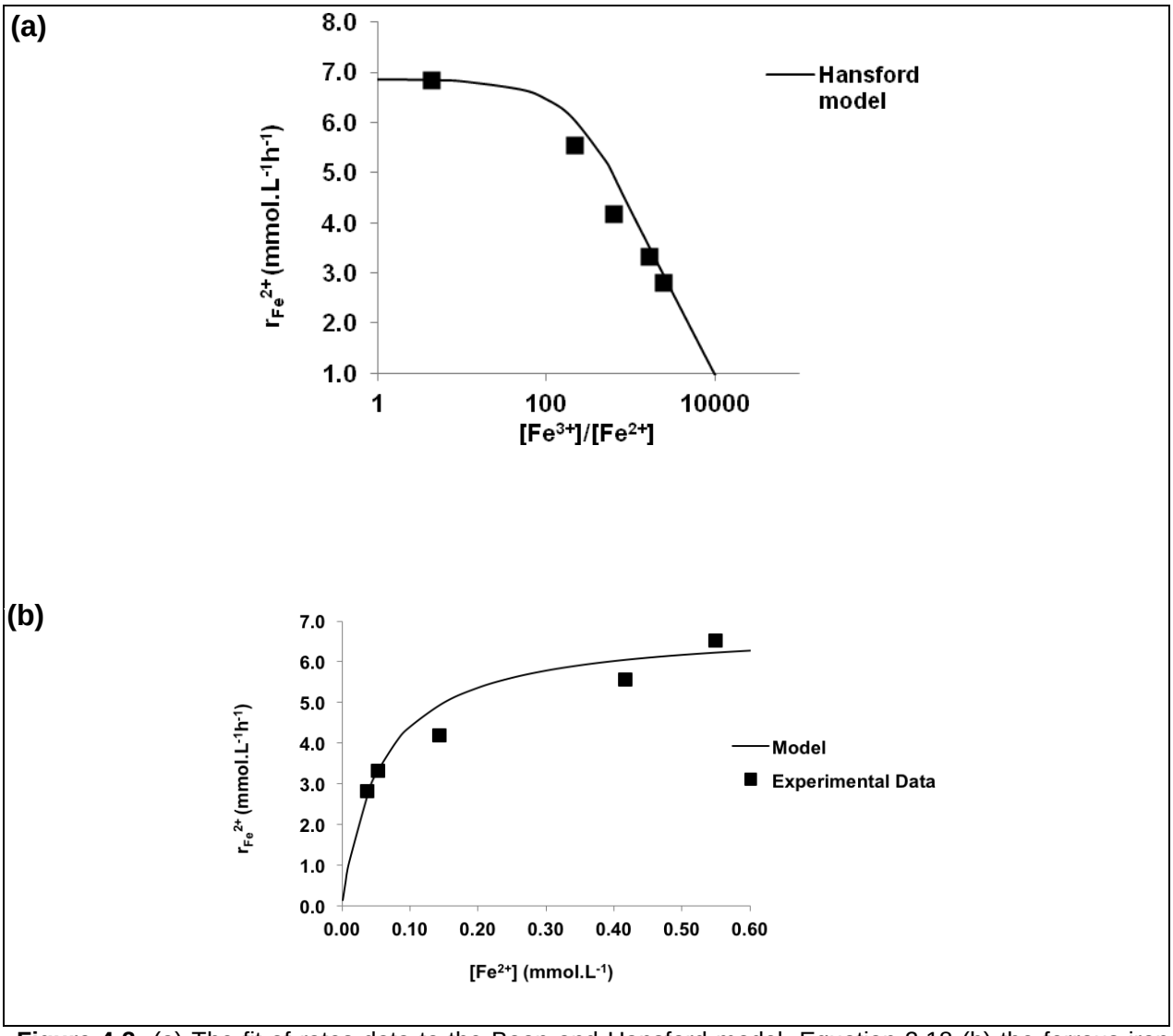


Figure 4:3: (a) The fit of rates data to the Boon and Hansford model, Equation 2.18 (b) the ferrous-iron oxidation rate versus residual ferrous-iron in relationship to the trend of the Monod model, Equation 2.19.

Table 4.5: Kinetic parameters at the pH of 1.3 ± 0.05 to 1.45 ± 0.05, respectively

Temperature (° C)	pH	Hansford Model		Monod Model		Average	Reference
		$r_{Fe^{2+}}^{max}$	$K'_{Fe^{2+}}$	$r_{Fe^{2+}}^{max}$	$K_{Fe^{2+}}$	$r_{Fe^{2+}}^{max}$	
38.6	± 1.3	6.84	0.001	6.85	0.006	6.85	Present study
35	± 1.45	15.09	0.047	15.10	3.91	15.10	Chowdhury (2012)

*Units: $r_{Fe^{2+}}^{max}$ (mmol $Fe^{2+}.L^{-1}$ h^{-1}), $K'_{Fe^{2+}}$ is dimensionless, $K_{Fe^{2+}}$ (mmol $Fe^{2+}.L^{-1}$)

Chapter 5

5. Conclusion and the Recommendations

5.1 Conclusions

This present study attempted to manage and control the formation of jarosite through the manipulation of the feed pH, and the ferrous-iron biooxidation kinetics at pH of 1.3, the lowest at which the amount of accumulated jarosite was minimal. This study has shown that amount of jarosite/ferric precipitate that accumulates in the bioreactor increases with an increase in the time of continuous operation of the reactor.It shows that jarosite precipitate accumulation during microbial ferrous-iron oxidation can be managed and controlled such that it does not become problematic by manipulating bioreactor solution pH. The results show that by operating the bioreactor at pH 1.7 for 10 days and with a subsequent reduction of the pH to 1.5 and 1.3 after the 10th day for another five days of continuous operation, about 33 and 52% reduction in jarosite accumulation can be achieved. The kinetics of microbial oxidation of ferrous-iron at pH 1.3 and 38.6°C in the novel bioreactor can be described fairly accurately using both Monod and Hansford models (Equations 2.15 and 2.16). Although the maximum overall microbial ferrous-iron oxidation rate for *At. ferrooxidans* is lower than that of *L. ferriphilum,* the result shows that *At. ferrooxidans* have higher affinity for the substrate when compared to *L. ferriphilum* in a previous study. The results of this study would certainly have a positive implication toward the management and control of jarosite precipitate in a typical bioleach heap system or at least packed-column reactor. It would also find application in the design of such systems.

$$-r_{Fe^{2+}} = \frac{r^{\max}_{Fe^{2+}}}{1 + K'_{Fe^{2+}} \dfrac{[Fe^{3+}]}{[Fe^{2+}]}} \qquad 2.18$$

$$-r_{Fe^{2+}} = \frac{r^{max}_{Fe^{2+}}[Fe^{2+}]}{K_{Fe^{2+}} + [Fe^{2+}]} \qquad 2.19$$

5.2 Recommendations for future studies

The management and control of jarosite/ferric precipitated were presented in this study. Although the research was conducted in a novel packed-column reactor which simulated in the real solution flow dynamics in a typical bioleach operation, it should be noted that in the real heap system interaction between pyrite (ferrous source) oxidation and metal dissolution cannot be separated. The oxidised ferric is used up for metal dissolution. Understanding of this interaction might lead to better insight into the kinetics of both metal and ferrous-iron biooxidation.

The temperature, aeration rate and the pH at required set points were simultaneously controlled in this study. This is far from the true realities within the heap bed, as mentioned earlier. The successful homogenous distribution of all key operating parameters within any system depends upon effective mass and heat transfer phenomenon, as demonstrated in Chapter 2 under Section 2.11. This mass and heat transfer phenomenon within the heap system is very complex due to a variety of factors such as unequal distributions of the concentrates, different ore particles sizes which cause channelling within heap bed and the height of heap. These factors impact negatively on mass and heat transfer, resulting in wide gradient variations in key operating parameters such as pH, temperature and aeration rates within the heap bed (Section 2.5.1).

It must be noted that certain mineral ores contain some gangue materials (gypsum, carbonates) and during the process of bioleaching, the acid dissolves gangue materials and releases these ions (anions and cations) in the leaching solution. These ions may impose negative effects on the *Acidithiobacillus ferroxidans* microbial strain. Although some studies have investigated the effect of specific dissolved cation on the microbial kinetics, studying the effect of dissolved gangue materials in a simulated PLS may be important to accurate kinetic prediction.

The effect variation of residence time on the jarosite management could also be taken into account for the future studies as this study was conducted at a single residence time. The outcome may provide additional insights on how the appropriate residence time of the

bioreactor could be achieved in relation to the management of the jarosite. The literature has revealed that both the pH and temperature define the formation of the jarosite; however the present study only restricted itself to pH. It is against this background that future studies may be directed toward studies that will encompass both the manipulation of pH and temperature simultaneously to determine the best combination for achieving the least jarosite formation. The temperature is crucial in the bioleaching process, not only for the purpose of the cell activities of the microorganisms but for defining the solubility of oxygen (O_2) in bioleaching solution.

Future studies should be directed toward investigating effect of pH on microbial ferrous-iron oxidation kinetics rates in packed-columns that simulate the real flow dynamics of a typical heap set up. This statement follows the previous studies by Kinnunen and Puhakka(2005) who discovered that within the pH range of 0.9 to 1.6, the oxidation rate does not change significantly. This may not be applicable to the bioreactors with the solution flow dynamics that simulate the actual heap system.

Chapter 6

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APPENDICES

Appendix A

A1.1: A theoretical approach for the jarosite quantification

The following conditions were applied at every new set point of pH used in all the approaches in this present research on the quantification of the jarosite.

- The experimental conditions (temperature, aeration rate) were kept constant for all the experimental trials conducted at different pH values.
- The working volume of growth medium in the bioreactor was kept constant throughout the experimental trials.
- The quantity of jarosite produced in the bioreactor increased with an increase in the operation pH; therefore the bio-film formation was not proportional at different pH values.
- The significantly lower reduction-oxidation potentials (mV) recorded at other pH values other than the pH of 1.3 confirm the diffusion barriers inhibiting the flow of the nutrients in the bioreactor caused by precipitations layers.

A1.2 Calculation of the jarosite accumulation ($g.L^{-1}$) by the AAS (Atomic Absorbance Spectrometry)

- The iron content in the sample was determined by the use of the non-linear method embedded within the AAS software components. This method allowed a calibration of iron from 6 ppm to 30 ppm.
- All the samples analysed were diluted by a dilution factor of 200/250 to fall within the calibration range.
- All the final results were multiplied by their dilution factors to obtain the final values ($g.L^{-1}$).

A1.2: The experimental results of jarosite accumulation ($g.L^{-1}$) and redox potentials (mV) at constant dilution rate and temperature of 0.05 h^1 and 38.6 °C respectively.

Table A1.1: Jarosite formation experimental results obtained at different pH levels

Days	pH									
	1.3		1.5		1.7		Change of pH after 10 days from 1.7 to 1.3		Change in the pH after 10 days from 1.7 to 1.5	
	Mass of Jarosite	mV	Mass of Jarosite	mV	Mass of Jarosite	mV	Mass of Jarosite	mV	Mass of Jarosite	mV
10	4.95	678	5.89	630	7.08	610	-	-	-	-
15	5.50	711	7.90	645	9.98	634	4.78	635	6.60	625

*mV= redox potential, $g.L^{-1}$ = mass of jarosite

Appendix B

B1.1: A theoretical development for the kinetics of microbial ferrous –iron oxidation

The overall cell and substrate mass balances must be established for a packed reactor at different residence times so as to establish the kinetics and the yield parameters for the Fe^{2+} oxidising culture. Therefore, the following general assumptions are valid for this present experimental study:

- The reaction in the bioreactor occurs at a constant aeration rate, temperature the microbial concentration due to the fact that at steady-state the rate of cell growth is equal to the cell removal in the bioreactor.
- The experiment is conduct at a constant working volume.
- The process is carried out in a continuous, stead-state mode.
- The liquid in the bioreactor attains a near perfect mixing, due to the intensive mixing caused by high aeration rate.
- The solution is sterile.
- There is no presence in the bioreactor of any growth-inhibiting factors.
- The culture is substrate-limited in relation to ferrous-iron.
- There is no diffusion limitation of the substrate caused by the jarosite precipitates layers on a thin bio-film because of a small drop of the concentration of the limiting substrate.
- The glass balls (the packing material) offer a surface on which the microorganisms uniformly immobilise themselves, thereby enhancing their growth within the solution.
- The growth rate of cells is faster than the cell death rate. Consequently, it is assumed to be negligible.

The derivation of the rate equations is well-explained in Ojuma*et al.* (2006). While the simplification of the Hansford and Monod models used in the present research is well-explained inChowdhury (2012) (unpublished).

Appendix C

C1.1: The determination of the dilution rate by the weight decrease of the feed vessels

The dilution rate, also known as the inverse of the residence time (τ^{-1}), was determined by the weight decrease of the vessel using Equation C1.1:

$$D = \frac{1}{\tau} = \frac{(m_{initial} - m_{final})}{V_{bioreactor}\rho_{feed}(t_{final} - t_{initial})} \quad . \qquad \textbf{C1.1}$$

Where D : The dilution rate (h^{-1})

τ : The residence time (h)

$m_{initial} - m_{final}$: The weight decrease of the feed vessel (g)

$t_{final} - t_{initial}$: The time interval for the weight decrease (h)

ρ_{feed} : The density of the feed solution (gL^{-1})

C1.2: Theoretical aspect of the calibration using the Nernst Equation

The Nernst Equation (Equation C1.2) defines the relationship between the redox potential (E_h), the standard redox potential ($E^0{}_h$) and the ferric to ferrous iron concentration [Fe^{3+}] / [Fe^{2+}].

$$E_h = E^0{}_h + \frac{RT}{nF}\ln\frac{[a_{Fe^{3+}}]}{[a_{Fe^{2+}}]} \qquad \text{C1.2}$$

The standard redox potential ($E^0{}_h$) for the half cell reaction $Fe^{2+} \rightarrow Fe^{3+} + e$ is 770 mV. This phenomenon is derived from the thermodynamic data and is applied to a situation whereby the activities of both ferric denoted by $a_{Fe^{3=}}$ and for the ferrous iron $a_{Fe^{2+}}$ are both equal, measured by the Standard Hydrogen Electrode (SHE). The compound activity i, a_i is equal to its concentration only if the ionic strength of the solution is zero. Therefore, for the ionic strength greater than zero, $a_i = y_i C_i$, whereby y_i is the activity coefficient. It is for this reason that the actual value of E_h at equal ferric and ferrous iron concentrations would change with increasing ionic strength as a result of the influence of the presence of the other cations and the anions. The previous study(Nagpal and Dahlstrom, 1994)[1] revealed that the presence of complexing agents such as $SO_4{}^{2-} OH^-$ caused a decreased of the ferrous and the ferric ions. This phenomenon has been proven by the simulation of computing programs such as the Visual Minteq and HRC Chemistry software, elaborating on how stronger complexes are formed with ferric rather than the ferrous-iron. Consequently, equation could be written as:

$$E_h = E'_h + \frac{RT}{nF} \ln \frac{[Fe^{3+}]}{[Fe^{2+}]}$$

Whereby $E'_h = E^0{}_h + \frac{RT}{nF} \ln \frac{y_{Fe^{3+}}}{y_{Fe^{2+}}}$

It is for this reason that the term E'_h is defined as a solution potential measured at equal ferric and ferrous- iron concentrations. It defines the activity coefficients Fe^{3+} and Fe^{2+}, the formations of the complexes with Fe^{3+} and $Fe^{2+}{}^{+}$ as well the type of electrode. The commonly used Nernst Equation relates the measured solution potential (E_h), the standard redox potential and the ration between the total concentrations of ferric and the ferrous-ions. Consequently, for a specific electrode (E'_h), the values may be obtained from the intercept of plot E_h versus ([Fe^{3+}] / [Fe^{2+}]) and the slope gives $\frac{RT}{nF}$.

Table C1.1: Parameters determined from the standard calibration curve for redox probes used in the present study

Temperature (°C)	E_h' **(mV)**	$\frac{RT}{nF}$ **(cJ.mol^{-2})**	**R^2**
38.6	440.10	25.12	0.9934

1. Nagpal, S. &Dahlstrom D. 1994. A mathematical model for the bacterial oxidation of a sulphide ore concentrate. *Biotechnology and Bioengineering,* 43: 357-364.

Appendix D

Statistical analysis: The relationship between sum of squares and correlation coefficient

D1.1: Sum of squares

Consider two quantities: y_n, the measured data and $\hat{y}_n$, the predicted data which may be represented according to this regression line $\hat{y}_n = a + bx$ whereby a and b are the intercepts and the slope of the regression line respectively, while the sum of squares error (SSE) is the difference between the observed quantity and the predicted quantity as shown by Equation D1.1.

$$SSE = \sum (y_n - \hat{y}_n)^2 = \sum (y_n - a - bx_n)^2) \qquad \text{D1.1}$$

This quantity will be small if the observed values $\hat{y}_n$, fall close to the regression line (i.e. $\hat{y}_n = a + bx$) and will be vice versa large if they do not fall close to the regression line.

The term $y_n - \hat{y}_n$ in Equation D1.1 represents the error or residual for the nth observation. By substituting $a = \bar{y} - b\bar{x}$ into Equation D1.1 then SSE may be expressed as follows:

$$SSE = \sum (y_n - \bar{y} + b\bar{x} - \bar{x}_n)^2 = \sum ((y_n - \bar{y}) - b(\bar{x}_n - \bar{x}))^2$$

$$= \sum (y_n - \bar{y})^2 - 2b\sum (y_n - \bar{y})(x_n - \bar{x}) + b^2 \sum (x_n - \bar{x})^2 \qquad \text{D1.2}$$

$$= SS_y + 2bSS_{xy} + b^2 SS_y$$

However, $b = SS_{xy} / SS_x$ and it is for reason that SSE may be written as Equation D1.3

$$SSE = SS_Y - bSS_{xy} \quad \text{D1.3}$$

The first term on the right-hand side of Equation D1.3 represents the total sums of squares and is denoted by SST, so that $SST = SS_y$.

The second term determines by how much the total variability is reduced by the regression line $\hat{y}_n = a + bx$. Thus the term bSS_{xy}, denoted by SSR, is known as the sum of squares due to regression. Consequently, Equation D1.3 may be written as follows:

$$SSE = SST - SSR \quad \text{D1.4}$$

Equation D1.4 is important due to the fact that it demonstrates how SST may be decomposed into SSR and the error sum of squares, SSE. The decomposition is shown by the regression. Equation D1.4 may be written as follows:

$$\frac{SSE}{SST} = 1 - \frac{SSR}{SST} \quad \text{D1.5}$$

Where

$$\frac{SSR}{SST} = \frac{bSS_{XY}}{SS_Y} = \frac{SS^2}{SS_{XY} SS_y} = R^2$$

$$SS_x = \sum x^2 - \frac{\left(\sum x\right)^2}{n}$$

$$SS_y = \sum y^2 - \left(\sum y\right)^2$$

$$SS_{xy} = \sum xy - \frac{\left(\sum x\right)\left(\sum y\right)}{n}$$

Consequently, applying Equation D1.5, the error sum of squares (SSE) and the coefficient of regression (R^2) may be related as demonstrated by Equation D1.6.(Keller and Warrack, 1999)[2]

$$R^2 = 1 - \frac{SSE}{SST} \qquad \text{D1.6}$$

This correlation will be used for an error analysis between the modelled and measured data which were obtained in the present experiment (refer to section D1.2).

D1.2 Error analysis between modelled and measured data

The average error percentage (shown in Table D1.1) obtained using Equation D1.1, after the data was fitted both to the Hansford and Monod models:

Table D1.1: Error analysis of ferrous ironbiooxidation experimental data at constant temperature and pH of 38.6 °C and 1.3

Measured data	Hansford model	Monod model
2.8	2.7	2.9
3.3	3.4	3.2
4.2	4.9	4.0
5.5	6.0	4.4
6.8	6.8	5.0
Average % Error	6.2	11.0

2. Keller, G. &Warrack, B. 1999. Statistics: for Management and Economics. (5th Edition) Duxbury Thomson Learning, USA. pp. 626-673

Appendix E

Determination of concentration of iron species

E1.1: Reagent preparation

E1.1.1: Spekker acid

The spekker acid solution was prepared by mixing equal of concentrated sulphuric acid (98% H_2SO_4) and the phosphoric acid (85%) with distilled water in the ratio of 3:4.

- Measure 600 mL distilled water using a 2L beaker.
- Carefully add 225 mL of concentrated sulphuric acid (98%) and 225 mL of phosphoric acid (85%) by slowly pouring against the wall of the beaker (Caution: rapid pouring of concentrated acid may result in intensive heat of mixing that can prove to be harmful).
- The mixture should be cooled to the room temperature before transferring into the storage bottle.

E1.1.2: Ferric acid

The ferric acid solution was prepared from the spekker acid:

- Measure 600 mL distilled using in a 2L beaker.
- Slowly and carefully add 150 mL of spekker acid, followed by the addition of 300 mL of concentrated hydrochloric acid (32% HCL) to the distilled water.
- Agitate the mixture using a magnetic stirrer and allow the mixture to cool to room temperature before transferring the product into a storage bottle.

E1.1.3: Stannous chloride solution ($Sncl_2$)

- Weigh out 30 g of the stannous chloride in a 200 mL beaker.
- Add 100 mL of concentrated hydrochloric acid (32%) and then agitate at a temperature of 50°C until it dissolves completely.
- Allow to cool to room temperature and then dilute with 200 mL distilled water.
- Add a small amount of the granular tin to the solution in order to retard precipitationE1.1.4: Mercury Chloride solution ($HgCl_2$)
- Weigh out 50g of mercury chloride in a 2 L beaker.
- Add 1 L of distilled water and agitate until the solute completely dissolves (about 2 hours).
- Add a spatula tip of $HgCl_2$ and stir for two hours before storage.

E1.1.5: Potassium Dichromate solution (0.0149 M $K_2Cr_2O_7$)

- Dry approximately 10g of $K_2Cr_2O_7$ having a molar mass of 294.20 $g.mol^{-1}$ in an oven at a temperature of 105-110 °C for a duration of 1-2 hours, followed by the cooling process in a desiccator.
- Accurately weigh out 8.78 g of the dried $K_2Cr_2O_7$ into a 100 mL beaker.
- Transfer quantitatively into a 2 L beaker.
- Add 1.5 L of the distilled water and agitate until complete dissolution.
- Transfer quantitatively into a 2 L standard volumetric flask and fill to the 2 L mark with distilled water.

E1.1.6: Barium DiphenyllamineSulphonate (BDS) solution ($C_{24}H_{20}BaN_2O_6S_2$)

- Weigh out 1.0 g of barium diphenylamine sulphonate in a 250 mL beaker and add 100 mL of concentrated sulphuric acid (98%). Agitate the solute until complete dissolution.

E1.2: Determine the ferrous-iron concentration by titrating with potassium dichromate solution.

- Pipette 5 mL of the required aliquote solution into a 125 mL of conical flask.
- Add 10 mL of spekker acid solution.
- Add 2-3 drops of the BDS indicator.

- Titrate with the potassium dichromate (0.0149 M $K_2Cr_2O_7$) solution until the first permanent colour change from yellow to intense purple is obtained.

Ferrous-iron may also be calculated using the chemical Equation E1.1

$$\left[Fe^{2+}\right] = \frac{[K_2Cr_2O_7] \times V_T \times (55.84 \times 6)}{V_{Solution}}$$

Whereby

$\left[Fe^{2+}\right]$ = Ferrous-iron concentration ($g.L^{-1}$)

$K_2Cr_2O_7$ = $K_2Cr_2O_7$ concentration (i.e 0.0149 M $K_2Cr_2O_7$)

V_T = Titration volume (mL), containing the amount of the 0.0149 M $K_2Cr_2O_7$ added.

$V_{Solution}$ = Volume of the aliquot (mL).

E1.3: The determination of the total iron concentration by titrating with potassium dichromate solution

1. Filter 5 mL aliquot of sample solution.
2. Pipette the required amount of aliquot (i.e 5 mL) into a 125 mL conical flask.
3. Add 10 mL of spekker acid solution and heat until the mixture boils.
4. Add stannous ($SnCl_2$) solution drop wise until the yellow colour disappears completely. Add one extra drop and take note of the number of drops of the stannous chloride added; this is done in order to give an approximation of the anticipated amount of $SnCl_2$ to be added when conducting a series of duplicate samples for titrations.
5. Allow the solution to cool to room temperature and then add 10 mL of mercury chloride ($HgCl_2$) solution. A silky-white precipitate should appear. If no precipitate forms then too little stannous chloride was added in step 4. However, if the precipitate formed is heavy and grey or black then too much stannous chloride was added. If either of the instances occurs, the experiment must be aborted and repeated.

6. Add 3-4 drops of barium diphenylamine indicator solution (BDS) and titrate with the potassium dichromate solution until the first permanent colour change, yellow to intense purple, is achieved.

Total iron concentration may be calculated using Equation E1.1

$$[Fe_T] = \frac{[K_2Cr_2O_7] \times V_T \times (55.84 \times 6)}{V_{Solution}} \qquad \text{E1.1}$$

Whereby

$[Fe_T]$ = Total iron concentration ($g.L^{-1}$)

$K_2Cr_2O_7$ = $K_2Cr_2O_7$ concentration (i.e 0.0149 M $K_2Cr_2O_7$).

V_T = Titration volume (mL), containing the amount of the 0.0149 M $K_2Cr_2O_7$ added.

$V_{Solution}$ = Volume of the aliquot (mL).

E1.4:Vishniac Trace Metal Solution

Viashniac Trace Metal Solution was prepared according to the method suggested by Vishniac and Santer(1957).

1. Prepare 6% potassium hydroxide (KOH) by weighing 15 g KOH and dilute to 250 Ml with distilled water.
2. Dissolve 50 g EDTA (Ethylene diaminetetra acetic acid disodium salt dehydrate) in 200 mL of 6% KOH using a magnetic stirrer.
3. In a separate 500 mL beaker, weigh the salts listed below and dissolve in 400 mL of distilled water for 30 minutes using a magnetic stirrer.

$ZnSO_4.7H_2O$	22 g
$CaCl_2.2H_2O$	9.23 g

$MnCl_2.4H_2O$	5.06 g
$FeSO_4.7H_2O$	5 g
$(NH_4)_6Mo_7O_{24}.4H_2O$	1.1 g
$CuSO_4.5H_2O$	1.58 g
$CoCl_2.6H_2O$	1.62 g

4. Transfer the solution in step 2 quantitatively into the solution prepared in step 3 and fill up to 1 L with distilled water by rinsing the 500 mL beaker with 400 mL of distilled water. A greenish brown solution result.

Appendix F

Experimental data at a constant temperature and pH of 38.6 °C and ± 1.3

Table F1.1: Ferrous-iron biooxidation experimental data

Dilution rate (h^{-1})	$[Fe_T]_{in}$	$[Fe_T]_{out}$	Precipitation of Fe^{3+}	% of Jarosite formation	$[Fe^{2+}]_{in}$	$[Fe^{2+}]_{out}$
0.03	89.53	88.28	1.25	1.40	84.16	0.04
0.04	89.53	87.74	1.74	1.94	82.91	0.05
0.05	89.53	87.62	1.91	2.13	83.62	0.14
0.07	89.53	88.28	1.25	1.40	83.62	0.42
0.10	89.53	89.53	0.00	0.00	85.41	16.90

Units: $[Fe_T]_{in}$ ($mmol.L^{-1}$), $[Fe_T]_{out}$ ($mmol.L^{-1}$), $[Fe^{2+}]_{in}$ ($mmol.L^{-1}$), $[Fe^{2+}]_{out}$ ($mmol.L^{-1}$)