

BIOLEACHING OF LOW-GRADE NICKEL SULPHIDE ORE AT ELEVATED pH

By

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Abstract

This thesis examines the bioleaching of six different Canadian nickel sulphide ores at pH levels above what is generally considered optimum (~ 2). The majority of work discussed in this thesis was conducted with a low-grade metamorphosed ultramafic nickel sulphide ore from Manitoba, Canada (Ore 3), which is not currently exploitable with conventional technologies. The ore contains 21% magnesium and 0.3% nickel. Nickel is the only significant metal value, and is present primarily as pentlandite. A substantial fraction of the magnesium is present as the serpentine mineral lizardite, making processing difficult with conventional pyro- and biohydrometallurgical techniques. The work with this ore has two equally important objectives: to minimize magnesium mobilization and to obtain an acceptable level of nickel extraction. Batch stirred-tank bioleaching experiments were conducted with finely ground ore ($-147\ \mu\text{m}$) with temperature and pH control. The first phase of experimentation examined the effect of pH (2 to 6) at $30\ ^\circ\text{C}$, and the second phase examined all combinations of three pH levels (3, 4 and 5) and five temperatures (5, 15, 22.5, 30, and $45\ ^\circ\text{C}$).

The initial rate of nickel extraction from pentlandite was observed to be inversely correlated to acidity at all temperatures, while the final nickel extraction after five weeks was determined to be moderately correlated to acidity at high temperatures and negatively correlated to acidity at low temperatures. The advantage of elevated-pH bioleaching was most evident at $5\ ^\circ\text{C}$, at which the final nickel extraction at pH 5 was approximately 250% greater than at pH 3. Electron probe X-ray microanalysis of the post-leach residues revealed that un-reacted lizardite was enriched with nickel during experiments conducted at pH 5, and the extent of enrichment was a strong function of temperature. The undesirable extraction of magnesium exhibited a strong negative pH*temperature interaction and the consumption of sulphuric acid directly tracked magnesium extraction over all experimental conditions. Bioleaching at elevated pH substantially increased the ratio of nickel to magnesium in the leachate, and resulted in a substantial reduction in sulphuric acid consumption.

A third phase of bioleaching experiments with Ore 3 was conducted, in which six conditions from the second phase were repeated, in order to characterize the bacterial

community structure as a function of time over a wide range of pH (3 to 5) and temperature (5 to 45 °C) conditions. A combination of classical microbiological and molecular biological techniques was used to identify and enumerate the members of the bacterial consortia. DGGE analysis revealed the presence of at least 16 distinct 16S rRNA gene sequences, two of which are not closely related to existing GenBank sequences and may be from novel species. Thirteen sequences are related to gene sequences of genera that have previously been detected in bioleaching environments (*Acidithiobacillus*, *Leptospirillum*, *Sulfobacillus*, *Acidiphilium*, *Ferrimicrobium*, and *Acidimicrobium*). *Acidithiobacillus* spp. were dominant at all temperatures except 45 °C, at which *Sulfobacillus* spp. were dominant. Many of the acidithiobacilli are most closely related to strains of *Acidithiobacillus ferrooxidans*, and different strains were dominant under different experimental conditions, indicating considerable phenotypic heterogeneity within the species.

Additional stirred-tank bioleaching experiments were conducted with five other nickel sulphide ores from different geographical locations across Canada. Mineralogical and chemical examination revealed considerable variability between the samples, particularly in the silicate phases. The ores contain 0.3 to 1% nickel, primarily in pentlandite and secondarily in pyrrhotite. The ores were subjected to the same crushing and grinding procedure, and bioleached under the same conditions for three weeks with a mixed culture of iron- and sulphur-oxidizing bacteria. In general, the presence of the bacteria resulted in a statistically significant increase in nickel, cobalt, and copper extraction, and oxidation-reduction potential; whereas their presence resulted in a statistically significant decrease in acid consumption. Nickel extraction from pentlandite and pyrrhotite during bioleaching at pH 2 and 3 was generally good (49 to 86% after three weeks). All six ores showed a similar response to a change in pH; an increase in pH from 2 to 3 resulted in approximately the same nickel and cobalt extraction (within statistical error), and a statistically significant reduction in sulphuric acid consumption. In light of the results obtained in this study, it is recommended that bioleaching studies with nickel sulphide ores and concentrates consider a wider pH range than what is generally considered optimum, particularly if a low-discharge process is desirable.

Résumé

Cette thèse examine la biolixiviation de six minerais Canadien de sulfures de nickel, testée à des niveaux de pH au-dessus de ce qui est généralement considéré comme optimal (~2). La plus grande partie du travail décrite dans cette thèse a été effectuée sur du minerai métamorphosé ultramafique à faible teneur en sulfure de nickel provenant du Manitoba (minerai 3). Ce dernier n'est d'ailleurs pas exploitable à partir de technologies conventionnelles. Ce minerai contient 21% de magnésium et 0.3% de nickel. Le nickel est le seul métal ayant une certaine valeur économique importante et est présent sous forme de pentlandite. Une grande partie du magnésium présent est sous la forme de lizardite, un minéral de serpentine, ce qui rend d'ailleurs difficile le traitement du minerai à partir des techniques de pyro- et de bio-hydrrométallurgie conventionnelles. Le travail fait à partir de ce minerai comporte deux objectifs tout aussi importants l'un que l'autre: soit de minimiser la mobilisation du magnésium et obtenir un niveau acceptable d'extraction de nickel. Des tests de biolixiviation sur du minerai finement moulu (-147 μm) et agité en mode séquentiel en réacteurs ont été entrepris tout en contrôlant la température et le pH. La première phase de l'expérimentation a permis de vérifier l'effet du pH (2 à 6) à 30 °C et la deuxième phase de l'étude a permis d'examiner toutes les combinaisons de trois niveaux de pH (3, 4, et 5) et de cinq températures (5, 15, 22.5, 30 et 45 °C).

Il a été observé que le niveau initial d'extraction du nickel à partir de la pentlandite était inversement corrélé à l'acidité à toutes les températures étudiées. Par contre, l'extraction finale du nickel après cinq semaines d'essais était modérément corrélée à l'acidité aux températures élevées et négativement corrélée à l'acidité aux températures basses. L'avantage de la biolixiviation à pH élevé était plus remarquable à 5 °C, température à laquelle l'extraction finale de nickel à pH 5 était approximativement 250% plus élevée que celle obtenue à pH 3. Une analyse par micro-sonde électronique sur un résidu lixivie a montré que la lizardite restante s'était enrichie en nickel au cours des tests effectués à pH 5. L'étendue de cet enrichissement était en fonction de la température. L'extraction involontaire du magnésium a démontré une interaction négative pH*température. La consommation d'acide sulfurique était directement reliée à l'extraction du magnésium sous toutes les

conditions expérimentales testées. La biolixiviation à pH élevé a considérablement augmenté le ratio de nickel versus le magnésium dans le lixiviat, et en conséquence, a réduit considérablement la consommation d'acide sulfurique.

Une troisième phase d'étude de biolixiviation sur le minerai 3 a été entreprise, au cours de laquelle six conditions étudiées au cours de la phase 2 ont été répétées. Le but était de caractériser la structure de la communauté bactérienne en fonction du temps et d'une variété de conditions de pH (3 à 5) et de températures (5 à 45 °C). Une combinaison de techniques de microbiologie classique et de biologie moléculaire a été utilisée afin d'identifier et d'énumérer les membres de ces consortiums bactériens. L'analyse par électrophorèse sur gel en gradient dénaturant ou DGGE a révélé la présence d'au moins 16 séquences distinctes du gène de l'ARNr 16S, dont deux qui ne sont pas rapprochées aux séquences existantes de la GenBank et pourraient provenir de nouvelles espèces. Treize séquences sont apparentées aux séquences de gènes de genres qui ont été antérieurement détectés dans des environnements propices à la biolixiviation (*Acidithiobacillus*, *Leptospirillum*, *Sulfobacillus*, *Acidiphilium*, *Ferrimicrobium*, et *Acidimicrobium*). *Acidithiobacillus* spp. était dominante à toutes les températures excepté à 45 °C, à laquelle *Sulfobacillus* spp. était dominante. Plusieurs des acidithiobacilles étaient plus apparentés aux souches *Acidithiobacillus ferrooxidans*. Différentes souches étaient dominantes sous différentes conditions expérimentales, indiquant une hétérogénéité phénotypique considérable parmi les espèces.

Des essais additionnels de biolixiviation dans des réacteurs agités ont été effectués sur d'autres minerais de sulfure de nickel provenant de différentes locations géographiques situées au Canada. Une analyse minéralogique et chimique présente une variabilité considérable entre les échantillons, en particulier parmi les phases de silicates. Les minerais contiennent de 0.3 à 1% de nickel, principalement dans la pentlandite et deuxièmement dans la pyrrhotite. Les minerais ont tous été broyés et moulus selon la même procédure et biolixiviés sous les mêmes conditions durant trois semaines à partir de cultures bactériennes mixtes capables d'oxyder le fer et le soufre. En général, la présence de bactéries a augmenté de manière statistiquement significative l'extraction du nickel, du cobalt et du cuivre ainsi que le potentiel d'oxydo-réduction, mais a entraîné une réduction significative de la consommation d'acide. L'extraction du nickel à partir de la pentlandite et de la pyrrhotite

durant la biolixiviation à pH 2 et 3 était généralement bonne (49 à 86% après trois semaines d'essais). Tous les six minerais testés ont réagi de façon similaire à un changement de pH. Une augmentation de pH de 2 à 3 a permis une extraction assez similaire du nickel et du cobalt (dans la limite de l'erreur statistique), et une réduction statistiquement significative de la consommation en acide sulfurique.

Selon les résultats obtenus lors de cette étude, il est recommandé que des essais de biolixiviation sur des minerais et des concentrés de sulfure de nickel soient effectués sur une plus grande étendue de pH que celui considéré comme optimal, surtout si un procédé de traitement sans rejet est souhaitable.

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Dedication

I would like to dedicate this thesis to my wife, Allison Larin-Cameron, and my two boys, Charles G. Cameron and Fraser R. Cameron. They have provided me with the inspiration and the ‘financial incentive’ to complete my degree.

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Nomenclature

A	Arrhenius frequency factor	$\text{mol}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$, s^{-1} , $\text{L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$
A_1	Constant in Equation 5.2	dimensionless
A_2	Constant in Equation 5.2	dimensionless
A_3	Constant in Equation 5.2	dimensionless
A_4	Constant in Equation 5.2	dimensionless
$a^{\text{Fe}^{3+}}$	Activity of ferric ion	dimensionless
$a^{\text{Fe}^{2+}}$	Activity of ferrous ion	dimensionless
b	Stoichiometric reaction coefficient	dimensionless
B_1	Constant in Equation 5.2	dimensionless
B_2	Constant in Equation 5.2	dimensionless
B_3	Constant in Equation 5.2	dimensionless
c	Concentration factor in Equation C.2	dimensionless
C_A	Concentration of A	$\text{mol}\cdot\text{L}^{-1}$
d_l	Liberation diameter	m
d_N	Nominal crush size	m
D	Impeller diameter	m
D_e	Diffusivity of A	$\text{m}^2\cdot\text{s}^{-1}$
E	Activation energy	$\text{J}\cdot\text{mol}^{-1}$
E_h	ORP versus a standard hydrogen electrode	V
f	Shape factor	dimensionless
g	Granulometric factor	dimensionless
k	Kinetic rate constant	$\text{mol}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$, s^{-1} , $\text{L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$
k'	Mass transfer coefficient through stagnant film	$\text{m}\cdot\text{s}^{-1}$
k''	First order reaction rate constant	s^{-1}
l	Liberation factor	dimensionless
M_L	Lot mass	kg
M_S	Sample mass	kg
N_R	Reynolds number	dimensionless
n	Impeller rotational speed	revolutions $\cdot\text{s}^{-1}$

R_x	Rate of increase of metal concentration in solution	$\text{mol}\cdot\text{L}^{-1}\cdot\text{s}^{-1}$
R	Ideal gas constant	$8.314\text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1}$
R_o	Initial particle radius	m
Sa	Salinity	$\text{g}\cdot\text{kg}^{-1}$
S_{AB}	Similarity coefficient	dimensionless
T	Temperature	$^{\circ}\text{C}$, K
T_2	Bacterial doubling time	time
T_0	Initial condition	time
T_7	After 7 days	time
T_{35}	After 35 days	time
t	Time	time
v_{bar}	Average particle volume	m^3
v_N	Volume of the nominal particle	m^3
Y	Predicted value in Equation 5.1	%
X_B	Fraction of reacted mineral converted	dimensionless

Greek Letters

β_0	Regression constant	%
β_1	Regression constant	$\%\cdot\text{K}^{-1}$, %
β_2	Regression constant	%
β_{11}	Regression constant	$\%\cdot\text{K}^{-2}$
β_{22}	Regression constant	%
β_{12}	Regression constant	$\%\cdot\text{K}^{-1}$, %
α	Fraction of the mineral of interest	dimensionless
ε°	Half-cell reduction potential	V
ρ	density	$\text{kg}\cdot\text{m}^{-3}$
ρ_B	Molar density of B in solid	$\text{mol B}/(\text{m}^3 \text{ solid})$
τ_p, τ_f, τ_r	Constant, defined as the time required for complete conversion	s
σ_{FSE}	Fundamental error	dimensionless

μ_f	Dynamic viscosity of fluid	Pa·s
μ	Specific growth rate coefficient	s ⁻¹

List of Acronyms and Abbreviations

Abio:Bio	Abiotic to biotic ratio
AES	Atomic emission spectroscopy
ANOVA	Analysis of variance
ARD	Acid rock drainage
ATCC	American type culture collection
Att	ATCC media 23
BSE	Backscattered electron
CI	Confidence interval
DGGE	Denaturing gradient gel electrophoresis
DO	Dissolved oxygen
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide
EDS	Energy dispersive spectra
EDTA	Ethylenediaminetetraacetic acid
EPMA	Electron probe X-ray microanalysis
EPS	Extracellular polymeric substance
HPLC	High performance liquid chromatography
ICP	Inductively coupled plasma
MgO	High-magnesium gangue minerals
MLA	Mineral analyser
MPN	Most probable number
mTK	Modified TK media
%Ni:%Mg	Percent nickel extracted to percent magnesium extracted ratio
%Ni:%Co	Percent nickel extracted to percent cobalt extracted ratio
NP	Neutralization potential

ORP	Oxidation/reduction potential
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PGE	Platinum group elements
pH _{opt}	Optimum pH for bacterial growth
PLS	Pregnant leaching solution
RNA	Ribonucleic acid
RPM	Revolutions per minute
SEM	Scanning electron microscope
SHE	Standard hydrogen electrode
STP	Standard temperature and pressure
STR	Stirred-tank reactor
TAE	Tris acidic acid EDTA buffer
TE	Tris EDTA buffer
T _{opt}	Optimum temperature for bacterial growth
thermo	Media used for thermophilic bacteria
UPGMA	Unweighted pair group method using arithmetic average
UV	Ultraviolet
WDS	Wavelength-dispersive X-ray analyses
XRD	X-ray diffraction

Chapter 1 – Introduction

1.1. Project History

This research was conducted as part of the CANMET-MMSL core project 603071, entitled “Development of a Bioleaching Process for Nickel Production from Canadian Ores.” The project’s objective is to develop a heap bioleaching circuit for low-grade and recalcitrant nickel sulphide ores. The major tasks include:

1. Conducting a comprehensive literature review on the state of the art of nickel sulphide bioleaching;
2. Acquiring a number of low-grade Canadian nickel sulphide ores;
3. Chemical and mineralogical characterization of the ore samples;
4. Assessing the amenability of the ore samples to bioleaching;
5. Selecting one ore for a more intensive kinetic study, and further testing in small bioleaching columns;
6. Developing genetic microbiology identification and enumeration techniques; and
7. Developing a hydrometallurgy circuit to recover nickel from a wide range of pregnant leaching solutions (PLS).

This thesis addresses tasks 1 to 5. Six ores of differing mineralogy were acquired for this research project. Shake flask and stirred-tank reactor (STR) experiments were used to assess the amenability of the different ores to bioleaching, develop a broad understanding of the bioleaching of the primary nickel-containing sulphide minerals pentlandite and

pyrrhotite, and develop correlations between mineralogical/chemical content and (bio)leachability.

Ore 3 from Manitoba was selected for the more intensive study after considering a number of factors, including: the company's attempts at developing a pyro- or hydrometallurgical circuit to recover nickel from this ore have been exhausted (i.e. economical interest); and bioleaching this ore presents a unique challenge not typical of the other ores in this study. Most importantly from an academic perspective, it was believed that a novel approach to bioleaching would be required in order to achieve the desired performance objectives. The primary hypothesis of this work was developed after the results of some preliminary shake flask and stirred-tank work (Chapter 3), and is focused on challenges specific to Ore 3.

Mineralogical characterization is frequently omitted from published bioleaching studies due to budget constraints, as scanning electron microscopy (SEM), X-ray diffraction (XRD), and electron probe X-ray microanalysis (EPMA) can be cost prohibitive. A substantial portion of this project was dedicated to mineralogical analysis, which was used to make comparisons between the ore and the leach residues. This information was essential in elucidating micro-scale mechanisms that occurred during bioleaching. The mineralogical analyses were also used to identify the minerals that were actively being leached, and identify secondary phases that formed as a result of bioleaching.

1.2. Primary Problem Definition and Hypothesis

The company that provided Ore 3 has indicated that they are interested in exploring the prospect of heap bioleaching for that ore; however, the mineralogy presents a significant

challenge, and magnesium management was identified as a major obstacle that must be addressed prior to commercialization. Detailed mineralogical and chemical characterization of Ore 3 is given in Chapter 4. In brief, Ore 3 is a metamorphosed ultramafic-dominated low-grade nickel sulphide ore, characterized by low sulphide mineral content ($< 3\%$), high magnesium silicate mineral content ($> 80\%$), containing 0.3% nickel, and 21% magnesium. Bioleaching an ore with such an assemblage of gangue minerals is challenging, as many magnesium silicate minerals are reactive in acidic media, consuming sulphuric acid, which can be a major operational cost to a heap bioleaching operation. The removal of magnesium from the pregnant liquor and its disposal can represent a significant expense, as the market for magnesium salts is limited, and the price of magnesium metal does not allow for economical recovery. Furthermore, excessive loss of mass during heap bioleaching could result in pile slumping, which reduces heap permeability and negatively impacts heap performance. It is believed that the applicability of heap bioleaching to Ore 3 might ultimately be determined by the behaviour of the magnesium silicate gangue minerals, since their reactivity has a direct effect on two of the major operating costs: sulphuric acid consumption and waste management.

The primary hypothesis of this thesis is that bioleaching Ore 3 at elevated pH (≥ 3) can substantially reduce magnesium mobilization and sulphuric acid consumption, while maintaining an acceptable nickel extraction. This is a novel approach to mineral processing, and may be applicable to other low-grade nickel sulphide deposits of similar mineralogy. A comprehensive kinetic study using batch STR experiments was used to support the primary hypothesis. Six experimental conditions were repeated for the purpose of characterizing the bacterial consortia present at six different combinations of

pH and temperature. A combination of classical and molecular biological techniques was used to identify and enumerate the members of the bacterial consortia three times over the course of each five-week experiment. These experiments have provided valuable information on the species present (and their growth characteristics) during bioleaching of a low-grade nickel sulphide ore over a wide range of temperature (5 to 45 °C) and pH conditions (3 to 5).

1.3. Secondary Problem Definition and Hypothesis

Conventional bioleaching wisdom dictates that a low-pH environment (pH ~ 2) is generally favourable to the dissolution of sulphide minerals; however, a comprehensive review of the technical literature revealed that the dissolution of the primary nickeliferous minerals (pentlandite and pyrrhotite) is less dependent on a low-pH environment, and several studies have reported an inverse relationship between (bio)leaching rates and acidity. **The secondary hypothesis of this thesis is that bioleaching ores in which the primary metal value is nickel at elevated pH (≥ 3) may result in savings related to solution management, sulphuric acid consumption, and in some cases may result in favourable kinetics.**

Five additional ores of varying mineralogy from across Canada were bioleached in STR experiments in order to assess the general effect of pH on nickel extraction from pentlandite and pyrrhotite during bioleaching. This was done in order to develop a broader understanding of the degradation of these minerals under moderately acidic bioleaching conditions. The ores were subjected to the same crushing and grinding procedure, and subjected to the same stirred-tank bioleaching tests with bacteria that were enriched from the same source. This is the only study in the technical literature that has examined the bioleaching of many different sulphide ores (nickel or otherwise) that have been prepared

according to the same crushing and grinding protocol and bioleached under the same experimental conditions, with the same mixed culture of bacteria.

1.4. Experimental Plan

The original experimental plan for this project, as set out in the CANMET-MMSL project proposal (603071) planned for using shake flask bioleaching experiments to assess the effect of bioleaching on ten Canadian nickel sulphide ores as part of the initial amenability study. A total of seven ores were acquired for this study, although only six ores were characterized and used during the bioleaching experiments. A substantial quantity of asbestiform fibres was observed during comminution of one ore; consequently, that ore was not processed further for health and safety reasons. The other six ores were extensively chemically and mineralogically characterized, and the results are discussed in the introduction to Chapter 7. The same crushing and grinding procedure was used for all samples, the details of which and the accompanying theory of representative sample division are discussed in Appendix C.

Ores 1 and 2 were the first two ores to be acquired, in October 2006. As originally planned, shake flask experiments were used to assess the ores' amenability to bioleaching and to assess the effect of bacteria, nutrient concentration, and pH on the bioleaching of nickel. To the best of this author's knowledge, no prior bioleaching work has been done with samples from these two ore bodies; therefore the preliminary shake flask work was exploratory in nature, with no clear experimental objective other than maximizing nickel extraction.

Ore 3 was acquired in June 2007. There was a more defined experimental objective with this ore, as the company had conducted bioleaching work with this ore at conventional pH levels and determined that the undesirable extraction of magnesium was unacceptably high (unpublished data provided by the company). In the present study, the initial shake flask experiments with Ore 3 resulted in adequate nickel solubilisation, but were deemed unsatisfactory due to poor pH control. For this reason, half of the shake flask experiments with Ore 3 were cancelled. It was concluded that STR experiments with pH control was a superior experimental apparatus for bioleaching Ore 3, particularly since pH manipulation was believed to be the most effective way to achieve the dual experimental objective of achieving an acceptable level of nickel extraction, while minimizing magnesium extraction. The conditions examined with Ores 1 and 2 were repeated in STRs and subsequent experiments with all six ores were conducted in STRs.

Most nickel sulphide ores contain copper and cobalt, and often contain platinum group elements (PGEs) as secondary by-product credits. Their relative value to the overall economics of a process is dependent on both the relative abundance of the metals within the ore and the relative selling price of the metals. All the ores used in this study contain copper and cobalt in varying concentrations (PGEs were not assayed). The primary focus of this study was to investigate the bioleaching of nickel from sulphide ores; however, the extraction of both cobalt and copper have been followed and discussed where appropriate, since the extractions of these two metals could be an important consideration in determining the potential economics of a commercial bioleaching process.

1.5. Thesis Organization

This thesis is presented as a series of four papers (Chapters 4 to 7), in addition to a literature review (Chapter 2), preliminary experiments that were used to develop the hypotheses (Chapter 3), and conclusions (Chapter 8). At the time this thesis was submitted, the work had been disseminated in three articles of the peer-reviewed journal *Hydrometallurgy* (Chapters 4, 5, and 6); one conference poster (Chapters 4 and 5); and one conference oral presentation and the corresponding proceedings (Chapter 7). Chapter 7 has also been submitted for consideration to a special Bio&Hydrometallurgy 2010 edition of the peer-reviewed journal *Minerals Engineering*. Chapter 2 is an abridged version of a comprehensive literature review on the bioleaching of nickel sulphide ores and concentrates, which has been published as CANMET-MMSL Internal Report MMSL 07-008 (LS). The chapters that have been written as journal articles contain a preface indicating where the material has been disseminated or submitted.

1.6. Statement of Contributions of Collaborators

This work was conducted as part of a multidisciplinary project that involved a number of collaborators at CANMET-MMSL, the University of Ottawa, and the Biotechnology Research Institute, NRC. The original nickel bioleaching project was proposed as an internal CANMET-MMSL project by Dr. Saviz Mortazavi. The first major project task was a comprehensive literature review written by the PhD candidate, which identified a number of potential research areas that could be included under the umbrella of the proposed nickel bioleaching project. Several research scientists, engineers, and technicians at CANMET-MMSL have contributed to this project; their contributions have been acknowledged in the list of authors in the preface to each chapter.

The primary contributions of the collaborators include the mineralogy characterization that is presented in all chapters of this thesis, which was conducted in collaboration with Drs. Rolando Lastra and Yves Thibault, and the molecular biological work that is presented in Chapter 6, which was conducted in collaboration with Drs. William Yeung and Charles Greer.

Chapter 2 – Literature Review

2.1. Introduction

Conventional extractive metallurgy of nickel from sulphide minerals consists of a combination of pyrometallurgical and hydrometallurgical processes. Nickel sulphide minerals are concentrated primarily by froth flotation and magnetic separation. After beneficiation, concentrates may be subjected to a number of hydrometallurgical and/or pyrometallurgical processes. Pyrometallurgical processes are used on over 90% of global nickel sulphide concentrates (Kerfoot *et al.*, 1997). Low-grade and complex ores can be recalcitrant to conventional concentration technologies, leading to poor quality concentrates, which can incur high smelter penalties. Furthermore, processing low-grade ores leads to an increased amount of rejection material, along with its accompanying environmental liability, and loss of potentially recoverable nickel.

Traditional pyrometallurgical processing techniques are coming under increasing public and regulatory scrutiny due to environmental concerns, as the environment has become a major public and political issue in recent years. Bioleaching operations have a significantly lower environmental impact in comparison to conventional pyrometallurgical operations. The long-term liabilities associated with waste-rock piles are significantly reduced, as the acid-generating minerals are consumed during the bioleaching process. Exposed sulphide minerals are readily oxidized under a controlled environment, depleting the reduced-sulphur content of the ore, thus reducing the potential to produce acid rock drainage (ARD).

Heap bioleaching is particularly attractive for treating small and low-grade ore bodies, complex ores that are recalcitrant to conventional technologies, and high-grade ore bodies located in remote areas. Mechanical comminution requirements of a heap bioleaching operation are substantially less compared to conventional technologies, as froth flotation requires a particle diameter several orders of magnitude smaller than is required for heap bioleaching. High selectivity during froth flotation requires highly liberated material, which necessarily requires finely ground ore.

The importance of heap leaching in the copper industry has steadily grown since the late 1970s. Since 1977, at least twenty-three commercial heap leaching operations have been commissioned for processing copper oxide and secondary copper sulphide ores (Watling, 2006). In 1999, an estimated 15% of the world's copper came from heap (bio)leaching (Readette, 1999). In the last decade, extensive efforts have been made to expand heap bioleaching technology to primary copper sulphide ores, and to a lesser extent nickel sulphide ores. There have been heap bioleaching pilot trials with nickel sulphide ores in Australia (Hunter, 2002), Finland (Riekkola-Vanhanen, 2007), and China (Wen *et al.*, 2006). The first commercial application of nickel sulphide heap bioleaching began production at Talvivaara, Finland in October 2008 (Talvivaara, 2009).

Leaching of sulphide minerals was long believed to be a purely chemical process, until Colmer and Hinkle (1947) discovered the first iron-oxidizing bacteria. Since then, a substantial number of papers have been published on the bioleaching of sulphide minerals. It is commonly accepted that the presence of iron- and sulphur-oxidizing bacteria can substantially increase the observed leaching rates, and that bioleaching involves numerous chemical, electrochemical, and biochemical processes that contribute to the observed rate of

metal release. The relative importance of those processes is system-specific, and depends on the mineral of interest and the solution chemistry.

As the first task associated with this research project, a comprehensive literature review on the bioleaching of nickel sulphide ores and concentrates was completed in November 2007 (Cameron, 2007), entitled “Bioleaching in the nickel mining industry – A literature review.” For the sake of brevity, the literature review presented in this thesis is an abridged version, which highlights the factors that are considered and/or investigated in this thesis.

2.2. Economically Significant Nickel Sulphide Minerals

More than 50% of world nickel production comes from sulphide ores (Eramet, 2006). Pentlandite is the most economically significant nickel sulphide mineral, and usually contains 32 to 39% nickel by mass. Other sulphide minerals frequently found in association with pentlandite include pyrrhotite, chalcopyrite, and pyrite. Nickeliferous pyrrhotite is generally the most abundant sulphide phase in nickel sulphide ores, and typically contains 0.2 to 0.5% nickel in solid solution, in addition to very finely divided pentlandite inclusions (Habashi, 1997). Nickel sulphide ores frequently contain copper, cobalt, and numerous platinum group elements (PGE), all of which may represent significant values (Kerfoot *et al.*, 1997).

Pentlandite grains often form by exsolving from molten pyrrhotite during the cooling phase of orebody formation. Consequently, the two minerals are usually found in intimate contact and are frequently intertwined. This intimacy can have consequences for the leaching process. If the two minerals are in direct physical contact, it is anticipated that pentlandite

would be passivated by the presence of pyrrhotite due to galvanic interactions (discussed in Section 2.4.3.6).

2.3. Heap Bioleaching

A full-scale copper heap contains 1 to 20 million tonnes of crushed ore, stacked 6 to 10 meters high on a lined pad. A heap bioleaching operation generally includes the following processes/unit operations: the ore is irrigated with recycled leachate containing the oxidant, bacteria, and nutrients; the leachate trickles through the ore bed and reacts with the sulphide minerals, which releases the metals into solution; the pregnant leaching solution (PLS) is collected in a leachate pond at the base of the heap; a portion of the leachate is subjected to a hydrometallurgical process to recover the metal values; and the barren solution is mixed with PLS and re-circulated to the top of the heap. The pH of the leachate may be adjusted prior to application to the top of the heap, and nutrient levels are adjusted as required. Low pressure blowers are usually placed at the bottom of the pile in order to supply the oxygen and carbon dioxide required for bacterial growth and to maintain an oxidative environment within the heap.

The crush size, irrigation rate, heap height, and aeration rate are project-specific and are usually determined during pilot testing. In many cases, the ore is agglomerated with sulphuric acid prior to stacking in order to limit the migration of fines and provide an acid pre-conditioning stage. After leaching, fresh ore may be stacked on top of the leached ore; the leached ore may be removed from the pad for further processing or application to a tailings area; or the leached ore may be left in place, while the pad is extended and new ore is stacked adjacent to the old heap.

2.4. Bioleaching Fundamentals

2.4.1. Bioleaching microorganisms

Sulphide mineral bioleaching operations usually involve a consortium of iron- and sulphur-oxidizing bacteria. *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*) was long believed to be the sole microorganism involved, but it is now known that other microorganisms such as *Leptospirillum ferrooxidans* (*L. ferrooxidans*), *Acidithiobacillus thiooxidans* (*At. thiooxidans*), moderately-thermophilic and thermophilic species, and heterotrophic species play an important role.

Acidithiobacillus spp are autotrophs that grow chemolithotrophically on energy liberated from the oxidation of reduced-sulphur compounds. Some species have the ability to grow by oxidizing ferrous ion or hydrogen. *Acidithiobacillus* spp fix carbon from carbon dioxide via the Benson-Calvin cycle. Their optimum pH for growth is species-specific, but is generally less than 4.0. The optimum growth temperature for mesophilic species is between 30 and 35 °C, and up to 45 °C for moderately-thermophilic species. They are motile by means of one or more polar flagella and they have no known resting stage. They are ubiquitous in nature and may be found in any acidic, sulphide-rich environment (Kelly and Wood, 2000).

At. ferrooxidans derives its energy from the oxidation of elemental sulphur, thiosulphate, and ferrous ion. The reported optimum pH for iron oxidation is approximately 2, in a range from 1.5 to 6. Traditionally, *At. ferrooxidans* has been regarded as a mesophile, growing in a temperature range from 15 to 37 °C, with an optimum around 30 °C (Leduc and Ferroni, 1994). Psychrotolerant strains have been isolated that exhibited exponential growth

on ferrous ion at temperatures as low as 2 °C (Leduc *et al.*, 1993). Generally considered to be aerobic, *At. ferrooxidans* is capable of anaerobic growth on sulphur under specific circumstances, using ferric ion as a terminal electron acceptor (Das *et al.*, 1992; Pronk *et al.*, 1992).

At. thiooxidans derives energy solely from the oxidation of reduced-sulphur compounds including elemental sulphur, thiosulphate, and tetrathionate. It is a mesophile with an optimum growth temperature in the range from 25 to 30 °C. It is the most acid-tolerant of the sulphur-oxidizing species, with a pH growth range of 0.5 to 4.0 (Gould and Kapoor, 2003).

Acidithiobacillus caldus (*At. caldus*) is genotypically and phenotypically similar to *At. thiooxidans*, but some strains of *At. caldus* have been reported to grow mixotrophically using yeast extract or glucose (Rawlings, 2002). It metabolizes reduced-sulphur compounds, but not ferrous ion. It is moderately thermophilic, with an optimum growth temperature of 45 °C, an optimum growth pH of 2 to 2.5, but is able to tolerate acidity as low as pH 1.0. *At. caldus* has been shown to be one of the dominant organisms in many commercial operations, particularly stirred-tank gold biooxidation processes above 40 °C (Hallberg and Lindstrom, 1994; Hallberg *et al.*, 1996).

L. ferrooxidans is an acidophilic, obligate chemolithotrophic autotroph, capable of ferrous ion oxidation, but not able to utilize reduced-sulphur compounds (Hutchins *et al.*, 1986). Frequently regarded as moderately thermophilic, the optimum growth temperature is usually above 30 °C with activity up to 45 °C. They tend to grow more slowly than *At. ferrooxidans* on ferrous sulphate media in batch culture, but at comparable rates in

continuous culture at higher ferrous ion levels. They exhibit similar resistance to pH (pH 1.5 to 4.0), and ferrous and ferric ion levels (Hippe, 2000; Hutchins *et al.*, 1986; Norris, 1990).

Small organic molecules such as pyruvate and glucose can inhibit the obligate chemolithotrophs, and it is believed that heterotrophic microorganisms such as *Acidithiobacillus acidophilus* (*At. acidophilus*) metabolize the organic waste products produced by the iron- and sulphur-oxidizers. The autotrophs flourish in a less toxic environment, resulting in an increase in the observed oxidation rates (Paiment *et al.*, 2001; Rawlings, 2002).

Until recently, there was little published data on the consortium mix that exists in full-scale heap bioleaching operations. The widespread application of molecular biological techniques has led to significant advances in the understanding of the bacterial species present in acidic mining-related environments and bioleaching operations in the last decade. Several studies have applied molecular techniques to examine the bacteria community structure of acidic mining-related environments such as acid rock drainage (ARD) sites (Bond *et al.*, 2000; Hallberg and Johnson, 2003; Xiao *et al.*, 2009), mine water sites (He *et al.*, 2007; Xiao *et al.*, 2009), mine tailings sites (Mendez *et al.*, 2008), and acidified metal-laden waterways (Gonzalez-Toril *et al.*, 2003).

Molecular techniques have also been used to identify and enumerate the prevalent bacterial species in a number of bioleaching environments, although those applications are usually targeted to specific microorganisms of interest (i.e. sulphur- and iron-oxidizers) and reveal less information on the total bacterial community structure. These studies include bench-scale stirred-tank bioleaching experiments (Xingyu *et al.*, 2009, 2010), commercial stirred-tank bioleaching operations (Coram and Rawlings, 2002), column bioleaching

experiments (Coram-Uliana *et al.*, 2006; Halinen *et al.*, 2009), and heap bioleaching operations (Coram-Uliana *et al.*, 2006; Demergasso *et al.*, 2005; Qin *et al.*, 2009).

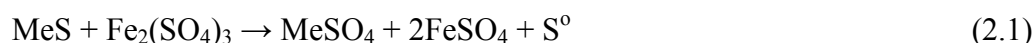
2.4.2. Leaching mechanisms

Historically, three leaching mechanisms for sulphide minerals have been prevalent in the technical literature: direct leaching, indirect leaching, and galvanic dissolution. Direct leaching requires bacterial adhesion to the sulphide mineral surface, and enzymatically-catalyzed disruption of the crystal lattice. Indirect leaching is a chemical process whereby the sulphide mineral is solubilized by either sulphuric acid or an oxidant (usually ferric ion). Bacterial involvement during indirect leaching is limited to the production of ferric ion and sulphuric acid in the leachate. Although galvanic dissolution has been considered a leaching mechanism in many bioleaching publications, it is more appropriately described as an electrochemical phenomenon, and has been discussed in Section 2.4.3.5.

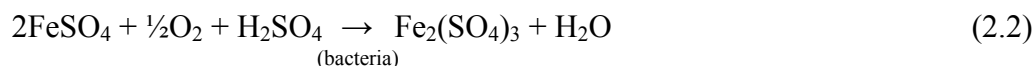
The existence of a truly direct mechanism has been the subject of much debate for several decades. According to Sand *et al.* (1999), no enzyme or factor necessary for directly metabolizing the crystal lattice has ever been isolated. Crundwell (1996) studied *At. ferrooxidans* and *L. ferrooxidans* during biofilm formation on pyrite, and concluded that the extracellular polymeric substance (EPS) layer between the bacterial cell wall and the mineral surface creates a microenvironment in which the chemical and electrochemical conditions are different than in the bulk solution (i.e. redox potential and pH), thereby increasing mineral dissolution rates. Evidence by Schippers and Sand (1999) suggests that sulphide minerals degrade via two chemical pathways based on the minerals' acid solubility. It is generally accepted that the presence of certain bacteria on the mineral surface may

expedite the naturally-occurring dissolution process, but bacteria merely facilitate the appropriate chemical and electrochemical environment promoting chemical dissolution.

At low pH, ferric ion is usually found in high concentrations, and is usually considered to be the primary oxidant in most bioleaching systems. Most sulphide minerals produce elemental sulphur as a by-product after undergoing oxidative dissolution. The overall stoichiometric equation describing the chemical oxidation of a simple bivalent metal sulphide by ferric sulphate is given in Equation 2.1. The end products are its corresponding metal sulphate, ferrous sulphate, and elemental sulphur.



A cycle of acid generation and ferric ion regeneration may occur, providing the presence of iron- and sulphur-oxidizing bacteria and suitable growth conditions within the leachate. Iron-oxidizing microorganisms such as *At. ferrooxidans* and *L. ferrooxidans* regenerate ferric ion in the bulk solution by enzymatically oxidizing ferrous ion according to Equation 2.2. Chemical oxidation of ferrous ion by molecular oxygen also occurs, however conversion rates in the presence of *At. ferrooxidans* may be higher by up to six orders of magnitude at low pH (Singer and Stumm, 1970).



Elemental sulphur may be metabolized by species such as *At. ferrooxidans* and *At. thiooxidans* to produce sulphuric acid according to Equation 2.3. Microbial sulphur oxidation is deemed crucial in order to sustain a low-pH environment, as ferric ion regeneration is an acid-consuming process. Furthermore, continual sulphur removal from the mineral surface prevents the formation of a passivating layer.



A cycle may form whereby ferric ion oxidizes the metal sulphide, which releases ferrous ion. Ferrous ion is bacterially oxidized to form more ferric ion, which in turn oxidizes more metal sulphide. Meanwhile, the pH of the system is maintained by the microbial oxidation of the sulphur by-products.

2.4.3. Factors affecting bioleaching

2.4.3.1. Temperature

Temperature can have a profound effect on the observed rate of mineral dissolution, as temperature affects both the kinetic rate constant of chemical dissolution and the rate of microbial growth (which controls the solution chemistry). The optimum growth temperature for a particular microorganism is species-specific and different strains of the same species can have different optimum growth temperatures. The mesophilic bacteria that are typical of heap bioleaching operations tend to grow over a wide temperature range, and are completely inhibited or killed at 35 to 45 °C. Psychrotolerant strains have been isolated growing on ferrous ion at temperatures as low as 2 °C (Leduc *et al.*, 1993), and thermophilic species are able to survive at temperatures ranging from 45 to 80 °C.

Chemical leaching generally follows Arrhenius' law, which describes the reaction rate constant (k) as a function of temperature (T) and activation energy (E) according to Equation 2.4, where A is a frequency factor, and R is the ideal gas constant.

$$k = A * e^{\frac{-E}{RT}} \quad (2.4)$$

Experimentally observed apparent activation energies for the oxidative dissolution of pentlandite and pyrrhotite are listed in Table 2.1. It should be noted that the apparent activation energy observed by Dutrizac and MacDonald (1974) is expected to reflect the combined resistance of at least surface-reaction and stagnant-film control due to the low-mixing environment associated with column leaching.

Table 2.1. Experimentally observed apparent activation energies for the oxidative dissolution of pentlandite and pyrrhotite.

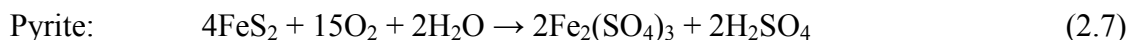
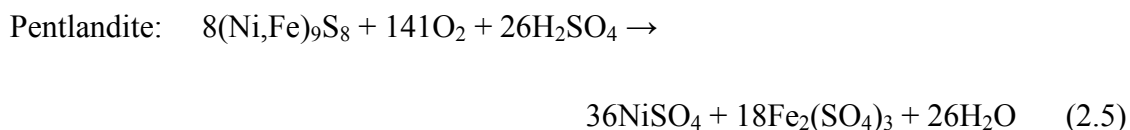
Mineral	Apparent activation energy (kJ/mol)	Reactor type	Reference
Pentlandite	38	Small column	Dutrizac and MacDonald (1974)
Pentlandite	61	Stirred tank	Corrans and Scholtz (1976)
Pentlandite	59	Pressure oxidation	Shneerson <i>et al.</i> (1966)
Nickeliferous pyrrhotite	40	Shake flask	Ahonen and Tuovinen (1991)

The temperatures experienced during heap bioleaching may vary considerably, depending on the sulphide content of the ore, regional climate, aeration rate, and irrigation rate. It is impossible to operate a heap at a uniform temperature; however, the rates of aeration and irrigation may be manipulated in order to maintain the temperature within a desired range. Generally, reported temperatures are 20 to 60 °C, and as high as 85 °C (Hunter, 2002) in one instant.

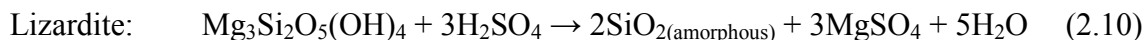
2.4.3.2. Acid balance

Acid consumption can be a major processing cost to a heap bioleaching operation (Watling, 2006); consequently, the acid balance is an important consideration. Numerous chemical and biologically-mediated reactions are involved in the overall acid balance during bioleaching. The simplified stoichiometric equations describing the oxidative dissolution of

pentlandite, pyrrhotite, pyrite, as well as a simple generic bivalent-metal sulphide are given in Equations 2.5 to 2.8 respectively. For simplicity, the formula for pyrrhotite has been written as FeS; although it is actually characterized by an iron-deficient crystal lattice (Fe_{1-x}S). These equations include the biological oxidation of elemental sulphur and ferrous ion. In the absence of ferric precipitation, the complete oxidative dissolution of all sulphide minerals except disulphides (e.g. pyrite) is either acid consuming or acid neutral.

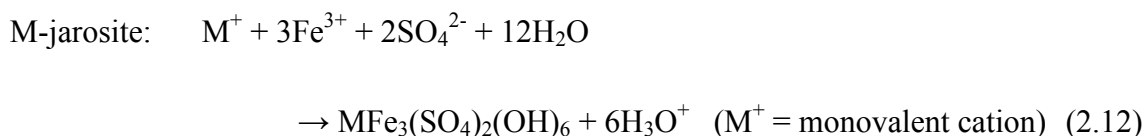
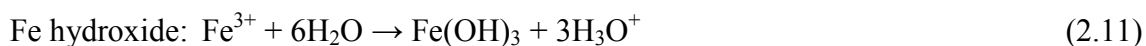


Many gangue minerals such as dolomite (a common carbonate) and lizardite (a common silicate) dissolve under mildly acidic conditions and consume acid (Equations 2.9 and 2.10 respectively).



Biologically-mediated reactions include the oxidation of elemental sulphur (Equation 2.3), which produces acidity, and the oxidation of ferrous ion, which consumes acidity (Equation 2.2). In addition, many microorganisms have the ability to oxidize reduced-sulphur compounds such as thiosulphate, which can be intermediates in the degradation of sulphide minerals (Schipper and Sand, 1999).

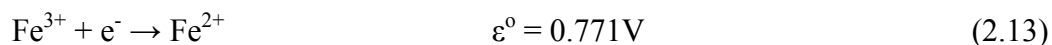
The precipitation of secondary ferric phases such as ferric hydroxide (Equation 2.11) and jarosite (Equation 2.12) produce acidity. It is interesting to note that the precipitation of ferric hydroxide-type compounds produces more acidity on a per mole of ferric ion basis than does jarosite.



Typically, the initial phase of a batch bioleaching process is acid consuming as the gangue minerals and the component sulphide minerals dissolve, while the later phase may be acid generating as secondary ferric compounds precipitate and reduced-sulphur by-products are oxidized by the bacteria.

2.4.3.3. Oxidation-reduction potential

Ferric ion is a strong oxidizing agent, and the relative abundance of ferric and ferrous ions in solution is the principal factor determining a bioleaching solution's oxidation-reduction potential (ORP) at low pH. Equation 2.14 is used to determine the ORP at 25 °C, when the dominant redox couple is ferric/ferrous (Equation 2.13), where $a^{\text{Fe}^{3+}}$ and $a^{\text{Fe}^{2+}}$ are the activities of ferric and ferrous ion respectively, and are approximated by their concentrations in dilute solutions (Natarajan, 1990; Rossi, 1990).



$$E_h = 0.771 + 0.059 \log \frac{a^{\text{Fe}^{3+}}}{a^{\text{Fe}^{2+}}} \approx 0.771 + 0.059 \log \frac{[\text{Fe}^{3+}]}{[\text{Fe}^{2+}]} \quad (2.14)$$

E_h is defined as the ORP when the standard hydrogen electrode (SHE) is used as the reference electrode. Numerous correction factors exist in order to convert the ORP measured with different reference electrodes to E_h . The maximum E_h experienced during bioleaching is a strong function of pH, as the solubility of ferric ion is limited above pH 3. Reported E_h values are in the range of 700 to 900 mV at pH 2.

2.4.3.4. Oxidant concentration

Both oxygen and ferric ion have been determined to be suitable oxidants for oxidizing many metal sulphide minerals. Ferric ion is widely considered to be the primary oxidant during bioleaching at low pH; however, the solubility of ferric compounds is limited when $\text{pH} > 3$. At 25 °C, the solubility product of ferric hydroxide is 1.1×10^{-36} and the dissociation constant of water is 1.0×10^{-14} (Oxtoby and Nachtrieb, 1990). Using these values, the maximum solubility of ferric hydroxide as a function of pH is displayed in Table 2.2. This would predict precipitation of ferric hydroxides even at relatively low concentrations of ferric ion beginning at $\text{pH} \geq 3.0$.

Table 2.2. Solubility of ferric ion as a function of pH at 25°C.

pH	[Fe ³⁺] (Mol/L)
2	1.1
3	1.1×10^{-3}
4	1.1×10^{-6}
5	1.1×10^{-9}

2.4.3.5. Rest potential of metal sulphide minerals and galvanic interactions

Galvanic dissolution is an electrochemical process that accelerates the oxidation of one mineral, while retarding the oxidation of another. Essentially, a galvanic cell is initiated

as two adjacent metal sulphide minerals establish contact through the leach solution. The more active mineral (lower rest potential) will spontaneously become the anode, which will expedite its dissolution, while the nobler mineral (higher rest potential) will become the cathode. The cathodically protected mineral will exhibit a retarded oxidation rate (Natarajan, 1990; Rossi, 1990).

In a leaching system composed of more than one sulphide mineral, galvanic interaction will necessarily occur. Individual base-metal sulphide minerals can be arranged in a galvanic series according to their rest potentials. Rossi (1990) presents a number of rest potential measurements obtained under varying experimental conditions. Measurements obtained by Yakhontova (1985) in sulphuric acid solution at pH 2.5 most resemble typical bioleaching conditions (Table 2.3). The two primary nickel-containing sulphide minerals have been shaded.

Table 2.3. Measured rest potential of some common sulphide minerals.

Mineral	Rest Potential vs. SHE (mV)
Pyrite	550-600
Pentlandite	550
Pyrrhotite	450
Chalcopyrite	400
Sphalerite	350
Galena	300

In nickel sulphide ores, pentlandite and pyrrhotite are frequently found in intimate contact. According to the observed rest potentials, one would expect the presence of pyrrhotite to retard the dissolution of pentlandite due to galvanic interactions. Pyrrhotite has frequently been observed to dissolve faster than pentlandite, but no study has offered conclusive evidence to suggest that galvanic interactions were involved.

2.4.3.6. Passivation and solid-phase alterations during leaching

Passivation of ore particles during bioleaching can have a detrimental effect on process performance. Passivation is caused by the precipitation of secondary solid phases during operation. Precipitate build-up on the minerals' surface can create an additional resistance to mass transfer, slowing the diffusion of products and reactants in/out of the individual particles. Possible secondary phases include elemental sulphur, metal polysulphides, jarosite, covellite, Fe(III)-oxyhydroxides, Fe(III)-oxyhydroxysulfates, schwertmannite ($\text{Fe}_8\text{O}_8(\text{OH})_6\text{SO}_4$), ferrihydrite ($\text{Fe}_5\text{HO}_8 \cdot 4\text{H}_2\text{O}$), and ferric hydroxide (Ahonen and Tuovinen, 1994).

The nature of the precipitate formed depends on the combination of minerals present, solution chemistry, electrochemical properties of the solution, and acidity. The solution chemistry of a bioleaching operation is extremely complex and varies between operations. Consequently, it is difficult to accurately predict precipitate formation based solely on thermodynamic and solution chemistry considerations. As such, much of the available information concerning precipitates and passivation are a result of direct observations.

Ahonen and Tuovinen (1994) examined the solid-phase alterations and iron transformations in a number of column bioleaching experiments with a complex base-metal sulphide ore. Solid-state precipitates were identified by XRD (X-ray diffraction) and SEM (scanning electron microscope) analysis of the leach residues. They concluded that ferric ion solubility was controlled by the precipitation of jarosite at $\text{pH} < 2.5$, and by the precipitation of ferric hydroxide-type compounds at $\text{pH} > 2.5$. Figure 2.1 is a recreation of a schematic representation provided by Ahonen and Tuovinen (1995), depicting the precipitation of secondary solid phases during bioleaching. Features include: a compact ferric oxide/

oxyhydroxide layer on the pyrrhotite surface; intact pentlandite inclusions protruding from the pyrrhotite; a layer of elemental sulphur covering the pyrrhotite surface; covellite precipitation on pyrrhotite surfaces; residual sphalerite located within a fracture in the pyrrhotite; and relatively recalcitrant pyrite grains containing chalcopyrite veins.

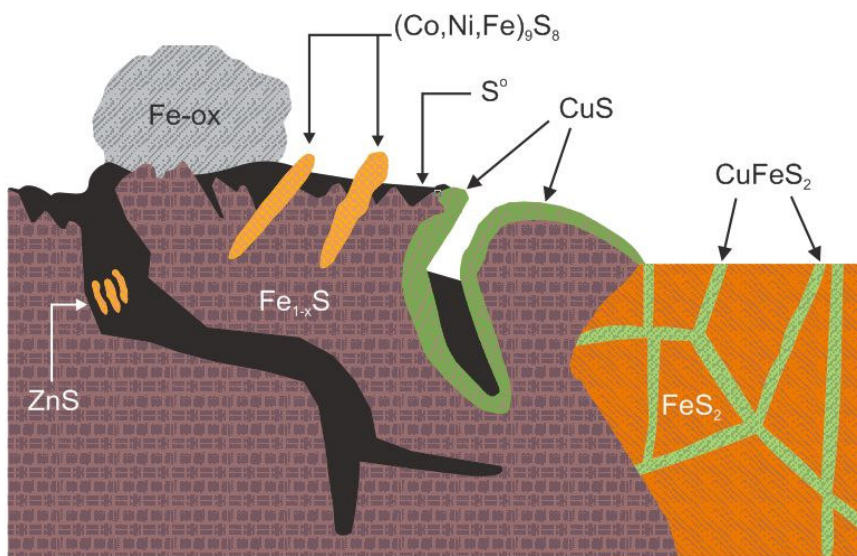


Figure 2.1. Schematic diagram of mineralogical observations of bioleaching residues (adapted from Ahonen and Tuovinen, 1995).

2.4.3.7. Nutrients and toxicity

Autotrophic bioleaching microorganisms require carbon dioxide, oxygen, ammonium nitrogen, and phosphate, as well as several other nutrients and micronutrients. In commercial heap-bioleach operations, the ambient air supplies carbon dioxide and oxygen; nitrogen and phosphorus are supplemented; and the ore supplies the remaining nutrients. Nitrogen and phosphorus may be supplied by the addition of $(\text{NH}_4)_2\text{SO}_4$ and either H_3PO_4 or KH_2PO_4 (Schnell, 1997).

Studies have shown bioleaching microorganisms to be inhibited by a range of organic chemicals and metal cations. The list of organic compounds includes solvent extraction compounds (Torma and Itzkovitch, 1976), surfactants (Torma *et al.*, 1976), metabolic products (Tuttle and Dugan, 1976), as well as many simple organic compounds (Frattoni *et al.*, 2000; Rossi, 1990). Bacterial inhibition inevitably leads to a loss of process performance and lower metal recovery.

Reported tolerances to metal cations vary considerably. Strain variability and experimental conditions appear to have a considerable effect on the reported tolerance levels. Typically, as a bacterial culture is acclimated to a particular ore, its tolerance to the metals present in that ore increase due to selective pressures. Li and Ke (2001) studied the effects of Mg and Cu on the Fe-oxidation ability of a Ni-adapted strain of *At. ferrooxidans*. A wild strain of *At. ferrooxidans* was adapted to 30 g/L of Ni by serial sub-culturing over a period of one year. The effect of binary and ternary combinations of Mg, Cu, and Ni were examined. Li and Ke (2001) concluded inhibition was determined by a combination of the number of metal species present as well as their corresponding concentrations.

2.5. Bioleaching Models

Bioleaching models contain both mechanistic and empirical expressions used to mathematically represent the various sub-processes. Robust modeling of a bioleaching process is inherently difficult, due in part to its heterogeneous nature and biological involvement. Typically, bioleaching models involve a two-part solution: a microbial growth model describing the production of ferric ion in the bulk solution, and an abiotic particle-leaching model. The conversion of ferrous ion in the bulk solution is generally modeled with

Monod or Monod-type kinetics. The most prevalent abiotic particle-leaching model applied to low-grade ores is the shrinking core model.

2.5.1. Shrinking core model

The shrinking core model is the most prevalent particle leaching model applied to low-grade ores. Conceptually, the model is based on a reaction zone that topochemically moves inward as leaching progresses, leaving only inert gangue and completely reacted mineral residue behind. The reaction zone is narrow, thus the reaction is deemed to be occurring on a surface that progressively gets smaller. The model assumes that although the particle properties change as leaching progresses, the size of the individual particle remains constant. The shrinking core model further assumes: the ore sample can adequately be described by a single particle diameter; transport of the bulk solution is not limiting; steady-state diffusion of the oxidant through the reacted outer region; first-order reaction at the mineral surface; and the reaction rate inside the reaction zone is equal to the rate of reactant supplied by diffusion.

Three types of kinetic control and their combinations are possible within the context of shrinking-core kinetics: surface-reaction control, product-layer diffusion control, and stagnant-film diffusion control. Mathematical derivations and conceptual explanation of the different limiting situations may be found in Levenspiel (1999), and references therein. The integrated forms of the different equations are listed in Table 2.4, for the generic heterogeneous reaction in Equation 2.15.



Table 2.4. Mathematical description of the shrinking-core model (Levenspiel,1999).

Control Mechanism	Model Variation	Definition of the time constant (τ)	Constants and Parameters
Product-layer diffusion	$1 - 3(1 - X_B)^{2/3} + 2(1 - X_B) = \frac{t}{\tau_p}$	$\tau_p = \frac{\rho_B R_o^2}{6bD_e C_A}$	X_B : fraction of B converted, R_o : initial particle radius, ρ_B : molar density of B in the solid, C_A : concentration of A in the leachate, b : stoichiometric reaction coefficient, D_e : diffusivity of A through the product layer, k' : mass transfer coefficient of A through film, and k'' : 1 st order reaction rate constant.
Stagnant-film diffusion	$X_B = \frac{t}{\tau_f}$	$\tau_f = \frac{\rho_B R_o}{3bk' C_A}$	
Surface-reaction	$1 - (1 - X_B)^{1/3} = \frac{t}{\tau_r}$	$\tau_r = \frac{\rho_B R_o}{3bk'' C_A}$	

In the case of changing temperature or oxidant concentration, the differential form of each equation (dX_B/dt) must be used. In the case of surface-reaction control, $k''C_A$ may be replaced with any function of C_A if the reaction rate is not first order. The overall fraction reacted is determined by summation of the weighted reacted fraction in each weight fraction.

2.5.2. Micro-scale electrochemical considerations

Sulphide minerals tend to be good conductors, and the oxidation reactions that occur at their surfaces are electrochemical in nature. At the micro-scale, the leaching kinetics of such reactions have been found to be adequately described by electrochemical processes analogous to electrode chemistry. Based on electrochemical considerations, Dixon (2000) demonstrated that the kinetics of electrochemical reactions which are limited by the rate of charge transfer may be grouped into two common types of rate-limiting situations shown in Equations 2.16 and 2.17, where: R_x is the rate of reaction; k is the reaction rate constant; and $a^{Fe^{2+}}$ and $a^{Fe^{3+}}$ are the activities of ferrous and ferric ion in the leachate respectively.

$$\text{Type 1: } R_x \propto k \left(a^{Fe^{3+}} \right)^{0.5} \propto k [Fe^{3+}]^{0.5} \quad (2.16)$$

$$\text{Type 2: } R_x \propto k \left(\frac{a^{Fe^{3+}}}{a^{Fe^{2+}}} \right)^{0.5} \propto k \left(\left[\frac{Fe^{3+}}{Fe^{2+}} \right] \right)^{0.5} \quad (2.17)$$

Type 1 leaching is characterized by slow reduction of the oxidant at the mineral's surface. Type 2 is characterized by fast reversible reduction at the mineral's surface, and is proportional to $([Fe^{3+}]/[Fe^{2+}])^{0.5}$ (i.e. some function of ORP) when leaching occurs at low pH in a ferric ion rich environment. A third type of leaching (Type 3) exists, where the reaction at the mineral's surface proceeds quickly, and the reaction rate is limited by the supply of the oxidant. In this case, the reaction rate is controlled by diffusion rather than an electrochemical reaction and is simply a function of the activity of the oxidant in the leachate (Equation 2.18).

$$\text{Type 3: } R_x \propto k(a^{Fe^{3+}}) \propto k[Fe^{3+}] \quad (2.18)$$

According to Dixon (2000), pressure oxidation with dissolved oxygen is almost universally Type 1, and the rate is proportional to $[O_2]^{0.5}$.

2.6. Bioleaching of Nickel Sulphide Ores

There have been hundreds of papers written on the bioleaching of metal sulphide ores, although only a handful are directly applicable to the bioleaching of pyrrhotite and pentlandite. This section only considers bioleaching studies with nickel sulphide ores and concentrates that have examined pH as a factor during bioleaching, as the primary hypotheses of this thesis are related to the effect of pH on the bioleaching of nickel sulphides.

2.6.1. Effect of pH on nickel extraction from pentlandite and pyrrhotite

The technical literature indicates that nickel-bearing sulphide minerals are amenable to bioleaching under common heap leaching conditions. A typical heap bioleaching operation maintains a solution pH between 1.5 and 2.5 (Plumb *et al.*, 2008), which is deemed necessary to obtain adequate sulphide mineral dissolution rates. Solution pH can have a dramatic effect on the formation of secondary ferric phases, microbial growth rates, and the solubility of copper. Maximum dissolution rates of sulphide minerals are generally correlated to low solution pH and high ORP (Ahonen and Tuovinen, 1995); however, nickel extraction from pentlandite and pyrrhotite has been shown to be less dependent on pH.

Dutrizac and MacDonald (1974) column leached a low-grade pentlandite ore with acidic ferric sulphate, with and without bacteria. They observed that the rate of nickel extraction was first order with respect to $[\text{Fe}^{3+}]$ and proportional to $[\text{H}_2\text{SO}_4]^{-0.14}$ in the range of $0 < \text{pH} < 2$ during leaching with ferric sulphate, and determined an apparent activation energy of 38 kJ/mol.

Ahonen and Tuovinen (1995) summarized the results of 31 column bioleaching experiments they conducted with a low-grade sulphide ore, which contained sulphides of zinc, copper, lead, and nickel. Nickeliferous pyrrhotite was the primary source of nickel. They observed that nickel extraction was least sensitive to pH of all metals, and showed little dependency on pH, ORP, or ferric ion concentration.

As part of the feasibility study for the Talvivaara pilot bioheap in Finland, Riekkola-Vanhanen *et al.* (2001) bioleached the black-schist ore in stirred-tank reactors in the pH range of 1.5 to 3. Pentlandite and violarite constituted approximately 80% of the nickel content. Over 90% of the nickel was extracted in two weeks, and showed limited dependency

on pH. Results of these bench-scale experiments were used to establish baseline operating conditions for two 450 kg columns with ore from the same deposit (Riekkola-Vanhanen and Heimala, 1999). It was concluded that operation at pH 3 resulted in satisfactory nickel recovery, while minimizing the solubilisation of silicate gangue minerals during the large column experiments.

The leaching of a pentlandite concentrate with acidic ferric sulphate, with and without bacteria in shake flasks was performed by Corrins and Scholtz (1976). They determined that the leaching of pentlandite involved two simultaneous processes, one dependant on the concentration of ferric ion and one dependant on the concentration of dissolved oxygen. The observed rate was proportional to approximately $[O_2]^{0.5}$, $[Fe^{3+}]$, and nearly independent of pH ($\propto [H^+]^{0.12}$) in the range $1.5 < pH < 4$. They calculated an apparent activation energy of 61 kJ/mol for the oxidative dissolution of pentlandite. A similar apparent activation energy and dependency on oxygen was reported for autoclave leaching of a pentlandite concentrate with molecular oxygen by Schneerson *et al.* (1966). The half order dependency on molecular oxygen observed by Corrins and Scholtz (1976) and Schneerson *et al.* (1966) is consistent with Type 1 leaching as discussed in Section 2.5.2.

2.7. Conclusions

The most economically significant nickel-bearing sulphide mineral is primarily pentlandite and secondarily pyrrhotite. Conventional extractive metallurgy of nickel from sulphide minerals consists of a combination of pyrometallurgical and hydrometallurgical processes. Heap (bio)leaching is an accepted processing route for copper oxide ores and secondary copper sulphides ore, and there appears to be both economic and environmental incentives to adapt heap bioleaching practices to nickel sulphide ores.

Temperature, solution pH, and nutrient concentration (primarily ammonium and phosphate) are the most frequently studied variables and are often found to have the most impact on process performance. Generally, the ore supplies the nutrient required by the autotrophic sulphur- and iron-oxidizing bacteria, with the exception of nitrogen and phosphorous, which are usually supplemented in laboratory experiments as ammonium and phosphate respectively. Bioleaching microorganisms are inhibited by a number of inorganic cations and anions, and organic molecules. Adaptation via constant selective pressures has been successfully demonstrated.

Sulphuric acid can be a major expense to a bioleaching operation. The mineralogy of the ore is the principal factor determining acid consumption; acid consuming minerals include carbonates, oxides, clays, and many silicates. The solution pH would be expected to have a considerable effect on the dissolution rate of the gangue minerals. In addition, the dissolution of the gangue minerals may have an effect on the costs associated with solution maintenance and waste management. As the gangue minerals dissolve, a host of undesirable ions are released into solution (i.e. Al, Si, Mg, Ca, etc.), which may be toxic to the bacteria and cause downstream processing problems. There are costs associated with removing those impurities from the leachate, with their subsequent storage or disposal. As mining operations increasingly look for ways to reduce their environmental footprints, the cost associated with leachate management will undoubtedly increase. Few studies have examined the dissolution of different gangue minerals as a function of pH during (bio)leaching, and the resulting effect on acid consumption.

A review of the technical literature indicated that the bioleaching of nickel sulphide ores and concentrates is generally good. High nickel extractions have been achieved over a

wide range of temperature and pH levels. Nickel extraction from pentlandite and pyrrhotite is less dependent on pH compared to many other sulphide minerals. It is believed that bioleaching at pH levels higher than what is generally considered optimum (~ 2) may offer cost advantages related to the consumption of sulphuric acid and waste management, and may result in favourable kinetics in some cases.

2.8. References

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Chapter 3 – Preliminary Bioleaching Experiments

3.1. Introduction

The original experimental plan was designed to subject each ore to the same preliminary bioleaching experiments in order to identify correlations between amenability to bioleaching and mineralogical and/or chemical composition. A review of the technical literature on bioleaching revealed that temperature, solution pH, and nutrient concentration (primarily ammonium and phosphate) are the most frequently studied variables and are often found to have the most impact on process performance. Considering the number of experiments required to study the bioleaching of ten different ores, it was deemed necessary to eliminate at least one of these three variables. Temperature is often found to be the most important factor affecting the bioleaching of sulphide ores, which is a process that is generally characterized by high apparent activation energies. For this reason, it was believed that either nutrient concentration or pH was the most logical choice for elimination. Many of the experiments discussed in this chapter were designed to eliminate either media type or pH as a variable for subsequent bioleaching experiments. The primary hypothesis of this thesis (based on pH) was developed after the preliminary experiments discussed in this chapter.

Ores 1 and 2 were the first two ores to be acquired. Shake flask experiments were used to initially assess the ores' amenability to bioleaching and to assess the effect of bacteria, nutrient concentration, and pH. Ore 3 was acquired in June 2007, and the initial shake flask experiments with Ore 3 resulted in adequate nickel solubilisation, but they were deemed unsatisfactory due to poor pH control, as the pH was observed to increase rapidly in between pH adjustments for several days following start-up. For this reason, it was

concluded that a stirred-tank reactor (STR) experiment with pH control was a superior experimental apparatus, and approximately half of the planned shake flask experiments with Ore 3 were not completed.

The preliminary experiments with Ores 1 and 2 were exploratory in nature, with no specific experimental objective other than maximizing nickel extraction. The experimental objective with Ore 3 was more defined, as previous bioleaching work (unpublished data provided by the company) with this ore at conventional pH levels resulted in unacceptably high magnesium extraction. The work with Ore 3 had two equally important objectives: to achieve an acceptable level of nickel extraction; and to minimize magnesium mobilization. Furthermore, the consumption of sulphuric acid was also a consideration, as acid consumption can be a major operational cost to a bioleaching operation.

Magnesium extraction during bioleaching of Ores 1 and 2 was not a major concern, but the fate of magnesium in those two systems was followed in order to develop insight into the leaching of the gangue minerals. Magnesium is a major component in many acid consuming silicate gangue minerals, it is stable in solution over a wide range of pH conditions, and does not precipitate in the presence of sulphide. For these reasons, magnesium has been viewed as a chemically stable indicator of gangue dissolution.

The experiments discussed in this chapter were conducted before the experiments presented in subsequent chapters of this thesis; however, this chapter was written after most of the other chapters. In order to avoid unnecessary repetition, this chapter contains several references to subsequent chapters for experimental details and further discussion. The results and discussion section of this chapter has been divided into two sections: results and discussion related to the shake flask experiments with Ores 1 to 3; and results and discussion

related to STR bioleaching experiments conducted with Ore 3. This was done in order to independently discuss the results in terms of the different experimental objectives with the different ores.

3.2. Materials and Methods

3.2.1. Nickel sulphide ores

All the stirred-tank and shake flask experiments discussed in this thesis were conducted with ore ground such that 95% passed 100 Tyler mesh ($\sim 147\ \mu\text{m}$). It was desired to conduct the experiments under surface reaction controlled conditions, which requires the sulphide grains to be liberated and accessible to the leaching solution. In order to have well-liberated sulphide grains, the maximum particle diameter must be approximately equal to the diameter of the average sulphide grain. Due to time constraints, experiments were scheduled to being several months before the results of liberation analyses were available, therefore the average sulphide grain diameter had to be estimated and the maximum particles size was selected based on that assumed average sulphide grain diameter. The average sulphide grain diameter was assumed to be $\sim 100\ \mu\text{m}$; therefore, it was further assumed that the sulphide grains were well-liberated if the maximum particle diameter was $147\ \mu\text{m}$ (100 Tyler mesh). The results of the liberation analyses (Lastra *et al.*, 2007a; 2007b; 2008) showed that sulphide grains were indeed well-liberated in particles that were $147\ \mu\text{m}$ in diameter, which validated the average sulphide grain diameter assumption. The ore processing protocol is described in Appendix C.

Lastra *et al.* (2007a, 2007b, 2008) reported on the mineralogical characterization and liberation analysis of Ores 1 to 3 respectively. The materials and methods for the

mineralogical characterization are described in Section 4.2.1.1. The mineralogical and chemical composition of the nickel sulphide ores used in these experiments have been discussed and compared in the introduction of Chapter 7. In brief, Ores 1 and 2 are both from the Sudbury, Ontario region and are mineralogically and chemically similar, whereas Ore 3 is from Manitoba and is quite different from the other two ores. The most abundant mineral phases, the nickel content of the primary nickel-containing minerals, and the chemical composition of the three ores is given in Tables 3.1, 3.2, and 3.3 respectively.

Table 3.1. Mineralogical composition of Ores 1, 2, and 3.

Mineral or mineral group	Ore 1	Ore 2	Ore 3
Amphibole/pyroxene	19.6	36.4	6.2
Chalcopyrite	0.7	1.9	tr
Chlorite	1.7	1.5	9.1
Oxides (magnetite, hematite, ilmenite, chromite)	9.0	2.5	12.2
Feldspar	23.2	19.3	0.1
Pentlandite	3	2.0	0.7
Pyrrhotite	33	25.2	0.9
Pyrite	0.1	0.1	0.5
Serpentine	nd	nd	64.4

All values quoted in %mass; tr: trace; nd: not detected.

Table 3.2. Chemical composition of the primary nickel-bearing phases and distribution of nickel in Ores 1, 2, and 3.

Mineral		Ore 1	Ore 2	Ore 3
Pentlandite	Ni	36.3 ± 0.7	36.2 ± 0.7	39 ± 2
	Co	0.8 ± 0.2	1.4 ± 0.3	1.1 ± 0.4
Pyrrhotite	Ni	0.7 ± 0.1	0.8 ± 0.2	0.7 ± 0.2
Pyrite	Ni	N/A	N/A	0.03 ± 0.01
Proportion of Ni reporting to pentlandite		83	77	97
Proportion of Ni reporting to pyrrhotite		16	22	2

All values quoted in %mass ± 1 standard deviation

Table 3.3. Chemical composition of Ores 1, 2, and 3.

Element	Ore 1	Ore 2	Ore 3
Ni	0.789 ± 0.02	0.675 ± 0.03	0.305 ± 0.005
Mg	2.74 ± 0.02	3.76 ± 0.02	21.2 ± 0.1
Cu	0.224 ± 0.003	0.629 ± 0.01	0.016 ± 0.002
Co	0.0274 ± 0.0001	0.0289 ± 0.0003	0.013 ± 0.0004
Fe	14.0 ± 0.2	16.1 ± 0.1	7.22 ± 0.1

All values quoted in %mass ± 1 standard deviation

In addition to nickel, it is important to consider the relative importance of the secondary metal values in each of the ores when evaluating the success of the bioleaching experiments. Both copper and cobalt are generally associated with nickel sulphide deposits but the relative economic significance of each may vary considerably between ores. In terms of economic significance, copper and cobalt are significant with Ores 1 and 2, whereas only cobalt is significant with Ore 3.

3.2.2. Bacterial culture

The original microbial culture used in this study was derived from water and soil samples collected during a site visit to mining-related locations in Sudbury, Ontario in October 2006. A total of 16 samples were taken from locations with a range of acidity (pH 3.2 to 6.5) and oxidation-reduction potential (125 to 438 mV vs. Ag/AgCl).

Each water and soil sample was tested for the presence of both sulphur- and iron-oxidizing bacteria. The test consisted of placing one gram of solid (soil samples) or one mL of liquid (water samples) in a temperature-sterilized 250 mL Erlenmeyer flask with 100 mL of filter-sterilized media, followed by incubation in a rotary shaker at 30 °C for 28 days. Media designated mTK and Att (Table 3.4) were used to detect the presence of iron- and

sulphur-oxidizing microorganisms respectively. A positive test for iron-oxidizing bacteria was indicated by the formation of an orange precipitate, whereas a positive test for sulphur-oxidizing bacteria was indicated by a drop in pH (0.5 units below the average value of the control flasks). All samples tested positive for both iron- and sulphur-oxidizing bacteria.

Table 3.4. Composition of the growth media used during enrichment, culture maintenance, and bioleaching experiments.

	Modified TK (mTK)	McCready	ATCC Medium 23 (Att)
Nutrients (g/L)	0.5 (NH ₄) ₂ SO ₄ 0.5 K ₂ HPO ₄ 0.5 MgSO ₄ ·7H ₂ O	0.0661 (NH ₄) ₂ SO ₄ 0.0174 K ₂ HPO ₄ 0.123 MgSO ₄ ·7H ₂ O	0.1 NH ₄ Cl 3.0 KH ₂ PO ₄ 0.2 MgCl ₂ ·6H ₂ O 0.1 CaCl ₂
Energy Source (g/L)	33.4 FeSO ₄ ·7H ₂ O	33.4 FeSO ₄ ·7H ₂ O	5.0 Na ₂ S ₂ O ₃ ·5H ₂ O or
Initial pH	2.1	2.1	4.2
Reference	Modified from Tuovinen and Kelly (1973)	McCready <i>et al.</i> (1986)	Modified from Gherna <i>et al.</i> (1989)

Both iron- and sulphur-oxidizing microorganisms were enriched from each sample using media designated mTK and Att respectively (Table 3.4). All the enrichment cultures were combined and the resulting mixed culture was acclimated to Ores 1 and 2 by serial sub-culturing at 30 °C in a temperature-controlled rotary shaker at 150 RPM, in 250 mL Erlenmeyer flasks at 5% pulp density (mass/vol), with ore ground to minus 147 µm, with mTK media at pH 2.1 ± 0.1. The pH of the leachate was measured regularly and was adjusted as required with 10% H₂SO₄ (vol/vol) or 10% NaHCO₃ (mass/vol). A new sub-culture (i.e. maintenance culture) was started by inoculating 5 g of ore in 95 mL of media with 5 mL of slurry from the previous sub-culture (well shaken). When a new ore was obtained, a maintenance culture was started with that ore by taking a 5 mL slurry sample from each of the existing maintenance cultures.

No magnesium supplement or energy source was added during growth on sulphide ores. Magnesium was omitted because the ores supplied enough dissolved magnesium, and iron was omitted in order to encourage the bacteria to attach to the ore and utilized the sulphide minerals as an energy source. Initial molecular biological characterization by Dinardo and Mohapatra (2008) revealed that the original mixed culture contained microorganisms closely related to *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, and *Acidiphilium* spp.

Three different media were used in the experiments discussed in this chapter (Table 3.4). Att is an ATCC media, which is used for growing autotrophic sulphur-oxidizing mesophiles, and was used only for the original culture enrichment and the bacterial enumerations (discussed in Section 3.2.4.). The two nutrient media that were tested as factors in the shake flask and stirred-tank experiments are mTK (rich) and McCready (lean). The mTK media is a slightly richer version of the TK media that was developed by Tuovinen and Kelly (1973), which has been widely used for laboratory bioleaching experiments at mesophilic temperatures. McCready media is a minimal nutrient media that was formulated to maximize uranium solubilisation during bioleaching with *Acidithiobacillus ferrooxidans*, and is more representative of the nutrient levels that would be expected during heap bioleaching.

3.2.3. Shake flask experiments

The eight-week shake flask experiments were conducted in a temperature-controlled rotary shaker at 150 RPM, in 250 mL Erlenmeyer flasks at 10% pulp density (mass/vol), with ore ground such that 95% was minus 100 Tyler mesh (147 μm). Aliquots of the leachate were removed for analysis at $t = 0$, and weekly thereafter. Each flask was weighed after

inoculation, and evaporative losses were made up with deionized water prior to sampling. The solids were allowed to settle for 5 minutes prior to sample removal and the volume removed for metal analysis was replaced with an equal volume of fresh media. The flask weights were kept constant and only leachate was removed for metal analysis in order to not lose solid material and maintain a constant pulp density. All shake flask experiments were conducted in triplicates.

Shake flasks containing 10 g of ore and 95 mL of media were inoculated with 5 mL of leachate (no solids) from a 14 to 21 day old maintenance culture grown on the same ore. Only leachate was used as inoculum in order to avoid variability between replicates based on the amount of solid transferred. The pH was measured regularly and was adjusted as required. The pH was adjusted to the desired value only if it was determined to be ± 0.1 from the desired value.

Abiotic control experiments at the baseline pH value of 2.1 were conducted with thymol (0.9 g/L) as bactericide and 5% methanol (Meline *et al.*, 1996) to assess the effect of the presence of the bacteria. Of the sterilization methods described by Brickett *et al.* (1995), thymol was considered to be the preferred method of controlling bacterial growth in this study. Other sterilization methods such as heat treatment and irradiation were deemed unsatisfactory due to the risk of recontamination due to the ubiquitous distribution of mesophilic sulphur- and iron-oxidizing bacteria in the environmental laboratories at CANMET-MMSL.

3.2.3.1. Factorial design experiments to assess the effect of pH and nutrient media

A two factor, two level factorial design was used to examine the effect of pH and nutrient level during bioleaching. Experimental conditions are listed in Table 3.5.

Table 3.5. Experimental design and coding for shake flask experiments with Ores 1 and 2.

Factor	Level (coded value)	
	Low (-1)	High (+1)
pH	2.1	3.0
Nutrient media	McCready (lean)	mTK (rich)

3.2.4. Preliminary stirred-tank reactor experiments

Stirred-tank reactions were conducted at 30 °C in a jacketed 2.0 L glass reaction vessel with temperature control, pH control, aeration, and continuous stirring. Aeration was set at ~ 500 mL/min at the beginning of all experiments; however, the aeration rate gradually decreased over the course of the experiments due to fouling. Aerators were cleaned or replaced as required, in order to maintain the dissolved oxygen near saturation in all experiments. Sulphuric acid was kept in a graduated cylinder and pumped into the reaction vessel with a peristaltic pump as required by the pH controller. The volume of acid remaining in the graduated cylinder was recorded during each sampling session, and the volume used was mathematically converted to a weight equivalent of concentrated H₂SO₄. Acid consumption has been consistently quoted on a g/kg ore basis. A picture of the experimental apparatus is provided in Figure 3.1.



Figure 3.1. Photograph of the stirred-tank reactor setup.

The Reynolds number was calculated ($N_R \sim 30,000$) according to Equation 3.1 (Tchobanoglous *et al.*, 2003), where D is diameter of the impeller (7.62 cm), n is the stirring speed (230 RPM), ρ is the density of the leachate (approximated by water at 30 °C), and μ_f is the dynamic fluid viscosity of the leachate (approximated by water at 30 °C, 0.798 mPa·s).

$$N_R = \frac{D^2 n \rho}{\mu_f} \quad (3.1)$$

The inoculum for individual experiments was developed by combining 5 g of ore, 100 mL of media, and 8 mL of 10% H₂SO₄ (vol/vol). After 48 hours in an orbital shaker at 30 °C, the pH was adjusted to 3, and inoculated with 5 mL of slurry from a well-shaken

maintenance culture. The resulting inoculum was maintained in an orbital shaker for 10 to 12 days at pH 3, and then combined with 145 g ore in the reaction vessel and made up to a total volume of 1.5 L with media. Aliquots of the leachate were taken periodically, and evaporative losses were volumetrically made up with distilled water prior to sampling. The sample volume was replaced with an equal volume of fresh media. After each experiment, the residue was filtered, washed with distilled water, and dried at 45 °C for one week. The residue was gently broken up with a mortar and pestle, and sample splits for chemical characterization were pulverized prior to analysis.

Maintaining the inoculum in an orbital shaker prior to the commencement of each STR experiment ensured an adequate population of actively growing bacteria at the beginning of each experiment. It was determined that after 10 to 12 days at pH 3, each inoculum shake flask (~ 100 mL) prepared with McCready media according to the procedure described above contained approximately 5.5×10^9 sulphur-oxidizing bacteria and approximately 3.2×10^9 iron-oxidizing bacteria.

Bacterial determinations were accomplished with a five-tube most-probable-number (MPN) method (Cochran, 1950). All tubes were incubated in the dark for 28 days at room temperature. The energy source in the growth media was used to distinguish between iron- and sulphur-oxidizing populations, which were enumerated with media designated mTK and Att respectively (Table 3.4). A positive test for iron-oxidizing bacteria was indicated by the formation of an orange precipitate, whereas a positive test for sulphur-oxidizing bacteria was indicated by a drop in pH (0.5 units below the average value of the control tubes). Each enumeration experiment was run concurrently with 10 un-inoculated control tubes.

Oxidation-reduction potential (ORP), conductivity, acid level, and the dissolved oxygen were measured and recorded during each sampling session. Dissolved oxygen and conductivity were measured with an Orion Star-5 benchtop multimeter, ORP (vs. Ag/AgCl) with a WTW Multiline P4 handheld meter, and the pH was controlled with a Eutech alpha-pH800 controller. In order to ensure consistency between pH controllers, the pH of each reactor was measured with a portable meter (Orion Star 3), and compared to the reading of the respective pH controller. In the event of a discrepancy, appropriate action was taken to identify and correct the problem. The Orion Star 3 handheld meter was verified against buffers of pH 4 and 7 daily, and re-calibrated when necessary. All other probes were maintained as necessary according the manufacturers' instruction.

A single STR experiment was conducted by using elemental sulphur as the only source of acidity. In that experiment, 9 grams of elemental sulphur (S^0) was added to the mixture at time zero, and the pH was monitored rather than controlled. The experiment was inoculated on day 0 with 5 mL of slurry from a flask of neutrophilic sulphur-oxidizing bacteria grown on a combination of Ore 3 and elemental sulphur, and then re-inoculated with 5 mL of the regular maintenance culture on day 14 (shown by an arrow in Figure 3.6). The STR experiments conducted as part of the preliminary experiments discussed in this chapter were conducted without replicates.

The culture of neutrophilic sulphur-oxidizing bacteria was developed by serial sub-culturing with Ore 3 and elemental sulphur at 30 °C, without pH control (i.e. no 8 mL of 10% H_2SO_4). In the absence of sulphuric acid addition, the pH of the solution was observed to increase to greater than 8 within minutes of mixing the ore and the media, and the pH slowly decreased to 4 to 5 after approximately one month of incubation in the rotary shaker.

A new sub-culture was developed by combining 5 g of ore and 1 g of elemental sulphur in 100 mL of mTK and inoculating the mixture with 5 mL of slurry from the previous sub-culture (well-shaken).

3.2.5. Chemical analysis

All chemical determinations were done by the ISO 9001 certified Analytical Services Group at CANMET-MMSL's laboratories in Ottawa, Canada. Aqueous metal determinations were carried out by ICP-AES using a two-point calibration (0 and 10 ppm). Ore and solid residue samples were digested with a four-acid preparation method (Donaldson, 1974) prior to metal analysis by ICP-AES. Total sulphur (S_{total}) was determined by pyrolysis in a Leco furnace, and sulphate ($S_{sulphate}$) by HPLC. Elemental sulphur ($S_{elemental}$) was extracted with toluene and quantified spectrophotometrically at 300 nm (Donaldson, 1974), and sulphide ($S_{sulphide}$) was determined by applying a total sulphur mass balance (Equation 3.2).

$$S_{total} = S_{sulphate} + S_{sulphide} + S_{element} \quad (3.2)$$

3.2.6. Data analysis

Minitab release 14 was used to analyze the factorial experiments and identify significant factors using a linear function of pH and media type (Equation 3.3), where β_0 , β_1 , and β_{12} are the regression constants and Y is the predicted response.

$$Y = \beta_0 + \beta_1 pH + \beta_2 media + \beta_{12} pH * media \quad (3.3)$$

The data points in the graphs presented in this chapter are the mean value of the replicates (when applicable), and metal extraction curves have been presented as the percent metal extracted as calculated from the leachate determinations. A mass balance approach has

been used to modify the calculated extractions to account for dilution caused by sample removal (see Appendix E for a sample calculation). The metal extractions calculated from the post-leach solid residues have been compared to the final metal extractions calculated from the leachate determinations. There was generally good agreement between the final metal extractions calculated from the leachate assays and the final metal extractions calculated from the solid residue assays, and significance testing of both sets of data yielded similar conclusions.

3.3. Results and Discussion of the Shake Flask Experiments with Ores 1, 2, and 3

3.3.1. Shake flask experiments with Ores 1 and 2

The average metal extraction curves during the shake flask experiments with Ores 1 and 2 are shown in Figures 3.2 and 3.3 respectively (each condition was done in triplicate). Abiotic experiments were conducted at pH 2.1 in order to assess the effect of the presence of the mixed-culture of iron- and sulphur-oxidizing bacteria on the extraction of nickel and copper. Only the concentrations of nickel and copper were determined in the leachates during abiotic leaching because those metals were believed to be the primary metal values in both Ores 1 and 2. After reviewing the chemical composition of Ores 1 and 2 (Table 3.3), it was determined that cobalt was also a significant value in both ores, and future experiments were modified accordingly. The abiotic experiments were terminated after 35 days (compared to 56 days for the biotic experiments) due to equipment failure, and a decision was made not to repeat the experiments after the rotary shaker was repaired, as the experiments were successful in providing evidence that the bacteria have a significant effect

on metal extraction from both ores. With both ores, the presence of bacteria produced a statistically significant (95% confidence interval) increase in both nickel and copper extraction after 35 days of (bio)leaching at pH 2.1.

Each shake flask was weighed after inoculation, and evaporative losses were made up with deionized water prior to sample removal; however, the weight of acid addition for pH control was greater than the weight loss due to evaporation during the first few weeks. For this reason, the first few weeks of metal extraction should be interpreted with caution as a result of 5 to 10% dilution error. Experiments conducted at the lower pH were more affected, as they required more acid addition. The metal extraction curves have not been corrected for this dilution. All shake flasks returned to their original weights after three to four weeks of operation. The final extractions after 56 days are not affected, as they were calculated on a mass balance basis.

Of the three primary metal values (Figures 3.2 and 3.3), the extractions of nickel and cobalt were generally good, whereas the extraction of copper was generally poor (< 30%). Low copper extraction at 30 °C is not unexpected, as copper reports primarily to chalcopyrite in both Ores 1 and 2. The bioleaching of chalcopyrite is widely considered problematic and is characterized by low extractions under mesophilic conditions, the theories for which have been thoroughly reviewed in Watling (2006).

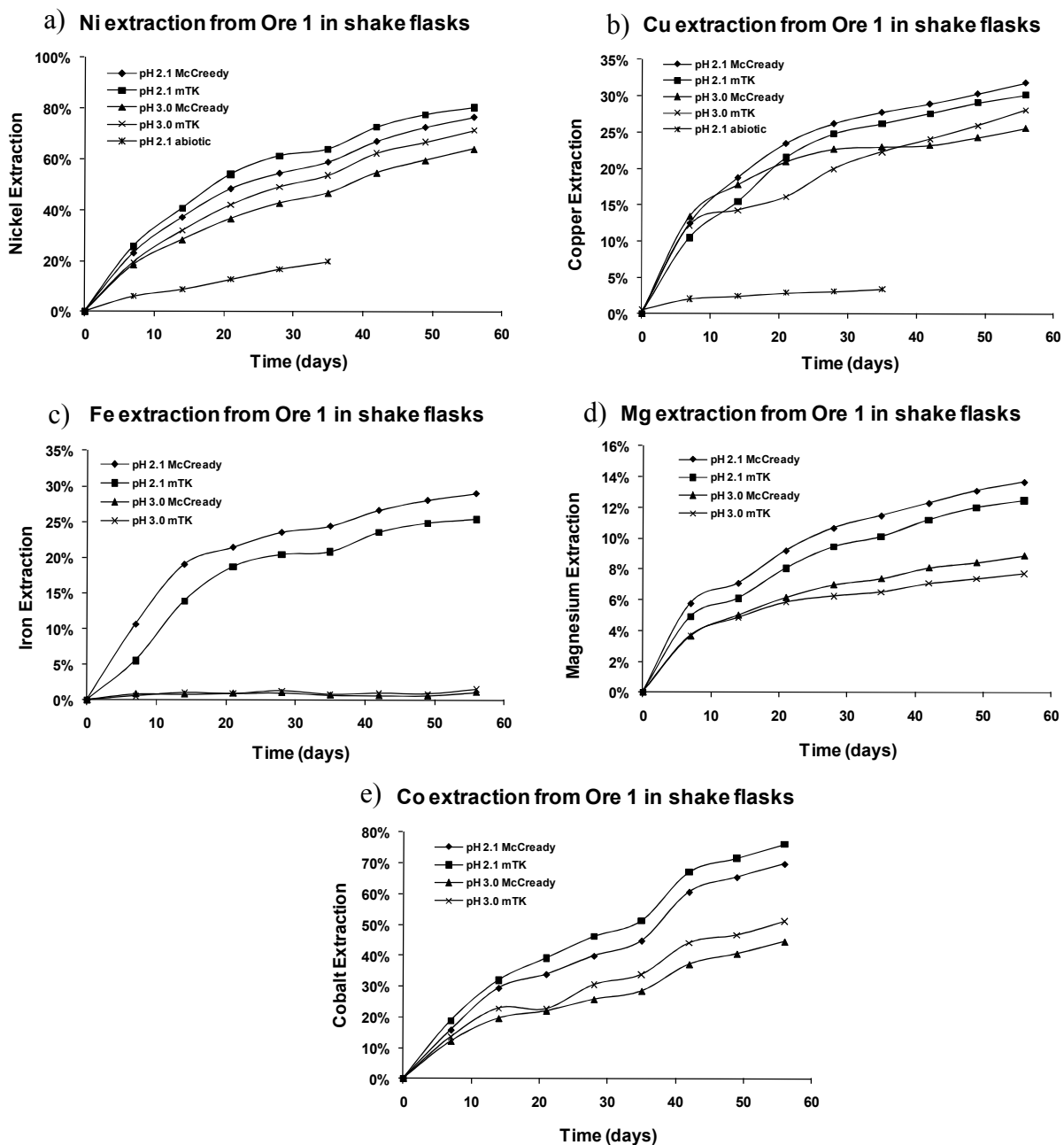


Figure 3.2. Extraction of a) nickel, b) copper, c) iron, d) magnesium, and e) cobalt as a function of time during shake flask (bio)leaching experiments with Ore 1.

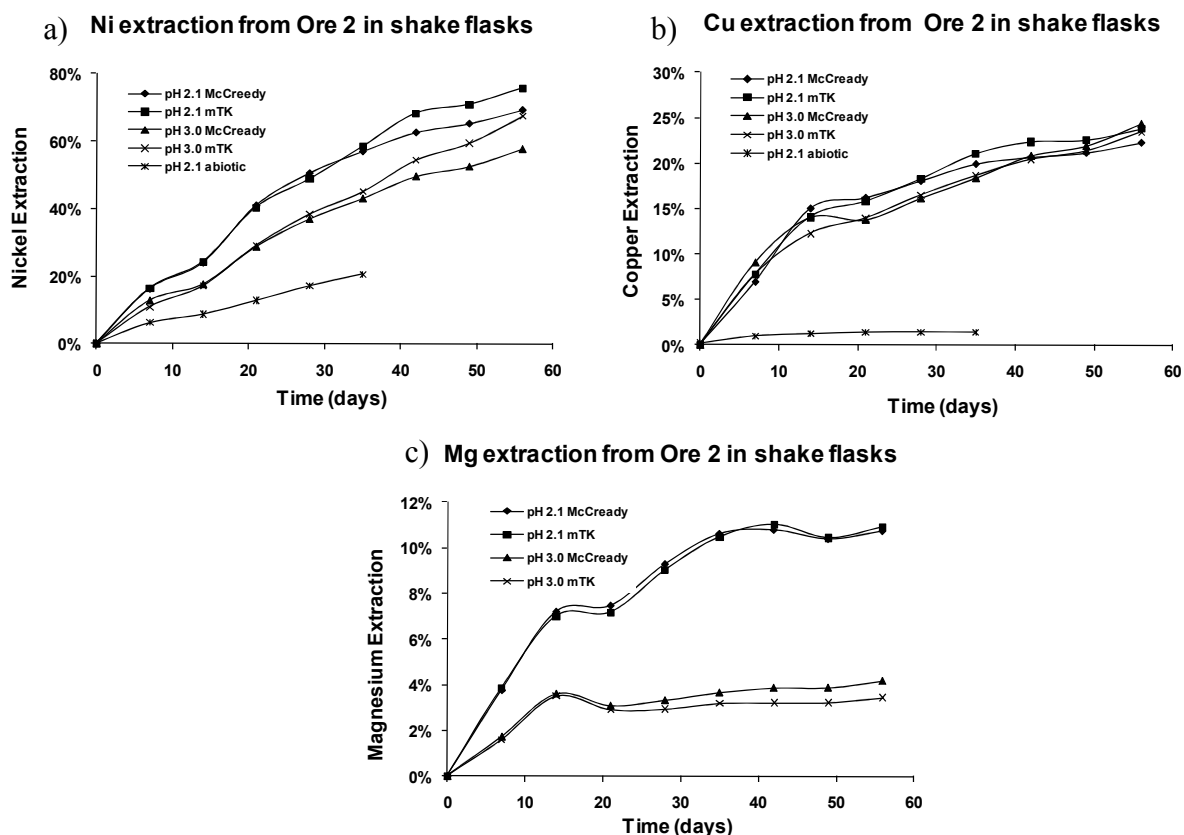


Figure 3.3. Extraction of a) nickel, b) copper, and c) magnesium as a function of time during shake flask (bio)leaching experiments with Ore 2.

Significant terms (i.e. pH, media, and media*pH interaction) affecting the response variables (i.e. metal extractions) with 90 and 95% confidence intervals (CI) during shake flask bioleaching of Ores 1 and 2 have been identified in Tables 3.6 and 3.7 respectively. The data was tested with two different CIs in order to avoid dismissing marginally significant effects since these experiments were conducted as preliminary work. When interpreting Tables 6 and 7: “all” implies that the term was significant for the response variable on all sampling days; and “none” implies that the term was not significant for the response variable on all sampling days. In intermediate cases, the sampling days on which the term had a significant effect on the response variable has been identified. For example, during the

bioleaching of Ore 1 (Table 3.6), with a 95% CI, the pH*media interaction had a significant effect on Mg extraction only on day 7; whereas the pH*media interaction term was significant on both days 7 and 21 with a 90% CI. The two cells in Table 3.6 have been shaded.

Table 3.6. Significant factors and interactions during bioleaching of Ore 1.

Response variable	pH		media		pH*media interaction	
	90% CI	95% CI	90% CI	95% CI	90% CI	95% CI
Ni extracted	all	all	7, 42, 49, 56	none	none	none
Mg extracted	all	all	all	14 to 56	7, 21	7
Cu extracted	21 to 56	21 to 56	21, 28	21	56	none
Co extracted	all	all	7, 56	7	none	none
Fe extracted	all	all	all	all except 21	all	all except 21

CI: confidence interval

Table 3.7. Significant factors and interactions during bioleaching of Ore 2.

Response variable	pH		media		pH*media interaction	
	90% CI	95% CI	90% CI	95% CI	90% CI	95% CI
Ni extracted	all	all	49, 56	56	none	none
Mg extracted	all	all	none	none	49, 56	56
Cu extracted	21, 28, 35	28	none	none	none	none

CI: confidence interval

Analysis of the factorial designs reveals that the relative order of importance for the factors are pH > media > pH*media. Table 3.8 lists the average effects (in terms of percent metal extraction) of the factors and the interaction term after 56 days of bioleaching with 90% CI. Effects that are not statistically significant (90% CI) have been omitted from Table 3.8.

Table 3.8. Average effect of pH, media, and pH*media on the metal extractions from Ores 1 and 2 after 56 days of bioleaching.

Average effect in the response variable	pH		media		pH*media interaction	
	Ore 1	Ore 2	Ore 1	Ore 2	Ore 1	Ore 2
Ni extracted (%)	-10.8	-10.0	5.6	8.2	-	-
Mg extracted (%)	-4.8	-7.0	-1.2	-	-	-0.5
Cu extracted (%)	-4.2	-	-	-	2.1	-
Co extracted (%)	-25.1	N/A	6.4	N/A	-	N/A
Fe extracted (%)	-25.8	N/A	-1.6	N/A	2.0	N/A

All effects are quoted in terms of % extraction. 90% CI.

The pH of the leachate was determined to have the most impact on the extraction of metals (Table 3.8, “pH” column has been shaded). After 56 days of bioleaching, the effect caused by pH was almost always significant, with higher pH consistently resulting in lower metal extractions (i.e. the effect was always negative). The extraction of Fe was most negatively affected by higher pH, which is most likely related to the low solubility of ferric-containing compounds (discussed further in Chapter 7). Of the five elements followed during bioleaching of Ore 1, the two nuisance elements (iron and magnesium) were more negatively affected by higher pH compared to the valuable metals (nickel, cobalt, and copper), on a relative basis (Table 3.8).

The effect of media was often significant; although, the magnitude of the effect was consistently less than the magnitude of the effect caused by pH (Table 3.8). The effect of media was positive on both nickel and cobalt extraction; negative on iron and magnesium extraction from Ore 1; and not significant on magnesium extraction from Ore 2 or copper from either ore. The positive effect of the richer media on nickel and cobalt extraction may be explained in terms of increased biological activity, and the negative effect on iron may be

explained in terms of increased rate of iron precipitation as ferric-containing compounds, as a result of an increased rate of biological ferrous ion oxidation. There is currently no conclusive explanation for the negative effect on magnesium extraction. The acid dissolution of magnesium-containing silicate minerals is generally considered to be a non-oxidative reaction, which should not depend on the ferric ion concentration or ORP, therefore would be expected to be independent of biological activity.

The pH*media interaction generally had little effect (Table 3.8). After 56 days of bioleaching, the pH*media interaction effect was not significant during nickel extraction with either ore, significant during magnesium extraction with Ore 2 (negative), and significant during both copper and iron extraction (both positive) with Ore 1.

3.3.2. Shake flask experiments with Ore 3

Ore 3 contains a substantial fraction of acid-consuming minerals (~ 64% serpentine), and the ore's initial acid demand had to be determined prior to the commencement of the first maintenance culture. Initial acid demand at 30 °C in a rotary shaker at 150 RPM was determined by combining 5 g ore in 100 mL mTK, and adjusting the pH to 2.1 twice a day by drop-wise addition of 10% H₂SO₄. From the total number of drop consumed, it was determined that approximately 8 mL of 10% H₂SO₄ was required to satisfy the ore's immediate acid demand to produce a relatively pH-stable environment. The experiment was repeated by adding the acid in one addition and monitoring the pH over 48 hours. Following the addition of 8 mL of 10% H₂SO₄ to 5 g ore in 100 mL mTK, the pH of the leachate was observed to immediately decrease to ~ 1.4 and then increase to ~ 2.7 after 48 hours. This modest increase in pH (relative to the original pH of the media ~ 2.1) was considered

acceptable and this protocol was adopted to condition both the maintenance cultures and the inocula for all the STR experiments conducted with Ore 3.

Acid conditioning Ore 3 prior to inoculation with the mixed culture of iron- and sulphur-oxidizing microorganisms had the additional benefit of applying a constant selective pressure for magnesium tolerance. It was determined that after acid conditioning, the leachate of a shake flask contained approximately 5 to 6 g/L magnesium. Li and Ke (2001) demonstrated that a wild strain of *At. ferrooxidans* was able to develop a high tolerance to both nickel and magnesium by slowly increasing the concentration of those metals during serial batch sub-culturing.

The protocol that was developed to satisfy the immediate acid demand for Ore 3 for use with maintenance cultures and STR inocula was not suitable to be used for conditioning the ore prior to the commencement of the shake flask experiments with Ore 3. A large addition of sulphuric acid would have caused the dissolution of large quantities of magnesium silicates, which would have resulted in significant dissolved magnesium. Minimizing magnesium dissolution was a primary experimental objective with this ore. For this reason, the shake flask experiments with Ore 3 proceeded with twice-daily (morning and late afternoon) pH adjustment with 10% H₂SO₄. Even with frequent pH adjustments, there were excessive pH fluctuations regardless of the pH of the experiments (i.e. pH 2.1 or 3.0). Initially after start-up, the pH was observed to increase to greater than 6 in the 16 hours (afternoon until next morning) following pH adjustment. It was believed that large pH fluctuations of this nature would have resulted in bacterial inhibition and/or death, so the shake flasks were not inoculated until the pH was observed to increase less than + 1 pH unit from the set-point in the overnight period. As a result, the shake flask experiments with

Ore 3 were inoculated 13 days after the combination of the ore and the media. Inoculation was accomplished with 5 mL of leachate from a maintenance culture. The shake flask experiments with Ore 3 lasted a total of 48 days (35 days after the 13 day acid conditioning stage). The cumulative time in the metal extraction curves in Figure 3.4 include the 13 days of conditioning, and the first sample was taken on day 13 after inoculation.

Before the completion of the first set of shake flask experiments with Ore 3, it was clear that shake flasks were not an appropriate reactor design for bioleaching experiments with this ore; however, the results of the shake flask experiments with Ore 3 were encouraging and have been presented for this reason (Figure 3.4). The higher pH level resulted in a statistically significant (95% CI) reduction in nickel, copper, magnesium, and iron. The encouraging observation was that the higher pH resulted in a modest decrease in nickel extraction (average of 84 and 65% at pH 2.1 and 3.0 respectively), whereas there was a substantial reduction in the magnesium extraction (average of 43 and 11% at pH 2.1 and 3.0 respectively). In terms of the dual experimental objective with this ore, bioleaching at the higher pH level appeared to be advantageous.

Experiments with the low-nutrient media (McCready, Table 3.4) were performed first, and in light of the difficulties associated with pH control and the length of the acid conditioning phase, the experiments with the high-nutrient media (mTK) and the abiotic experiments were cancelled. The effect of the richer media and the effect of bacteria on the bioleaching of Ore 3 were tested in the STR experiments, which have been discussed in Section 3.4.

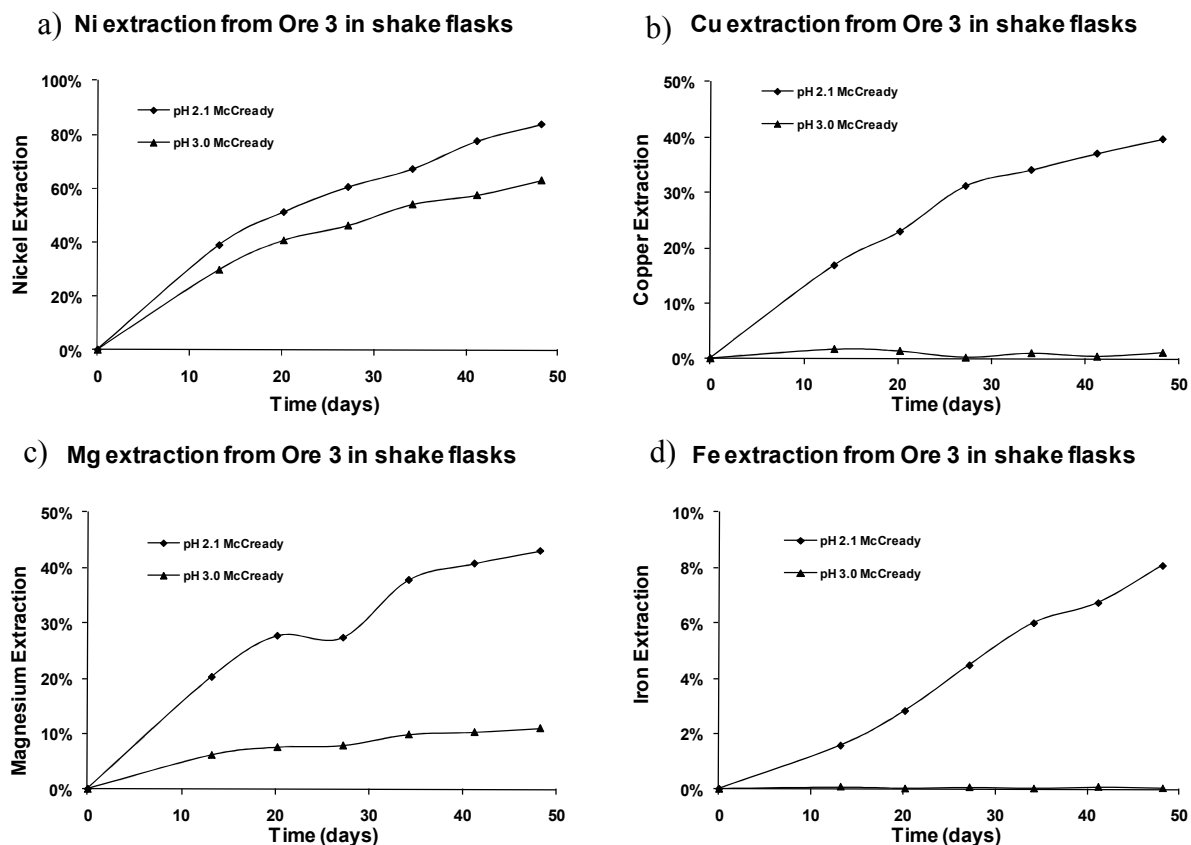


Figure 3.4. Extraction of a) nickel, b) copper, c) magnesium, and d) iron as a function of time during shake flask bioleaching experiments with Ore 3.

In addition to pH control, the use of STRs for bioleaching offers several advantages compared to shake flask experiments. The consumption of sulphuric acid during bioleaching may be an important factor to consider, as it can be a major operational cost to a heap bioleaching operation. Accurately measuring the acid consumption during a shake flask experiment is difficult, as counting the number of drops of acid while adjusting the pH of a shake flask is cumbersome and time consuming, whereas the amount of sulphuric acid consumed during an STR experiment can be accurately and easily determined by drawing the required acid from a graduated cylinder. STR experiments also result in superior mass transfer compared to shake flask experiments, as a result of a higher Reynolds number (i.e.

more turbulent flow). Furthermore, the ability to continuously sparge the leachate with air in order to ensure an adequate supply of oxygen is highly desirable.

3.3.3. Conclusions of the shake flask experiments

Three ores were subjected to shake flask (bio)leaching at 30 °C as part of an amenability study with different nickel sulphide ores from three locations in Canada. The effects studied were the presence of bacteria, leachate pH, and growth media type (ammonium and phosphate levels). The effect caused by the presence of bacteria at pH 2.1 was determined by comparing the extraction of nickel and copper during biotic experiments to the extraction of nickel and copper during control experiments in which thymol was used as a bactericide. A two factor, two level factorial design was used to assess the relative effects of leachate pH, media type, and the pH*media interaction. It was desired to use the results of these preliminary experiments to eliminate either leachate pH or media as a factor to be studied during subsequent experiments with other ores. Shake flasks were determined to be an unsuitable reaction vessel for evaluating the amenability of Ore 3 to bioleaching due to poor pH control. Consequently, the planned biotic experiments with mTK media and the abiotic experiments with thymol were cancelled.

The presence of the mixed-culture of sulphur- and iron-oxidizing bacteria had a substantial positive effect on the extraction of both nickel and copper during shake flask experiments with Ores 1 and 2, after 35 days of bioleaching. Analysis of the factorial designs revealed that the relative order of importance for the factors are $\text{pH} > \text{media} > \text{pH} * \text{media}$. Of the five elements followed during bioleaching of Ore 1, the two nuisance elements (iron and magnesium) were more negatively affected by higher pH compared to the valuable metals

(nickel, cobalt, and copper), on a relative basis. The interaction term was determined to have an insignificant effect on the extraction of nickel from both Ores 1 and 2.

The mineralogy of Ore 3 presented a challenge not common to the other two ores. In addition to maximizing the desirable extraction of nickel, minimizing the undesirable extraction of magnesium was of equal importance. Due to the long acid conditioning phase that was required prior to flask inoculation and the large pH fluctuations throughout the duration of the shake flask experiments, it was determined that shake flask tests were an inappropriate reaction vessel for conducting bioleaching experiments with Ore 3. However, in terms of the dual experimental objective, bioleaching Ore 3 at the higher pH level appeared to be advantageous. Bioleaching at the higher pH level resulted in a modest decrease in nickel extraction, whereas there was a substantial reduction in the magnesium extraction.

3.4. Results and Discussion of the STR Experiments with Ore 3

The inoculum for an individual batch STR experiment was incubated for 10 to 12 days in a rotary shaker at 150 RPM at pH 3 prior to the commencement of the STR experiment. It was determined that after 10 to 12 days at pH 3, each inoculum shake flask (~ 100 mL) prepared with McCready media according to the procedure described in Section 3.2.4 contained $\sim 5.5 \times 10^{10}$ sulphur-oxidizing bacteria and $\sim 3.2 \times 10^{10}$ iron-oxidizing bacteria. These cell densities were determined by calculating the average of the cell densities after 10, 11, and 12 days of incubation (Table 3.9) using the MPN method (Cochran, 1950) with mTK and Att media for enumerating iron- and sulphur-oxidizing bacteria respectively. Each incubation period was done in duplicate.

Table 3.9. Density of iron- and sulphur-oxidizing bacteria in the STR inocula at 30 °C with Ore 3.

Incubation time (days)	Concentration of iron-oxidizing bacteria (cells/mL)	Concentration of sulphur-oxidizing bacteria (cell/mL)
10	2.3×10^8	7.9×10^8
10	7.9×10^8	1.3×10^9
11	1.1×10^8	3.3×10^8
11	1.3×10^8	3.3×10^8
12	3.3×10^8	3.3×10^8
12	3.3×10^8	2.3×10^8

The first set of shake flask experiments with Ore 3 appeared to produce encouraging results (Figure 3.4); however, it was believed the experiments should be repeated under constant pH conditions. The preliminary STR experiments with Ore 3 were designed to: confirm the observations obtained during shake flask experiments; establish an appropriate experiment duration for future experiments; determine if the richer media has a beneficial effect; and establish an appropriate pH range for the first set of experiments to be conducted at 30 °C. Four STR experiments with Ore 3 are discussed in this chapter, the conditions of which are listed in Table 3.10.

Table 3.10. Experimental conditions during the preliminary STR experiments with Ore 3.

Experiment #	Temperature (°C)	pH	Media	Duration (days)
STR 1	30	2	McCready	62
STR 2	30	3	McCready	62
STR 3	30	3	mTK	35
STR 4	30	not controlled	McCready with 9 g of S ^o	35

The first two STR experiments with Ore 3 (STR 1 & 2) were conducted with McCready media at pH 2 and 3 at 30 °C in order to confirm the observations from the shake

flask experiments. The concentrations of nickel, cobalt, and magnesium were determined 13 times over the course of the 62-day experiments (Figure 3.5). The third experiment (STR 3) was conducted with the richer media to assess the effect of media at pH 3. Considering the results of the first two experiments, the richer media was not tested at the lower pH level because of the excessively high levels of magnesium extraction and sulphuric acid consumption at pH 2 with McCready media.

The results of the first two experiments were used to determine an appropriate duration for future STR experiments with this ore. The concentrations of the two primary metal values (nickel and cobalt) were observed to plateau after approximately 21 days (Figures 3.5a and 3.5c). A slightly longer duration of 35 days was selected for future experiments based on the anticipation of conducting future experiments at lower temperatures (an initial temperature range of 15 to 45 °C was considered). STR experiments 3 & 4 were conducted with the shorter duration of 35 days and a modified sampling schedule (samples on days 0, 2, 7, 14, 21, 28, and 35).

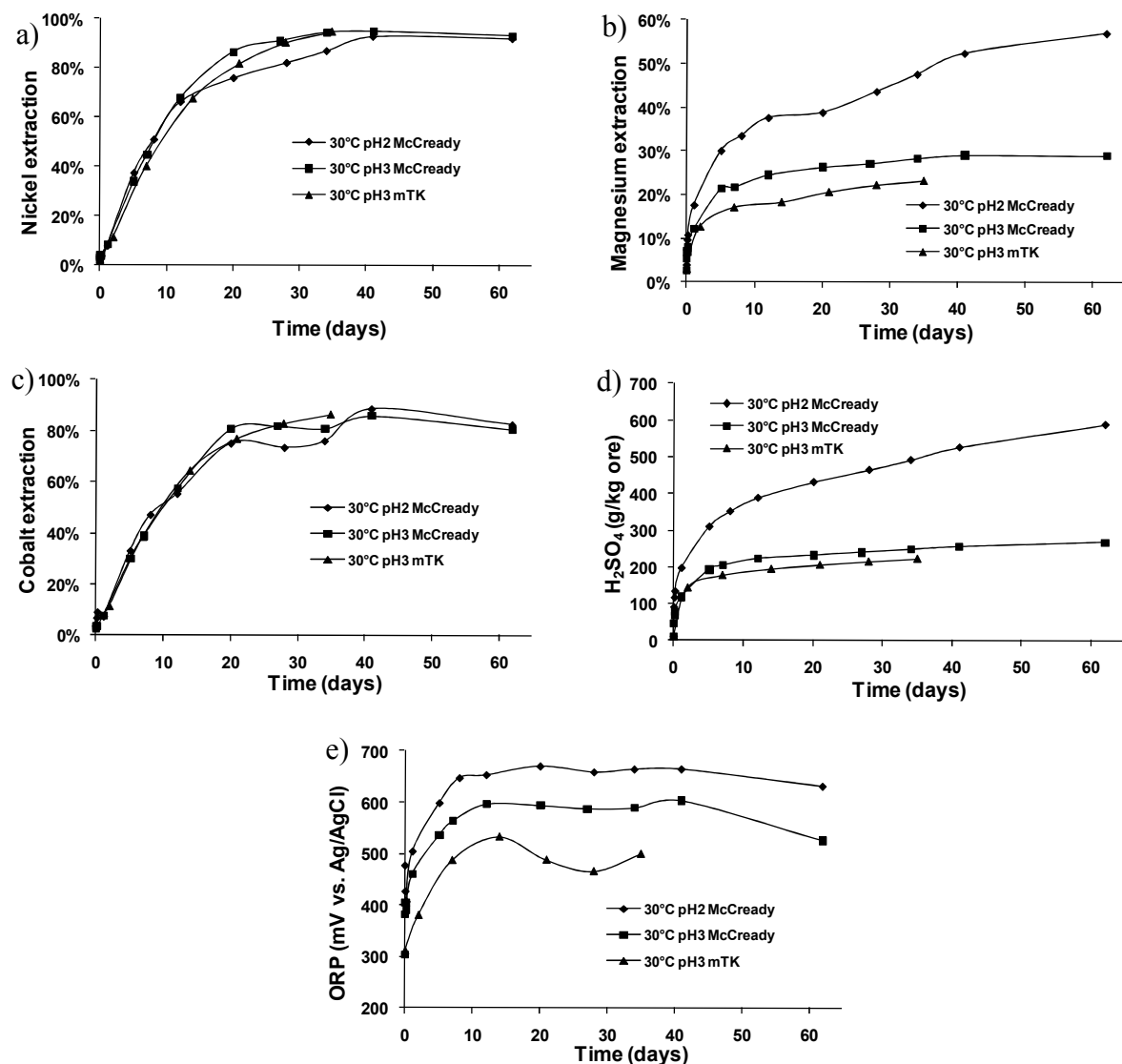


Figure 3.5. Extraction of a) nickel, b) magnesium, c) cobalt, d) the consumption of sulphuric acid, and e) ORP as a function of time during bioleaching of Ore 3.

The results of STR 1 and STR 2 exhibited the same encouraging trends as the shake flask experiments discussed in the previous section. Both nickel and cobalt extraction were largely unaffected by the pH of the leachate, whereas there was a significant reduction in both magnesium extraction and sulphuric acid consumption (~ 50% reduction in both) at pH 3 compared to pH 2 (Figures 3.5). The results of these two experiments were viewed as

very encouraging and it was decided that further investigation was warranted. The experiment conducted with the richer media (mTK) did not produce superior metal extraction (Figure 3.5, series 'mTK').

The fourth STR experiment with Ore 3 (STR 4) was conducted in order to establish an appropriate pH range for future STR experiments (Figure 3.6). This experiment was commenced by mixing 150 g of ore, 1.5 L of McCready media, 9 g of elemental sulphur, and inoculated with 5 mL of slurry from a culture of neutrophilic sulphur-oxidizers. The reactor was re-inoculated with 5 mL of the regular maintenance culture on day 14 (shown by an arrow in Figure 3.6).

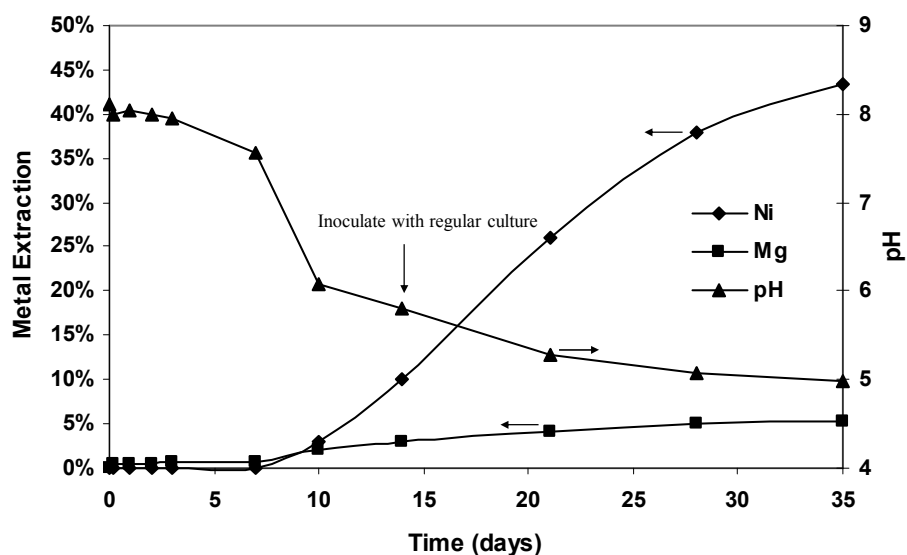


Figure 3.6. Extraction of nickel, magnesium, and the pH as a function of time during STR bioleaching of Ore 3 at 30 °C with the addition of elemental sulphur.

The results of STR 4 show that nickel leaching from Ore 3 began abruptly as the pH of the leachate fell below 6 (Figure 3.6). At the time this experiment was conducted, it was unclear if this observation was related to an increase in biological activity at $\text{pH} < 6$ or if it

was a result of a chemical solubility-related process (i.e. nickel precipitation above pH 6). Based on the results of this experiment, pH 6 was selected as the upper limit for further STR experiments with Ore 3, the results of which are presented in Chapters 4, 5, and 6. The results of STR 4 clearly demonstrate that the culture of sulphur-oxidizing microorganisms used in this study is able to oxidize elemental sulphur over a wide pH range. Furthermore it was shown that biological sulphur oxidation can be used successfully to control pH and possibly eliminate the need for sulphuric acid (as previously demonstrated by Salo-Zieman *et al.*, 2006).

3.4.3. Conclusions of the preliminary STR experiments with Ore 3

The mineralogy of Ore 3 presents a unique challenge for the application of bioleaching, as the ore contains a substantial fraction of readily acid-soluble magnesium silicate minerals. Prior to the commencement of the bioleaching experiments with this ore, discussions with the company identified minimizing magnesium extraction as a primary performance objective, in addition to maximizing nickel extraction. Furthermore, the consumption of sulphuric acid was also considered important, as sulphuric acid addition can be a major operational cost to a commercial heap bioleaching operation.

Four STR experiments were conducted as part of the preliminary bioleaching study with Ore 3. The first two experiments repeated the conditions tested during the shake flask experiments with the lean media (McCready) at pH 2 and 3. The extraction of the valuable metals (nickel and cobalt) were relatively unaffected by an increase in pH from 2 to 3, whereas both the consumption of sulphuric acid and the extraction of magnesium were reduced by ~ 50% by increasing the pH from 2 to 3, after 56 days of bioleaching. These

results were viewed as very encouraging and it was believed that further bioleaching experiments at higher pH levels were warranted.

The third STR experiment with Ore 3 was conducted to establish whether there was a substantial advantage to using a media with higher levels of ammonium and phosphate. The experiment conducted with the richer media (mTK) did not produce superior nickel or cobalt extraction. The fourth STR experiment was conducted in order to establish an appropriate pH range for future STR experiments with Ore 3. Based on the results of this experiment, pH 6 was selected as the upper limit for further STR experiments with Ore 3. Furthermore, the results of STR 4 clearly show that the mixed culture of sulphur-oxidizing microorganisms used in this study is able to oxidize elemental sulphur over a wide pH range.

3.5. General Discussion and Conclusions

The results of the preliminary experiments described in this chapter were used to make a number of decisions that were applied to future experiments, including: selection of a nutrient media, and developing a protocol for acid-conditioning Ore 3 prior to starting an inoculum or a new shake flask sub-culture. Although the extractions of several metals were followed because of their potential value as by-products or their potential for complicating down-stream processes, the extraction of nickel (and magnesium with Ore 3) was the primary consideration when making decisions.

During the shake flask experiments with Ores 1 and 2, the richer media resulted in a modest average increase in nickel extraction of 6 and 8% respectively, after 56 days of bioleaching. In the STR experiments with Ore 3, the richer media did not appear to have any effect on nickel extraction, although it should be noted that it is not possible to conclude the

effect is not significant with any degree of confidence, since the preliminary STR experiments were conducted without replicates. Since there was no overwhelming advantage when using the richer media in any of the experiments, a decision was made to conduct future experiments with the leaner media (McCready). This decision was primarily based on the assumption that it contained nutrient levels more representative of a commercial heap bioleaching operation.

A method for satisfying the initial acid demand prior to commencing an inoculum or a new shake flask sub-culture with Ore 3 was developed by measuring the amount of sulphuric acid required to maintain the pH below 3 for 48 hours. The protocol that was developed consisted of combining 5 g of ore, 100 mL of McCready media, and 8 mL of 10% H_2SO_4 (vol/vol). After 48 hours, the pH was adjusted to 3, and inoculated with 5 mL of slurry from a well-shaken maintenance culture. This protocol was used to prepare all the new maintenance sub-cultures and inocula for the STR experiments with Ore 3 that have been discussed in Chapters 4 to 7 in this thesis.

3.6. References

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Chapter 4 – Bioleaching of Ore 3 in Stirred-Tank Reactors at Elevated pH

Preface

A total of 107 stirred-tank reactor experiments ranging from ten days to twelve weeks were conducted with six different nickel sulphide ores for this thesis and have been discussed in Chapters 3 to 7. A complete list of the experimental conditions and the purpose of each experiment has been given in Appendix B.

Chapters 4 to 6 are exclusively dedicated to the bioleaching of Ore 3. This chapter has been published in a peer-reviewed journal: Cameron, R.A., Lastra, R., Mortazavi, S., Bedard, P.L., Morin, L., Gould, W.D., Kennedy, K.J., 2009. Bioleaching of a low-grade ultramafic nickel sulphide ore in stirred-tank reactors at elevated pH. *Hydrometallurgy* **97**, 213-220.

Abstract

The purpose of the present work is to investigate the technical feasibility of applying elevated-pH bioleaching to a low-grade ultramafic nickel sulphide ore from Manitoba, Canada, which is not currently exploitable with conventional technologies. The ore contains 21% magnesium and 0.3% nickel. Nickel is the only significant metal value, and is present primarily as pentlandite. A substantial fraction of the magnesium is present as lizardite, making processing difficult with conventional pyro- and biohydrometallurgical techniques. This work has two equally important objectives: to obtain an acceptable nickel extraction and to minimize magnesium mobilization. Five-week stirred-tank bioleaching experiments were

conducted with finely ground ore ($-147\ \mu\text{m}$) at $30\ ^\circ\text{C}$ to study the effect of pH (2 to 6) on nickel and magnesium extraction, and the consumption of sulphuric acid. The rate of nickel extraction from pentlandite was found to be relatively insensitive to acidity at low pH and positively correlated to acidity at high pH. During the first three weeks of bioleaching, nickel was extracted at similar rates for experiments conducted at $\text{pH} \leq 5$, with over 70% of the nickel extracted in that timeframe. The leaching of magnesium showed a greater dependency on pH, gradually decreasing from 70 to 10% at pH 2 and 5 respectively, after five weeks. Bioleaching at elevated pH substantially increased the ratio of nickel to magnesium in the leachate, and resulted in substantially less sulphuric acid consumption.

4.1. Introduction

Heap bioleaching practices have the potential to enable the development of deposits that are not currently economically viable using conventional mineral processing technologies. Since 1977, over twenty commercial heap/dump (bio)leaching operations have been commissioned for processing copper oxide and secondary copper sulphide ores (Watling, 2006). In the last decade, extensive efforts have been made to expand the technology to primary copper sulphide ores, and to a lesser extent nickel sulphide ores. There have been heap bioleaching pilot trials with nickel sulphide ores in Australia (Hunter, 2002), Finland (Riekkola-Vanhanen, 2007), and China (Wen *et al.*, 2006). The first commercial application of nickel sulphide heap bioleaching began production at Talvivaara, Finland in October 2008 (Talvivaara, 2009).

More than 50% of the world nickel production comes from sulphide ores (Eramet, 2006), pentlandite being the most economically significant nickel sulphide mineral. Other sulphide minerals frequently found in association with pentlandite include pyrrhotite,

chalcopyrite, and pyrite. Nickeliferous pyrrhotite is generally the most abundant sulphide phase in nickel sulphide ores, and typically contains 0.2 to 0.5% nickel in solid solution, in addition to very finely divided pentlandite inclusions (Habashi, 1997). Nickel sulphide ores frequently contain copper, cobalt, and numerous platinum group elements, all of which may represent significant values (Kerfoot *et al.*, 1997).

The technical literature indicates that nickel-bearing sulphide minerals are amenable to dissolution under common heap bioleaching conditions. A typical heap bioleaching operation maintains a solution pH between 1.5 and 2.5 (Plumb *et al.*, 2008), which is deemed necessary to obtain adequate sulphide mineral dissolution rates. Solution pH can have a dramatic effect on the formation of secondary ferric phases, microbial growth rates, and the solubility of certain metal species. Maximum dissolution rates of sulphide minerals are generally correlated to low solution pH and high oxidation-reduction potential (Ahonen and Tuovinen, 1995); however, nickel extraction from pentlandite and pyrrhotite has been shown to be less dependent on pH.

Ahonen and Tuovinen (1995) column bioleached a low-grade multi-sulphide ore in which nickel was present primarily as pyrrhotite. They observed that the extraction of nickel was least sensitive to pH. Nickel leaching from pentlandite has been observed to be adversely affected by low pH in some instances. Dutrizac and MacDonald (1974), and Corrins and Scholtz (1976) determined that nickel extraction from pentlandite was inversely correlated to acidity during acidic ferric sulphate leaching at low pH.

As part of the Talvivaara research program, Riekkola-Vanhanen *et al.* (2001) bioleached their multi-sulphide black-schist ore in stirred-tank reactors in the range of pH 1.5 to 3. Pentlandite and violarite constituted approximately 80% of the nickel content. Over

90% of the nickel was extracted in two weeks, and showed limited dependency on pH.

Results of these bench-scale experiments were used to establish baseline operating conditions for two 450 kg columns with ore from the same deposit (Reikkola-Vanhanen and Heimala, 1999). It was concluded that operation at pH 3 resulted in satisfactory nickel recovery.

A low-grade ultramafic-dominated nickel sulphide ore from Manitoba was acquired for this study. Ultramafic nickel sulphide ores of this nature frequently contain a substantial fraction of magnesium silicate minerals such as serpentines, amphiboles, biotite, chlorite, and talc (collectively called MgO). Some of these gangue minerals are naturally floatable, leading to high-MgO concentrates, which must be smelted at higher temperatures, resulting in reduced furnace life (Muinonen, 2006). Furthermore, the presence of hydrophilic MgO particles may interfere with the flotation of the sulphide minerals, reducing flotation rates (Bremmell *et al.*, 2005).

Bioleaching high-MgO ores is also challenging, as many magnesium silicate minerals are reactive in mildly acidic media, consuming sulphuric acid, which can be a major operational cost to a heap bioleaching operation (Watling, 2006). Furthermore, the removal of magnesium from the pregnant liquor and its disposal can represent a significant expense and logistical challenge, as hydrated magnesium sulphate salts are highly voluminous, and the price of magnesium metal does not allow for economical recovery.

In the present study, the low-grade nickel sulphide ore was subjected to stirred-tank bioleaching at pH levels from 2 to 6. The purpose of this study was to assess the amenability of the ore to bioleaching at elevated pH. The objectives were to minimize magnesium mobilization and to maintain an acceptable level of nickel extraction. Bioleaching nickel

sulphide minerals at $\text{pH} > 3$ has not been reported in the technical literature, and is potentially a novel approach for recovering nickel from select low-grade ultramafic-dominated nickel sulphide ores. Furthermore, the reaction of magnesium silicate gangue minerals under different levels of acidity has widespread application to the leaching of ultramafic sulphide ores.

4.2. Methods and Materials

4.2.1. Low-grade sulphide ore

A 400 kg sample of low-grade nickel sulphide ore consisting of drill cores was obtained. The entire sample was crushed to -12.7 mm, and after thorough mixing, a sub-sample of approximately 10 kg was crushed to -6.35 mm. From the 10 kg sub-sample, a portion was used for mineralogical analysis, and the remainder was pulverized to -147 μm (100 Tyler mesh) and used for stirred-tank bioleaching tests and culture maintenance. Large representative sub-samples were obtained with a Jones riffle, while the sub-samples for the individual reactions were obtained by coning and quartering or by using a rotary riffle.

4.2.1.1. Mineralogical characterization

Mineralogical characterization of the ore was performed in the mineralogical laboratories at CANMET-MMSL in Ottawa, Canada. Lastra *et al.* (2008a) reported on the mineralogical characterization of the ore, including details of the procedure and complete liberation analysis. In brief, a portion of the sample was further crushed and sieved into 11 size fractions ranging from 38 to 850 μm . A sub-sample was taken from each size fraction and pulverized to -38 μm (400 Tyler mesh) with a McCrone micronising mill, and subjected to X-ray diffraction (XRD) analysis, using a Rigaku Rotaflex rotating anode powder

diffractometer equipped with a Cu anode, operating at 50 kV, 180 mA, with a 4 degrees/min scan speed.

A polished section was prepared for each size fraction, and studied using a mineral analyzer (MLA, JKTech) interfaced to a JEOL 733 electron microprobe equipped with two detectors for energy dispersive (EDS) X-ray analysis. All the analyses were performed at an acceleration voltage of 20 kV and 15 nA of constant electron beam current. The samples were studied with the GXMAP measurement mode of the MLA. In this mode, the backscattered electron (BSE) image is used to discriminate between grains of different BSE grey levels, then each grain is automatically scanned with the electron beam to obtain its EDS spectrum. In addition, grains with either the EDS spectra of pentlandite or the EDS spectra of chalcopyrite or a combination of both were scanned with a spacing of two pixels to obtain the EDS spectra at each point. This was done to separate pentlandite and chalcopyrite that have similar BSE grey level. Matching software (MLA-Particle X) was used to identify each grain by comparing its EDS spectra with the EDS spectra of known minerals. The information from the XRD study was used to identify and set up the EDS spectra for minerals constituents greater than 2% by mass. Minerals of less than 2% were identified using solely the EDS spectra.

The ore used in this study consists primarily of the magnesium silicate minerals lizardite, clinocllore, hypersthene, enstatite, and berthierine. The hydrated magnesium iron phyllosilicate mineral lizardite (a serpentine mineral) constitutes more than 60% of the sample. The ore contains less than 3% total sulphide, primarily pyrrhotite, pentlandite, and pyrite, in addition to a trace amount of chalcopyrite. The relative abundance of all the sulphide minerals and the most abundant gangue minerals in the original ore and the post-

leach residues are listed in Table 4.1. The concentration of most sulphide minerals in the post-leach residues were determined to be unreliable because they were too low and near the detection limit of the image analysis technique (0.1 wt%), and have been omitted. Lastra *et al.* (2008b) provides complete analysis of the post-leach residues.

Table 4.1. Mineral composition of Ore 3 and the post-leach residues according to MLA results.

Mineral	Content (%mass)					
	Ore	pH6	pH5	pH4	pH3	pH2
Sulphides						
Pentlandite	0.7	-	-	-	-	-
Pyrrhotite	0.9	-	-	-	-	-
Pyrite	0.5	-	-	-	-	-
Chalcopyrite	tr	-	-	-	-	-
Gangue						
Lizardite	64	62	59	41	30	3.5
Other Mg-silicates (hypersthene, enstatite, berthierine, clinocllore)	9.1	9	10	9	10	13
Magnetite or Fe-oxides	12	15	14	14	16	29
Carbonates (ankerite, dolomite)	2.1	tr	tr	tr	tr	tr
Quartz (+ extremely Mg-depleted lizardite or Si-oxide phase)	0.1	tr	tr	tr	tr	13
Talc (+ Mg-depleted lizardite)	3.3	4	15	36	36	19

Electron probe X-ray microanalysis (EPMA) was accomplished by wavelength-dispersive X-ray analyses (WDS) with a JEOL JXA 8900 EPMA, operated with an accelerating voltage of 25 kV, probe current of 50 nA, and counting times ranging from 20 to 100 seconds on peak and background. A few small grains of pentlandite were analyzed at 20 kV to keep the source of the X-rays within the grain. The K α characteristic X-ray was used for all elements investigated (Fe, Ni, Co, S) and a correction was applied for the overlap of Fe K β on Co K α . Matrix corrections were made using the $\phi(\rho z)$ program provided by JEOL.

EPMA was used to determine the nickel content of pentlandite, pyrrhotite, and the major gangue phases: lizardite, magnetite, clinocllore, tremolite, and actinolite. Pentlandite and pyrrhotite were determined to contain 39 ± 2 and $0.65 \pm 0.2\%$ nickel by mass respectively. None of the major gangue phases contained a substantial fraction of nickel. EPMA revealed that this ore has a complex mineralogy. Figure 4.1 shows a BSE image of an Fe-rich magnesium silicate (stoichiometrically consistent with an Fe-rich lizardite) phase intimately associated with lizardite. The Fe-rich magnesium silicate is speckled with micro Ni-Fe-S inclusions, which are stoichiometrically consistent with pentlandite and appear to be 0.5 to 2 μm in diameter. Many similar inclusions were observed to be present in other similar Fe-rich magnesium silicate grains; however, too few particles were examined to quantify the fraction of nickel that can be attributed to these inclusions. It can be concluded that pentlandite and the pentlandite-consistent inclusions (collectively referred to as pentlandite in the remainder of this article) are the primary source of nickel in this ore.

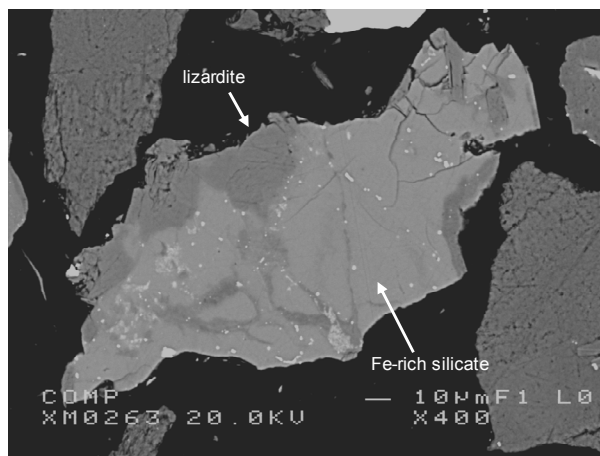


Figure 4.1. BSE image showing Ni-Fe-S inclusions throughout an Fe-rich magnesium silicate phase in Ore 3.

4.2.1.2. Chemical analysis

All chemical determinations were done by the ISO 9001 certified Analytical Services Group at CANMET-MMSL's laboratories in Ottawa, Canada. Aqueous metal determinations were carried out by ICP-AES. Ore and solid residue samples were digested with a four-acid preparation method (Donaldson, 1974) prior to metal analysis by ICP-AES. Total sulphur was determined by pyrolysis in a Leco furnace, and sulphate by HPLC. Elemental sulphur was extracted with toluene and quantified spectrophotometrically, and sulphide was determined by applying a total sulphur mass balance. The chemical composition of the ore is listed in Table 4.2.

Table 4.2. Chemical composition of Ore 3.

Ni (%)	Mg (%)	Co (%)	Cu (%)	Fe (%)	Sulphide S (%)	Elemental S (%)	Total S (%)
0.305 ± 0.004	21.2 ± 0.1	0.0130 ± 0.0004	0.016 ± 0.002	7.2 ± 0.1	0.57 ± 0.03	0.08 ± 0.04	0.65 ± 0.01

Average of four replicates ± 1SD

4.2.2. Microbial culture

The microbial culture used in this study was derived from water and soil samples collected during an October 2006 site visit to mining-related locations in Sudbury, Ontario. Iron- and sulphur-oxidizing microorganisms were enriched from each sample using media designated mTK and At.t respectively (Table 4.3).

Table 4.3. Composition of the growth media used during enrichment, culture maintenance, and bioleaching experiments.

	Modified TK (mTK)	McCready	ATCC Medium 23 (At.t)
Nutrients (g/L)	0.5 (NH ₄) ₂ SO ₄ 0.5 K ₂ HPO ₄ 0.5 MgSO ₄ ·7H ₂ O	0.0661 (NH ₄) ₂ SO ₄ 0.0174 K ₂ HPO ₄ 0.123 MgSO ₄ ·7H ₂ O	0.1 NH ₄ Cl 3.0 KH ₂ PO ₄ 0.2 MgCl ₂ ·6H ₂ O 0.1 CaCl ₂
Energy Source (g/L)	33.4 FeSO ₄ ·7H ₂ O	33.4 FeSO ₄ ·7H ₂ O	5.0 Na ₂ S ₂ O ₃ ·5H ₂ O
Initial pH	2.1	2.1	4.2
Reference	Modified from Tuovinen and Kelly (1973)	McCready <i>et al.</i> (1986)	Modified from Gherna <i>et al.</i> (1989)

All enrichment cultures were combined, and the resulting mixed culture was maintained on a finely-ground low-grade nickel sulphide ore from the Sudbury region by serial sub-culturing. Microbial characterization including polymerase chain reaction (PCR) with specific primers revealed the resulting mixed culture contained *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, and at least one *Acidiphilium* species (Dinardo and Mohapatra, 2008). The culture was maintained in an orbital shaker in a 250 mL Erlenmeyer flask at 5% pulp density (mass/vol), @150 RPM, pH 2.2 ± 0.2, and 30 °C in mTK media. A maintenance culture was acclimated to the low-grade ore used in this study for several months by serial sub-culturing prior to the commencement of the bioleaching experiments. During growth on sulphide ores, both the magnesium supplement and the energy source were omitted from the growth media.

The ore had a high acid-consuming capacity, which required significant acid addition prior to inoculating a new maintenance sub-culture. In the absence of acid addition, the pH was observed to increase from 3 to almost 8 within minutes after combining the media and the ore. It was determined that 5 g of ore (-147 µm) required 8 mL of 10% H₂SO₄ (vol/vol) to satisfy the ore's immediate acid demand in order to maintain the pH below 3 for 48 hours.

4.2.3. Bioleaching experiments

Eleven stirred-tank bioleaching experiments and four abiotic control experiments were conducted at 30 °C. Table 4.4 shows the conditions of the individual experiments, and indicates which post-leach residues were mineralogically characterized. Both biotic and abiotic experiments at the baseline condition of pH 3 were carried out in triplicate, whereas most of the other experiments were done in duplicate.

Table 4.4. Experimental conditions for the (bio)leaching experiments with Ore 3 and the results after 35 days of bioleaching at 30 °C.

Experiment number	pH	Mineralogical characterization	Ni extraction after 35 days ¹ (%)	Mg extraction after 35 days ¹ (%)	H ₂ SO ₄ consumption (g/kg ore)	Residual weight (g)
1	2	Yes	90	76	727	89
2	3	Yes	88	29	238	127
3	3	Yes	91	30	250	126
4	3	No	85	30	255	126
5	4	Yes	83	22	170	132
6	4	No	84	22	167	132
7	4	No	75	21	188	131
8	5	Yes	69	10	73	139
9	5	Yes	67	10	71	142
10	6	Yes	37	5.9	47	143
11	6	No	22	7.1	40	143
12	3 Control	Yes	46	42	358	118
13	3 Control	Yes	45	38	350	122
14	3 Control	No	52	53	393	119
15	5 Control	No	46	31	98	139

Stirred-tank reactions were conducted in a jacketed 2.0 L glass reaction vessel with temperature control, pH control, aeration, and continuous stirring for five weeks. Aeration was adequate to maintain the dissolved oxygen near-saturation in all experiments. Sulphuric acid was kept in a graduated cylinder and pumped into the reaction vessel with a peristaltic

pump as required by the pH controller. The volume of acid remaining in the graduated cylinder was recorded during each sampling session, and the volume used was mathematically converted to a weight equivalent.

The inoculum for individual experiments was developed by combining 5 g of ore, 100 mL of McCready media (Table 4.3), and 8 mL of 10% H₂SO₄ (vol/vol). After 48 hours, the pH was adjusted to 3, and inoculated with 5 mL of slurry from a well-shaken maintenance culture. The resulting inoculum was maintained in an orbital shaker for ten to twelve days at pH 3, and then combined with 145 g ore in the reaction vessel and made up to a total volume of 1.5 L with McCready media. Aliquots of the leachate were taken periodically, and evaporative losses were volumetrically made up with distilled water prior to sampling. The sample volume was replaced with an equal volume of fresh McCready media. After each test, the residue was filtered, washed with distilled water, and dried at 45 °C for one week. The residue was gently broken up with a mortar and pestle, and sample splits were mineralogically and chemically characterized. Sample splits for chemical characterization were pulverized prior to analysis.

Maintaining the inoculum in an orbital shaker prior to the commencement of each stirred-tank experiment ensured an adequate population of actively growing bacteria at the beginning of each experiment. It was determined that after ten to twelve days at pH 3, each inoculum shake flask (~ 100 mL) prepared according to the procedure previously described contained approximately 5.5×10^{10} sulphur-oxidizing bacteria and 3.2×10^{10} iron-oxidizing bacteria. Bacterial determinations were accomplished with a five-tube most-probable-number (MPN) method (Cochran, 1950). All tubes were incubated in the dark for 28 days at room temperature. The energy source in the growth media was used to distinguish between iron-

and sulphur-oxidizing populations, which were enumerated with media designated mTK and Att respectively (Table 4.3). A positive test for iron-oxidizing bacteria was indicated by the formation of an orange precipitate, whereas a positive test for sulphur-oxidizing bacteria was indicated by a drop in pH (0.5 units below the average value of the control tubes).

Abiotic control experiments were conducted with thymol as bactericide in 5% methanol. The concentration of thymol was periodically determined by a Ultraspec 4300 pro UV spectrophotometer at 273.5 nm (Meline *et al.*, 1996), and evaporative losses were made up with a fresh thymol/methanol solution. Trial and error was used to determine a suitable thymol concentration range that resulted in a linear relationship between absorbance and the concentration of thymol. For the calibration curve that was used for all the abiotic experiments discussed in this thesis, the instrument was calibrated using a zero point and four concentrations of thymol (0 to 0.082 g/L). The four concentrations were made by dilution with deionized water from an original stock solution of 1.0296 g thymol in 1L of 5% methanol and 95% McCready media. The four concentrations and their corresponding absorbance readings are listed in Table 4.5. The calibration curve with the fitted linear relationship between absorbance and the concentration of thymol is given in Figure 4.2. During STR experimentation, a sample was initially diluted 10:1 with deionized water prior to thymol determination. If the absorbance was determined to be > 0.8 , the sample was further diluted until the absorbance reading was within the calibration range.

Table 4.5. Thymol calibration data.

Dilution factor from stock solution (1.0296 g/L)	Concentration of thymol (g/L)	Absorbance
0	0	0
0.02	0.02059	0.245
0.04	0.04118	0.505
0.06	0.06178	0.823
0.08	0.08237	1.062

The presence of thymol has been linked to the suppression of abiotic leaching when saturated (Perdicakis *et al.*, 2001). For this reason, the thymol concentration was maintained in a range (0.3 to 0.9 g/L) below the saturation concentration (~ 1 g/L in water), and above the concentration that causes complete inhibition of *Acidithiobacillus ferrooxidans* (Meline *et al.*, 1996). Of the sterilization methods described by Brickett *et al.* (1995), thymol was considered to be the preferred method of controlling bacterial growth in the current study.

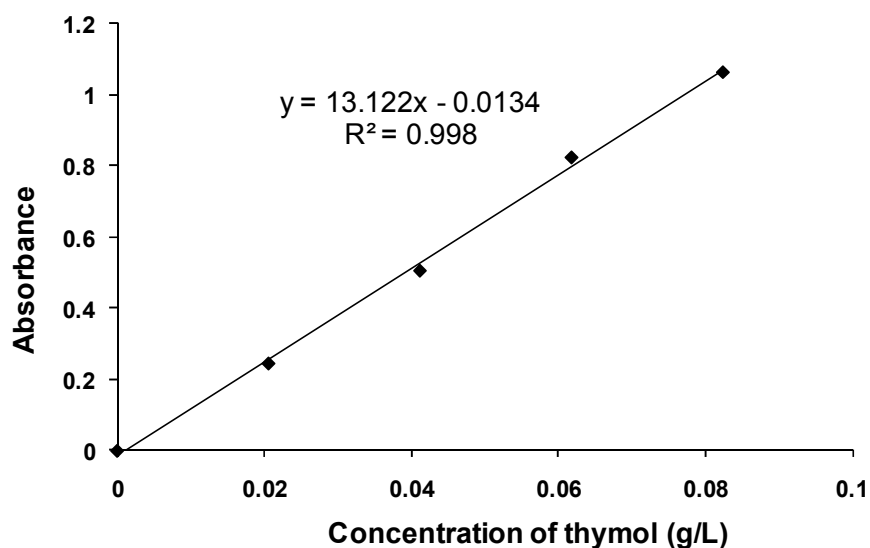


Figure 4.2. Thymol calibration curve used to determine the concentration of thymol during abiotic STR experiments.

Aliquots of the leachate were analyzed for nickel, magnesium, cobalt, copper, iron, and silicon. Solid samples were analyzed for those same elements, and the following sulphur species: sulphide-S, total-S, elemental-S, and sulphate-S. Oxidation-reduction potential (ORP), conductivity, acid level, and the dissolved oxygen were measured and recorded during each sampling session. Dissolved oxygen and conductivity were measured with an Orion Star-5 benchtop multimeter, ORP (vs. Ag/AgCl) with a WTW Multiline P4 handheld meter, and the pH was controlled with a Eutech alpha-pH800 controller.

Although pyrrhotite is present in this ore, its contribution to the total nickel content is estimated to be less than 2%, therefore the nickel contribution from pyrrhotite may be neglected with minimal error. The rate of appearance of nickel in the leachate was used as an indicator for the rate of pentlandite dissolution. Since magnesium is distributed throughout numerous magnesium silicate minerals in the ore, it is not possible to assign the appearance of magnesium in the leachate to the leaching of a single mineral. The extent of dissolution of the individual magnesium silicate minerals was determined based on the mineralogical characterization of the post-leach residues.

Significance testing was performed by analyzing the data using the one-way analysis of variance (ANOVA) function in Minitab release 14, with 95% confidence intervals unless otherwise indicated. Data points in the graphs presented in this report are the mean value of the replicates where applicable, and error bars have been plotted as the 95% confidence intervals based on the standard error of the mean. Metal extraction curves have been modified by the appropriate dilution factors to account for sample removal. Metal analyses presented as a function of time are based on solution assays, whereas determinations presented as final values after 35 days of bioleaching are based on the analysis of pre- and

post-leach solid residue assays, except where otherwise indicated. There was generally good agreement between the final metal extractions calculated from the leachate assays and the final metal extractions calculated from the solid residue assays, and significance testing of both sets of data yielded the same conclusions.

4.3. Results and Discussion

4.3.1. Bacterial growth curves

Bacteria can affect leaching rates by a number of different mechanisms, including: by increasing the solution ORP by oxidizing ferrous ion; by oxidizing elemental sulphur thereby preventing byproduct passivation and producing acidity; and by directly attaching to the mineral surface. A detailed description of these mechanisms is beyond the scope of this paper, but may be found elsewhere, including in recent reviews by Rohwerder *et al.* (2003), Watling (2006), and Suzuki (2001).

The dominant species that were present in the original mixed culture are generally considered to be obligate acidophiles (acidithiobacilli and leptospirilli); therefore, bacterial growth experiments were conducted to ensure that the bacteria were able to survive and reproduce at elevated pH. Growth experiments were conducted with the same low-grade ore according to the same protocol as the bioleaching experiments, except only 5 mL of slurry from a well-shaken maintenance culture was used as inoculum. Growth curves at pH 3 and 5 were constructed by enumerating both sulphur- and iron-oxidizing bacteria seven times over the course of the ten days (Figure 4.3). Slurry samples were taken from the reactors while stirring, so the bacterial density determinations represent a combination of both planktonic and sessile bacteria.

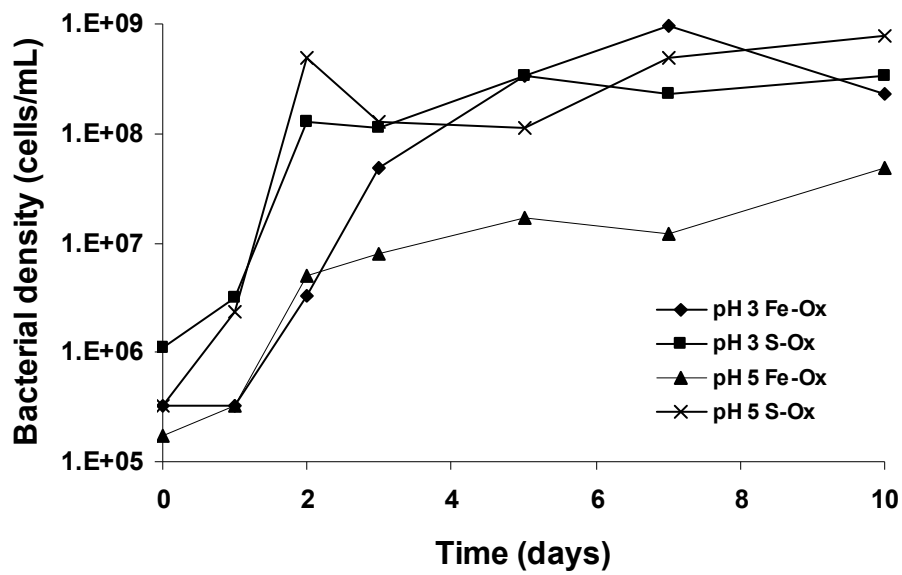


Figure 4.3. Density of iron- (Fe-Ox) and sulphur-oxidizing (S-Ox) bacteria at pH 3 and 5 during ten-day growth experiments at 30 °C with Ore 3.

Figure 4.3 shows that both sulphur- and iron-oxidizing bacteria were able to reproduce at both pH levels tested. The population density of the sulphur-oxidizing bacteria increased by approximately three orders of magnitude at both pH levels. The population density of the iron-oxidizing bacteria increased by approximately two and three orders of magnitude at pH 5 and 3 respectively. Slow growth of the iron-oxidizing bacteria at pH 5 is expected, as the total dissolved iron concentrations during both biotic and abiotic experiments at pH 5 were frequently below 1 ppm. Due to the nature of the enumeration technique, the bacterial density determinations are considered to be semi-quantitative.

4.3.2. Effect of pH on metal extraction from sulphide minerals

The leaching of copper from chalcopyrite is most affected by pH. Leachate determinations of copper were frequently below the detection limit in experiments conducted at pH > 3. Consequently, the behaviour of copper and copper-bearing minerals will not be

discussed further in this report. It is recognized that elevated-pH leaching is not appropriate for sulphide ores containing a substantial amount of copper. Although copper is generally associated with nickel sulphide deposits, the nickel-to-copper ratio (mass/mass) in this ore is 18, making nickel the primary metal of value.

The solubility of ferric compounds is highly dependent on pH, and secondary ferric phases such as jarosite and ferric hydroxide readily precipitate above pH 2.5 to 3. The total iron concentrations in this study were determined to be very low in all biotic experiments except at pH 2. The total dissolved iron averaged approximately 20, 5, and 1 ppm at pH 3, 4, and 5 respectively over the duration of the five-week experiments.

Figure 4.4 displays nickel and magnesium extraction, and total acid consumed (grams concentrated H_2SO_4 per kg ore) after 35 days of bioleaching at 30 °C. The data shows a gradual reduction in nickel extraction with increasing pH, and a more pronounced drop above pH 5. The final extraction of nickel at all pH levels was compared, and the differences were statistically significant between the following pH pairs: 2&5, 2&6, 3&5, 3&6, 4&6, and 5&6. Even with 80% confidence intervals (i.e. narrower error bars), the differences between pH 2&3, 2&4, and 3&4 were not statistically significant. The differences between the abiotic experiments at pH 3 and each of the biotic experiments at pH 2, 3, 4, and 5 were also determined to be statistically significant.

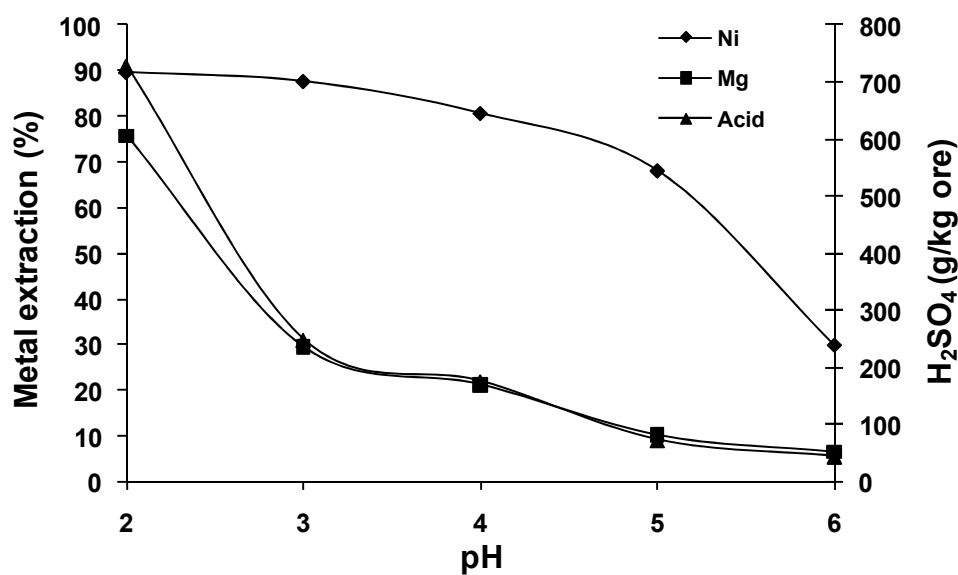


Figure 4.4. Nickel and magnesium extraction, and total acid consumption after 35 days of bioleaching at 30 °C with Ore 3.

Nickel extraction as a function of time for all experiments is presented in Figure 4.5. During the first two weeks, nickel bioleached from pentlandite at approximately the same rate at all pH levels (except pH 6). Other authors have reported either low sensitivity to pH or an inverse relationship between leaching rates and acidity during the leaching of nickel from pentlandite. Dutrizac and MacDonald (1974) reported a reaction rate constant inversely proportional to acidity (rate constant $\propto [\text{H}_2\text{SO}_4]^{-0.14}$) when the concentration of sulphuric acid was from 0.01 to 1.0 M during acidic ferric sulphate column leaching of a low-grade nickel sulphide ore (in which pentlandite contained the majority of the nickel); Corrans and Scholtz (1976) observed that the leaching of nickel was negatively correlated to acidity in the range of pH 0.3 to 1.7 (rate constant $\propto [\text{H}^+]^{-1}$), and observed a moderately positive correlation in the range of pH 1.7 to 3.7 (rate constant $\propto [\text{H}^+]^{0.12}$) during acidic ferric sulphate stirred-tank leaching of a pentlandite concentrate; and Riekkola-Vanhanen *et al.* (2001) reported that

nickel extraction showed limited dependency on pH during stirred-tank bioleaching tests with their multi-sulphide black-schist ore in the range of pH 1.5 to 3.

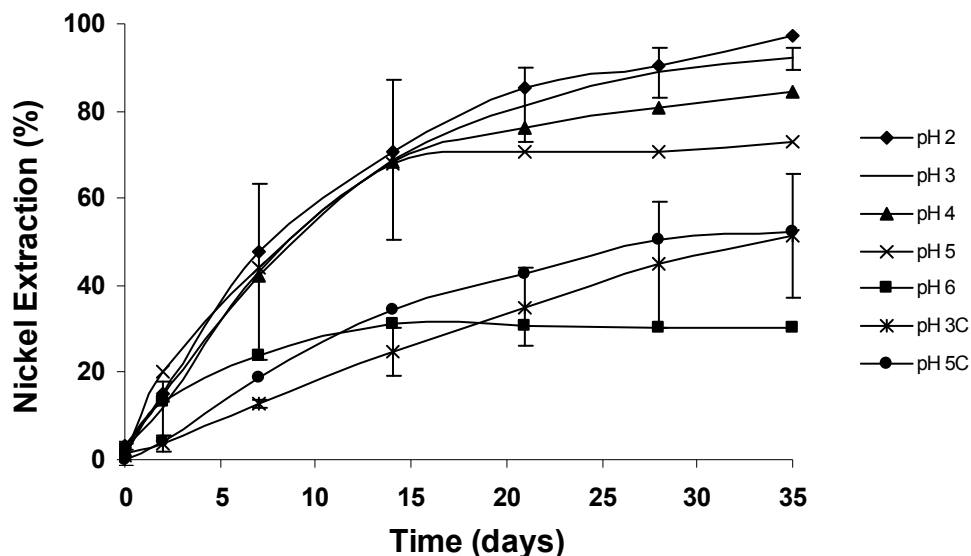


Figure 4.5. Nickel extraction as a function of time at different pH levels at 30 °C with Ore 3. C = abiotic.

All biotic experiments at $\text{pH} \leq 5$ leached more nickel compared to both of the abiotic control experiments (Figure 4.5, series ‘pH 3C’ and ‘pH 5C’), particularly during the first two weeks (Figure 4.5). At pH 6, the presence of the iron- and sulphur-oxidizing bacteria was initially beneficial; however, tests at pH 6 resulted in lower nickel extraction at the end of five weeks when compared to the abiotic tests at pH 3 and 5. Nickel extraction after two days of bioleaching at pH 5 is significantly greater than nickel extraction after two days of bioleaching pH 3 (95% CI).

The presence of the bacteria significantly reduced the consumption of sulphuric acid. After five weeks of (bio)leaching at pH 3, an average of 248 and 367 g H_2SO_4 per kg ore were consumed during biotic and abiotic experiments respectively. At pH 5, an average of 72

and 98 g H₂SO₄ per kg ore were consumed during biotic and abiotic experiments respectively. Bacteria can reduce sulphuric acid consumption by oxidizing elemental sulphur, which has been observed as a byproduct during oxidative dissolution of many sulphide minerals, including pentlandite (Dutrizac and MacDonald, 1974) and pyrrhotite (Bhatti *et al.*, 1993).

Three types of kinetic control and their combinations are possible within the context of shrinking-core kinetics: surface reaction control, product-layer diffusion control, and stagnant film control. At any time during a reaction, all three resistances are working in series; however, at a particular time, one is usually dominant and is considered to be the rate-limiting mechanism. Mathematical derivations and conceptual explanation of the different limiting conditions may be found in Levenspiel (1999), and references therein. The shrinking-core model was derived based on the reaction of mono-sized particles. The finely ground ore used in this study was not screened to have a narrow particle size distribution, so mathematical application of the shrinking-core model without incorporating a particle size distribution could produce erroneous conclusions (Gbor and Jia, 2004). It is still useful to qualitatively discuss the data obtained in this study in terms of the shrinking-core model as a conceptual aid.

The Reynolds number during the stirred-tank leaching experiments was calculated according to Tchobanoglous *et al.* (2003) to be within the turbulent regime ($\sim 30,000$); as such, it was assumed that bulk diffusion and mass transfer through the stagnant layer surrounding the particles were not limiting. Surface reaction control at the beginning of leaching is expected at moderate temperatures considering: the turbulent flow regime; the aeration was sufficient to maintain the dissolved oxygen level near saturated at all times; the

sulphide grains in the ore are well liberated at 147 μm (Lastra *et al.*, 2008a); and the magnitude of the activation energy reported for the oxidative dissolution of pentlandite (61 kJ/mol by Corrans and Scholtz, 1976). The ore used in this study primarily consists of gangue minerals (less than 3% sulphides). In the absence of the significant gangue dissolution, one would expect to see a shift to product-layer diffusion control as the readily available pentlandite on the surface of the ore particles is depleted. The reaction would be expected to become more mass transfer limited as the reaction zone topochemically moves inwards and the diffusional path of the products and/or reactants increases as they must diffuse through the un-reacted gangue.

The rate law for the leaching of a particular mineral is expected to be a function of the activation energy and the solution properties (oxidant concentration, pH, etc) during surface reaction controlled leaching. Figure 4.5 shows that nickel extraction during the first two weeks of bioleaching (expected to be surface reaction controlled) appears to be independent of pH (except at pH 6). This suggests that the rate law for the dissolution of pentlandite under surface reaction controlled conditions is not a strong function of pH. The effect of pH was evident after the second week, as indicated by the divergence of the nickel extraction curves in Figure 4.5. It is believed that this divergence is consistent with a change in rate control rather than a dependency on pH; an effect which is more pronounced with increasing pH. This could potentially be explained in terms of the dissolution of the gangue minerals and the resulting impact on the porosity of the remaining particles. Less gangue mineral dissolution means less porosity, necessarily resulting in more resistance to diffusional mass transfer. The amount of gangue dissolution is a function of pH, as evident by the lizardite amounts in Table 4.1 and the residual weights in Table 4.4. It is probable that

the experiments conducted at high pH were more diffusion limited because less of the gangue minerals had dissolved. The reduced reaction rate observed after the second week could be a result of a decrease in the rate of mass transfer through the reacted material rather than a chemical dependency on pH.

4.3.3. Gangue mineral dissolution

The behaviour of the magnesium silicate gangue minerals is of particular interest when considering the economics of a heap bioleaching operation with an ultramafic ore of this nature. From a processing perspective, the undesirable solubilisation of magnesium is as important as the desirable solubilisation of nickel. The applicability of heap bioleaching to this ore might ultimately be determined by the behaviour of the magnesium silicate gangue minerals, since the dissolution of gangue minerals has a direct effect on two major operating costs, which are sulphuric acid consumption and waste management/magnesium mitigation. Consequently, the behaviour of the gangue minerals as a function of pH was investigated in detail.

Little information on sulphuric acid leaching of magnesium silicate minerals as a function of pH under mildly acidic conditions is available in the technical literature. One may use the published neutralization potential (NP) values as a guide to infer acid consumption of minerals under bioleaching conditions. According to Jambor *et al.* (2007), the NP potential of ultramafic ores is dominated by the amount of olivine and serpentine. In fact, the incorporation of serpentine minerals into passive acid mine drainage treatment systems to generate alkalinity has been proposed because of their reactivity in dilute acidic solutions (Bernier, 2005). Since the ore used in this study contains ~ 64% lizardite (a serpentine mineral), it is reasonable to anticipate that lizardite will account for a significant

portion of the total acid consumption during bioleaching. The ore also contains ~ 2% carbonate minerals, which would be expected to completely dissolve at all pH levels tested in this study.

Figure 4.6 clearly shows that pH has a profound effect on magnesium extraction. Contrary to the release of nickel, the final extraction of magnesium was determined to be a strong function of pH at high acidity levels, and a weaker function of pH under moderately acidic conditions. The final extraction of magnesium during bioleaching at different pH levels was compared, and the differences were determined to be statistically significant for all pH pairs except 3&4, 4&5, 5&6. The difference between the biotic experiments at pH 3 and the abiotic experiments at pH 3 was determined to be statistically significant. This result was surprising because it was assumed that the leaching of the magnesium silicate minerals was strictly a chemical process. It is possible that biofilm formation on the surface of the silicate minerals provides additional mass transfer resistance, slowing the acid dissolution of the magnesium silicate gangue minerals.

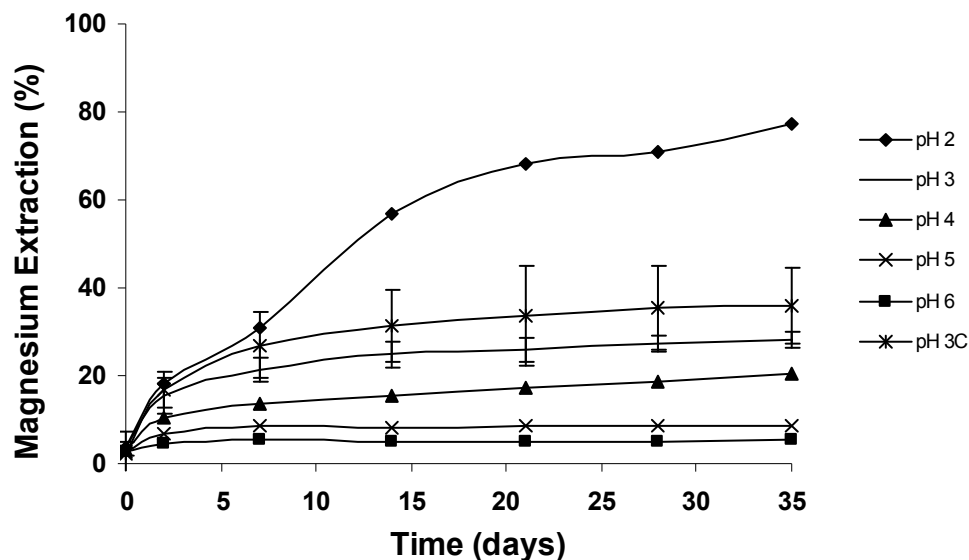


Figure 4.6. Magnesium extraction as a function of time at different pH levels at 30 °C with Ore 3. C = abiotic.

The post-leach residues were mineralogically characterized with XRD and SEM as part of the post-leach examination. As expected, the carbonate minerals were completely dissolved in all experiments (Table 4.1). According to the MLA, lizardite was determined to be the only major silicate phase that showed significant signs of dissolution, the extent of which was highly correlated to pH (Table 4.1). After five weeks of stirred-tank bioleaching, the lizardite was mostly pristine at pH 6 and almost completely dissolved at pH 2, and was altered to varying degrees at intermediate pH levels. The fraction of altered lizardite is a near-linear function of pH (Figure 4.7).

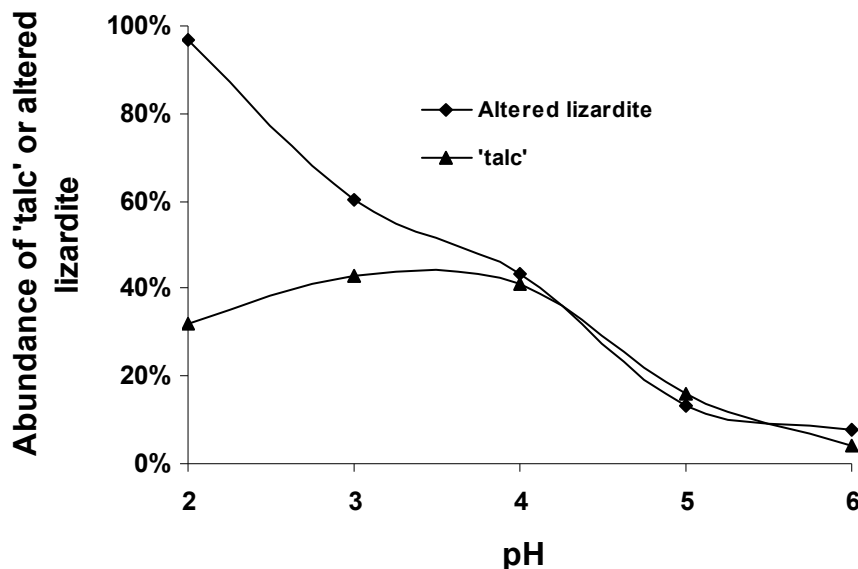


Figure 4.7. Abundance of altered lizardite and 'talc' in the post-leach residues after 35 days of bioleaching at 30 °C with Ore 3.

There were discrepancies between the XRD and MLA results with regard to the lizardite ($\text{Mg}_3\text{Si}_2\text{O}_5(\text{OH})_4$), talc ($\text{Mg}_3\text{Si}_4\text{O}_{10}(\text{OH})_2$), and quartz (SiO_2) determinations. XRD results showed lizardite as the major phase in all residues except pH 2, whereas the MLA results showed a gradual depletion of lizardite and an increase of talc, with decreasing pH (Figure 4.7). Understanding the reason behind the discrepancy provides insight into the behaviour of lizardite during (bio)leaching. The identification of a mineral by the MLA is made by comparing the EDS ratio of the constituent elements of the unknown mineral to that of known minerals, whereas the identification of a mineral by XRD is based on comparing X-ray diffraction patterns (crystal structure) of the unknown mineral to that of known minerals.

The cause of this discrepancy was the non-stoichiometric dissolution of lizardite, whereby the magnesium content of the lizardite was depleted without affecting the remaining

crystalline structure. If magnesium is moderately depleted, the MLA would identify the phase as ‘talc’, and if severely depleted, the phase would be identified as ‘quartz’. For reference, the EDS spectra for lizardite, talc, and a lizardite grain that has been attacked by the leachate and classified as ‘talc’ by the MLA have been provided in Figures 4.8a to 4.8c respectively. The EDS spectrum for talc (Figure 4.8b) was taken from a pure specimen, whereas the EDS spectrum for lizardite (Figure 4.8a) was taken from the pristine ore used in this study (Ore 3).

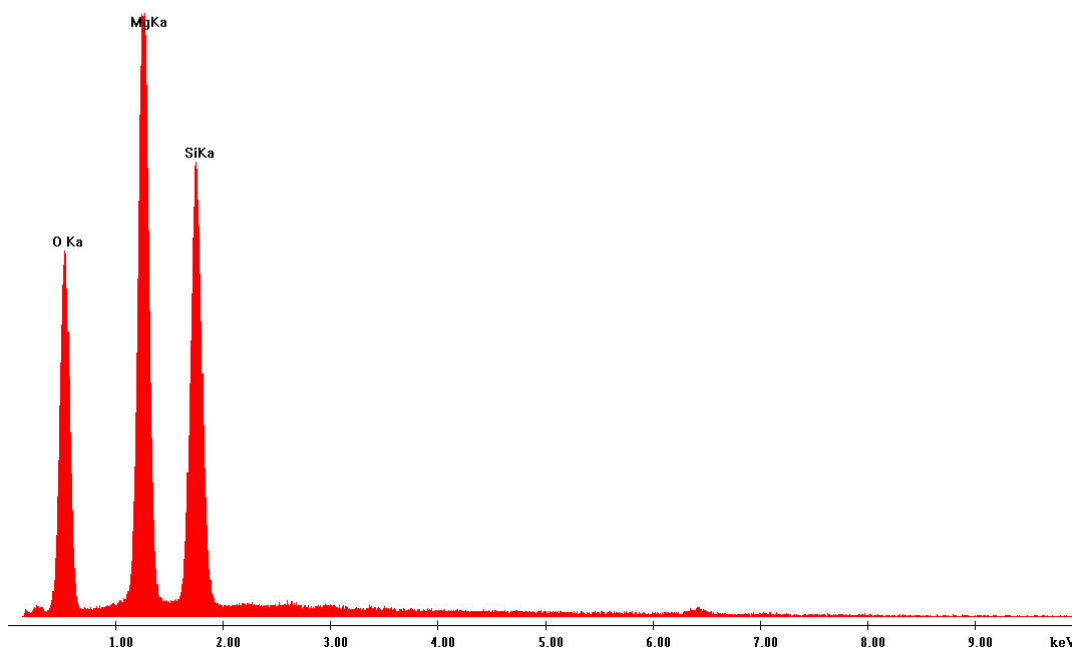


Figure 4.8a. EDS spectrum for lizardite in the pristine ore (Ore 3).

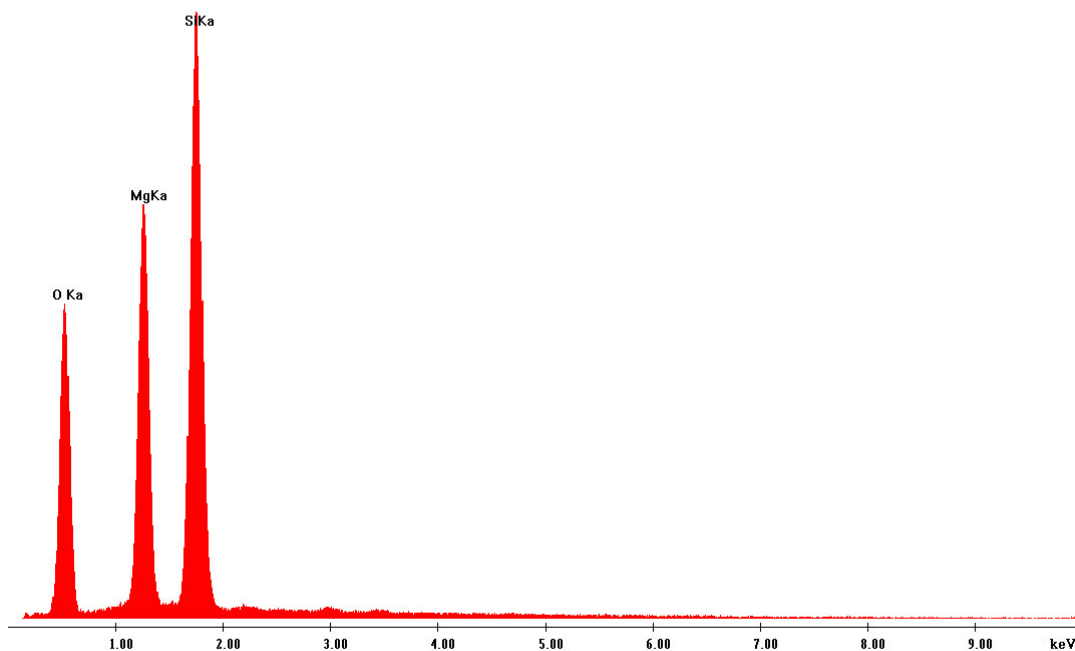


Figure 4.8b. EDS spectrum for a pure specimen of talc.

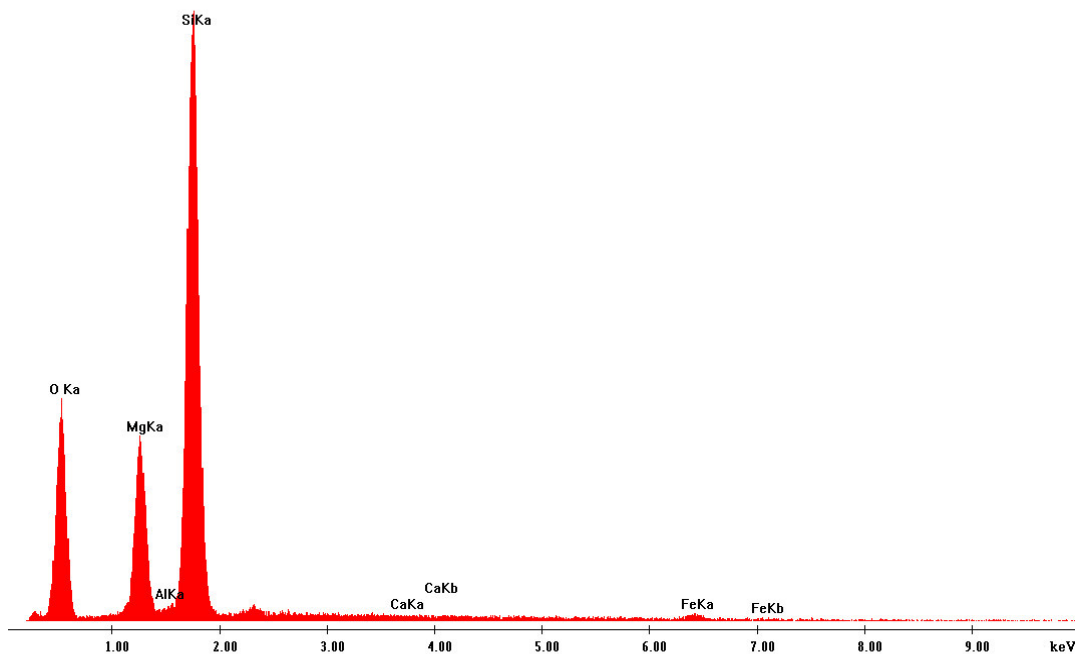


Figure 4.8c. EDS spectrum of a lizardite grain attacked by the leachate and classified as 'talc' by the MLA.

The disappearance of lizardite tracks the appearance of ‘talc’ at $\text{pH} \geq 4$ (Figure 4.7). The MLA data indicated that at $\text{pH} < 4$, Mg-depleted lizardite began to lose its structural integrity and transformed into amorphous silica at pH 2. Furthermore, XRD observations at pH 2 showed a substantial increase in the background at d-scale values between 3 and 4, which is consistent with an increase of amorphous silica. This increase in the background is evident in Figure 4.9, which compares the XRD spectra of the post-leach residues at pH 2 (highly altered) and 6 (near pristine).

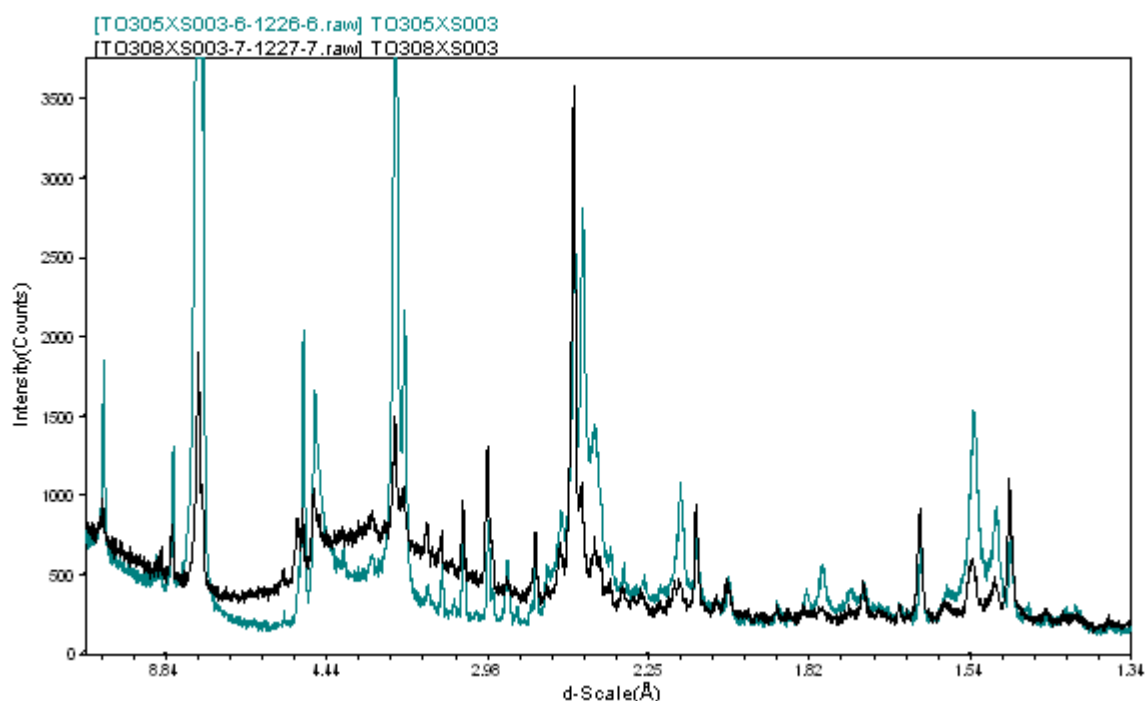
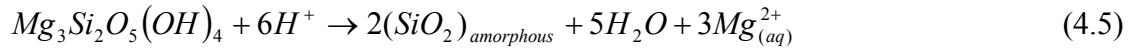
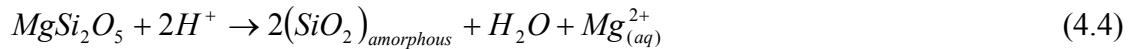
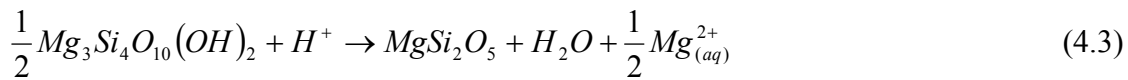
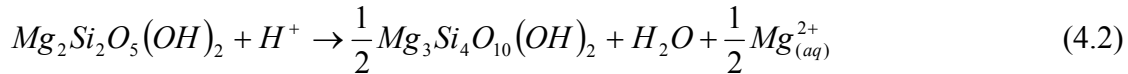
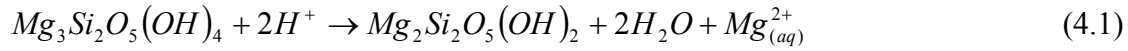


Figure 4.9. Comparison of the XRD spectra of the post-leach residues at pH 6 (series ‘T0305XS003’) and 2 (series ‘T0308XS003’) with Ore 3 at 30 °C.

The EDS spectra of the altered lizardite grain (Figure 4.8c) shows that both Mg and O are depleted relative to Si, compared to unaltered lizardite (Figure 4.8a). The loss of an Mg^{2+} cation is charge-balanced by the loss of two hydroxyl groups (OH^-). A possible pathway for the stepwise degradation of lizardite to amorphous silica by acid dissolution has

been proposed in Equations 4.1 to 4.4; the silicate reaction product in Equation 4.2 is stoichiometrically consistent with talc. The complete reaction of lizardite to amorphous silica by attack with sulphuric acid is presented in Equation 4.5.



Non-stoichiometric dissolution of silicate minerals has been documented in the technical literature (Dutrillac *et al.*, 2000; Terry, 1983). Dutrillac *et al.* (2000) studied the dissolution of asbestos tailings (>90% serpentine) with HCl, in which the serpentine contained a mixture of antigorite and lizardite. They determined that magnesium was preferentially leached from the serpentine, leaving an amorphous silica residue.

4.3.4. Processing considerations

For the purpose of process optimization, a combined metric of the percent nickel extracted to percent magnesium extracted (%Ni:%Mg ratio) was used for comparison purposes. The %Ni:%Mg ratio was chosen to reflect the two experimental objectives: minimizing magnesium extraction, and achieving an acceptable level of nickel extraction. Figure 4.10 clearly demonstrates that manipulating the pH is an effective tool for controlling the %Ni:%Mg ratio. Operating at elevated pH substantially increased the %Ni:%Mg ratio:

approximately 1.2 and 6.7 (an increase of ~ 450%), at pH 2 and 5 respectively, while maintaining an acceptable level of nickel extraction ($\geq 70\%$ in five weeks, Figure 4.5). The consumption of sulphuric acid directly tracked magnesium extraction over the entire pH range tested (Figure 4.4). The total consumption of sulphuric acid was decreased by an order of magnitude by increasing the pH from 2 to 5; 727 and 72 g H_2SO_4 per kg ore, at pH 2 and 5 respectively. Considering that a conventional heap bioleaching operation maintains the leachate pH between 1.5 and 2.5, the potential cost savings by operating at pH 5 are substantial.

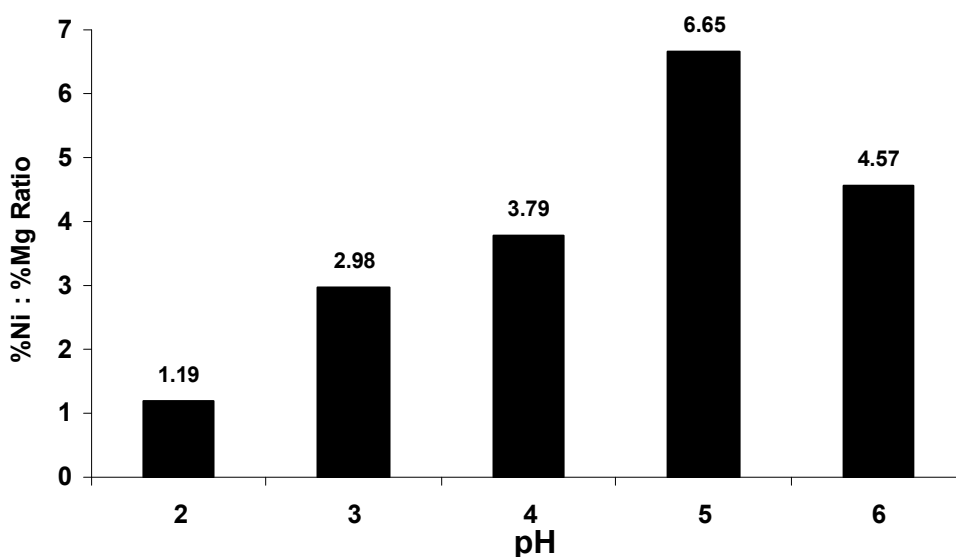


Figure 4.10. Final %Ni:%Mg ratio at different pH levels after 35 days of bioleaching at 30 °C with Ore 3.

4.4. Conclusions

A low-grade ultramafic nickel sulphide ore was acquired from Manitoba, Canada. It is not currently economically feasible to process this ore with conventional concentration and

smelting techniques. The ore is characterized by high magnesium content and low nickel content. Nickel is the primary metal value, and it is present mainly as pentlandite.

Stirred-tank experiments were conducted at 30 °C to study the effect of pH (2 to 6) on nickel and magnesium extraction. Nickel extraction from pentlandite was found to be relatively insensitive to acidity at low pH and sensitive at high pH. The opposite trend was observed while examining the extraction of magnesium. During the first three weeks of bioleaching, nickel was extracted at similar rates for experiments conducted at $\text{pH} \leq 5$, with over 70% of the nickel extracted in that timeframe. Leaching of magnesium showed a greater dependency on pH, gradually decreasing from 70% at pH 2 to 10% at pH 5, after five weeks. Operating at elevated pH substantially increased the %Ni:%Mg ratio in the leachate and reduced sulphuric acid consumption. The results of this study show that bioleaching at elevated pH may be an appropriate processing route for this particular ore, and that further investigation is warranted.

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Chapter 5 – Elevated-pH Bioleaching of Ore 3 in Stirred-tank Reactors at 5 to 45 °C

Preface

An abridged version of this chapter has been published in a peer-reviewed journal: Cameron, R.A., Lastra, R., Mortazavi, S., Gould, W.D., Thibault, Y., Bedard, P.L., Morin, L., Kennedy, K., 2009. Elevated-pH bioleaching of a low-grade ultramafic nickel sulphide ore in stirred-tank reactors at 5 to 45 °C. *Hydrometallurgy* **99**, 77-83. In addition, a portion of this chapter has been presented as a technical poster at a conference: Cameron, R.A., Lastra, R., Mortazavi, S., Gould, W.D., Thibault, Y., Bedard, P., Morin, L., Kennedy, K., 2009. Bioleaching of a low-grade ultramafic nickel sulphide ore in stirred-tank reactors at elevated pH. MetSoc COM2009, Sudbury, Ontario, August 23-26.

A substantial amount of data that was collected during the experiments that have been discussed in Chapters 4 and 5 has not been included in the main body of this thesis. The complete time series (days 2, 7, 14, 21, 28, and 35) of response surface models for the extraction of nickel, magnesium, and cobalt, the percent nickel extracted to percent magnesium extracted ratio (%Ni:%Mg), the percent nickel extracted to percent cobalt extracted ratio (%Ni:%Co), and the consumption of sulphuric acid are available in Appendix A. Details of the response surface methodology and all the model parameters are also available in Appendix A.

Abstract

This study is a continuation of previous work designed to assess the effect of elevated-pH bioleaching on a low-grade ultramafic nickel sulphide ore from Manitoba, Canada. The ore contains 21% magnesium and 0.3% nickel. Nickel is the only significant metal value, and is present primarily as pentlandite. A substantial fraction of the magnesium is present as lizardite, making processing the ore difficult with conventional pyro- and biohydrometallurgical techniques. This work has two objectives: to maximize nickel extraction, and to minimize magnesium mobilization. Five-week stirred-tank bioleaching experiments were conducted with finely ground ore ($-147\ \mu\text{m}$) at three pH levels (3, 4 and 5) and five temperatures (5, 15, 22.5, 30, and 45 °C). The initial rate of nickel extraction from pentlandite was observed to be inversely correlated to acidity at all temperatures, while the final extraction of nickel after five weeks was determined to be moderately correlated to acidity at high temperatures and negatively correlated to acidity at low temperatures. The advantage of elevated-pH bioleaching was most evident at 5 °C, in which the final extraction of nickel at pH 5 was approximately 250% greater than at pH 3. Electron probe X-ray microanalysis of the post-leach residues revealed that the un-reacted lizardite was enriched with nickel during experiments conducted at pH 5, and that the extent of enrichment was a strong function of temperature. The undesirable extraction of magnesium exhibited a strong negative pH-temperature interaction and the consumption of sulphuric acid directly tracked the extraction of magnesium over all experimental conditions. Bioleaching at elevated pH substantially increased the ratio of nickel to magnesium in the leachate, and resulted in a substantial reduction in sulphuric acid consumption.

5.1. Introduction

The discovery of new high grade base-metal deposits is diminishing in frequency; consequently, mining companies are processing low-grade deposits in order to maintain production levels. Heap bioleaching practices have the potential to enable the development of some low-grade deposits that are not currently economically viable with conventional processing technologies. Since 1977, over twenty commercial heap/dump (bio)leaching operations have been commissioned for processing copper oxide and secondary copper sulphide ores (Watling, 2006). There have been heap bioleaching pilot trials with nickel sulphide ores in Australia (Hunter, 2002), Finland (Riekkola-Vanhanen, 2007), and two in China (Wen *et al.*, 2006; Qin *et al.*, 2009). The first commercial application of nickel sulphide heap bioleaching began production at Talvivaara, Finland in October 2008 (Talvivaara, 2009).

Nickel sulphide deposits that contain a high level of magnesium silicate gangue minerals (collectively called MgO) are frequently difficult to process with conventional flotation and smelting technologies. Many magnesium silicate minerals are naturally floatable, leading to high-MgO concentrates, which must be smelted at higher temperatures, resulting in reduced furnace life (Muinonen, 2006). Furthermore, the presence of hydrophilic MgO particles may interfere with the flotation of the sulphide minerals, reducing flotation rates (Bremmell *et al.*, 2005).

Bioleaching high-MgO ores is also challenging, as many magnesium silicate minerals are reactive in acidic media, consuming sulphuric acid, which can be a major operational cost to a heap (bio)leaching operation (Watling, 2006). The removal of magnesium from the pregnant liquor and its disposal can represent a significant expense, as the market for

magnesium salts is limited, and the price of magnesium metal does not allow for economical recovery. Furthermore, excessive loss of mass during heap (bio)leaching could result in pile slumping, which reduces heap permeability and negatively impacts heap performance.

In the present study, a low-grade ultramafic nickel sulphide ore from Manitoba, Canada, was subjected to stirred-tank bioleaching at pH levels from 3 to 5, and temperatures ranging from 5 to 45 °C. The purpose of this study was to assess the amenability of the ore to bioleaching at elevated pH, with two objectives: to achieve an acceptable level of nickel extraction; and to minimize magnesium mobilization. A strong emphasis was put on minimizing the dissolution of the magnesium silicate minerals because their dissolution has a direct impact on two major operating costs, which are acid consumption and waste management.

The present work is a continuation of a previous stirred-tank bioleaching study with the same ore, which examined the effect of pH from 2 to 6, at 30 °C (Cameron *et al.*, 2009). In that study, the rate of nickel extraction from pentlandite was found to be relatively insensitive to acidity at low pH and positively correlated to acidity at high pH. The extraction of magnesium was determined to be strongly correlated to acidity at low pH; low pH operation resulted in considerable magnesium extraction. Operating at elevated pH (4 to 5) substantially increased the ratio of nickel to magnesium in the leachate and reduced sulphuric acid consumption. It was concluded that high-pH bioleaching with a mixed bacterial culture adapted to moderately acidophilic conditions was an effective means to prevent the dissolution of the magnesium silicate minerals.

There is currently at least one other group working on a heap bioleaching process for an ore containing a similar assemblage of high-MgO gangue, in which the primary nickel-

bearing phase is pentlandite. The Jinuan Group has completed a work program consisting of several two meter columns (Zhen *et al.*, 2009) and a 500-ton pilot bioheap (Qin *et al.*, 2009) in northwest China with encouraging results. Their approach differs significantly from the approach taken in this study, as they used a low-pH pre-leaching phase before bioleaching. This conditioning phase was deemed necessary to dissolve the readily leachable magnesium silicate minerals in order to condition the ore for optimum microbial growth prior to inoculation with their mixed culture of acidophilic mesophiles.

5.2. Materials and Methods

5.2.1. (Bio)leaching experiments

A total of 43 stirred-tank experiments were conducted with this ore, including 32 bioleaching experiments and 11 abiotic control experiments. Bioleaching experiments were conducted at all combinations of three pH levels (3, 4, and 5) and five temperatures (5, 15, 22.5, 30, and 45 °C). Abiotic control experiments were conducted at two pH levels (3 and 5) and four temperatures (5, 15, 30, and 45 °C). All bioleaching experiments were performed in duplicate, whereas the control experiments (other than at 30 °C) were conducted without replicates.

All materials and methods were the same as in the previously reported stirred-tank reactor experiments at 30 °C (Cameron *et al.*, 2009), unless otherwise noted. A 400 kg sample of drill cores was crushed to -12.7 mm, and a representative sub-sample was crushed to -147 µm (100 Tyler mesh). Lastra *et al.* (2008) reported on the mineralogical characterization of the ore (Table 5.1). Electron probe X-ray microanalysis (EPMA)

determined pentlandite to be the primary nickel-bearing phase. Chemical analysis revealed the ore to contain 0.31% Ni, 21% Mg, 0.016% Cu, 7.2% Fe, and 0.65% total-S.

Table 5.1. Mineralogical content of Ore 3.

Mineral	Content (%mass)
Sulphides	
Pentlandite	0.7
Pyrrhotite	0.9
Pyrite	0.5
Chalcopyrite	tr
Gangue	
Lizardite	64
Amphiboles (actinolite, hornblende, tremolite)	6.6
Other Mg-silicates (hypersthene, enstatite, berthierine, clinocllore)	9.1
Magnetite	12
Carbonates (ankerite, dolomite)	2.1
Quartz	0.1
Talc	3.3

The microbial culture was enriched at 30 °C from water and soil samples collected from mining-related locations in Sudbury, Canada. Genetic microbial characterization revealed that the resulting mixed culture contained *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, and at least one *Acidiphilium* species (Dinardo and Mohapatra, 2008). The bacteria were adapted to the low-grade ore at each temperature for several months by serial sub-culturing prior to the commencement of the bioleaching experiments. Maintenance cultures at 15 to 45 °C were kept in temperature-controlled orbital shakers, whereas the maintenance culture at 5 °C was kept in a small stirred-tank reactor in a temperature-controlled room.

The inoculum for each experiment was developed by combining 5 g of ore, 100 mL of McCready media (McCready *et al.*, 1986), and 8 mL of 10% H₂SO₄ (vol/vol) in a 250 mL Erlenmeyer flask. After 48 hours in an orbital shaker at 30 °C, the pH was adjusted to 3 and brought to the temperature of the experiment in which it was to be used. The shake flask was then inoculated with 5 mL of slurry from a well-shaken maintenance culture grown at the appropriate temperature. The resulting inoculum was maintained in an orbital shaker (all temperatures other than 5 °C) for ten to twelve days at pH 3. The inoculum was then combined with 145 g of ore in the reaction vessel and made up to a total volume of 1.5 L with McCready media with no energy source and no magnesium supplement. Inocula at 5 °C were placed in the temperature-controlled room and aerated for 21 days at pH 3. A longer incubation was deemed necessary to offset the lower microbial growth rates at 5 °C.

Reactions were conducted in jacketed 2.0 L glass reaction vessels with temperature control, pH control, aeration, and continuous stirring for five weeks. Sulphuric acid was kept in a graduated cylinder and pumped into the reaction vessel with a peristaltic pump as required by the pH controller. Aeration was adequate to maintain the dissolved oxygen near-saturation in all experiments. Abiotic control experiments were conducted, in which thymol was added as a bactericide in 5% methanol (Meline *et al.*, 1996). Aliquots of the leachate were analyzed for nickel, magnesium, cobalt, copper, iron, and silicon. Solid samples were analyzed for those same elements, and the following sulphur species: sulphide-S, total-S, elemental-S, and sulphate-S. Oxidation-reduction potential (ORP), conductivity, acid level, and the dissolved oxygen (DO) were measured and recorded during each sampling session.

All chemical determinations were done by the ISO 9001 certified Analytical Services Group at CANMET-MMSL's laboratories in Ottawa, Canada. Aqueous metal determinations

were carried out by ICP-AES. Ore and solid residue samples were digested with a four-acid preparation method (Donaldson, 1974) prior to metal analysis by ICP-AES. Total sulphur was determined by pyrolysis in a Leco furnace, and sulphate by HPLC. Elemental sulphur was extracted with toluene and quantified spectrophotometrically, and sulphide was determined by applying a total sulphur mass balance.

5.2.2. Process modelling and significance testing

Statistical significance testing was performed by analyzing the data using the one-way analysis of variance (ANOVA) function in Minitab release 14, with 95% confidence intervals unless otherwise indicated. Data points in the graphs presented in this paper are the mean value of the replicates where applicable. Metal extraction curves have been modified by the appropriate dilution factors to account for sample removal.

Response surface modeling of the three by five experimental matrix was used to create the three dimensional graphs presented in this chapter using a quadratic function of pH and temperature, with all interaction terms. The general formula for the full second degree polynomial model is given in Equation 5.1, where β_0 , β_1 , β_2 , β_{11} , β_{22} and β_{12} are the regression constants and Y is the predicted response.

$$Y = \beta_0 + \beta_1 T + \beta_2 pH + \beta_{11} T^2 + \beta_{22} pH^2 + \beta_{12} TpH \quad (5.1)$$

Details of the response surface methodology, the numerical model parameters, and the full time series (days 2, 7, 14, 21, 28, and 35) of response surface plots for nickel extraction, magnesium extraction, and sulphuric acid consumption are given in Appendix A.

5.3. Results and Discussion

The solubility of ferric compounds is highly dependent on pH, and secondary ferric phases such as jarosite and ferric hydroxide-type compounds readily precipitate above pH 2.5 to 3. The total iron concentrations in this study were determined to be very low in all biotic experiments. The total dissolved iron averaged approximately 20 to 50, 2 to 5, and 0.2 to 1.5 ppm at pH 3, 4, and 5 respectively during bioleaching experiments, regardless of temperature. Appreciable amounts of dissolved iron were only detected during abiotic leaching at pH 3, and averaged 250 to 500 ppm, showing a slight positive correlation to temperature. Although iron was not speciated in this study, it can be assumed that the bulk of the iron detected during abiotic leaching at pH 3 was ferrous ion, as abiotic oxidation of ferrous ion is slow at pH 3 over the temperature range studied (Singer and Stumm, 1970).

Bioleaching data is traditionally interpreted in terms of the iron concentration and speciation, and the solution ORP, which are related by the Nernst equation; however, ORP is only meaningful when there is a single dominant redox couple in solution. The ORP data exhibited a considerable amount of scatter in experiments conducted at pH 4 and 5 at all temperatures. Considering the consistently low levels of dissolved iron and the scatter in the ORP data, it is believed that the ORP data collected in this study is not meaningful and therefore has not been presented. All the data collected has been provided in Appendix D.

5.3.1. Effect of pH and temperature on nickel extraction from pentlandite from Ore 3

In the last several years, there has been an increasing amount of attention paid to the (bio)leaching of nickel-bearing sulphide ores and concentrates. Most studies focus on a relatively narrow pH range, generally 1.8 to 2.2. This pH range is perceived necessary to obtain adequate sulphide mineral dissolution rates, as pH can have a dramatic effect on the

formation of secondary ferric and secondary copper phases, and microbial growth rates. Most studies conclude that a low-pH environment is beneficial; however, several studies on the (bio)leaching of nickel from pentlandite have reported either low sensitivity to acidity (Riekkola-Vanhanen *et al.*, 2001) or an inverse relationship between (bio)leaching rates and acidity (Corrans and Scholtz, 1976; Dutrizac and MacDonald, 1974). The current study examines a much wider pH range and significantly higher pH levels than any of the previous studies have reported.

Pentlandite is not the only sulphide mineral that has displayed an inverse relationship between leaching rates and acidity. McKibben and Barnes (1986) reported that the initial rate of pyrite oxidation by ferric ion in the absence of oxygen was proportional to $[H^+]^{-0.5}$ (pH range 1 to 2), and effectively independent of pH during oxidation with dissolved oxygen in the absence of ferric ion (pH range 2 to 4). Holmes and Crundwell (2000) studied the oxidative leaching of pyrite in acidic ferric sulphate and determined that the reaction was proportional to the inverse of acidity; the order of the reaction with respect to $[H^+]$ varied from -0.18 to -0.5, depending on the concentrations of iron and dissolved oxygen.

The extraction of nickel from Ore 3 as a function of time at the different combinations of temperature and pH is displayed in Figures 5.1a to 5.1e. The data provides conclusive evidence that the initial rate of nickel extraction from pentlandite during bioleaching is negatively correlated to acidity (pH 3 to 5) in the temperature range tested. The trend of elevated-pH bioleaching being beneficial to the initial nickel extraction rate is also evident in the abiotic experiments, although the effect is more pronounced in the experiments with bacteria.

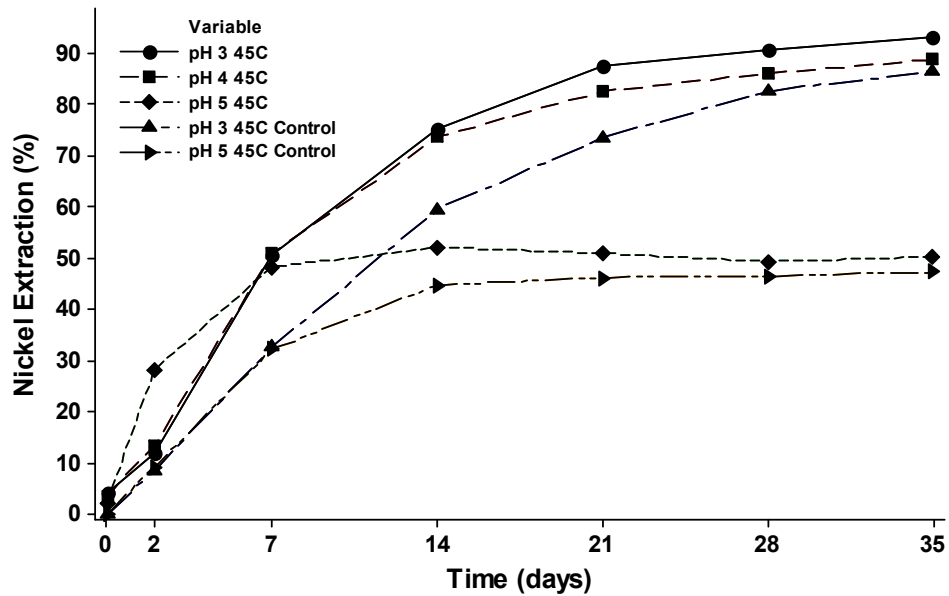


Figure 5.1a. Nickel extraction as a function of time and pH at 45 °C with Ore 3.

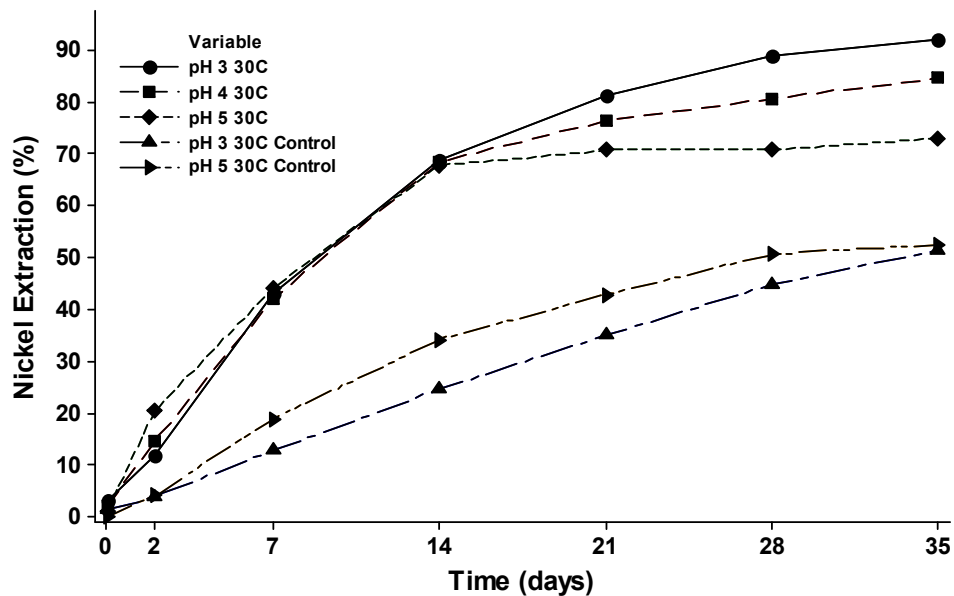


Figure 5.1b. Nickel extraction as a function of time and pH at 30 °C with Ore 3.

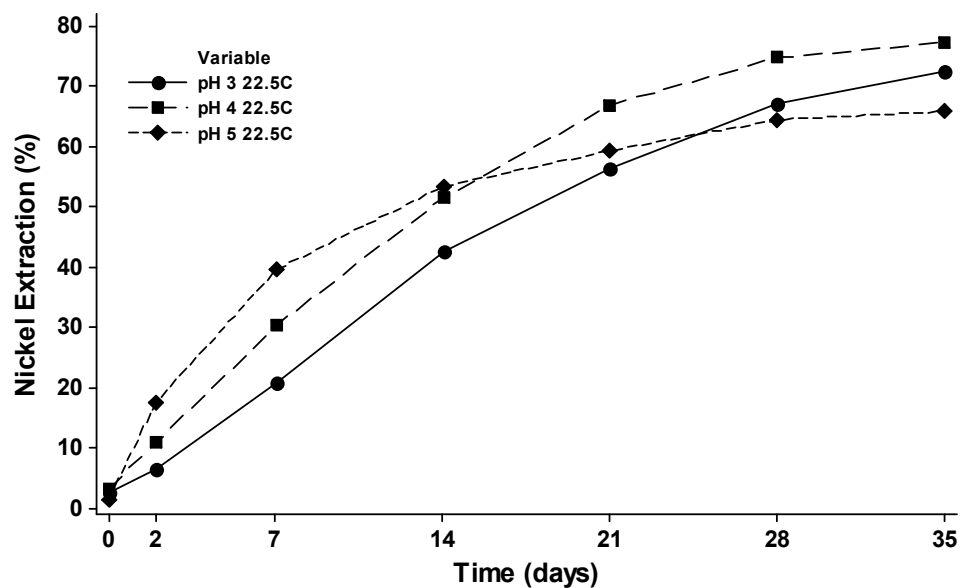


Figure 5.1c. Nickel extraction as a function of time and pH at 22.5 °C with Ore 3.

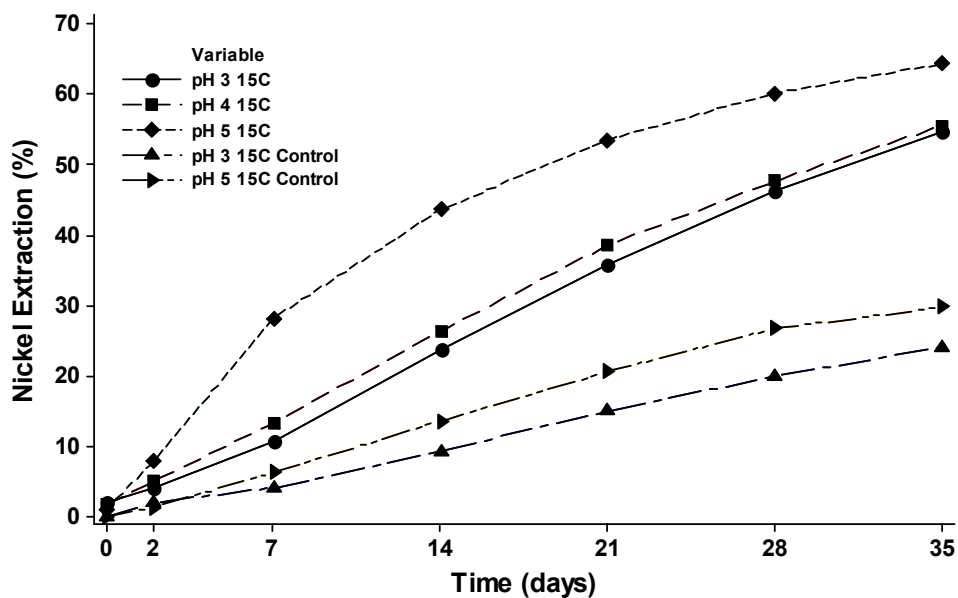


Figure 5.1d. Nickel extraction as a function of time and pH at 15 °C with Ore 3.

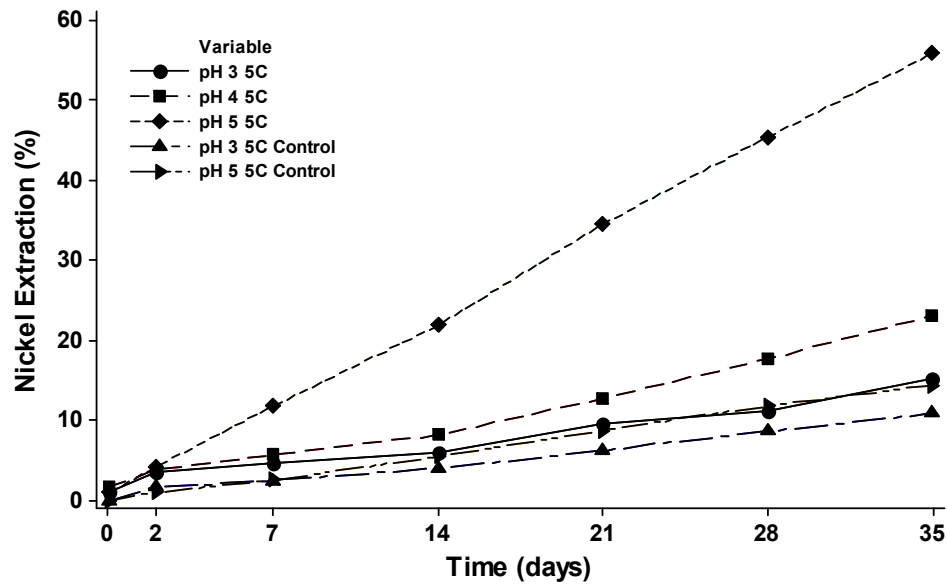


Figure 5.1e. Nickel extraction as a function of time and pH at 5 °C with Ore 3.

The trend of high pH being beneficial to the initial rate of nickel extraction is more evident at lower temperatures. At 5 °C, the nickel extraction during the first week of bioleaching at pH 5 was approximately 100% greater than at pH 3; and the final extraction of nickel after 35 days at pH 5 was more than 250% greater than the final nickel extraction at pH 3 (Figure 5.1e). Among the previous studies that have reported a negative correlation between the leaching of pentlandite and acidity, none have observed such large differences. The results at $T > 5$ °C indicate that although the initial rate of nickel extraction was greater at elevated pH, the ultimate extraction of nickel was correlated to low pH. This effect is not immediately evident at all temperatures higher than 5 °C, but is evident upon extrapolation of the nickel extraction curves beyond the length of the 35 day experiments using a second order polynomial (extrapolation not shown).

Three dimensional plots are useful to visualize the relative impact of pH and temperature on the extraction of nickel at any given time. Figures 5.2a and 5.2b are response surface plots of nickel extraction versus pH and temperature after 2 and 35 days respectively. The extraction of nickel after 2 days can be used as an indication of the initial rate of nickel extraction. It can be seen in Figure 5.2a that at all temperatures, the initial rate of nickel extraction is inversely proportional to acidity.

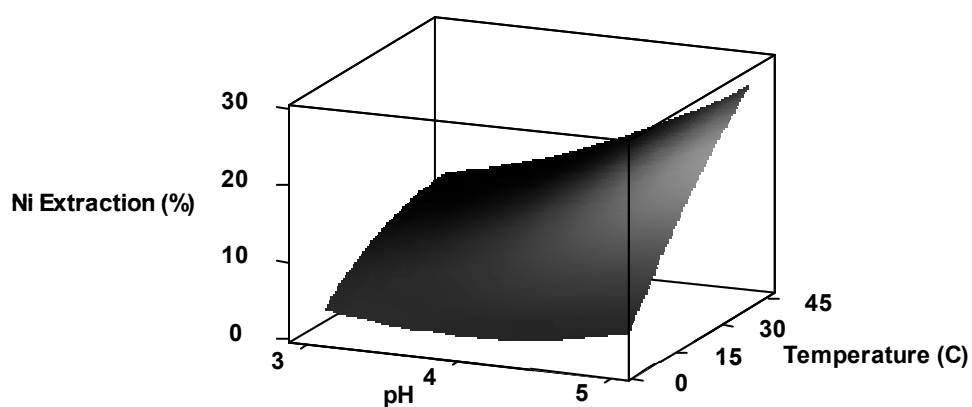


Figure 5.2a. Nickel extraction as a function of pH and temperature after 2 days of bioleaching with Ore 3.

The slope of the response surface along the nickel extraction/ temperature axis may be used as a measure of magnitude of the apparent activation energy for the bioleaching of pentlandite at any given pH. Figure 5.2a indicates that the relative magnitude of the apparent activation energy increases with increasing pH.

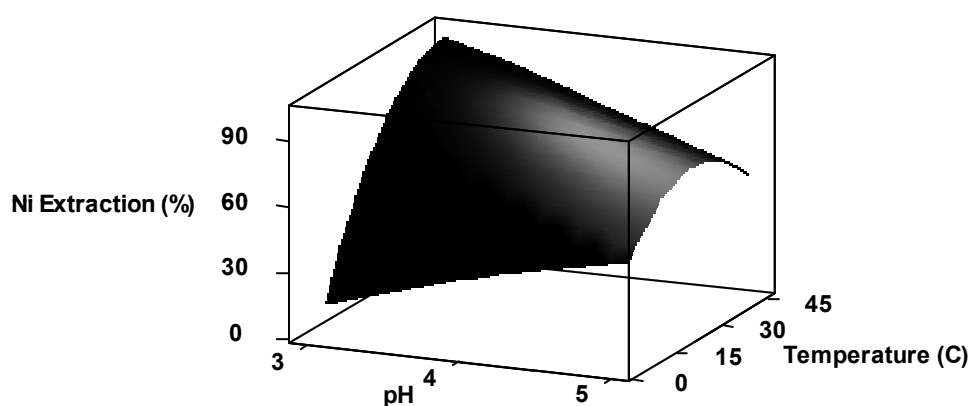


Figure 5.2b. Nickel extraction as a function of pH and temperature after 35 days of bioleaching with Ore 3.

At pH 3, the final nickel extraction is positively correlated to temperature as conventional bioleaching wisdom would predict (Figure 5.2b). The final extraction of nickel at pH 3 was compared and the differences were determined to be statistically significant for all temperature pairs, except 30 and 45 °C, in which nickel extraction approached 100%. Contrary to pH 3, there appears to be little advantage to operating at elevated temperature when operating at pH 5. The final extraction of nickel at pH 5 was compared and the differences were determined to be significant for the temperature pairs 30 and 5 °C, and 30 and 45 °C. Significance testing of the temperature pairs at pH 4 yielded intermediate results. At pH 5, the greatest final nickel extraction occurred at 30 °C, while the lowest at occurred at 45 °C.

A decision was made to continue one experiment at each of the pH levels tested at 5 °C past the 35-day termination date, considering: the unusual nickel extraction results obtained at that temperature; the cool climate in Manitoba where the ore body is located; and the low sulphide content of the ore, which is the primary source of heat generation during

heap bioleaching. Those experiments (STR 61 to 63) were continued with weekly sampling for 12 weeks (Figure 5.3). Even after 12 weeks of bioleaching, the extraction of nickel continued to increase, and the final nickel extraction after 12 weeks was ~ 75, 56, and 57% at pH 5, 4, and 3 respectively.

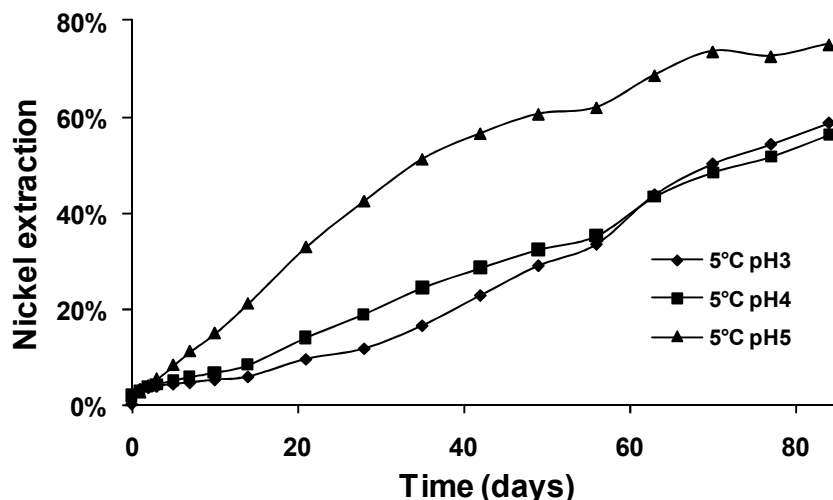


Figure 5.3. Nickel extraction as a function of pH and temperature during the extended bioleaching at 5 °C with Ore 3.

5.3.1.1. Mechanisms of pentlandite dissolution from Ore 3

Few mechanistic studies with pentlandite are available in the technical literature. To some degree, this is a result of the presence of a large fraction of pyrrhotite in pentlandite-containing ores, and the intimate nature of the two minerals, which makes obtaining a pyrrhotite-free concentrate difficult. The ore used in this study contains a relatively small fraction of pyrrhotite, making it possible to study the rate of pentlandite degradation based on the appearance of nickel in the leachate. It is estimated that ~ 97% of the nickel in this ore

reports to pentlandite, therefore the nickel contribution from pyrrhotite leaching may be neglected with minimal error.

Both chemical and biologically-mediated mechanisms contribute to the observed rate during (bio)leaching of sulphide minerals. The two possible chemical mechanisms of sulphide mineral degradation are acid dissolution and oxidative dissolution. With most sulphide minerals, the oxidative mechanism is usually dominant in the presence of a strong oxidant (i.e. ferric ion or molecular oxygen). The primary exception appears to be the leaching of pyrrhotite, as it reacts rapidly with acid, particularly in the absence of oxygen (Belzile *et al.*, 2004; Watling, 2008). Evidence by Schippers and Sand (1999) suggests that sulphide minerals degrade primarily via two indirect oxidative mechanisms based on the mineral's acid solubility. It is generally accepted that bacteria expedite the process by creating a more favourable chemical and/or electrochemical environment. The biologically-mediated mechanisms are generally considered to be either “direct” or “indirect” in nature (discussed in Section 2.4.2). It should be noted that the study conducted by Schippers and Sand (1999) did not investigate the leaching of the two primary nickel-bearing sulphide minerals pentlandite and pyrrhotite, as the authors were not able to obtain pure specimens of those minerals at the time the study was conducted (Wolfgang Sand, personal communications).

A comparison between the bioleaching experiments and their corresponding abiotic control experiments (Figures 5.1a, 5.1b, 5.1d, and 5.1e) indicates that the leaching of pentlandite from Ore 3 is a result of both chemical and biologically-mediated mechanisms. Under any set of conditions, the presence of bacteria make an obvious contribution to the rate of nickel extraction, but the abiotic contribution is always significant. In order to

quantify the bacterial contribution to the bioleaching process under different operating conditions, one may consider a ratio of the nickel extracted during the abiotic control experiments to the nickel extracted during the bioleaching experiments (Abio:Bio ratio). Since nickel extraction during bioleaching experiments is a combination of both chemical and biologically-mediated mechanisms, then the Abio:Bio is a measure of the chemical contribution measured as a fraction from 0 to 1. Abio:Bio near 1 implies a chemically-dominated mechanism, whereas a Abio:Bio near 0 implies a biologically-dominated mechanism. Figures 5.4a and 5.4b display the Abio:Bio ratio as a function of time at the different temperatures tested, at pH 5 and 3 respectively.

Generally, the bacterial contribution is greatest at the beginning of the experiments and gradually decreases with time, regardless of the operating conditions. The reactions conducted at pH 3 tend to be more chemically-dominated compared to the reactions conducted at pH 5, particularly at the beginning of the experiments. The bacterial contribution increases with decreasing temperature at pH 5, with the reaction mechanism being biologically-dominated at 5 °C, and almost entirely chemical at 45 °C. The initial impact of the bacteria at pH 5 is substantial at all temperatures. During both biotic and abiotic experiments at pH 5, the total dissolved iron averaged less than 10 ppm. Considering the consistently low levels of dissolved iron, the bacterial involvement in the process at pH 5 must be more “direct” in nature.

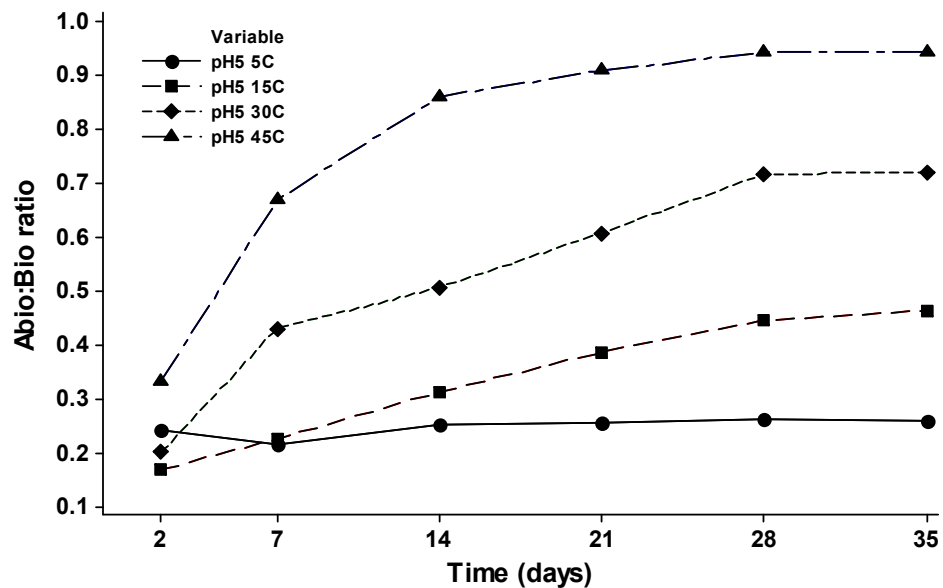


Figure 5.4a. Abio:Bio ratio as a function of time at different temperatures with Ore 3 at pH 5.

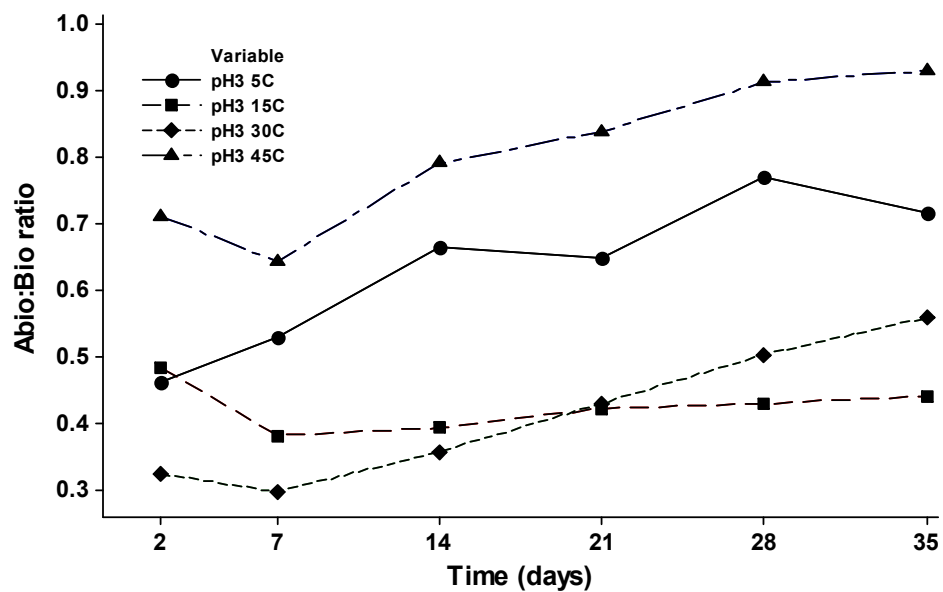


Figure 5.4b. Abio:Bio ratio as a function of time at different temperatures with Ore 3 at pH 3.

The maintenance cultures that were used to inoculate the STR experiments at the different temperatures were all derived from the original mixed culture that was enriched from the environmental samples at 30 °C (Section 5.2.1). Molecular biological analysis of the original enriched culture at 30 °C by Dinardo and Mohapatra (2008) revealed that the dominant iron- and sulphur-oxidizing microorganisms are generally considered to be mesophiles (*Acidithiobacillus* spp. and *Leptospirillum* sp.), while detailed molecular biological analysis in later experiments (Chapter 6) revealed that *Sulfobacillus* spp. were dominant in experiments conducted at 45 °C. Sulfobacilli are generally considered to be moderately-thermophilic mixotrophs, which are capable of either autotrophic or heterotrophic growth for a limited number of transfers; whereas they are capable of faster and extended growth on ferrous ion or reduced-sulphur compounds in the presence of yeast extract (Karavaiko *et al.*, 2006). The same nutrient media was used in all STR experiments discussed in this chapter (McCready with no magnesium and no iron). McCready media does not contain an organic amendment (i.e. yeast extract), which is adequate for growing *Acidithiobacilli* and *Leptospirilli* (both considered to be strict autotrophs); however, the lack of yeast extract may have significantly reduced the biological activity during the experiments at 45 °C. In light of this, it is not surprising that the experiments at 45 °C were generally chemically-dominated.

In terms of the oxidative dissolution mechanisms, ferric ion is generally considered to be the primary oxidant in most bioleaching systems; however, bioleaching does not require the presence of aqueous ferric ion for dissolution of all sulphide minerals. Torma and Sakaguchi (1978) reported that analytically pure samples of NiS, CoS, ZnS, CdS, CuS, and Cu₂S were degraded by *Acidithiobacillus ferrooxidans* in iron-free media. This observation

is consistent with the widely-accepted leaching mechanisms proposed by Schippers and Sand (1999), in which molecular oxygen may substitute for ferric ion in the polysulphide mechanism.

Corrans and Scholtz (1976) studied the dissolution of a pentlandite concentrate in stirred-tank reactors with acidic ferric sulphate. They determined that the leaching of pentlandite involved two simultaneous processes, one dependant on the concentration of ferric ion and one dependant on the concentration of molecular oxygen. The observed rate was proportional to approximately $[O_2]^{0.5}$, $[Fe^{3+}]$, and nearly independent of pH ($\propto [H^+]^{0.12}$) in the range of pH 1.5 to 4. It is reasonable to anticipate that both molecular oxygen and ferric ion contribute to the observed kinetics during bioleaching of Ore 3; however, the contribution of each is currently unknown and is most likely dependant on pH. There is not a single (bio)leaching study in the technical literature that has examined the bioleaching of pentlandite at pH 5. It seems unlikely that the kinetics of this system are controlled by the concentration of ferric ion at pH 5, as the total dissolved iron concentrations are often below 2 ppm (compared to several g/L with other ores at pH 2, Chapter 7).

The response of nickel (bio)leaching from Ore 3 to changes in pH is very unusual and quite contradictory to conventional bioleaching wisdom, which dictates that low pH is required. In order to differentiate between acid dissolution and oxidative dissolution at pH 5, two additional experiments were conducted at 30 °C: one in which the reactor was sparged (~ 500 mL/min) with air (STR 68); and one in which the reactor was sparged (~ 500 mL/min) with nitrogen gas (STR 69). These experiments were conducted abiotically in order to remove any possible effects related to the presence of bacteria. The experimental procedure was identical to the other abiotic control experiments that were conducted with

thymol and methanol as bactericide. The extraction of nickel during both experiments and the level of DO (as percent saturated) in the experiment that was sparged with N₂ gas are shown in Figure 5.5. Continuous sparging of N₂ was effective in maintaining a low level of DO throughout the experiment, with the exception of day 7 when the sparger became fouled and the N₂ line detached. This resulted in the DO reaching ~ 64% during sampling on day 7. It is unknown how long the gas line was detached; however, it was noted that the DO returned to less than 1% within minutes after re-attaching the N₂ supply line.

The experiment sparged with air in Figure 5.5 (STR 68) is not the same experiment conducted under the same condition, which is displayed in Figure 5.1b (STR 35). These two experiments were conducted with different sub-samples of ore, which were taken from different pails that were separated during the original sample preparation. The experiment at 30 °C and pH 5 was repeated in order to eliminate possible effects related to small differences in composition and/or particle size distribution.

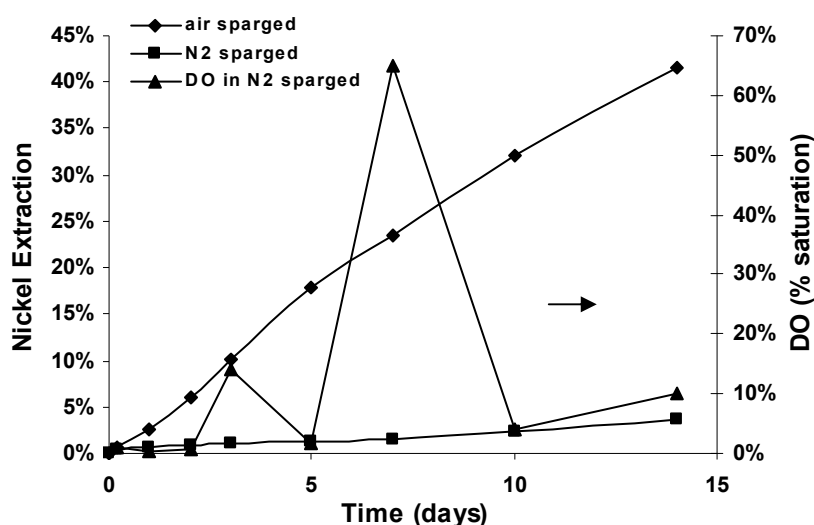


Figure 5.5. Nickel extraction and dissolved oxygen as a function of time during abiotic leaching of Ore 3 at pH 5, sparged with air and N₂ gas.

Oxygen is the final electron acceptor in this system, regardless of whether the primary oxidant is molecular oxygen or ferric ion, as the generation of ferric ion by oxidation of ferrous ion requires molecular oxygen. Consequently, removing molecular oxygen from the system should effectively eliminate oxidative dissolution. By process of elimination, the remaining dissolution of pentlandite would be expected to occur via acid dissolution. In the absence of molecular oxygen, the rate of nickel dissolution was dramatically reduced (Figure 5.5); > 40% and < 5% nickel extraction after 14 days with air and nitrogen respectively. This large difference in nickel extraction strongly implies the oxidative dissolution mechanism is dominant during the leaching of pentlandite at 30 °C and pH 5. In fact, Figure 5.5 overestimates the contribution of the acid dissolution mechanism as the DO was not completely removed from the system and reached at least 64% saturation due to mechanical failure in one instance.

5.3.1.1.1. Apparent activation energy for abiotic leaching of nickel from pentlandite from Ore 3

The nickel extraction curves during abiotic leaching of Ore 3 at 5, 15, 30, and 45 °C were used to calculate an apparent activation energy for the abiotic leaching of nickel from pentlandite at pH 3 and 5. The leaching curves were fitted to a second order polynomial (pH 3 and 5 in Figures 5.6a and 5.6b respectively) and the initial extraction rates were calculated from slope of the polynomials evaluated at $t = 0$. Arrhenius plots (natural logarithm of the initial extraction rates vs. $1000/T$) were used to determine an activation energy of 60 ± 7 and 51 ± 8 kJ/mol (80% CI) for the abiotic leaching of nickel from pentlandite from Ore 3 at pH 3 and 5 respectively (Figure 5.6c).

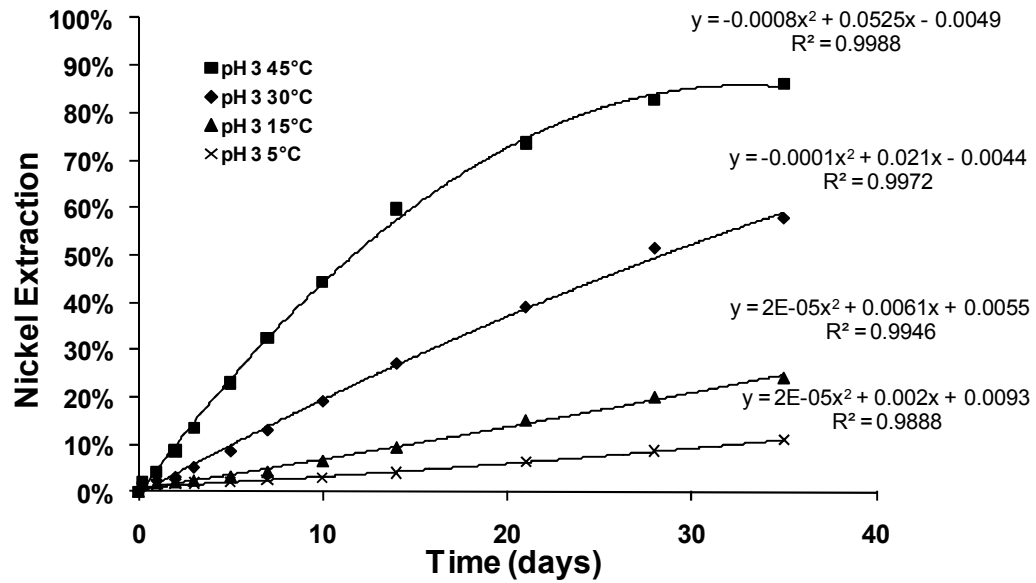


Figure 5.6a. Second order polynomial fit to abiotic leaching of nickel from Ore 3 at pH 3.

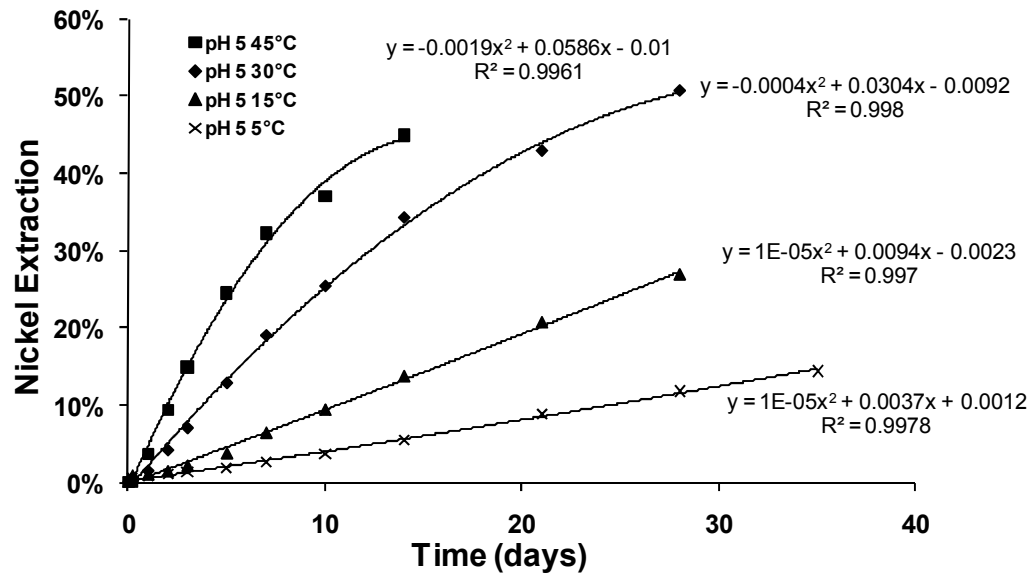


Figure 5.6b. Second order polynomial fit to abiotic leaching of nickel from Ore 3 at pH 5.

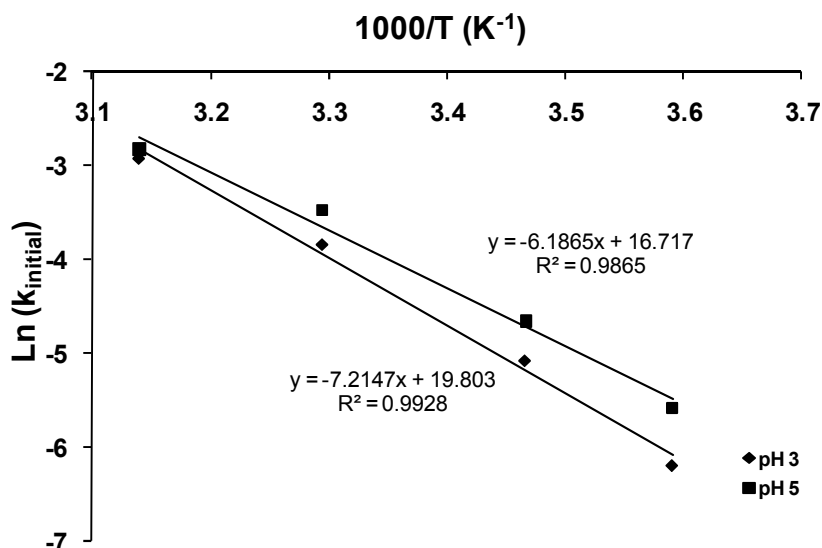


Figure 5.6c. Arrhenius plots for the leaching of pentlandite from Ore 3 at pH 3 and 5.

Two studies have reported an apparent activation energy for the dissolution of nickel from pentlandite. Corrans and Scholtz (1976) reported an apparent activation energy of 61 kJ/mol for the dissolution of nickel from pentlandite with a pentlandite concentrate in stirred-tank leaching experiments with acidic ferric sulphate. Although the conditions of these experiments most closely resemble the conditions experienced during the experiments conducted in this study, it is difficult to directly compare results as they did not report the pH, the partial pressure of oxygen, and the concentration of ferric ion at which the experiments were conducted. Dutrizac and MacDonald (1974) reported an apparent activation energy of 37.6 ± 2.5 kJ/mol for the dissolution of nickel from a low-grade nickel sulphide ore (in which pentlandite was the primary nickeliferous phase) during column leaching with acidic ferric sulphate. These results are not directly comparable to the results of the current study either, as column leaching would be expected to reflect the combined

resistance of at least surface-reaction and stagnant-film control due to the low-mixing environment; consequently, under-estimating the effect of temperature.

All experiments used to calculate the apparent activation energies in this study were conducted under near DO saturated conditions, and the maximum concentration of DO in an aqueous system is a strong function of temperature. Maximum DO levels can be estimated by using an empirical equation developed by Weiss (1970) for determining the maximum DO (ml/L) as a function of temperature and salinity for an aqueous system under atmospheric conditions (Equation 5.2), where T and Sa are the temperature (K) and salinity (g/kg) respectively. The calculated DO may be converted to mg/L by multiplying by 1.4276.

$$\ln(DO) = A_1 + A_2 * \frac{100}{T} + A_3 * \ln\left(\frac{T}{100}\right) + A_4 * \frac{T}{100} + Sa * \left[B_1 + B_2 * \frac{T}{100} + B_3 * \left(\frac{T}{100}\right)^2 \right] \quad (5.2)$$

Where,

$A_1 = -173.4292$	$B_1 = -0.033096$
$A_2 = 249.6339$	$B_2 = 0.014259$
$A_3 = 143.3483$	$B_3 = -0.001700$
$A_4 = -21.8492$	

According to Equation 5.2, the maximum DO in a low-salinity solution (i.e. $Sa = 0$) at 5 and 45 °C is 12.8 and 5.96 ppm respectively; indicating a considerable difference over the temperature range tested in this study. Since maximum DO is a function of temperature, it is not possible to independently evaluate the effects of temperature and DO on this process, while operating under DO-saturated conditions as experienced in these experiments. The abiotic leaching of pentlandite from Ore 3 was shown to be highly dependant on DO (Figure 5.5); consequently, the benefit of operating at higher temperature is at least partially offset by the lower level of DO. As a result, the apparent activation energy calculated from the data obtained in this study (Section 5.3.1.1.1) underestimates the effect of temperature.

The calculated apparent activation energy is lower than would be calculated from a set of experiments conducted at constant DO (on an absolute basis).

5.3.1.2. Mechanisms of nickel loss from the leachate during bioleaching of Ore 3

The initial rate of nickel extraction from Ore 3 at pH 5 and 45 °C was determined to be faster relative to the other four temperatures tested at pH 5; however, the final extraction of nickel at pH 5 and 45 °C was the lowest. This observation was interpreted as being indicative of at least one mechanism that was removing nickel from the leachate. Three possible mechanisms were considered: precipitation of a secondary nickel-containing phase; adsorption of nickel to one of the other minerals; and cation exchange, which would produce an existing phase that is enriched with nickel. Mineralogical examination of the post-leach residues with the MLA did not reveal the presence of any secondary nickel-containing phases.

The chemistry of lizardite in the pristine material (Ore 3) and the post-leach residues at several pH-temperature conditions was obtained by EPMA of 10 to 15 grains in each sample. Analytical details and complete results were reported in Thibault and Smith (2009). Figure 5.7 shows the nickel content of the analyzed lizardite in relation to the level of silicon, expressed in terms of Si^{4+} cations in a chemical formula charge-balanced with 7 O^{2-} anions. For reference, the end-member lizardite ($\text{Mg}_3\text{Si}_2\text{O}_5[\text{OH}]_4$) ideally contains 2 Si^{4+} based on a 7 O^{2-} charge equivalent (i.e. 5 O^{2-} and 4 OH^-). Lizardite in the pristine ore material contains slightly less than 2 silicon and very low levels of nickel. At pH 5, the silicon cation proportion of the lizardite grains is comparable to those in the pristine material, whereas bioleaching at pH 3 resulted in significant non-stoichiometric dissolution of lizardite, in which preferential magnesium extraction (charged balanced by the loss of OH^-) resulted in a

significant increase in the silicon proportion of the residual grains. The degree to which lizardite was altered at pH 3 is a function of temperature, and even experiments at 5 °C resulted in slight silicon enrichment (Figure 5.7).

The ‘unreacted’ lizardite grains at pH 5 are significantly enriched in nickel, and the extent of the enrichment is a strong function of temperature (Figure 5.7). The nickel content of the lizardite bioleached at 45 °C averaged approximately 0.3%, and exceeded 0.5% in one grain, whereas the nickel content of the lizardite bioleached at 5 °C approached that of pristine lizardite in Ore 3. It is possible this nickel-enrichment process at pH 5 resulted in a considerable amount of nickel being captured from the leachate. Interestingly, the altered lizardite grains at pH 3 do not show any significant nickel enrichment for all the temperatures investigated.

Possible mechanisms of nickel capture by the lizardite include precipitation, adsorption, absorption, and $\text{Ni}^{2+}/\text{Mg}^{2+}$ cation exchange. The mechanism is not definitive at this time; however, EPMA was not able to resolve a secondary nickel-containing phase on the lizardite (limit of resolution ~ nm), and both adsorption and absorption are processes that tend to have relatively small apparent activation energies. In contrast, the nickel capture by the lizardite was found to be greatly affected by temperature, suggesting a relatively high apparent activation energy. The results available at this time suggest $\text{Ni}^{2+}/\text{Mg}^{2+}$ cation exchange is a strong possibility.

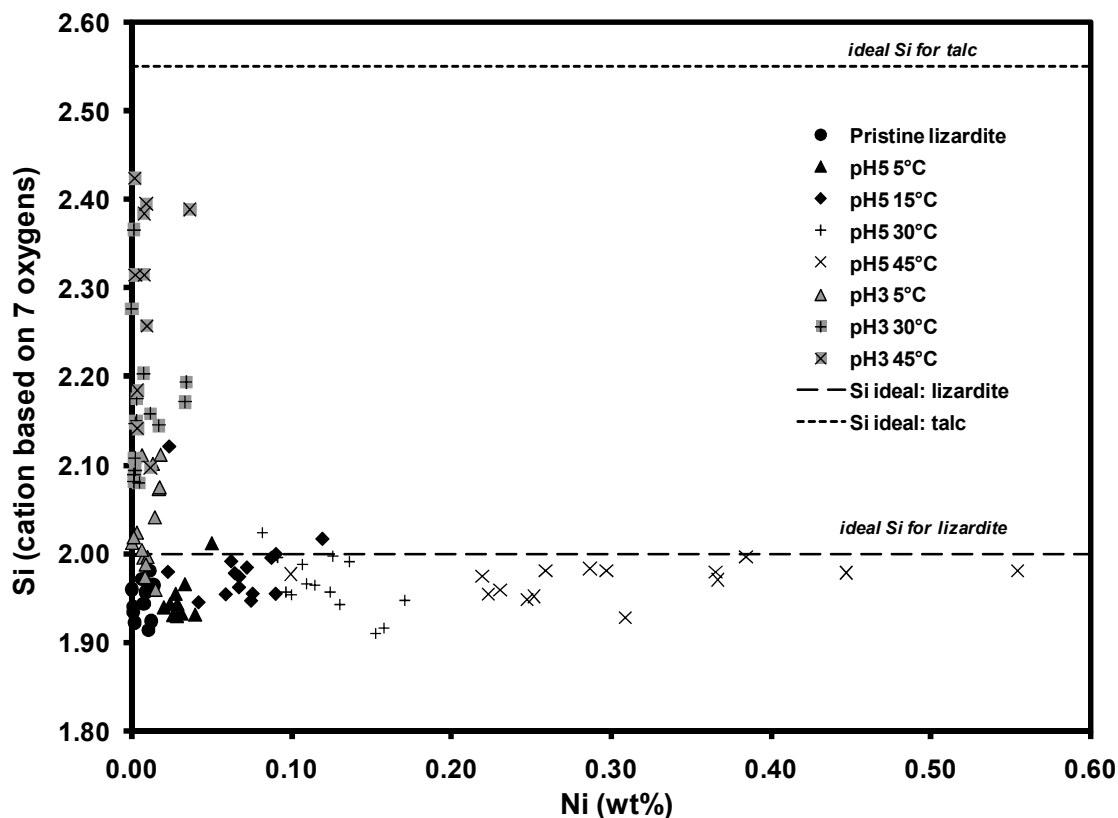


Figure 5.7. Nickel content (wt%) in relation to the proportion of Si^{4+} , expressed in terms of cations charge-balanced by 7 O^{2-} , for lizardite grains in Ore 3 and the post-leach residues.

The appearance of nickel in the leachate is the product of at least two competing reactions: the dissolution of pentlandite (acid and oxidative), which releases nickel into solution; and the capture of nickel by lizardite, which removes nickel from solution. The initial rate of pentlandite dissolution was relatively fast at pH 5 and 45 °C; however, the rate of appearance of nickel in the leachate was masked by the capture of nickel by the lizardite. Ignoring the chemical characteristics of the lizardite obtained by EPMA (Figure 5.7), it would be possible to erroneously conclude that the bioleaching of pentlandite was poor at pH 5 and 45 °C.

This enrichment process may possibly explain why the final extraction of nickel after 35 days of bioleaching was observed to be correlated to low pH at high temperatures. This observation was originally attributed to a change in chemical rate control, from surface reaction control at the beginning of the reaction to product-layer diffusion control towards the end (Section 4.3.2). Mineralogical examination of Ore 3 revealed the presence of a Fe-rich magnesium silicate speckled with micro Ni-Fe-S inclusions 0.5 to 2 μm in diameter (Cameron *et al.*, 2009). It was believed that in the absence of sufficient gangue mineral dissolution, many of these micro-inclusions would be locked and inaccessible to the leaching solution. In light of the EPMA data, it is probable that nickel capture by the lizardite at pH 5 is a significant contributing mechanism to explain why the final nickel extraction was correlated to acidity at high temperatures.

5.3.2. Effect of pH and temperature on the dissolution of the other metal values

Nickel sulphide ores often contain cobalt, copper, and PGEs as by-product credits. Ore 3 contains both copper and cobalt in comparable amounts ($0.016 \pm 0.002\%$ and $0.013 \pm 0.0004\%$ respectively). Cobalt is the most economically significant by-product in Ore 3 since its relative value is substantially higher than that of copper, and cobalt is usually recovered in the same unit operation as nickel in downstream processing, whereas copper recovery generally requires a separate unit operation. Copper was among the elements analyzed in the experiments discussed in this chapter but the results have been omitted since the total copper present in the leachate samples of the experiments conducted at $\text{pH} > 3$ were often less than 1 ppm, regardless of temperature. It is recognized that elevated-pH bioleaching is not a suitable process for ores that contain substantial levels of copper.

Cobalt is present in Ore 3 primarily as a lattice substitution in the pentlandite; the pentlandite in Ore 3 contains $1.13 \pm 0.37\%$ cobalt. Cobalt is often present as pyrite in nickel sulphide ores; however, Ore 3 contains little pyrite ($\sim 0.5\%$) and EPMA determined that the cobalt content of the pyrite in Ore 3 is quite low and often below the detection limit of the technique ($< 0.01\%$). To quantify the dissolution of cobalt relative to the dissolution of nickel, one may consider a ratio of the percent nickel extracted to the percent cobalt extracted (%Ni:%Co ratio). Since both metals are carried by the same mineral in Ore 3, one would expect the extraction of nickel and cobalt to be approximately equal on a percent extracted basis (i.e. constant %Ni:%Co ratio ~ 1). Figure 5.8a and 5.8b shows the %Ni:%Co ratio as function of temperature and pH after 2 and 35 days of bioleaching respectively.

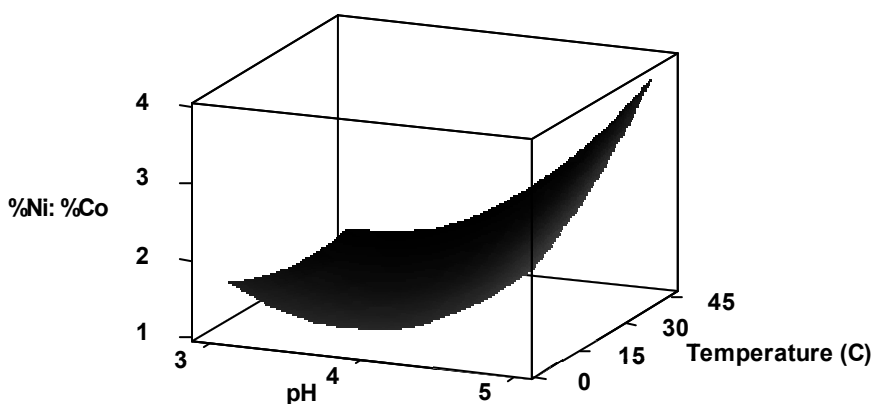


Figure 5.8a. %Ni:%Co ratio after 2 days of bioleaching with Ore 3 as a function of temperature and pH.

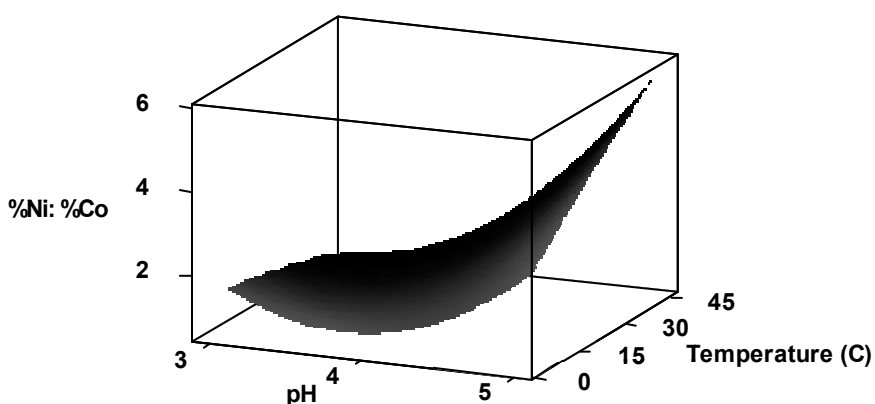


Figure 5.8b. %Ni:%Co ratio after 35 days of bioleaching with Ore 3 as a function of temperature and pH.

In both Figures 5.8a and 5.8b, the %Ni:%Co ratio is ~ 1 at low pH and low temperature; however, there is a substantial deviation from unity at high pH and elevated temperature. Comparison of Figures 5.8a and 5.8b indicate the trend was relatively consistent over the duration of the 35-day experiments. Possible explanations for this observation include: non-stoichiometric dissolution of pentandite; precipitation of a secondary cobalt phase; adsorption of cobalt to one of the other minerals; and cation exchange of cobalt with another cation in one of the other minerals. Currently, there is no conclusive explanation for this observation. A limited amount of resources were allocated to identifying the mechanism responsible for this observation due to resource constraints.

5.3.3. Effect of pH and temperature on magnesium dissolution from the gangue minerals

Magnesium extraction after 2 and 35 days of bioleaching with Ore 3 as a function of temperature and pH are displayed in Figures 5.9a and 5.9b respectively. The figures show that both temperature and pH were determined to affect the rate of magnesium extraction

from the low-grade ore, and that the relative impact of pH and temperature was found to be relatively consistent over the 35-day experiments (i.e. response surfaces have similar profiles). There is a strong negative pH-temperature interaction, resulting in considerable magnesium leaching at pH 3 and 45 °C. On relative basis, the magnesium extraction was determined to be neither a strong function of temperature at pH 5, nor a strong function of pH at 5 °C. It should be noted that the final magnesium extraction at 45 °C and pH 3 leached substantially less magnesium compared to 30 °C and pH 2 (Cameron *et al.*, 2009), 44 and 70% respectively.

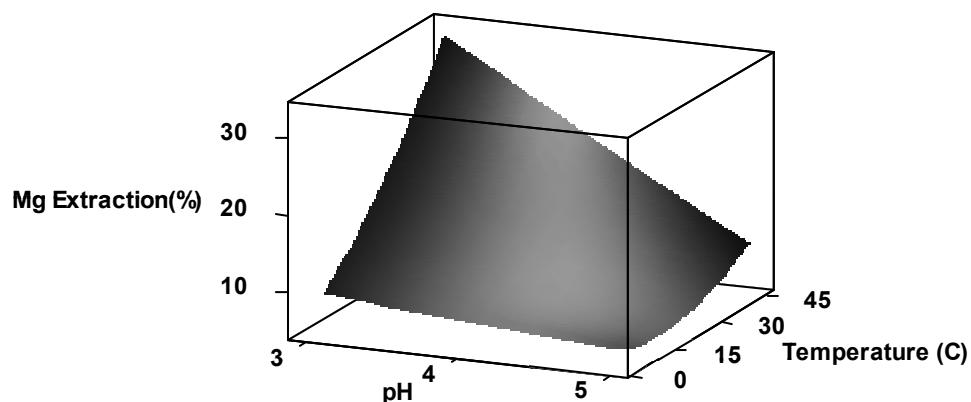


Figure 5.9a. Magnesium extraction after 2 days of bioleaching with Ore 3 as a function of temperature and pH.

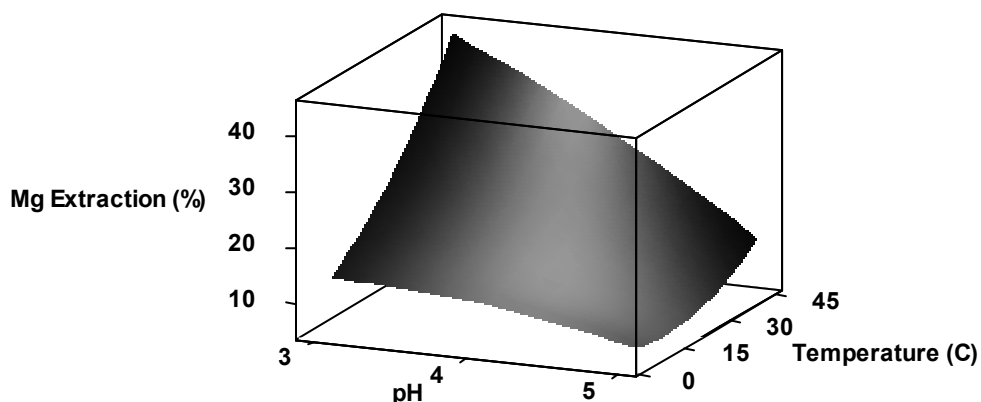


Figure 5.9b. Magnesium extraction after 35 days of bioleaching with Ore 3 as a function of temperature and pH.

5.3.3.1. Mechanisms of magnesium release from the gangue minerals

There are several potential sources of magnesium in the ore; dolomite and ankerite are both magnesium-containing carbonates, and there are numerous magnesium-containing silicate minerals. Mineralogical examination of the post-leach residues at 30 °C in the previous study revealed the carbonate minerals completely dissolved at all the pH levels tested, and lizardite was determined to be the only major silicate phase that showed significant signs of dissolution, the extent of which was highly correlated to pH. Translated to the release of magnesium into the leachate, there will necessarily be a minimum amount of magnesium leached, which can be attributed to the dissolution of the carbonates. Since lizardite constitutes such a large fraction of the ore, the incremental magnesium leached into the leachate can be attributed to the attack of the leaching solution on lizardite, which is related to pH.

The abiotic controls at pH 3 leached more magnesium compared to their respective biotic tests at 30 and 45 °C, whereas the abiotic magnesium extraction curves at all remaining temperatures are effectively superimposed on their corresponding biotic experiments. There is currently no conclusive explanation for this observation; however, one possible explanation is cation exchange between Fe^{2+} and Mg^{2+} in some of the silicate minerals. The reason to expect this phenomenon to occur at pH 3 would be related to the solubility and speciation of iron. In general, the total dissolved iron concentrations during abiotic leaching at pH 5 were < 2 ppm, whereas they were 300 to 500 ppm at pH 3. In the absence of iron-oxidizing bacteria, the dissolved iron at pH 3 would be expected to be primarily Fe^{2+} ; and Fe^{2+} is often observed to substitute for Mg^{2+} in silicate minerals, whereas Fe^{3+} is not.

The most likely phase would be expected to be lizardite, as it was seen to capture nickel at all temperatures tested at pH 5. The cations Mg^{2+} , Ni^{2+} , and Fe^{2+} often substitute for each other in silicate mineral series as all three cations have a similar atomic radius. The lizardite in Ore 3 was determined to be intimately associated with an Fe-rich silicate phase that is chemically consistent with an Fe-rich lizardite (Figure 4.1). Due to the variability in the iron content of the lizardite, it was not possible to utilize the EPMA to ascertain whether the lizardite was capturing Fe^{2+} .

5.3.4. Processing considerations

For the purpose of process optimization, a combined metric of the percent nickel extracted to percent magnesium extracted (%Ni:%Mg ratio) was used for comparison purposes because this metric reflects the two equally important experimental objectives. The %Ni:%Mg ratio was observed to gradually increase as time progressed, regardless of

experimental conditions. The %Ni:%Mg ratio after 2 and 35 days of bioleaching as a function of pH and temperature is shown in Figures 5.10a and 5.10b respectively. Upon completion of the 35-day experiments, the %Ni:%Mg ratio had either plateaued or began to decrease under all experimental conditions, with the exception of the experiments conducted at 5 °C, in which the %Ni:%Mg ratio continued to increase. In fact, the %Ni:%Mg ratio continued to increase after 84 days at all pH levels tested at 5 °C (Figure 5.11).

Figure 5.10a shows that the initial impact of high temperature and high pH (i.e. 45 °C and pH 5) on the %Ni:%Mg ratio is strongly positive; however the advantage of pH 5 at 45 °C gradually decreased as time progressed. At 45 °C, the %Ni:%Mg ratio was observed to be lowest at pH 5 after 35 days of bioleaching (Figure 5.10b). This is further evidence that strongly suggests that the capture of nickel by the lizardite in Ore 3 resulted in a substantial loss of nickel from the leachate at pH 5 and 45 °C.

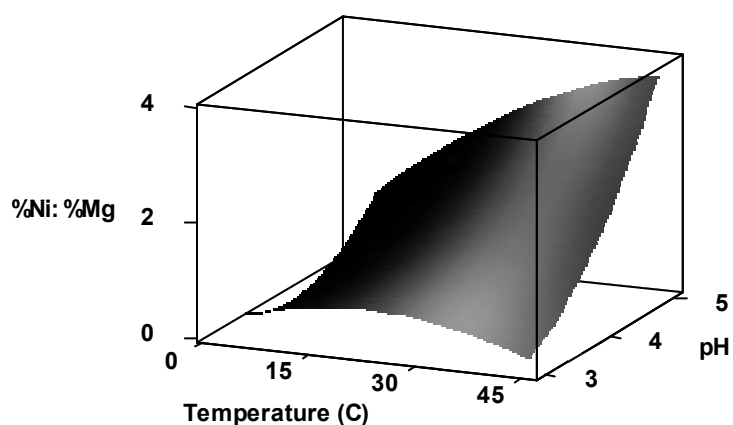


Figure 5.10a. %Ni:%Mg ratio after 2 days of bioleaching with Ore 3 as a function of temperature and pH.

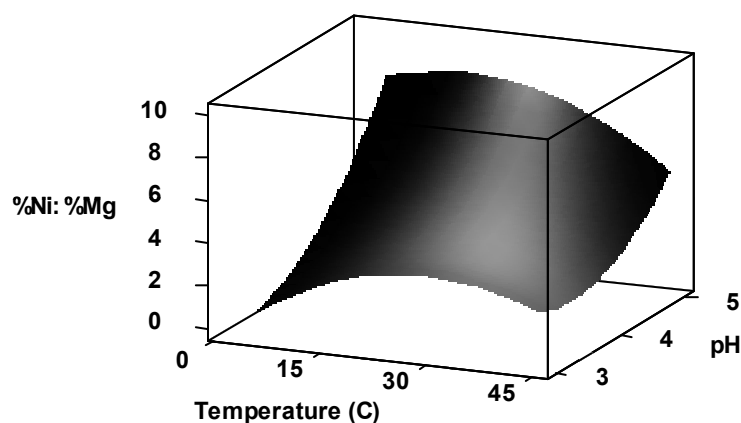


Figure 5.10b. %Ni:%Mg ratio after 35 days of bioleaching with Ore 3 as a function of temperature and pH.

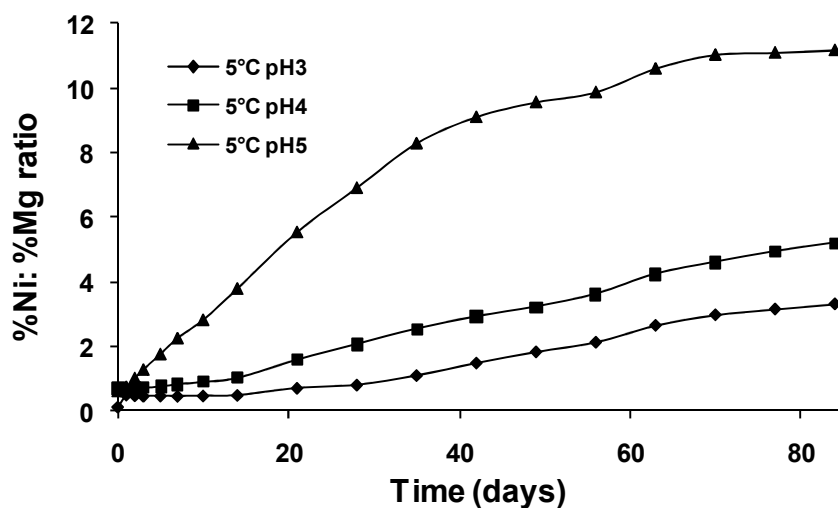


Figure 5.11. %Ni:%Mg ratio as a function of pH and temperature during the extended bioleaching at 5 °C with Ore 3.

The consumption of sulphuric acid after 2 and 35 of bioleaching during bioleaching of Ore 3 as a function of temperature and pH are shown in Figures 5.12a and 5.12b respectively. The figures show that both temperature and pH were determined to affect the

consumption of sulphuric acid, and the relative effect was independent of time (similar response surfaces). Comparison of Figures 5.9(a&b) and 5.12(a&b) indicates that acid consumption tracks the unwanted extraction of magnesium, and that the acid consumption exhibits the same strong negative pH-temperature interaction, which resulted in considerable acid consumption at pH 3 and 45 °C. Conversely, the acid consumption was determined to be neither a strong function of temperature at pH 5, nor a strong function of pH at 5 °C.

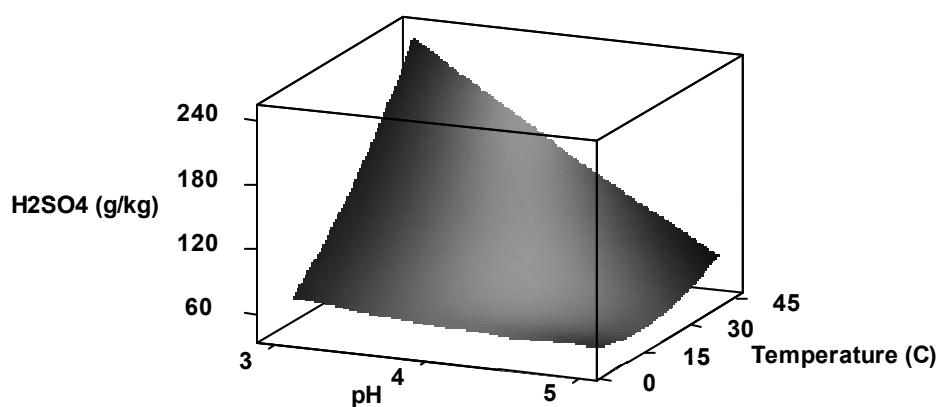


Figure 5.12a. Sulphuric acid consumption after 2 days of bioleaching with Ore 3 as a function of temperature and pH.

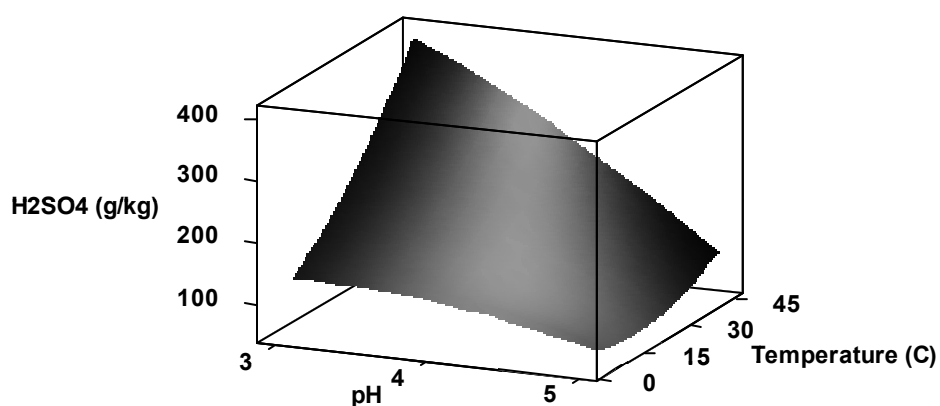


Figure 5.12b. Sulphuric acid consumption after 35 days of bioleaching with Ore 3 as a function of temperature and pH.

The results suggest that bioleaching at elevated pH and low temperature may be an appropriate processing route for this particular ore. This is convenient considering the low sulphide content of the ore and the cool climate in Manitoba where the ore body is located. The sulphide minerals constitute less than 3% (mass/mass) of the ore, which would result in little heat generation in the absence of augmenting the ore with a reduced-sulphur compound or a sulphide-rich waste rock.

Whether the beneficial effect of elevated pH on the (bio)leaching of pentlandite is universally applicable to all pentlandite-containing ores and concentrates is doubtful. It is recognized that an individual ore will behave in a unique manner under a given set of conditions because of differences in the mineral assemblage of the host rock; the habit of the individual sulphide blebs (i.e. massive or disseminated) and the degree of intergrowth between the sulphide minerals (i.e. pyrrhotite and pentlandite), which has a direct impact on the galvanic interactions; the constituents in the microbial consortium; and the reactivity of the individual sulphide minerals. The reactivity of the individual sulphide minerals may be

impacted by a number of factors, including the degree of solid solution and the degree of charge deficiency within the crystal lattice. Prosser (1996) identified in excess of thirty variables and phenomena that can affect the kinetics of a leaching process.

5.4. Conclusions

A low-grade ultramafic nickel sulphide ore was acquired from Manitoba, Canada, which is characterized by high magnesium and low nickel content, in which nickel is the primary metal value, and it is present mainly as pentlandite. It is currently not economically feasible to process this ore with conventional concentration and smelting technologies.

Stirred-tank bioleaching experiments were conducted with finely ground ore ($-147\ \mu\text{m}$) at three different pH levels (3, 4 and 5) and five different temperatures (5, 15, 22.5, 30, and 45 °C). The initial rate of nickel extraction from pentlandite was observed to be inversely correlated to acidity at all temperatures tested. The final extraction of nickel after five weeks was determined to be moderately correlated to acidity at high temperatures and negatively correlated to acidity at low temperatures. The advantage of elevated-pH bioleaching was most evident at 5 °C, at which the final extraction of nickel at pH 5 was determined to be approximately 250% greater than that at pH 3. The unwanted extraction of magnesium exhibited a strong negative pH-temperature interaction, resulting in considerable magnesium leaching at pH 3 and 45 °C. The final extraction of magnesium was neither a strong function of temperature at pH 5, nor a strong function of pH at 5 °C. The consumption of sulphuric acid tracked the extraction of magnesium over all conditions.

Bioleaching at elevated pH substantially increased the ratio of nickel to magnesium in the leachate, and resulted in significantly reduced sulphuric acid consumption; however,

the lowest final extraction of nickel at pH 5 was obtained at the highest temperature (45 °C). Electron probe X-ray microanalysis of the post-leach residues revealed that the remaining un-reacted lizardite was enriched with nickel during experiments conducted at pH 5, and that the extent of enrichment was a strong function of temperature. The results indicate that bioleaching at elevated pH and low temperature may be an appropriate processing route for this particular ore, which is convenient considering the low sulphide content of the ore and the cool climate in Manitoba where the ore body is located.

The appearance of nickel in the leachate is the product of competing mechanisms: the acid dissolution and oxidation dissolution of pentlandite, which both release nickel into solution; and the capture of nickel by lizardite, which removes nickel from solution. At pH 5 and 30 °C, the dominant mechanism responsible for releasing nickel into solution appears to be oxidative in nature. Considering the consistently low concentrations of iron in solution at pH 5, the results strongly imply that the primary oxidant under those conditions is dissolved oxygen.

A future goal of the ongoing nickel sulphide bioleaching program at CANMET-MMSL is to use column studies to assess the technical feasibility of applying heap bioleaching to Ore 3. It was deemed advisable to begin the research program with stirred-tank experiments in order to better understand the impact of temperature and pH on the desirable extraction of nickel and the undesirable extraction of magnesium from the low-grade ore. It is believed that the economics of a potential heap bioleaching process with this ore will largely be a function of acid consumption and magnesium waste management. Although the results of the stirred-tank studies will not directly scale-up to small columns, the micro-scale processes that have been identified in this chapter will also occur during

column and heap bioleaching. Results of these experiments have identified a range of temperature and pH conditions that will result in reasonable nickel extraction rates, without excessive magnesium extraction and sulphuric acid consumption. These results will be used to establish baseline operating conditions for the column work to follow.

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Chapter 6 – Bacterial Community Structure during Bioleaching of Ore 3 in Stirred-tank Reactors at Different Combinations of Temperature and pH

Preface

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Abstract

The focus of this study is to characterize the bacterial community structure present during stirred-tank bioleaching of a low-grade nickel sulphide ore at different temperatures (5 to 45 °C) and pH levels (3 and 5). This is a continuation of previous work, which was designed to assess the technical feasibility of applying elevated-pH bioleaching to this same ore from Manitoba, Canada. A combination of classical microbiological and molecular biological techniques has been used to identify and enumerate the members of the bacterial consortia over the course of the five-week experiments. DGGE analysis revealed the presence of at least 16 distinct 16S rRNA gene sequences, 14 of which are closely related to existing GenBank sequences. Two sequences were detected that are not closely related to existing GenBank sequences and may possibly be from novel species. Thirteen sequences are related to gene sequences of genera that have previously been detected in bioleaching

environments (*Acidithiobacillus*, *Leptospirillum*, *Sulfobacillus*, *Acidiphilium*, *Ferrimicrobium*, and *Acidimicrobium*). Members from the genus *Acidithiobacillus* were dominant at all temperatures except 45 °C, at which *Sulfobacillus* spp. were dominant. Many of the acidithiobacilli are most closely related to strains of *Acidithiobacillus ferrooxidans*, and different strains were dominant under different experimental conditions, indicating considerable phenotypic heterogeneity within the species.

6.1. Introduction

The widespread application of molecular biological techniques has led to significant advances in the understanding of the bacterial species present in acidic mining-related environments and bioleaching operations in the last decade. Several studies have applied molecular techniques to examine the bacteria community structure of acidic mining-related environments such as acid rock drainage (ARD) sites (Bond *et al.*, 2000; Hallberg and Johnson, 2003; Xiao *et al.*, 2009), mine water sites (He *et al.*, 2007; Xiao *et al.*, 2009), mine tailings sites (Mendez *et al.*, 2008), and acidified metal-laden waterways (Gonzalez-Toril *et al.*, 2003). Molecular techniques have also been used to identify and enumerate the prevalent bacterial species in a number of bioleaching environments, although those applications are usually targeted to specific microorganisms of interest (i.e. sulphur- and iron-oxidizers) and reveal less information on the total bacterial community structure. These studies include bench-scale stirred-tank bioleaching experiments (Xingyu *et al.*, 2009, 2010), commercial stirred-tank bioleaching operations (Coram and Rawlings, 2002), column bioleaching experiments (Coram-Uliana *et al.*, 2006; Halinen *et al.*, 2009), and heap bioleaching operations (Coram-Uliana *et al.*, 2006; Demergasso *et al.*, 2005; Qin *et al.*, 2009).

This study is a continuation of previous work, which was designed to assess the technical feasibility of applying elevated-pH bioleaching to a low-grade metamorphosed ultramafic-dominated nickel sulphide ore from Manitoba, Canada. Cameron *et al.* (2009a, 2009b) reported on the stirred-tank bioleaching of this ore at 30 °C (pH 2 to 6), and at 5 to 45 °C (pH 3 to 5) respectively. The ore is characterized by low nickel content and a high fraction of acid-soluble magnesium silicate gangue minerals. Attempts to bioleach this ore at conventional pH levels (~ 2) resulted in an unacceptable amount of solubilized magnesium and excess sulphuric acid consumption. It was concluded that operating at elevated pH (> 3) resulted in a substantial increase in the nickel to magnesium ratio in the leachate, and also resulted in a substantial reduction in the consumption of sulphuric acid.

In the previous work, the effect of the presence of bacteria and the effect of temperature and pH on the extraction of metals, the consumption of sulphuric acid, and the resulting solid state alterations were extensively studied. The focus of the present study is to characterize the microbial consortia involved in the bioleaching of the ore at six different combinations of temperature (5 to 45 °C) and pH (3 to 5). A combination of classical microbiological and molecular biological techniques was used to identify and enumerate the members in the consortia over the course of the five-week experiments.

The application of molecular biological techniques to bioleaching systems is not novel; however, there is currently no other study that comprehensively examines the microbial community structure under such controlled conditions and over such a wide range of temperature and pH. The effect bacteria have in the bioleaching environment has been well-documented; however, the effect of changing physico-chemical parameters on the bacterial community structure is poorly understood. This study furthers the understanding of

the microorganisms present during the bioleaching of sulphide ores and the potential impacts of temperature and pH on the bacterial community structure.

6.2. Materials and Methods

Cameron *et al.* (2009a) reported on the mineralogical and chemical characterization of the ore used in this study. The ore is characterized by a high content of magnesium silicate minerals (~ 64% lizardite) and a low content of sulphide minerals (< 3%). Pentlandite was determined to be the primary nickel-bearing phase. Chemical analysis revealed the ore to contain 0.31% Ni, 21% Mg, 0.016% Cu, 7.2% Fe, and 0.65% total-S.

6.2.1. *Original microbial consortium*

The original microbial culture used in this study was derived from water and soil samples collected from mining-related locations in Sudbury, Ontario in October 2006. A total of 16 samples were taken from locations with a range of acidity (pH 3.2 to 6.5) and oxidation-reduction potential (125 to 438 mV vs. Ag/AgCl). Iron- and sulphur-oxidizing microorganisms were enriched from each environmental sample using media designated mTK and Att respectively (Table 6.1).

Table 6.1. Composition of the growth media used during enrichment, culture maintenance, and bioleaching experiments.

	Modified TK (mTK)	McCready	ATCC Medium 23 (Att)	Thermophilic media (thermo)
Nutrients (g/L)	0.5 (NH ₄) ₂ SO ₄ 0.5 K ₂ HPO ₄ 0.5 MgSO ₄ ·7H ₂ O	0.0661 (NH ₄) ₂ SO ₄ 0.0174 K ₂ HPO ₄ 0.123 MgSO ₄ ·7H ₂ O	0.1 NH ₄ Cl 3.0 KH ₂ PO ₄ 0.2 MgCl ₂ ·6H ₂ O 0.1 CaCl ₂	1.3 (NH ₄) ₂ SO ₄ 0.05 CaCl ₂ ·2H ₂ O 0.28 K ₂ HPO ₄ 0.25 MgSO ₄ ·7H ₂ O 0.1 Yeast extract
Energy Source (g/L)	33.4 FeSO ₄ ·7H ₂ O	33.4 FeSO ₄ ·7H ₂ O	5.0 Na ₂ S ₂ O ₃ ·5H ₂ O or 0.5 Elemental sulphur	33.4 FeSO ₄ ·7H ₂ O, 5.0 Na ₂ S ₂ O ₃ ·5H ₂ O, or 0.5 Elemental sulphur
Initial pH	2.1	2.1	4.2	2.1 (Fe ²⁺) 4.2 (Na ₂ S ₂ O ₃ ·5H ₂ O or S ⁰)
Reference	Modified from Tuovinen and Kelly (1973)	McCready <i>et al.</i> (1986)	Modified from Gherna <i>et al.</i> (1989)	Modified from Salo-Zieman <i>et al.</i> (2006)

All enrichment cultures were combined, and the resulting mixed culture was maintained on a finely-ground low-grade nickel sulphide ore from the Sudbury region by serial sub-culturing in mTK media at pH 2.2. Initial molecular biological characterization revealed that the original mixed culture contained microorganisms closely related to *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, *Leptospirillum ferrooxidans*, and *Acidiphilium* spp. (Dinardo and Mohapatra, 2008). No magnesium supplement or energy source were added during growth on sulphide ores, unless otherwise noted.

A maintenance culture was acclimated to the ore used in this study for several months by serial sub-culturing at 30 °C and pH 3 before being used as inoculum for new maintenance cultures at 5, 15, and 45 °C. The maintenance cultures at the four different temperatures were acclimated to the ore used in this study for more than one year by serial sub-culturing prior to the commencement of the stirred-tank experiments. The introduction of new species by contamination from equipment cannot be ruled out, as sterile practices were not used during the stirred-tank reactor experiments. Furthermore, it is likely that the

ore contained bacteria, as iron- and sulphur-oxidizing bacteria are ubiquitous in nature. The ore also contained some organic material, which most likely included heterotrophic microorganisms. The ore was received as drill cores packed in burlap sacks, and several of the sacks contained nuts and/or seeds that had been deposited by animals during storage.

6.2.2. Bioleaching experiments and bacterial enumerations

Six stirred-tank experiments were conducted for this study at different combinations of temperature and pH: 5 °C (pH 3 and 5), 15 °C (pH 3), 30 °C (pH 3 and 5), and 45 °C (pH 3). The materials and methods were the same as in the previously reported stirred-tank reactor experiments (Cameron *et al.*, 2009a; 2009b), unless otherwise noted.

The inocula for each experiment was developed by combining 5 g of ore, 100 mL of McCready media (adapted from McCready *et al.*, 1986), and 8 mL of 10% H₂SO₄ (vol/vol) in a 250 mL Erlenmeyer flask. After 48 hours in an orbital shaker at 30 °C, the pH was adjusted to 3 and the flask was brought to the temperature of the experiment in which it was to be used. The shake flask was then inoculated with 5 mL of slurry from a well-shaken maintenance culture, which had been grown at the appropriate temperature, and the resulting inoculum was maintained in an orbital shaker for eleven days at pH 3. The inoculum was then combined with 145 g of ore in the reaction vessel and made up to a total volume of 1.5 L with McCready media.

The nature of these experiments required the periodic removal of large samples. Leachate samples (10 mL after 5 minutes settling) were taken for metal analysis on days 0, 2, 7, 14, 21, 28 and 35. Three 125 mL slurry samples were taken while mixing for molecular biological analysis on days 7, 21, and 35 (the samples taken on day 21 were not analyzed). Slurry samples (10 mL each) were taken while stirring on days 2, 7, 14, 21, and 35 for

bacterial enumerations. In order to maintain the original pulp density of 10% (mass/vol), evaporative losses were made up with deionised water prior to sampling and the volume of leachate taken for metal analysis was replaced with an equal volume of fresh media. The reactors were operated with a new volume after the removal of the large slurry samples; 1375 and 1250 mL after days 7 and 21 respectively. The dilution resulting from the removal of the slurry samples that were drawn for enumeration purposes was ignored, as it represents less than a 3% dilution error.

Bacterial determinations were accomplished with a five-tube most-probable-number (MPN) method (Cochran, 1950). The media used for the enumeration tests are listed in Table 6.1. Tubes from the experiments conducted at 30 and 45 °C were incubated in the dark for 28 days at their respective temperatures, whereas the tubes from the experiments conducted at 5 and 15 °C were incubated in the dark for 42 days in a temperature-controlled room at approximately 17 °C. Sterile techniques were used to ensure no false positive tests due to contamination. A positive test for sulphur-oxidizing bacteria was indicated by a drop in pH (0.5 units below the average value of the control tubes). At temperatures other than 45 °C, a positive test for iron-oxidizing bacteria was indicated by the formation of an orange precipitate. The rate of abiotic ferrous ion oxidation (and subsequent precipitation) at 45 °C was fast enough to make it difficult to visually differentiate between positive and negative tubes. For this reason, an increase in oxidation reduction potential (ORP) of + 50 mV relative to the average of the control tubes was used to identify positive tubes at 45 °C. There is no precedence in the technical literature to identify positive tubes in the context of an MPN test based on ORP; however, the average of the control tubes in this study was observed to be

very consistent within an individual rack of tubes (± 1 mV). Throughout the enumeration tests, not a single positive control tube was detected.

The bacterial consortium at each temperature was tested for the ability to oxidize ferrous ion and elemental sulphur. A test consisted of combining 1 mL of slurry from a well-shaken maintenance flask with 100 mL of filtered media (+ 0.5 g precipitated elemental sulphur when appropriate) in a 250 mL Erlenmeyer flask, which was then incubated in a temperature-controlled orbital shaker at 150 RPM. The flasks at 15 to 45 °C were incubated for one month, whereas the flasks at 5 °C were incubated for two months. A positive test for elemental sulphur-oxidizing bacteria was a drop in pH of 0.5 units below the sterile control flask.

An effort was made to ensure that experiments conducted at different pH levels reflect only the changes that can be attributed to differences in pH. For this reason, all six experiments were commenced and terminated on the same day and experiments conducted at the same temperature were inoculated with inocula prepared identically. All inocula were maintained at pH 3 for the eleven-day incubation period prior to the commencement of the stirred-tank experiments, making it as likely as possible that each experiment at a given temperature was inoculated with the same bacterial community structure (both species and cell densities); therefore, there were only four initial conditions (T_0 at 5, 15, 30, and 45 °C) for the six experiments conducted at distinct temperature and pH conditions.

Estimates of the bacterial community structure and of the cell densities on day 0 were obtained by enumeration and molecular biological analysis of inocula prepared according to the same protocol described above. The reported numbers for the cell densities are an arithmetic average of duplicates after applying the appropriate dilution factor of

approximately 100 mL in 1.5 L. It is recognized that it would have been preferable to determine the bacterial densities and community structure on day 0 from the same set of bioleaching tests; however, it was not logistically possible. Furthermore, it was thought that the 1:15 dilution on day 0 would have potentially caused detection problems during molecular biological analysis, particularly at 5 and 45 °C, at which the total community DNA was determined to be quite low.

6.2.3. Molecular biological techniques

6.2.3.1. DNA extraction

Prior to total community DNA extraction, the samples were vortexed for about 1 minute to re-suspend the settled ore material. One hundred mL of the sample was centrifuged at 5600 RPM (4000 x g) for 10 minutes at 4 °C. The pellet was washed with 30 mL of PBS (pH 7.4) and vortexed at maximum speed for about 1 minute. The sample was centrifuged again at 5600 RPM (4000 x g) for 10 minutes at 4 °C. The DNA extraction method made use of the MoBio PowerMax Soil DNA Extraction Kit and was performed following the manufacturer's instructions. The DNA on the white filter membrane was eluted with 3 mL of Solution C6 from the MoBio Soil Kit and incubated at room temperature for 10 minutes before the final centrifugation. The total community DNA was quantified using a NanoDrop instrument. For the samples with low DNA concentration, the total community DNA was further concentrated by ethanol precipitation. The DNA pellet was re-dissolved in 50 µL of TE (pH 8.0) before PCR amplification.

6.2.3.2. PCR amplification of the 16S rRNA gene

For PCR amplification of the 16S rRNA gene, the bacteria-specific forward primer F1 (5'-GAGTTTGATCCTGGCTCAG-3') and reverse primer 519R (5'-GTATTACCGCGGCTGCTGG-3') were used. These primers, complementary to conserved regions of the 16S rRNA gene, were used to amplify a 491-bp fragment corresponding to positions 27 to 519 of the *Escherichia coli* sequence and covered the variable regions V1 to V3. The bacteria-forward primer used for DGGE possessed a GC-clamp (5'-GGCGGGGCGGGGGCACGGGGGGCGCGGGCGGGGCGGGGG-3') at the 5' end. This GC-clamp stabilizes the melting behaviour of the amplified fragments (Sheffield *et al.*, 1989). Each 50 µL PCR mixture contained 1 µL of the template DNA (~ 1 ng/µL), 25 pmol of each oligonucleotide primer, 200 µM of each dNTP, 1 mM MgCl₂ and 2.5 units of Taq polymerase (Amersham Biosciences, Piscataway, NJ, USA) in 10X Taq polymerase buffer (100 mM Tris-HCl pH 9.0, 500 mM KCl, 15 mM MgCl₂). Briefly, after an initial temperature of 96 °C for 5 minutes and thermocycling at 94 °C for 1 minute, the annealing temperature was set to 65 °C for 1 minute and decreased by 1 °C every cycle for 10 cycles, and 3 minutes elongation time at 72 °C. Additional cycles (15 to 20) were performed with annealing temperatures of 55 °C. PCR products were loaded onto a 1% agarose gel with SYBR Safe (Molecular Probes, Eugene, OR, USA), using a 100-bp ladder (MBI Fermentas, Amherst, NY, USA) to determine the presence, size and quantity of the PCR products.

6.2.3.3. Denaturing gradient gel electrophoresis

The 16S rRNA gene PCR products from 3 to 8 PCR reactions were combined for each sample and concentrated by ethanol precipitation for DGGE analysis. About 500 ng of the 16S rRNA PCR product from each sample was applied to a lane, and was analyzed on an

8% polyacrylamide gel containing a gradient of 30-70% denaturant (7M urea and 40% deionized formamide were considered to be 100% denaturant). DGGE was performed with a DCode Universal Mutation Detection System (Bio-Rad). Electrophoresis was run at a constant voltage of 80 V for 16 hours at 60 °C in 1X TAE running buffer. The gels were then stained with VistaGreen (Amersham Biosciences, Piscataway, NJ, USA). The gels were imaged with the FluoroImager System Model 595 (Molecular Dynamics, Sunnyvale, CA, USA). The gel images were analyzed using GelCompar II v4.6 (Applied Maths, Sint-Martens-Latem, Belgium) to generate dendrogram profiles. The genotypes were visually detected based on the presence or absence of bands in each lane. A band was defined as “present” if its peak intensity was at least 2% of the most-intense band in the sample. After conversion and normalization of gels, the degree of similarity of DNA pattern profiles were computed using the Dice similarity coefficient (Dice, 1945), and dendrogram patterns were clustered by the Unweighted Pair Group Method using Arithmetic average (UPGMA) groupings with a similarity coefficient (S_{AB}) matrix.

6.2.3.4. Sequencing and phylogenetic analyses

Individual bands of the DGGE gels were excised and eluted with 25 µL of dH₂O for 2 to 3 days at 4 °C before being re-amplified with the same set of primers without the GC-clamp. One microliter of DNA was re-amplified with the appropriate primers without the GC-clamp as follows: an initial denaturation of 5 minutes at 96 °C; followed by 30 cycles of 94 °C for 1 minute; 65 °C for 30 seconds; and 72 °C for 1 minute. PCR products for sequencing were purified using the Illustra GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, Baie d’Urfe, QC). Sequencing was performed at the *Université Laval Plateforme d’analyses biomoléculaires* using a model ABI Prism 3130XL (Applied Biosystems,

Foster City, CA, USA) with their respective primers. Raw sequence data were assembled in BioEdit v7.0 (Hall, 1999). The sequences were manually aligned by comparing forward and reverse sequences. The occurrence of chimeric sequences was determined manually with the CHECK_CHIMERA function from the Ribosomal Database Project-II (<http://35.8.164.52/cgis/chimera.cgi?su=SSU>) (Cole *et al.*, 2003), and Bellerophon (<http://foo.maths.uq.edu.au/~huber/bellerophon.pl>) (Huber *et al.*, 2004). Close relatives of the final selection of different sequences (phylotypes) were tentatively identified by NCBI BLASTN search (<http://ncbi.nlm.nih.gov/blast/>). Sequences were aligned by the MacVector 9.0 software package (Accelrys) with both closely-related representatives from NCBI BLASTN and as well as novel complete and partial sequences obtained from GenBank. Additional manual alignment was done when necessary. Phylogenetic relationships were constructed with evolutionary distances (Jukes-Cantor distances) and the neighbor-joining method using the MacVector software package. The bootstrap analyses for the phylogenetic trees were calculated by running 1000 replicates for the neighbor-joining data.

The 16S rRNA gene sequences obtained in this study (BL-1 to BL-16) have been deposited in the GenBank database under accession numbers HM124428 to HM124443 respectively.

6.3. Results and Discussion

6.3.1. Effect of pH on nickel extraction from pentlandite

Nickel extraction from the low-grade ore as a function of time at the different combinations of temperature and pH is shown in Figure 6.1. The extraction of nickel at 5, 15, and 30 °C is similar to previously reported results (Cameron *et al.*, 2009a; 2009b); however,

the initial rate of nickel extraction at 45 °C in the current study appears to be slightly lower compared to the previously reported study, perhaps suggesting lower biological activity. The extraction of the other metals, acid consumption, and ORP measurements are comparable (data not shown).

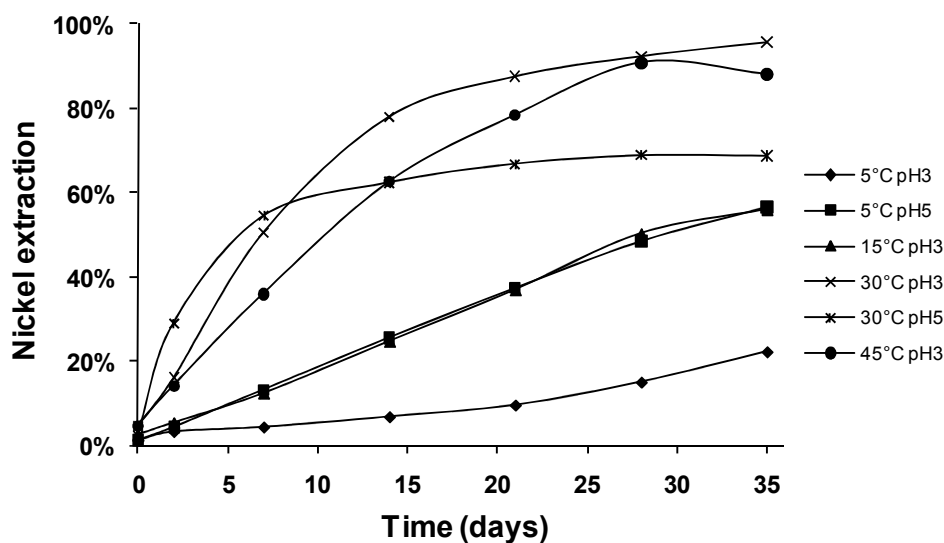


Figure 6.1. Nickel extraction from Ore 3 as a function of time at different combinations of temperature and pH.

6.3.2. Substrate utilization tests

Each bacterial consortium was tested for the ability to oxidize ferrous ion and elemental sulphur using media listed in Table 6.2. If the culture was able to oxidize a substrate in the absence of yeast extract, then the culture was not tested with yeast extract. Cultures at 5, 15, and 30 °C exhibited the ability to oxidize both substrates autotrophically, whereas the culture at 45 °C required yeast extract to oxidize both substrates.

Table 6.2. Substrate utilization tests with the maintenance cultures grown on Ore 3.

Test #	Substrate (media)	5 °C	15 °C	30 °C	45 °C
1	Ferrous ion (mTK)	Growth	Growth	Growth	No growth
2	Ferrous ion (thermo + FeSO ₄ ·7H ₂ O)	N/A	N/A	N/A	Growth
3	Elemental sulphur (Att + S ⁰)	Growth	Growth	Growth	No growth
4	Elemental sulphur (thermo + S ⁰)	N/A	N/A	N/A	Growth

6.3.3. Monitoring of the microbial consortium

6.3.3.1. DGGE analysis

Murray *et al.* (1996) determined there is a relationship between DGGE band intensity and the relative abundance of the corresponding phylotypes during DGGE analysis when using a mixture of DNA templates. More recent work (Fromin *et al.*, 2002) has suggested that the relative band intensity from DGGE could be use to estimate the relative density of the corresponding sequence type within the sample, strongly implying that band intensity has a diagnostic value in estimating the relative abundance of an organism in a sample.

The DGGE patterns for the four inocula (T₀ at 5, 15, 30, and 45 °C) and the DGGE patterns for the six experiments at days 0, 7, and 35 (T₀, T₇, and T₃₅ respectively) are given in Figures 6.2 and 6.3 respectively. Figure 6.3 reveals generally low similarity values (S_{AB}) between the T₀ samples and both the T₇ and T₃₅ samples at the same temperature, indicating large changes in the bacterial community structure. There are additional potential reasons for these apparent differences besides pH and the progression of time, including: the pulp density in the shake flask inocula was 5% (mass/vol), whereas the pulp density in the stirred-tank reactor experiments was 10% (mass/vol); and the Reynolds number in the stirred-tank

reactor experiments was substantially higher compared to the Reynolds number in the shake flask during incubation of the inocula. For this reason, it is more appropriate to compare the T_0 samples separately from the T_7 and T_{35} samples, and to rely on the comparison between the T_7 and T_{35} samples to evaluate shifts in the bacterial community structure on a time and pH basis.

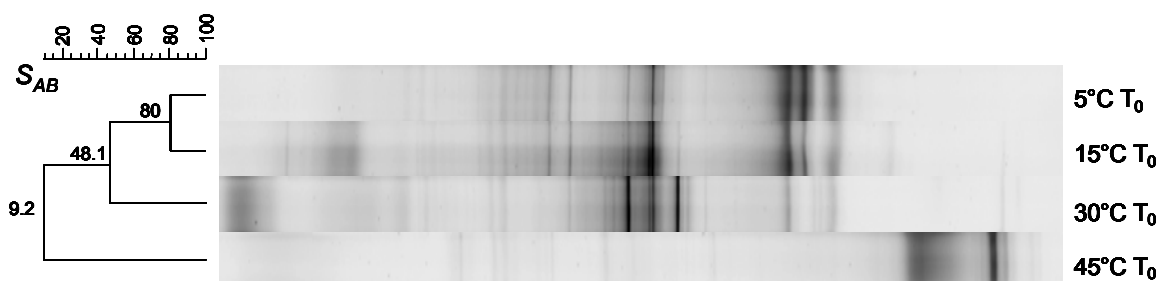


Figure 6.2. DGGE analysis of T_0 bioleaching samples using the 16S rRNA gene primer set F1 – 519R.

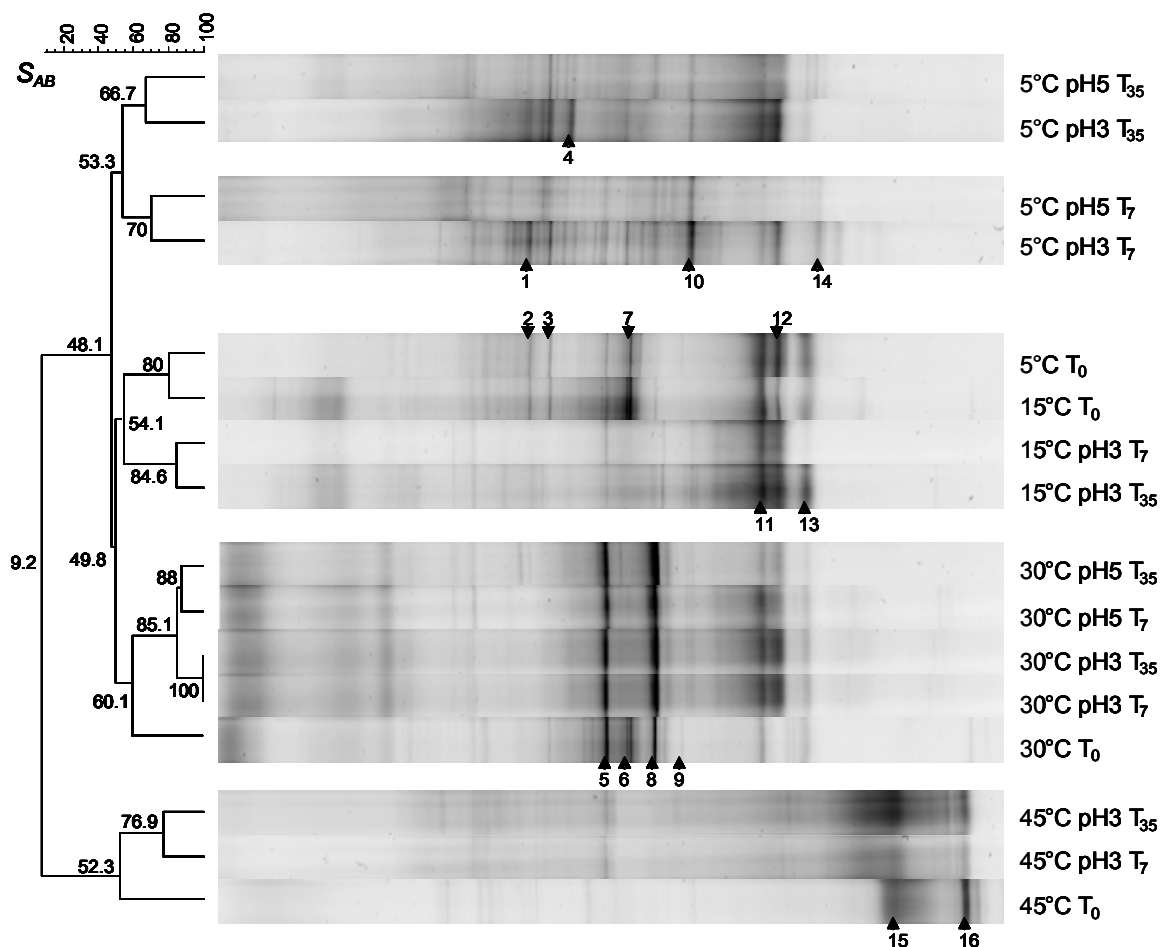


Figure 6.3. DGGE analysis of all the bioleaching samples using the 16S rRNA gene primer set F1 – 519R. Bands excised for sequencing are identified with numbers (1-16).

In general, temperature affected the bacterial community structure more than did pH. The T_0 samples at 5, 15, and 30 °C exhibited a great degree of similarity, whereas T_0 at 45 °C was substantially different. This suggests that the dominant bacteria at 5, 15, and 30 °C were mostly the same species, whereas the dominant species at 45 °C were unique. Upon comparison of the T_0 samples in the temperature range of 5 to 30 °C, it can be concluded that the sequences BL-5 and -8 decrease in relative abundance with decreasing temperature, whereas sequences BL-2, -3, -11, and -13 increase in relative abundance with

decreasing temperature (bands labelled in Figure 6.3), further suggesting a strong response to temperature. Surprisingly, the samples at 5 °C are more closely clustered by time rather than pH, suggesting that at 5 °C, time has more impact on the microbial community structure than does pH in this pH range. All 30 °C samples were clustered closely together, which suggests that the bacterial community structure at that temperature was relatively stable throughout the 35-day experiments. Sequences BL-10 and BL-4 were enriched only in the 5 °C samples.

6.3.3.2. Sequencing and phylogenetic analysis

The phylogenetic relationship of the 16 bacterial 16S rRNA gene sequences obtained from the bioleaching samples is given in Figure 6.4. All sequences, except BL-12 and BL-16 are closely related (> 97% identity) to sequences of previously-sequenced bacteria. All sequences, except BL-1, -10, and -14 are related to sequences of microorganisms that have previously been detected in bioleaching environments, belonging to the genera *Acidithiobacillus* (Halinen *et al.*, 2009; Xingyu *et al.*, 2009, 2010), *Leptospirillum* (Coram and Rawlings, 2002; Halinen *et al.*, 2009; Xingyu *et al.*, 2009, 2010), *Sulfobacillus* (Halinen *et al.*, 2009; Xingyu *et al.*, 2009, 2010), *Acidiphilium* (Coram-Uliana *et al.*, 2006), *Ferrimicrobium* (Halinen *et al.*, 2009), and *Acidimicrobium* (Cleaver *et al.*, 2007).

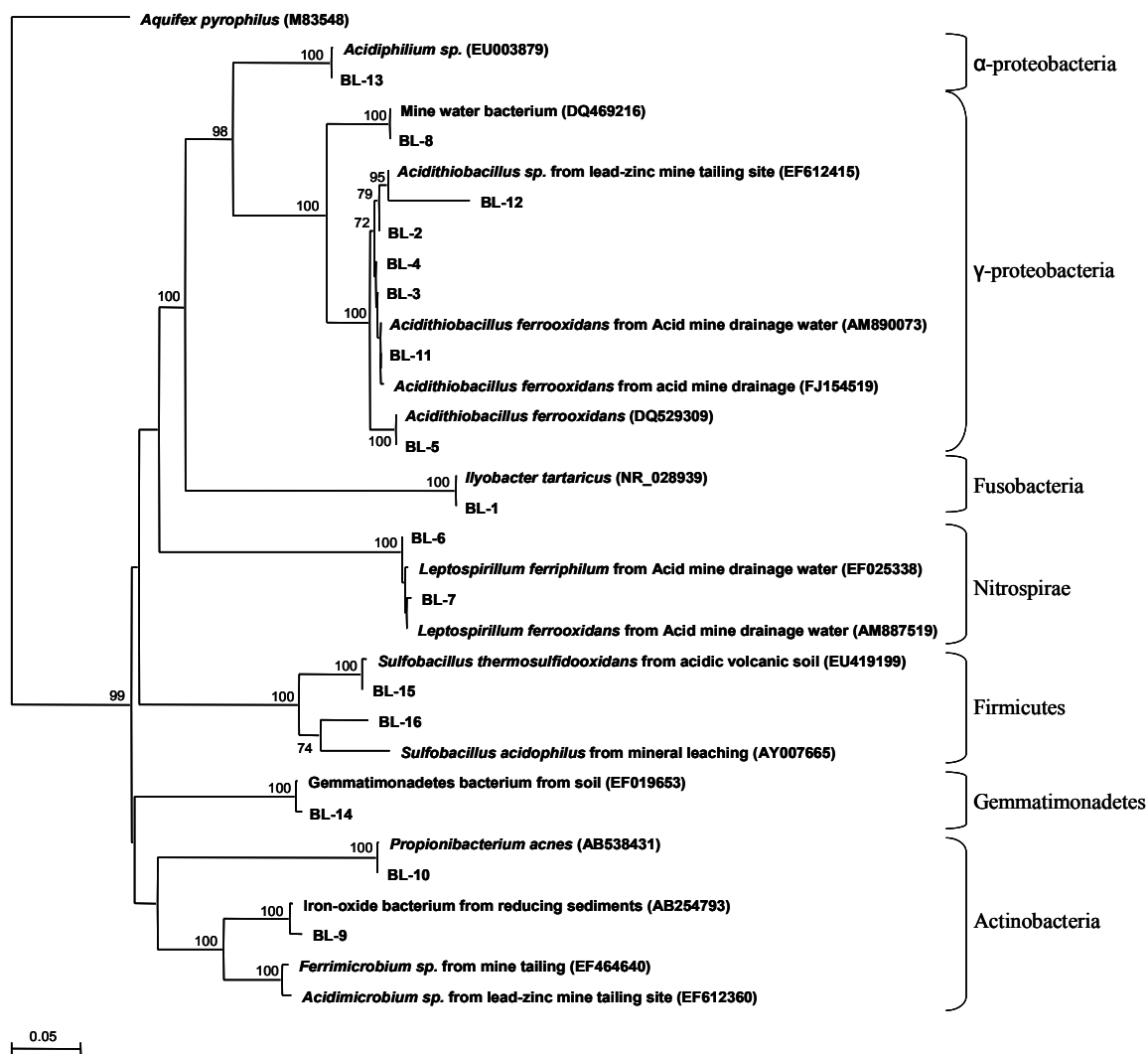


Figure 6.4. Phylogenetic relationship of the 16 bacterial 16S rRNA gene sequences obtained from the bioleaching samples. The bands were labelled bioleaching (BL) with their band numbers, as shown in Figure 6.3. The tree was inferred by neighbor-joining analysis of sequence from each band. *Aquifex pyrophilus* was used as the outgroup. Numbers on the nodes are the bootstrap values based on 1,000 replicates. The scale bar indicates the estimated number of base changes per nucleotide sequence position.

Two sequences were detected that are not closely related to existing GenBank sequences, and may possibly be from novel species: sequence BL-12, which is most closely related (93% identity) to the gene from an uncultured *Acidithiobacillus* sp. (EF612415) from a lead-zinc mine tailings site in Arizona (Mendez *et al.*, 2008); and sequence BL-16 that is

most closely related (90% identity) to the gene from *Sulfobacillus acidophilus* strain YTF-1 (AY007665), which is a microorganism that was isolated from an acidic geothermal site in Yellowstone National Park (Johnson *et al.*, 2001).

Most of the sequences that were detected are closely related to iron- and sulphur-oxidizing microorganisms that are commonly found in bioleaching environments. Members from the genus *Acidithiobacillus* were dominant at all temperatures except 45 °C, at which *Sulfobacillus* spp. were dominant. Sequences BL-6 and -7 both belong to the genus *Leptospirillum*, which virtually disappeared from all samples after T₀. There is no conclusive explanation for this observation, but it is possible these microorganisms were sensitive to either shear or metal concentration; however, this is inconsistent with observations that *Leptospirillum* spp. often dominate in commercial stirred-tank bioleaching operations (Rawlings *et al.*, 1999), which are typically characterized by high pulp densities and high shear.

The genus *Acidithiobacillus* are a group generally considered to be mesophilic, obligately acidophilic, autotrophic aerobes that oxidize numerous reduced-sulphur compounds, with some species having the ability to oxidize ferrous ion and/or hydrogen (Kelly and Wood, 2000). Their presence in all samples is expected, as all the inocula were grown at pH 3; however, one might expect to see the presence of some species that are widely considered to be moderate acidophiles in the experiments at pH 5, which are conditions that more closely resemble moderately-acidic ARD rather than a typical bioleaching operation. The dominance of *Acidithiobacillus* spp. at pH 5 may seem somewhat inconsistent with the perceived preferred habitat of *Acidithiobacillus* spp.; however recent work by Ni *et al.* (2008) suggests that the preferred habitat of certain *Acidithiobacillus* spp.

might be more diverse than originally thought. Ni *et al.* (2008) compared the growth characteristics of five unidentified sulphur-oxidizing *Acidithiobacillus* spp. isolated from different mining-related environments in China with the growth characteristics of two *Acidithiobacillus* type strains (ATCC 19377 and DSM 8584). They observed a wide range of optimum growth conditions (T_{opt} of 28 to 45 °C; and pH_{opt} of 2 to 5). Coincidentally, the pH_{opt} of the two type strains were towards the lower end of the range. There is clear evidence of this phenotypic heterogeneity in the current study. Sequences BL-2, -3, -4, -5, and -11 are all most closely related to strains of *Acidithiobacillus ferrooxidans*; however, they each show a different response to temperature.

The dominant iron- and sulphur-oxidizing species at 45 °C belong to the genus *Sulfobacillus*, which is comprised of moderately thermophilic spore-forming Gram-positive rods that are strictly aerobic mixotrophs capable of growth on ferrous ion and some reduced-sulphur compounds (Karavaiko *et al.*, 2006). Sulfobacilli are capable of either autotrophic or heterotrophic growth for a limited number of transfers; whereas they are capable of faster and extended mixotrophic growth in the presence of yeast extract (Karavaiko *et al.*, 2006). *Sulfobacillus acidophilus* strain YTF-1 (AY007665) is the fastest growing known iron-oxidizing microorganism (Johnson *et al.*, 2001), and is the species most closely related to sequence BL-16. Sequence BL-11 is closely related (99% identity) to the gene sequence from *Sulfobacillus thermosulfidooxidans* (EU491199). Sulfobacilli exhibit a number of characteristics that make them of interest for use in bioleaching; Watling *et al.* (2008) compared some characteristics of the four known *Sulfobacillus* spp., reviewed their occurrence in acidic and mining-related environments, and concluded that their versatility and resilience makes them valuable contributors in the bioleaching environment.

Heterotrophic microorganisms are often detected in bioleaching and acidic mining-related environments, and were detected in all samples other than at 45 °C in this study. Sequence BL-13 from the genus *Acidiphilium* was the dominant heterotroph in many of the sample at $T \leq 30$ °C. The genus *Acidiphilium* is composed of acidophilic, mostly obligately heterotrophic species (Johnson and Bridge, 2002) and *Acidiphilium* spp. are widely distributed throughout acidic mining-related environments. Sequence BL-10 (related to *Propionibacterium acnes*) was the dominant heterotroph in most of the samples at 5 °C. There was no detectable band for any obligately heterotrophic species at 45 °C; however, *Sulfobacillus* spp. are widely considered to be mixotrophic microorganisms (Karavaiko *et al.*, 2006).

Sequences BL-1, -10, and -14 are not typically found in bioleaching environments. Sequence BL-10 shows 99% homology to the gene from *Propionibacterium acnes*, which is an organism that is generally considered to be an anaerobic heterotroph and is known for causing the skin condition known as acne; however, it has been found in ARD (Hallberg and Johnson, 2003). Sequence BL-1 shows 99% homology to the gene from *Ilyobacter tartaricus*, which is a strict anaerobe capable of fermenting tartrate and has been found in numerous anoxic environments (Schink, 1984). Sequences BL-14 shows 99% homology to the gene from a bacterium of the phylum Gemmatimonadetes, which is a recently-discovered phylum, in which the first cultured species was *Gemmatimonas aurantiaca*; a rod-shaped Gram-negative aerobe capable of heterotrophic growth on a limited range of substrates in a minimal nutrient environment at mesophilic temperatures and near-neutral pH (Zhang *et al.*, 2003).

It is unclear the role (if any) played by these microorganisms (BL-1, -10, and -14) in the bioleaching process, nor how anaerobes were able to survive in a highly-aerated environment. It is possible these species were introduced by contamination during bioleaching (i.e. electrodes or glassware) or were present on the original ore sample. As previously mentioned, the ore was found to contain some organic material deposited by animals; however, it seems unlikely that the microorganisms were present on the original ore in sufficient numbers to be detected in DGGE analysis, which has a detection limit of approximately 1%.

6.3.3.3. Bacterial density measurements

Bacterial density curves were constructed by enumerating both iron- and sulphur-oxidizing bacteria using the MPN method six times over the course of the five-week experiments. Slurry samples were taken from the reactors while stirring, so the bacterial density determinations represent a combination of both planktonic and sessile bacteria. The first tube in each row was shaken vigorously in an attempt to dislodge sessile bacteria from the ore particles; however, no surfactant was used for fear it would negatively impact reproduction (Torma *et al.*, 1976). The bacterial growth curves for the experiments conducted at 45, 30, 15, and 5 °C are given in Figures 6.5a, 6.5b, 6.5c, and 6.5d respectively.

The MPN method estimates the population density of a particular group of microorganisms based on their ability to use a specific substrate. The MPN method does not differentiate between species like the molecular-based enumeration techniques; however, the MPN method offers the advantage of only enumerating live bacteria that are healthy enough to be capable of reproduction.

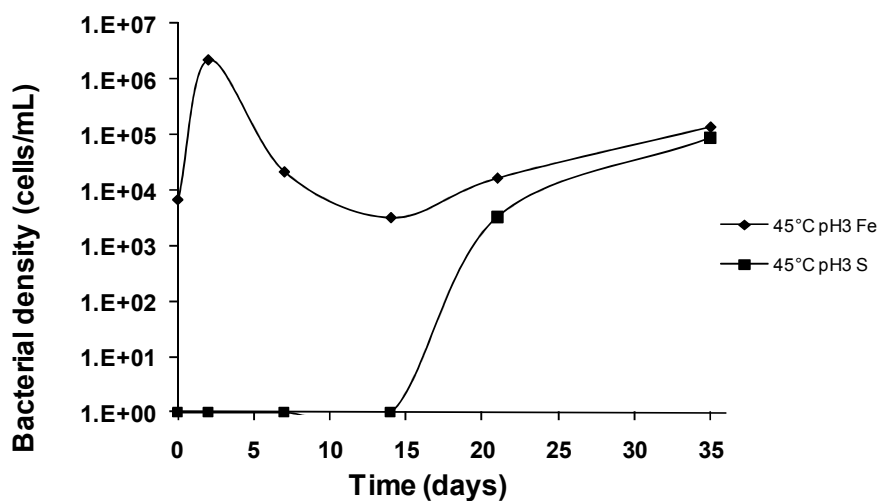


Figure 6.5a. Density of the iron- (Fe) and sulphur-oxidizing (S) bacteria during bioleaching of Ore 3 at pH 3 as a function of time at 45 °C.

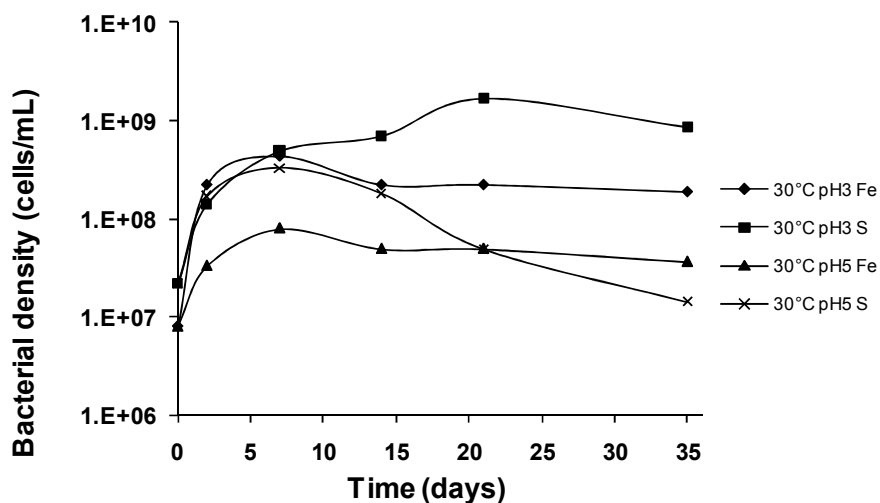


Figure 6.5b. Density of the iron- (Fe) and sulphur-oxidizing (S) bacteria during bioleaching of Ore 3 at pH 3 and 5 as a function of time at 30 °C.

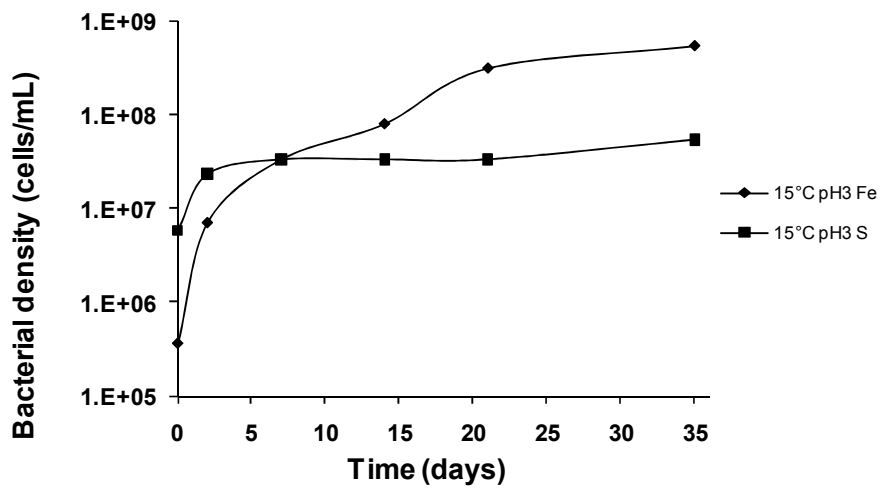


Figure 6.5c. Density of the iron- (Fe) and sulphur-oxidizing (S) bacteria during bioleaching of Ore 3 at pH 3 as a function of time at 15 °C.

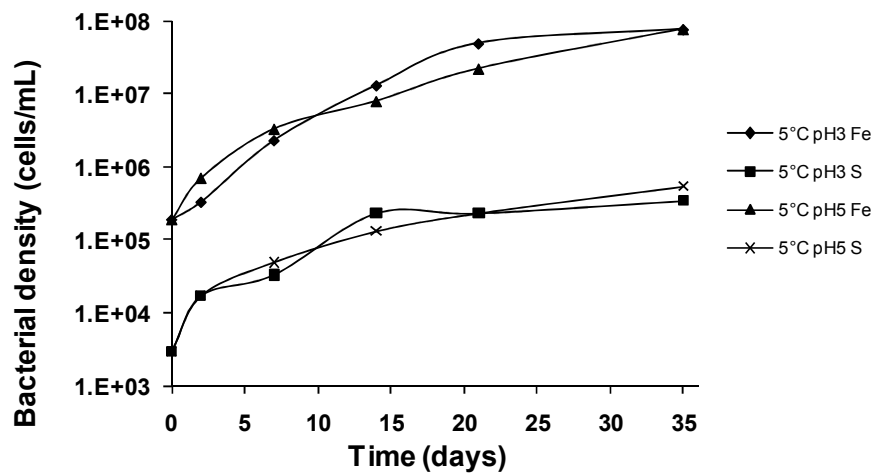


Figure 6.5d. Density of the iron- (Fe) and sulphur-oxidizing (S) bacteria during bioleaching of Ore 3 at pH 3 and 5 as a function of time at 5 °C.

Thiosulphate has been used for the purpose of enumerating sulphur-oxidizing bacteria in this study. Thiosulphate disproportionates in mildly acid media to produce

primarily elemental sulphur and sulphite. According to Xu and Schoonen (1995), the initial reaction proceeds rapidly according to Equation 6.1; however, the reaction does not go to completion and quickly achieves a steady-state, which results in a solution that contains varying amounts of sulphur, elemental sulphur, sulphite, and small quantities of other reduced-sulphur compounds.



Oxidation of any of these products would produce a drop in pH, which is the indicator for a positive tube in the MPN tests. Since it is not possible to conclude which reduced-sulphur compound is being oxidized, the results of the sulphur-oxidizing enumeration tests in this study have been interpreted as determining the total number of microorganisms capable of oxidizing a range of reduced-sulphur compounds, and have been consistently referred to as “sulphur-oxidizing bacteria” in this report.

Each enumeration experiment in this study was run concurrently with 10 un-inoculated control tubes. The initial pH of the media was set at 4.2; however, after incubation, the average pH of the un-inoculated tubes was always higher than 4.2, which is consistent with the consumption of protons during disproportionation (Equation 6.1). A tube was identified as positive for sulphur-oxidizing bacteria if the pH was found to be 0.5 units lower than the average of the control tubes. Generally speaking, positive tubes were determined to be pH < 2.5, making the identification of positive tubes unambiguous.

The results at 45 °C are unusual and generally characterized by low cell concentrations (Figure 6.5a). The concentration of iron-oxidizing bacteria exhibited a bimodal growth curve. There was no detectable presence of sulphur-oxidizing bacteria in the first 14 days, after which the concentration of sulphur-oxidizers increased and appeared to

track the concentration of iron-oxidizers over the last two enumeration periods. There is currently no conclusive explanation for these observations.

The experiments at 15 and 30 °C (Figures 6.5c and 6.5b respectively) exhibited similar trends, which are characteristic of bacterial growth in batch culture: a robust increase in cell concentrations in the first few days (log phase), followed by a period of constant cell concentrations (stationary phase), and then a decline in cell concentrations in some cases (death phase). It is not unexpected for cell concentrations to have declined as the substrate was exhausted (particularly at higher temperatures and pH 5), considering the ore contains less than 3% total sulphide mineral and the majority of the iron reports to either iron-containing oxides or iron-rich silicates, both of which were shown to be stable in this system at pH 5 (Table 4.1).

In the 30 °C experiments, iron-oxidizers at pH 3 and sulphur-oxidizers at both pH 3 and 5 multiplied quickly in the first few days, whereas the concentration of the iron-oxidizing bacteria at pH 5 exhibited less robust growth. Poor growth of the iron-oxidizing bacteria at pH 5 is somewhat expected considering the total dissolved iron determinations at 30 °C pH 5 were consistently in the range of 1 to 2 ppm. Similar cell densities and trends were observed in two different sets of growth experiments with the same ore: 35-day growth experiments at 30 °C pH 3 and 5 (unpublished data), and 10-day growth experiments at 30 °C pH 3 and 5 (Figure 4.3).

At 5 °C (Figure 6.5d), the iron-oxidizing bacteria consistently outnumbered the sulphur-oxidizing bacteria by approximately 2 to 3 orders of magnitude, and time appeared to be the most important factor affecting the bacterial densities. The observation that time appeared to be the most important factor at 5 °C is supported by the DGGE similarity

analysis, which clustered the 5 °C bands most closely by time rather than pH (Figure 6.3). It is surprising that a difference of two orders of magnitude in proton activity (i.e. pH 3 to 5) appeared to have little effect on the rate of growth at 5 °C.

6.3.3.3.1. Bacterial growth rates

Bacterial doubling times during exponential growth can be calculated from the growth curves. A plot of the natural logarithm of the bacterial densities during exponential growth versus time produces a straight line with a slope equal to the specific growth rate μ (time⁻¹), which is related to the doubling time T_2 (time) by equation 6.2.

$$T_2 = \frac{\ln 2}{\mu} \quad (6.2)$$

The plots of the natural logarithm of the bacterial densities versus time at 5 (pH 3 and 5) and 30 °C (pH 3 and 5) are given in Figures 6.6a&b and 6.7a&b respectively. Fe and S represent iron- and sulphur-oxidizing bacteria respectively. Only the lines that appeared to be linear over at least four times were considered. Of the growth experiments discussed in this chapter, only the lines at 5 °C appeared to be linear over four times. The doubling times at 30 °C were calculated from the 10-day growth experiments displayed in Chapter 4 (Figure 4.2), which had more frequent sampling and were inoculated with much smaller inoculums; 5 mL of slurry compared to 100 mL of slurry in the experiments in this chapter. The smaller inoculum allowed exponential growth to continue for a longer period of time.

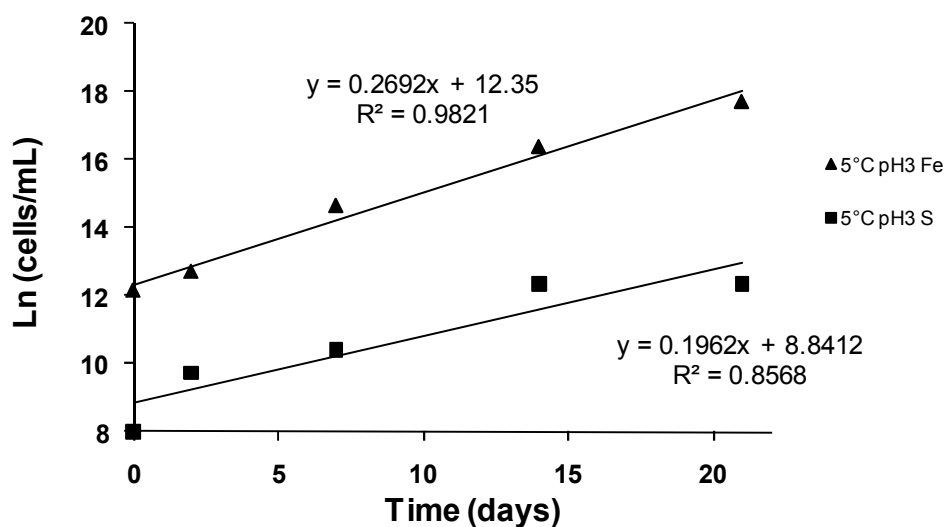


Figure 6.6a. Natural logarithm of bacterial density versus time during bioleaching of Ore 3 at 5 °C pH 3.

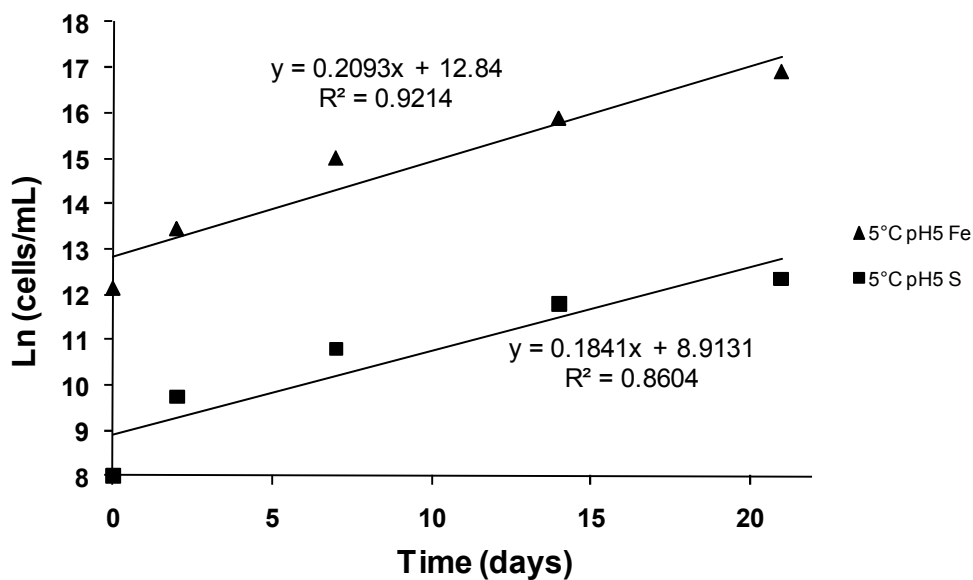


Figure 6.6b. Natural logarithm of bacterial density versus time during bioleaching of Ore 3 at 5 °C pH 5.

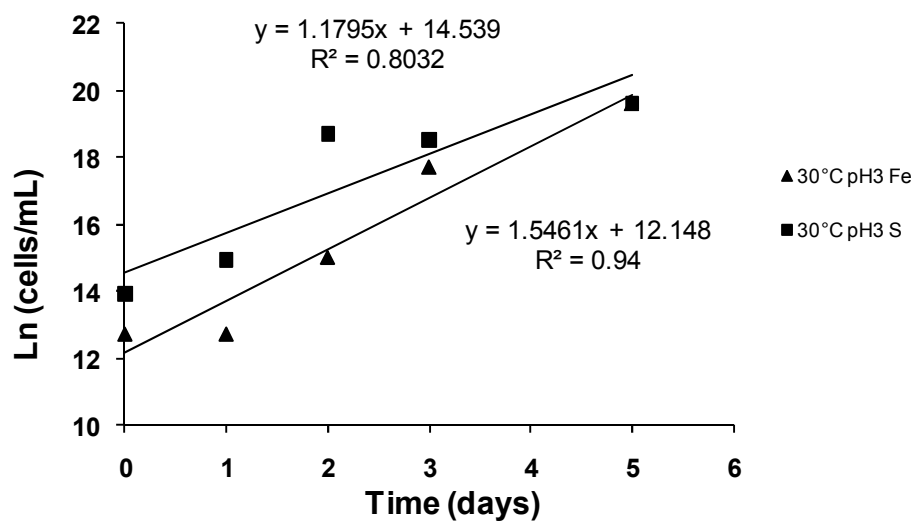


Figure 6.7a. Natural logarithm of bacterial density versus time during bioleaching of Ore 3 at 30 °C pH 3.

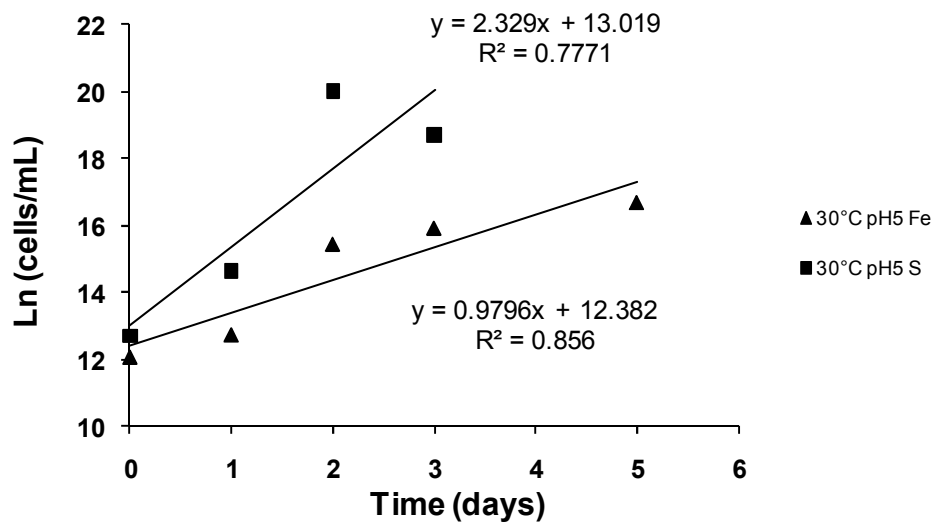


Figure 6.7b. Natural logarithm of bacterial density versus time during bioleaching of Ore 3 at 30 °C pH 5.

The calculated doubling times for the experiments at 5 and 30 °C are listed in Table 6.3. It should be noted that these doubling times were calculated from mixed populations of sulphur- and iron-oxidizing bacteria, and not from pure cultures. These experiments were not designed to calculate kinetic growth rates, and should be compared to pure-culture doubling times with caution.

Table 6.3. Calculated doubling times during exponential growth for S- and Fe-oxidizing bacteria at 5 and 30 °C with Ore 3.

Temperature (°C)	pH	Substrate	Doubling time (hours)	Coefficient of determination (R^2) of Ln(cells/mL) versus time
5	3	Iron	62 ± 8	0.98
		Sulphur	85 ± 39	0.86
	5	Iron	80 ± 24	0.92
		Sulphur	90 ± 40	0.86
30	3	Iron	11 ± 3	0.94
		Sulphur	14 ± 8	0.80
	5	Iron	17 ± 8	0.86
		Sulphur	7 ± 10	0.78

6.4. Conclusions

This report offers a characterization of the microbial consortia involved in the stirred-tank bioleaching of a low-grade nickel sulphide ore. The experiments were conducted over a range of temperature (5 to 45 °C) and pH (3 and 5) conditions. A combination of classical microbiological and molecular biological techniques was used to identify and enumerate the microorganisms. There was agreement between the results obtained by the classical enumeration tests and the molecular biological techniques.

DGGE analysis revealed the presence of at least 16 distinct 16S rRNA gene sequences, 14 of which are closely related to existing GenBank sequences (> 97%). The

other two sequences are not closely related to existing GenBank sequences, and may possibly be from novel species; they are most closely related to sequences from an *Acidithiobacillus* sp. and a strain of *Sulfobacillus acidophilus*. Thirteen sequences are related to gene sequences of genera that have previously been detected in bioleaching environments (*Acidithiobacillus*, *Leptospirillum*, *Sulfobacillus*, *Acidiphilium*, *Ferrimicrobium*, and *Acidimicrobium*), and one sequence that is most closely related to a gene sequence of a microorganism that has been previously detected in ARD (*Propionibacterium acnes*). At 5 °C, two sequences were detected that are not typical of either bioleaching or acidic mining-related environments, and are closely related to the species *Ilyobacter tartaricus* and to the phylum Gemmatimonadetes. The 16S rRNA gene sequences obtained in this study (BL-1 to BL-16) have been deposited in the GenBank database under accession numbers HM124428 to HM124443 respectively.

Substrate utilization tests determined that the mixed cultures at 5, 15, and 30 °C were able to oxidize elemental sulphur and ferrous ion in minimal nutrient media, whereas the culture at 45 °C required yeast extract in order to oxidize both substrates. Considering the low sulphide content of this ore, the growth curves generally show robust growth of both iron- and sulphur-oxidizing bacteria at all temperatures except 45 °C. A difference of two orders of magnitude in proton activity (i.e. pH 3 to 5) had an insignificant effect on the rate of bacterial growth at 5 °C.

DGGE analysis revealed large changes in the bacterial community structure as a function of temperature, pH, and time. This is a strong indication that the original microbial consortium that was enriched at 30 °C was quite diverse and robust. Members from the genus *Acidithiobacillus* were dominant at all temperatures except 45 °C, at which

Sulfobacillus spp. were dominant. Many of the acidithiobacilli were most closely related to strains of *Acidithiobacillus ferrooxidans*, and different strains were dominant under different experimental conditions, suggesting considerable phenotypic heterogeneity within the species.

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Chapter 7 – Bioleaching of Six Nickel Sulphide Ores with Differing Mineralogies in Stirred-tank Reactors at 30 °C

Preface

This chapter has been presented at a conference and published in the conference proceedings: Cameron, R.A., Lastra, R., Gould, W.D., Mortazavi, S., Thibault, Y., Bedard, P.L., Morin, L., Koren, D.W., Kennedy, K., 2010. MEI Bio&Hydrometallurgy 2010, Cape Town, South Africa, November 8 to 9, 2010. In addition, this manuscript is conditionally accepted pending minor revisions for a special edition of the peer-reviewed journal *Minerals Engineering* that has been reserved for papers presented at Bio&Hydrometallurgy 2010.

Abstract

A bioleaching study was conducted with six nickel sulphide ores from different geographical locations across Canada. Mineralogical and chemical examination revealed considerable variability between the samples, particularly in the silicate phases. The ores contain 0.3 to 1% nickel, primarily in pentlandite and secondarily in pyrrhotite. Copper was present primarily in chalcopyrite, and cobalt in pentlandite. The ores were subjected to the same crushing and grinding procedure, and bioleached under the same conditions for three weeks with a mixed culture of iron- and sulphur-oxidizing bacteria. Stirred-tank experiments with finely ground ore ($-147\ \mu\text{m}$) at 30 °C were conducted to assess the effect of pH (2 to 5) and the impact of the bacteria. In general, the presence of the bacteria resulted in a statistically significant increase in nickel, cobalt, and copper extraction, and oxidation-reduction potential; whereas their presence resulted in a statistically significant decrease in

acid consumption. Nickel extraction from pentlandite and pyrrhotite during bioleaching at pH 2 and 3 was generally good (49 to 86% after three weeks). All six ores showed a similar response to a change in pH; an increase in pH from 2 to 3 resulted in approximately the same nickel and cobalt extraction (within statistical error), and a statistically significant reduction in sulphuric acid consumption.

7.1. Introduction

The discovery of new high grade base-metal deposits is diminishing in frequency; consequently, mining companies are processing low-grade deposits in order to maintain production levels. Heap bioleaching practices have the potential to enable the development of some low-grade deposits that are not currently economically viable with conventional processing technologies. Since 1977, over twenty commercial heap/dump (bio)leaching operations have been commissioned for processing copper oxide and secondary copper sulphide ores (Watling, 2006). There have been heap bioleaching pilot trials with nickel sulphide ores in Australia (Hunter, 2002), Finland (Riekkola-Vanhanen, 2007), and China (Wen *et al.*, 2006; Qin *et al.*, 2009). The first commercial application of nickel sulphide heap bioleaching began production at Talvivaara, Finland in October 2008 (Talvivaara, 2009).

The primary objective of this study was to assess the amenability of several different nickel sulphide ores to bioleaching, and to identify broad trends with respect to mineralogical content; particularly with regard to the bioleaching of the primary nickel-bearing phases, pentlandite and pyrrhotite. The ores were subjected to the same crushing and grinding procedure, and subjected to the same stirred-tank bioleaching tests with bacteria that were enriched from the same source. This is the only study that has examined the bioleaching of several different ores under identical experimental conditions. Several of the ores were

determined to contain appreciable amounts of copper and cobalt; however, an emphasis was placed on the extraction of nickel.

Six ore samples were acquired from different geographic locations across Canada in order to study the bioleaching of nickel sulphide ores that contain a variety of mineralogical assemblages. The ore samples were subjected to a thorough mineralogical and chemical characterization. Ores 1, 2, and 4 are from different deposits in Sudbury, Ontario; Ore 3 is from Manitoba; Ore 5 is from the Ungava Peninsula, Quebec; and Ore 6 is from Labrador, Newfoundland.

All the experiments discussed in this paper were conducted at 30 °C, with pH as the only factor. Solution pH was selected as the only factor because a review of the technical literature indicated that pentlandite and pyrrhotite are amenable to bioleaching at pH levels higher than what is generally considered to be optimum (i.e. pH ~ 2) for bioleaching of copper sulphides. This point was verified during shake flask experiments with Ores 1 and 2, during which the bioleaching of nickel showed limited dependency on pH in the range of pH 2 to 3 (Chapter 3). It was then considered that operating at higher pH levels may have the potential to result in cost savings related to sulphuric acid consumption.

7.2. Materials and Methods

A total of 53 stirred-tank experiments at 30 °C were conducted (9 discussed in Chapters 4 and 5), including 40 bioleaching experiments and 13 abiotic experiments. The materials and methods were the same as in the previously reported stirred-tank reactor experiments conducted with Ore 3 (Cameron *et al.*, 2009a; 2009b), unless otherwise noted. There were some minor differences in the experimental procedures as a result of slightly

different experimental objectives. These differences were in the preparation of the inocula, the duration of the experiments, and the sampling frequency. The bioleaching experiments were performed in at least duplicate, with the exception of Ore 3 at pH 2, which was performed without a replicate. The abiotic experiments were performed without replicates, with the exception of Ore 3 at pH 3, which was performed in triplicate.

7.2.1. Characterization of the nickel sulphide ores

The ores were received in bulk samples ranging in size from 100 to 2000 kg. Each bulk sample was crushed to -12.7 mm, and after thorough mixing, a sub-sample of several kilograms was crushed to -6.35 mm. For each ore, a portion of the sub-sample was used for mineralogical characterization, and a portion was pulverized to -147 μm (100 Tyler mesh) and used for stirred-tank (bio)leaching experiments and bacterial culture maintenance. Lastra *et al.* (2007a; 2007b; 2008; 2009a; 2009b; 2010) reported on the mineralogical characterization of Ores 1 to Ore 6 (summarized in Tables 7.1 and 7.2).

Table 7.1. Mineralogical composition of the nickel sulphide ores.

Mineral or mineral group	Ore 1	Ore 2	Ore 3	Ore 4	Ore 5	Ore 6
Amphibole/pyroxene	19.6	36.4	6.2	32.6	17.4	11.6
Carbonates (calcite, dolomite, ankerite)	0.1	0.3	2.1	0.2	tr	nd
Chalcopyrite	0.7	1.9	tr	2.0	2.7	1.8*
Chlorite	1.7	1.5	9.1	1.1	16.9	nd
Oxides (magnetite, hematite, ilmenite, chromite)	9.0	2.5	12.2	1.0	4.1	6.2
Feldspars	23.2	19.3	0.1	21.8	0.2	51.7
Pentlandite	3.0	2.0	0.7	1.2	2.5	2.9
Pyrrhotite	33	25.2	0.9	13	15.7	15.3
Pyrite	0.1	0.1	0.5	2.5	tr	0.6
Quartz	7	4.7	0.1	13.2	0.1	0.5
Serpentine	nd	nd	64.4	3.9	39.9	nd
Sphalerite	tr	tr	tr	tr	0.2	tr
Talc	0.5	0.1	3.3	nd	nd	nd
Others (apatite, danalite, epidote, mica, titanite)	2.1	6.0	0.5	7.5	0.1	9.6

All values quoted in %mass; tr: trace; nd: not detected; and *: plus trace of cubanite.

As seen in Table 7.1, all the ores contain the same sulphide minerals, mainly pentlandite, pyrrhotite, and chalcopyrite. Pyrrhotite is the most abundant sulphide mineral in all the ores (up to 33% of Ore 1). Nickel is present primarily in pentlandite and secondarily in pyrrhotite, whereas copper is present primarily in chalcopyrite. There are significant differences in the silicate minerals, which are the main constituents of the ores. Ores 1, 2 and 4 are from different deposits in Ontario and contain similar silicate minerals, with major amounts of feldspars, amphiboles, and pyroxenes. Ore 3 (from Manitoba) and Ore 5 (from Quebec) contain major amounts of serpentine (~ 60 and 40% respectively), whereas Ore 6 (from Newfoundland) contains > 50% feldspars.

Electron probe X-ray microanalysis (EPMA) was used to determine the chemical composition of the pentlandite, pyrrhotite, and pyrite in each ore (Table 7.2). The nickel content of the pentlandite ranged from ~ 32% in Ore 5 to ~ 39% in Ore 3. The nickel content of the pyrrhotite exhibited considerable variability, ranging from ~ 0.2% in Ore 5 to ~ 0.8% in Ore 2. Pentlandite is the primary nickel-bearing phase in all six ores; ranging from ~ 97% of the nickel in Ore 3, to ~ 78% of the nickel in Ore 2. Pyrrhotite is the second most significant nickel-bearing phase in all the ores, and contains 22% of the nickel in Ore 2. Pyrite was determined to be a significant nickel-bearing phase only in Ore 4 (~ 12%). None of the other phases contained a significant quantity of nickel. Cobalt is primarily present in pentlandite in all the ores.

Table 7.2. Chemical composition of the primary nickel-bearing phases and distribution of nickel.

Mineral		Ore 1	Ore 2	Ore 3	Ore 4	Ore 5	Ore 6
Pentlandite	Ni (%)	36.3 ± 0.7	36.2 ± 0.7	39 ± 2	36.1 ± 0.8	31.9 ± 0.4	33 ± 1
	Co (%)	0.8 ± 0.2	1.4 ± 0.3	1.1 ± 0.4	1.2 ± 0.3	1.63 ± 0.09	1.6 ± 0.2
Pyrrhotite	Ni (%)	0.7 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	0.19 ± 0.06	0.30 ± 0.09
Pyrite	Ni (%)	~ 0	~ 0	0.03 ± 0.01	3 ± 2	~ 0	~ 0
Proportion of nickel reporting to pentlandite (%)		83	78	97	72	96	95
Proportion of nickel reporting to pyrrhotite (%)		16	22	2	15	3	5
Proportion of nickel reporting to pyrite (%)		~ 0	~ 0	~ 0	12	~ 0	~ 0

All values quoted in %mass ± 1 standard deviation

The chemical composition data listed in Table 7.3 are the averages of at least three replicates taken from different sub-samples. The nickel content of the individual ore samples varied considerably, ranging from 0.3% to 1% in Ores 3 and 6 respectively.

Table 7.3. Chemical composition of the nickel sulphide ores.

Element	Ore 1	Ore 2	Ore 3	Ore 4	Ore 5	Ore 6
Ni (%)	0.79 ± 0.02	0.68 ± 0.03	0.305 ± 0.005	0.59 ± 0.01	0.95 ± 0.01	0.99 ± 0.02
Mg (%)	2.74 ± 0.02	3.76 ± 0.02	21.2 ± 0.1	3.39 ± 0.04	13.2 ± 0.3	3.20 ± 0.006
Cu (%)	0.224 ± 0.003	0.63 ± 0.01	0.016 ± 0.002	0.721 ± 0.004	0.965 ± 0.006	0.602 ± 0.004
Co (%)	0.0274 ± 0.0001	0.0289 ± 0.0003	0.013 ± 0.0004	0.0252 ± 0.0007	0.042 ± 0.002	0.0439 ± 0.0008
Fe (%)	14.0 ± 0.2	16.1 ± 0.1	7.2 ± 0.1	14.13 ± 0.04	20.5 ± 0.4	21.5 ± 0.2

All values quoted in %mass ± 1 standard deviation

7.2.2. Microbial culture and (bio)leaching experiments

The enrichment procedure used to develop the mixed culture of iron- and sulphur-oxidizing bacteria that was used in this study, as well as the identification of the dominate species present in that culture, has been described in Cameron *et al.* (2010). The mixed

culture was adapted to each ore sample at 30 °C for several months by serial dilution in shake flasks prior to the commencement of the bioleaching experiments.

The inoculum for each individual experiment was developed by combining 5 g of ore and 100 mL of McCready media (McCready *et al.*, 1986) with no magnesium and no iron. In addition, inocula prepared for experiments with Ore 3 required 8 mL of 10% H₂SO₄ (v/v) in order to satisfy the ore's immediate acid demand (Cameron *et al.*, 2009a). After 48 hours in an orbital shaker at 30 °C, the pH was adjusted and the shake flask was inoculated with 5 mL of slurry from a well-shaken maintenance culture grown on the ore to be tested. The resulting inoculum was maintained in an orbital shaker for ten to twelve days (Ore 3), or eleven days (Ores 1, 2, 4, 5, and 6) at constant pH. All the inocula for the experiments with Ore 3 were maintained at pH 3 during incubation, whereas the inocula for the experiments with the other ores were maintained at different pH levels: pH 2 for experiments conducted at pH 2; and pH 3 for experiments conducted at pH 3 and 5.

The inocula were combined with 145 g of ore in the reaction vessel and made up to a total volume of 1.5 L with McCready media (no magnesium and no iron). Magnesium was omitted from the media because the ores supplied enough dissolved magnesium; and iron was omitted in order to encourage the bacteria to attach to the ore and utilize the sulphide minerals as an energy source. Reactions were conducted in jacketed 2.0 L glass reaction vessels with temperature control, pH control, aeration (~ 500 mL/min at STP), and continuous stirring for either three or five weeks. Sulphuric acid or a base solution (NaHCO₃ or Na₂CO₃) was kept in a graduated cylinder and pumped into the reaction vessel with a peristaltic pump as required by the pH controller.

Abiotic experiments were conducted, in which thymol was added as a bactericide to media containing 5% methanol (Meline *et al.*, 1996). The concentration of thymol was periodically determined by a UV spectrophotometer at 273.5 nm, and evaporative losses were made up with fresh thymol/methanol solution in order to maintain the concentration of thymol within a range of 0.3 to 0.9 g/L.

All chemical determinations were done by the ISO 9001 certified Analytical Services Group at CANMET-MMSL laboratories in Ottawa, Canada (Cameron *et al.*, 2009a; 2009b). Statistical significance testing was performed by analyzing the data using the one-way analysis of variance function in Minitab release 14, with 80% confidence intervals, unless otherwise indicated. An 80% confidence interval (CI) was considered appropriate, as most experiments were conducted with few replicates. Data points in the graphs presented in this report are the mean values of the replicates where applicable and metal extraction curves have been modified by the appropriate dilution factors to account for sample removal. The length of the individual experiments ranged from three to five weeks; although only three weeks of data are presented in this report for the purpose of comparison. For this reason, comparisons are based on the corrected leachate determinations after three weeks. Final metal extractions based on the leachate determinations and the post-leach solid residue determinations were compared, and there was generally good agreement.

7.3. Results and Discussion

7.3.1. Metal extractions, sulphuric acid consumption, and ORP during bioleaching

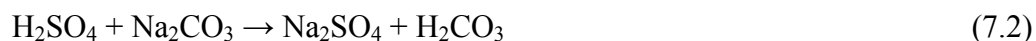
Most nickel sulphide ores contain copper and cobalt, and often platinum group elements (PGEs) as by-product credits. All the ores used in this study contain copper and

cobalt (PGEs were not assayed). The primary focus of this study was to investigate the bioleaching of nickel from sulphide ores; however, the extraction of both cobalt and copper was followed and discussed when appropriate, since the extractions of these two metals would undoubtedly be an important consideration in evaluating the economics of a commercial bioleaching process.

The extraction of nickel, copper, and magnesium, the concentration of iron, acid consumption ($\text{g H}_2\text{SO}_4$ per kg ore), oxidation-reduction potential (vs Ag/AgCl), and the ratio of the percent nickel recovered to percent cobalt recovered (%Ni:%Co ratio) during (bio)leaching of the six ores at 30 °C as a function of time at the different pH levels tested are given in Figures 7.1a to 7.1f. Bioleaching data is traditionally interpreted in terms of the iron concentration and speciation, and the oxidation-reduction potential (ORP); however, ORP is only meaningful when there is a single dominant redox couple in solution. For this reason, the ORP values at pH 5 should be interpreted with caution as the total dissolved iron was determined to be < 20 ppm after 21 days of bioleaching in all biotic experiments at pH 5.

Many of the experiments conducted with bacteria required the addition of base (either NaHCO_3 or Na_2CO_3) to neutralize excess acid production in order to maintain the desired pH. The sulphuric acid consumption data in Figures 7.1a to 7.1f has been mathematically corrected to reflect the addition of base as a negative acid addition. Correction factors were calculated using a proton mass balance based on the stoichiometry of Equations 7.1 and 7.2, and expressed as a weight equivalent of sulphuric acid. If NaHCO_3 was used, the correction factor is -0.584 g H_2SO_4 per g NaHCO_3 consumed, whereas if Na_2CO_3 was used, the correction factor is -0.925 g H_2SO_4 per g Na_2CO_3 consumed. Equations 7.1 and 7.2 describe

the reaction of sulphuric acid with sodium bicarbonate and sodium carbonate respectively, and Equation 7.3 describes the dehydration of carbonic acid.



Under certain conditions, carbonic acid (H_2CO_3) may proceed to lose as many as two protons, which would generate acidity; however, at the pH levels examined in this study (i.e. $\text{pH} \leq 5$), H_2CO_3 is the dominant carbonate species (Stumm and Morgan, 1996), and the protolysis reaction may be safely ignored. Under the conditions examined in this study, carbonic acid in excess of the maximum solubility will undergo dehydration (Equation 7.3) and be vented to the atmosphere as carbon dioxide, with no impact on the acid balance. Equilibration would be expected to occur rapidly given the high aeration rate ($\sim 500 \text{ mL/min}$) used in these experiments.

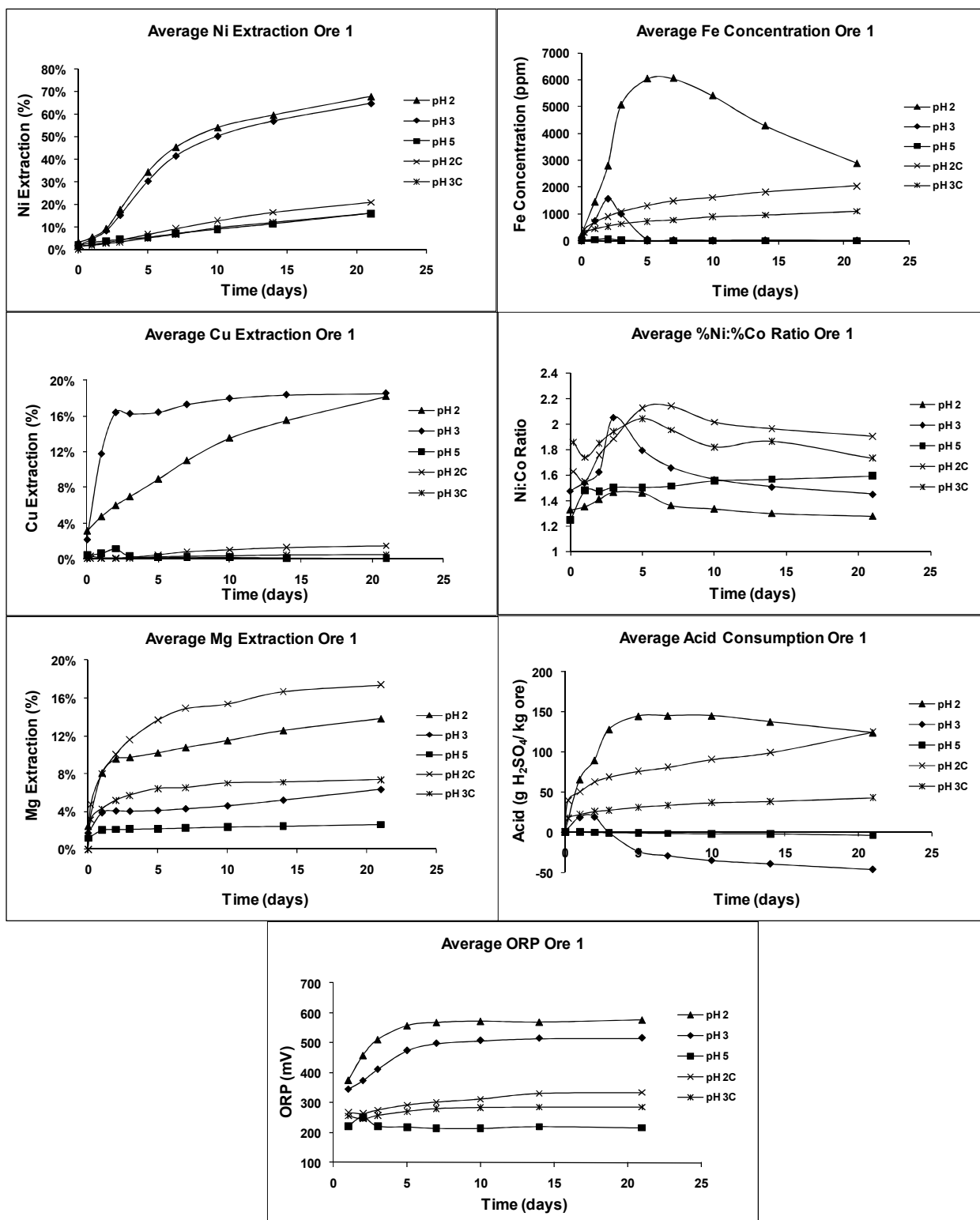


Figure 7.1a. Average metal extractions, average acid consumptions, and average ORP during (bio)leaching of Ore 1 as a function of time. C = abiotic conditions.

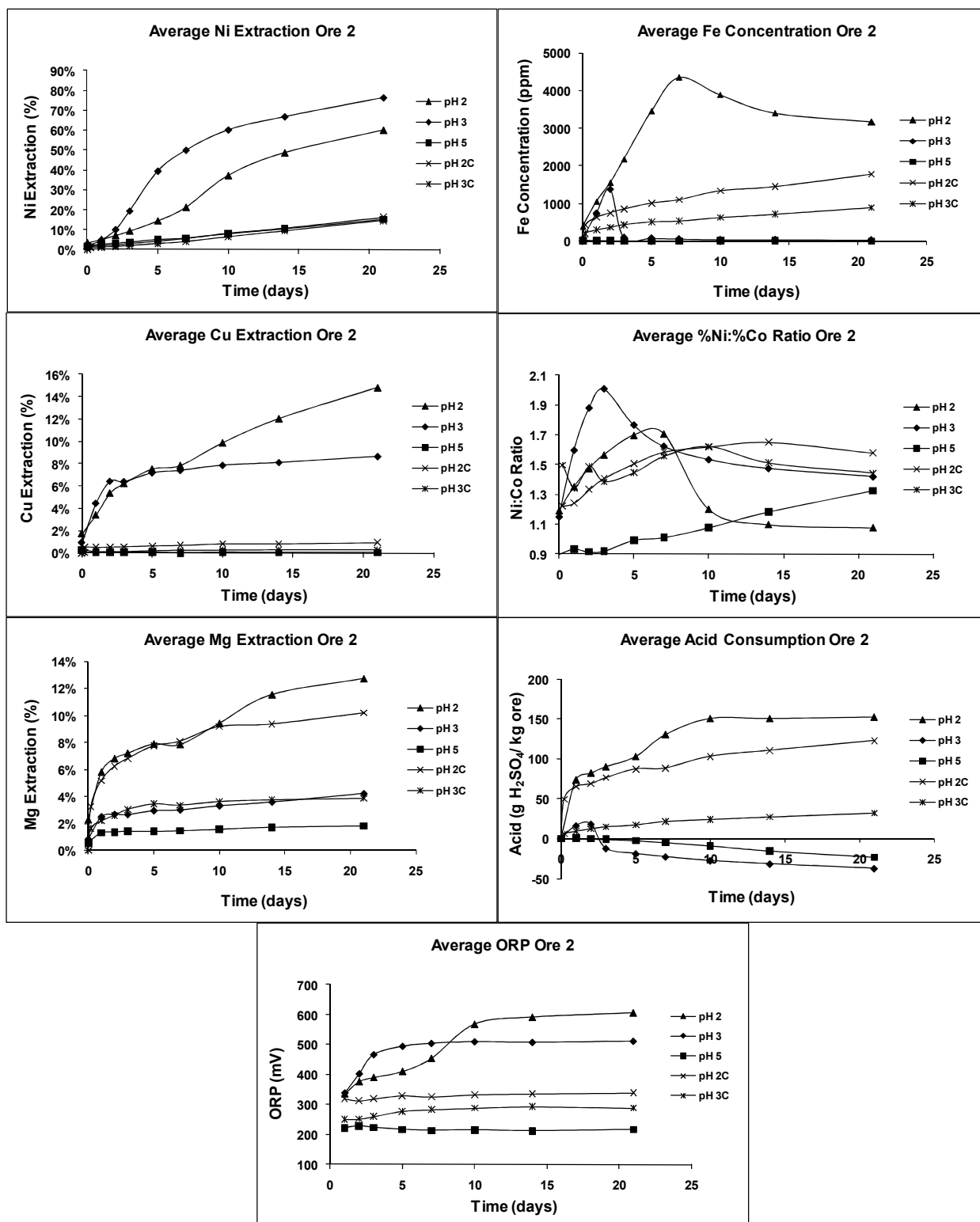


Figure 7.1b. Average metal extractions, average acid consumptions, and average ORP during (bio)leaching of Ore 2 as a function of time. C = abiotic conditions.

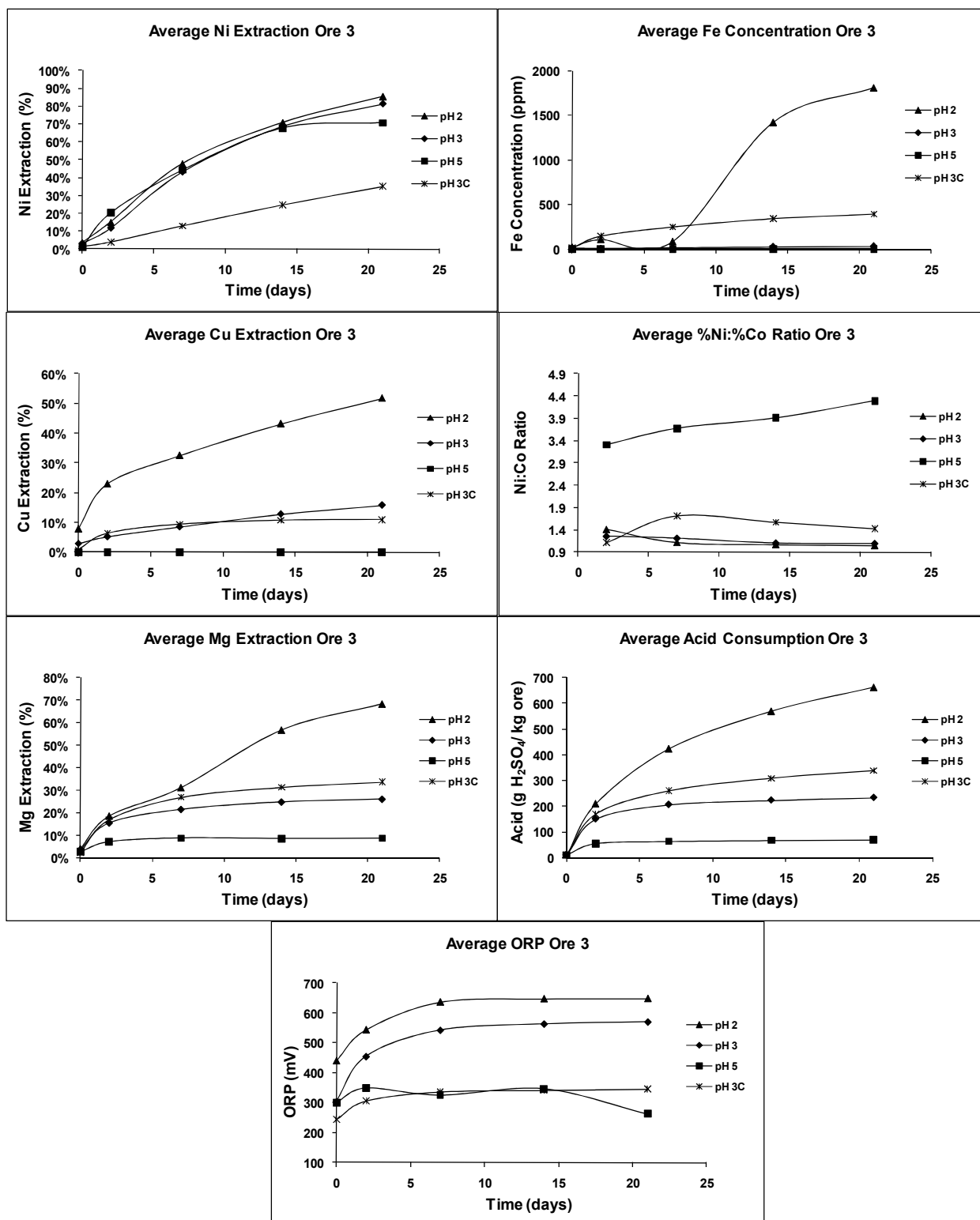


Figure 7.1c. Average metal extractions, average acid consumptions, and average ORP during (bio)leaching of Ore 3 as a function of time. C = abiotic conditions.

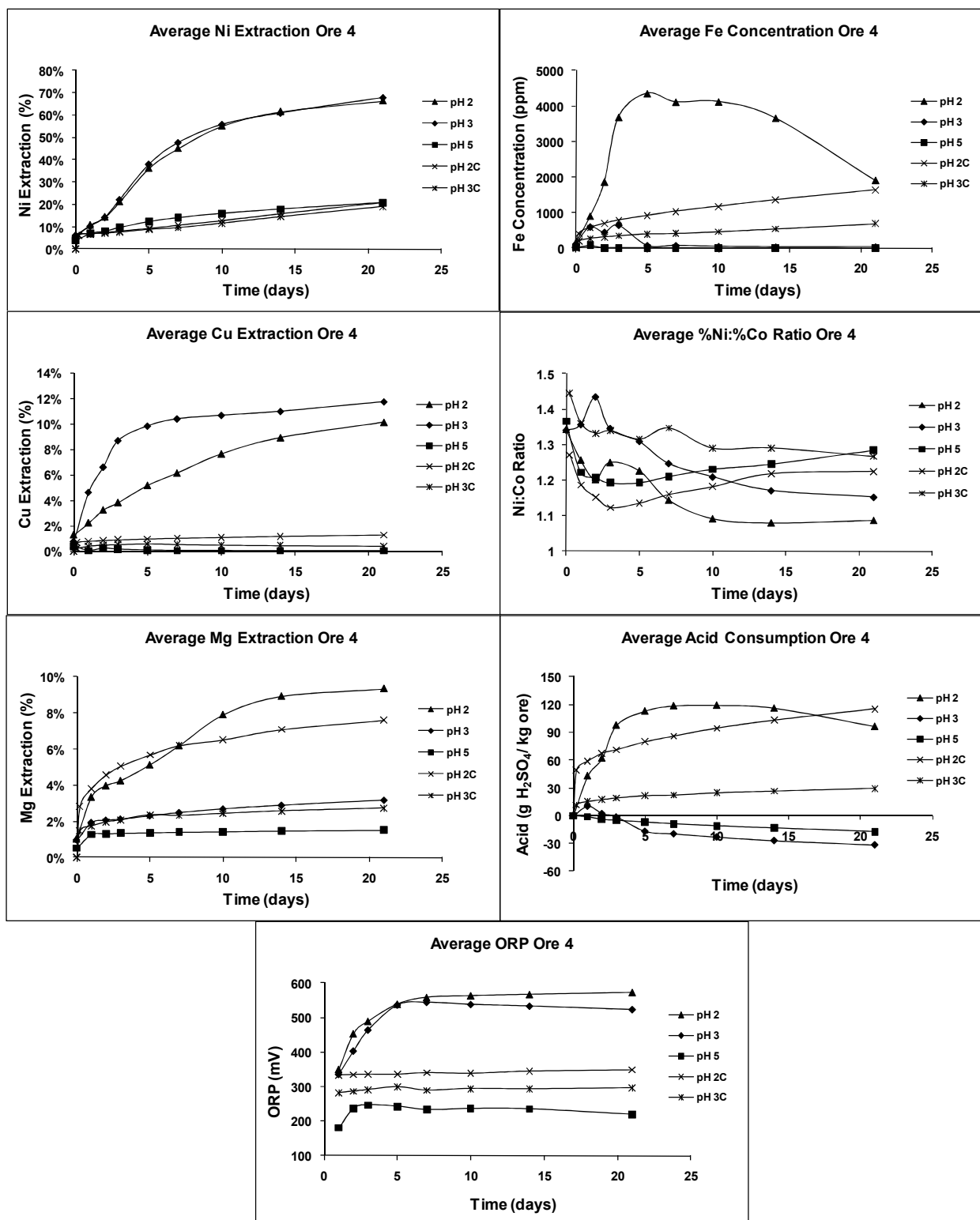


Figure 7.1d. Average metal extractions, average acid consumptions, and average ORP during (bio)leaching of Ore 4 as a function of time. C = abiotic conditions.

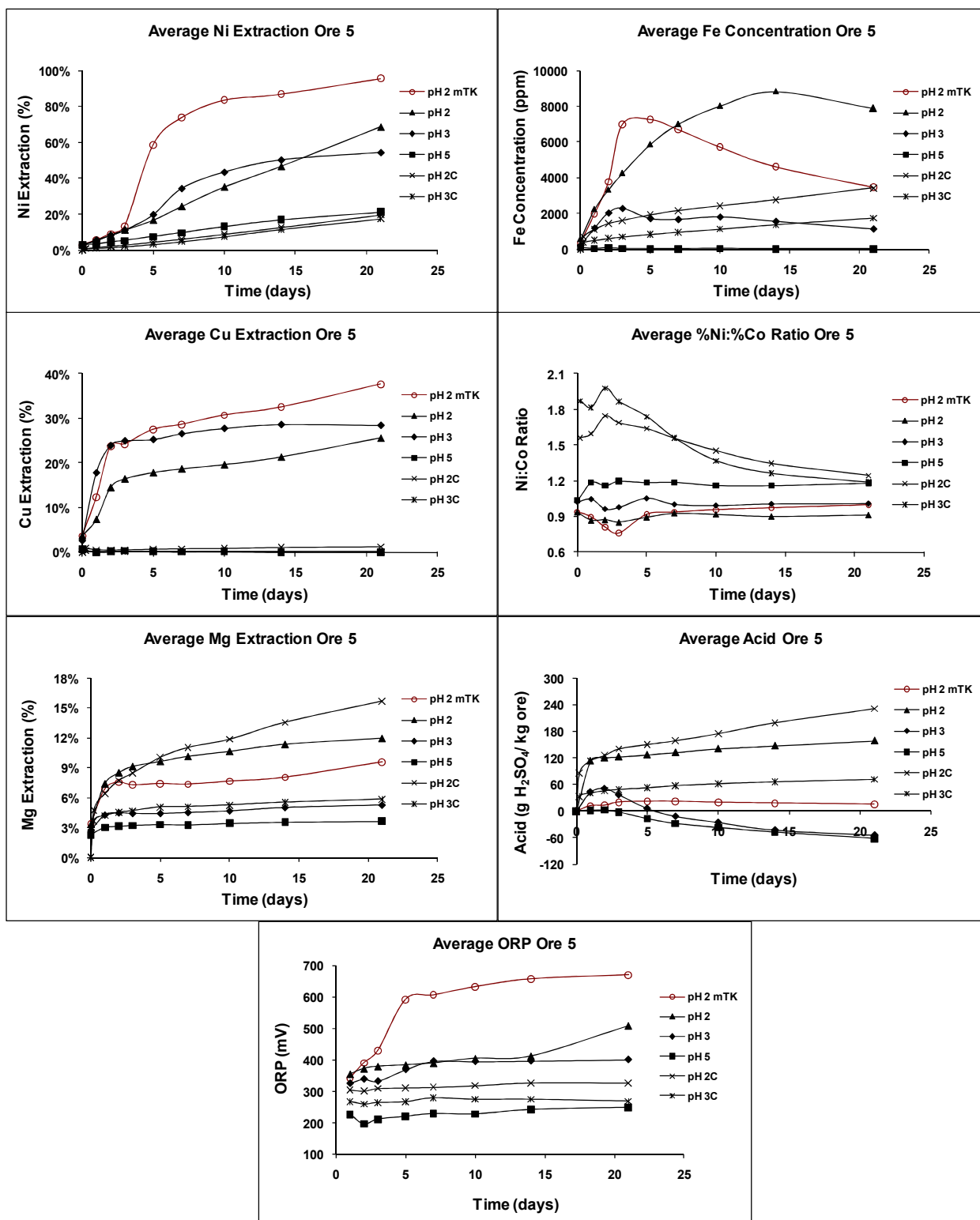


Figure 7.1e. Average metal extractions, average acid consumptions, and average ORP during (bio)leaching of Ore 5 as a function of time. C = abiotic conditions; mTK = modified TK media.

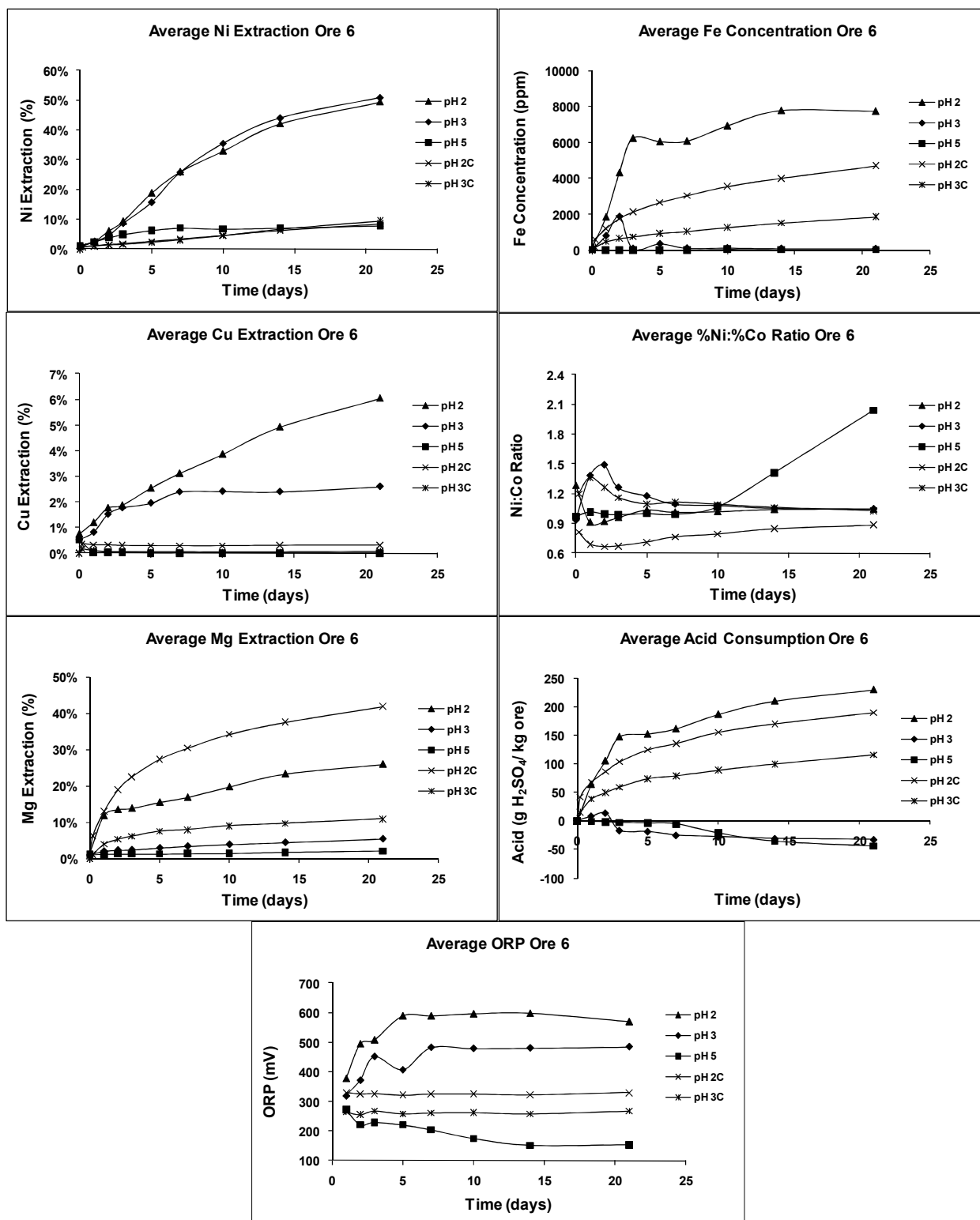


Figure 7.1f. Average metal extractions, average acid consumptions, and average ORP during (bio)leaching of Ore 6 as a function of time. C = abiotic conditions.

The highest, the lowest, and the mean values of the metal extractions and the sulphuric acid consumptions after 21 days of bioleaching are listed in Table 7.4. The mean acid consumption and magnesium extraction values have not been provided, as the coefficient of variation was calculated to be higher than 100% in most cases.

Table 7.4. High, low, and mean values the metal extractions and sulphuric acid consumption after 21 days of bioleaching at pH 2, 3 and 5.

	pH	High	Low	Mean (± 1 standard deviation)
Ni	2	86% (Ore 3)	49% (Ore 6)	66 $\pm$ 12%
	3	81% (Ore 3)	51% (Ore 6)	66 $\pm$ 12%
	5	71% (Ore 3)	7.8% (Ore 6)	25 $\pm$ 23%
Co	2	82% (Ore 3)	47% (Ores 6)	62 $\pm$ 13%
	3	74% (Ore 3)	45% (Ore 1)	56 $\pm$ 10%
	5	18% (Ore 5)	3.8% (Ore 6)	13 $\pm$ 5.3%
Cu	2	52% (Ore 3)	6% (Ore 6)	21 $\pm$ 17%
	3	28% (Ore 5)	2.6% (Ore 6)	14 $\pm$ 9%
	5	~ 0%	~ 0%	~ 0%
Mg	2	68% (Ore 3)	9.3% (Ore 4)	N/A
	3	26% (Ore 3)	3.2% (Ore 4)	N/A
	5	8.5% (Ore 3)	1.5% (Ore 4)	N/A
Acid consumption (g/kg ore)	2	661 (Ore 3)	96 (Ore 4)	N/A
	3	233 (Ore 3)	-54 (Ore 5)	N/A
	5	69 (Ore 3)	-62 (Ore 5)	N/A

7.3.2. Effect of the bacteria

Comparison of the experiments conducted with bacteria and their corresponding abiotic experiments at the same pH (2 and 3) reveals that in general, the presence of the bacteria produced a statistically significant increase in nickel, cobalt, and copper extraction, and ORP; while their presence produced a statistically significant decrease in acid

consumption. It should be noted that there were instances in which the difference was not statistically significant.

In general, the presence of the bacteria produced an increase in the level of dissolved iron at pH 2, whereas they produced a decrease in the level of dissolved iron at pH 3. The presence of the bacteria had an opposite effect at pH 2 and 3 due to the large difference in the solubility of ferric ion in that pH range. Secondary ferric phases such as jarosite and ferric hydroxide-type compounds readily precipitate above pH 2.5 to 3, resulting in maximum ferric ion concentrations in the order of g/L and ppm at pH 2 and 3 respectively. The effect that the bacteria have on the extraction of metals from sulphide minerals, ORP, and the consumption of sulphuric acid is straightforward and consistent with published studies in the technical literature.

7.3.3. Metal extraction from sulphide minerals during bioleaching

In general, replicate experiments closely tracked each other in terms of metal extractions, acid consumption, and ORP; however, the replicates with Ore 5 exhibited considerable variability. For this reason, an additional bioleaching experiment was conducted at each pH level with Ore 5. The ORP was recorded during each sampling session. On average, the ORP during bioleaching of Ore 5 was ~ 100 mV less compared to the average of the other five ores at both pH 2 and 3. The replicates with the higher ORP resulted in higher nickel and cobalt extraction.

The biological oxidation of iron appeared to be poor during experiments at pH 2 and 3 with Ore 5, as evident by the consistently low ORP. Furthermore, two of the three replicates at pH 3 had total iron concentrations of ~ 1400 and ~ 1900 ppm after 21 days of bioleaching, further suggesting poor biological iron oxidation at pH 3 (the average for the

other five ores at pH 3 was < 40 ppm). It was believed that for some unknown reason, the experiments with Ore 5 were nutrient-lacking. One additional experiment at pH 2 was conducted with a richer media (mTK, Table 3.4), which resulted in a substantially higher average ORP (+ 150 mV), and higher nickel and cobalt extractions (Figure 1e, 'pH 2 mTK' series).

7.3.3.1. Nickel and cobalt

Ore 3 is a low-grade metamorphosed ultramafic-dominated nickel sulphide ore that is characterized by low nickel content and a high fraction of acid-soluble magnesium silicate gangue minerals. Bioleaching this ore at conventional pH levels (~ 2) resulted in an unacceptable amount of solubilized magnesium and excess sulphuric acid consumption. Ore 3 was further subjected to a more intensive study that examined stirred-tank bioleaching at 30 °C (pH 2 to 6), and at 5 to 45 °C (pH 3 to 5), the results of which have been reported in Cameron *et al.* (2009a; 2009b; 2009c; 2010). During the first three weeks of bioleaching with Ore 3 at 30 °C, nickel was extracted at similar rates during experiments conducted at $\text{pH} \leq 5$, with over 70% of the nickel extracted in that timeframe. The initial rate of nickel extraction from pentlandite was observed to be inversely correlated to acidity at all temperatures tested. The most surprising results were obtained at 5 °C, in which the nickel extraction at pH 5 was approximately 250% greater than the nickel extraction at pH 3 after five weeks; an observation that is in contradiction to conventional bioleaching wisdom, which dictates that a low-pH environment is generally favourable to the dissolution of sulphide minerals. It was concluded that operating at elevated pH (≥ 3) resulted in a substantial increase in the nickel to magnesium ratio in the leachate, and also resulted in a substantial reduction in the consumption of sulphuric acid.

The original experimental plan in this study was designed to subject each ore to both bioleaching and abiotic leaching at pH 2 and 3. In light of the encouraging results obtained at pH 5 with Ore 3, the experimental plan with the other five ores was modified to include experiments at pH 5. Bioleaching Ores 1, 2, 4, 5, and 6 at pH 5 was undertaken not due to a perceived economic advantage but rather out of academic interest, as the bioleaching of Ore 3 produced results that have not been reported with other ores in the technical literature and it was of interest to test other nickel sulphide ores containing different mineral assemblages under similar conditions.

Nickel extraction from the six ores at pH 2 and 3 was generally good, whereas nickel extraction at pH 5 was poor with all ores except Ore 3. In general, the nickel extraction curves for the individual ores are clustered in two groups: biotic experiments at pH 2 and 3, which produced similar results; and the abiotic experiments at pH 2 and 3, and the biotic experiments at pH 5, which produced similar results (Figures 7.1a to 7.1f). The final nickel extractions after 21 days of bioleaching at pH 2 and 3 were compared and the difference was determined to be significant only with Ore 2, in which the final nickel extraction at pH 3 was significantly greater compared to pH 2 (average of 76 and 60% at pH 3 and 2 respectively). There was a noticeable lag in both the ORP and the nickel extraction curves (evident in both replicates) during bioleaching of Ore 2 at pH 2, after which nickel was extracted at similar rates at both pH 2 and 3 (Figure 7.1b). With each ore, the final nickel extraction after 21 days at pH 5 was significantly less compared to that for both pH 2 and 3 (80% CI). Figure 7.2 shows the average nickel extraction curves (all ores combined) at the different pH levels tested.

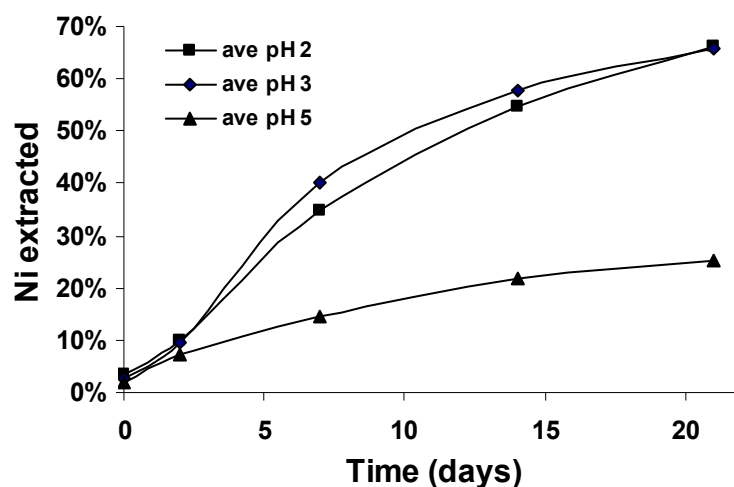


Figure 7.2. Average nickel extraction (all ores combined) as a function of time at different pH levels.

In terms of nickel extraction, the most obvious point of comparison between the ores is the nickel extraction at pH 5. Nickel extraction from Ore 3 demonstrates a response to pH that is not followed by the other five ores. The extraction of nickel at pH 5 after 21 days with Ore 3 ($70.7 \pm 0.5\%$) is substantially higher compared to the average of the other five ores ($16 \pm 5\%$). There is currently no conclusive explanation for this observation; however, there are a number of chemical and mineralogical differences between Ore 3 and the other ores that may have contributed to this observation. The chemistry and mineralogy of Ore 3 is quite different compared to the other ores. It contains the highest quantity of serpentine ($\sim 64\%$); the lowest quantity of pyrrhotite ($< 1\%$); the lowest quantity of chalcopyrite ($\sim 0\%$); and the pentlandite in Ore 3 was determined to contain the highest fraction of nickel ($\sim 39\%$).

With the ores that contain appreciable amounts of nickel in pyrrhotite (i.e. Ores 1, 2, and 4), comparing the %Ni:%Co ratio during bioleaching can provide some insight into the relative dissolution rates of pentlandite and pyrrhotite. Nickel is present in both pentlandite

and pyrrhotite, while cobalt is present primarily in pentlandite. Therefore, %Ni:%Co > 1 suggests relatively strong pyrrhotite dissolution. In general, the %Ni:%Co ratio is higher during the abiotic experiments compared to their corresponding biotic experiments with Ores 1, 2, and 4, indicating the relative rate of pyrrhotite-to-pentlandite dissolution is higher under abiotic conditions. This suggests the dissolution of pentlandite is more positively affected by the presence of the bacteria compared to pyrrhotite. This is in agreement with previous observations that pyrrhotite has been found to react rapidly with acid (Belzile *et al.*, 2004; Watling, 2008).

Cobalt extraction would be expected to closely track nickel extraction, as the primary carrier of both elements in all six ores is pentlandite. In general, cobalt extraction does track nickel extraction; however there is one exception. Significance testing of the final cobalt extraction after 21 days of bioleaching revealed similar results as nickel, both in terms of the positive influence of the bacteria and the insensitivity to pH in the range of pH 2 to 3. Cobalt extraction from the six ores after 21 days at pH 2 and 3 was generally good ($62 \pm 13\%$ and $56 \pm 10\%$ respectively), but it was poor at pH 5 with all ores. Cobalt extraction did not track nickel extraction at pH 5 with Ore 3; nickel extraction averaged 71%, while cobalt extraction averaged only 17%.

In general, cobalt extraction is more negatively affected by an increase in pH compared to nickel extraction (Table 7.4). This trend is reflected as an increase in the %Ni:%Co ratio with increasing pH. The final %Ni:%Co ratio at pH 5 was determined to be higher compared to that of both pH 2 and 3 by a statistically significant margin with all the ores tested. The effect was most pronounced with Ores 3 and 6 (Figures 7.1c and 7.1f

respectively). As previously stated, this trend was consistently observed during bioleaching of Ore 3 over a wide range of pH and temperature conditions (Chapter 5, Figure 5.8a&b).

7.3.3.2. Copper

Copper extraction was generally poor under all experimental conditions. Low copper extractions at 30 °C is not unexpected, as copper is present primarily in chalcopyrite in all the ores in this study. The bioleaching of chalcopyrite is widely considered problematic and is characterized by low copper extractions under mesophilic conditions.

The final copper extractions after 21 days of bioleaching at pH 2 and 3 were compared and the difference was determined to be statistically significant with Ores 2, 3, and 6, in which the final copper extraction at pH 2 was greater compared to pH 3. With all the ores, the final copper extraction at pH 5 was ~ 0%. The initial rate of copper extraction during bioleaching of Ores 1, 4, and 5 was observed to be greater at pH 3 compared to pH 2. Low-pH conditions are generally considered to be favourable for the dissolution of chalcopyrite; however, Riekkola-Vanhanen *et al.* (2001) observed copper extraction from chalcopyrite to be faster at pH 3 compared to both 2 and 1.5 during stirred-tank bioleaching experiments with their low-grade black schist ore.

The final copper extraction after 21 days of bioleaching at pH 2 appears to be inversely correlated to the amount of feldspars in the ore (Figure 7.3). Ores 3 and 5 have 0.1 and 0.2% feldspars respectively (two lowest), and have the highest copper extractions, whereas Ore 6 has 52% feldspars (highest), and has the lowest copper extraction after 21 days. Further investigation of this is needed.

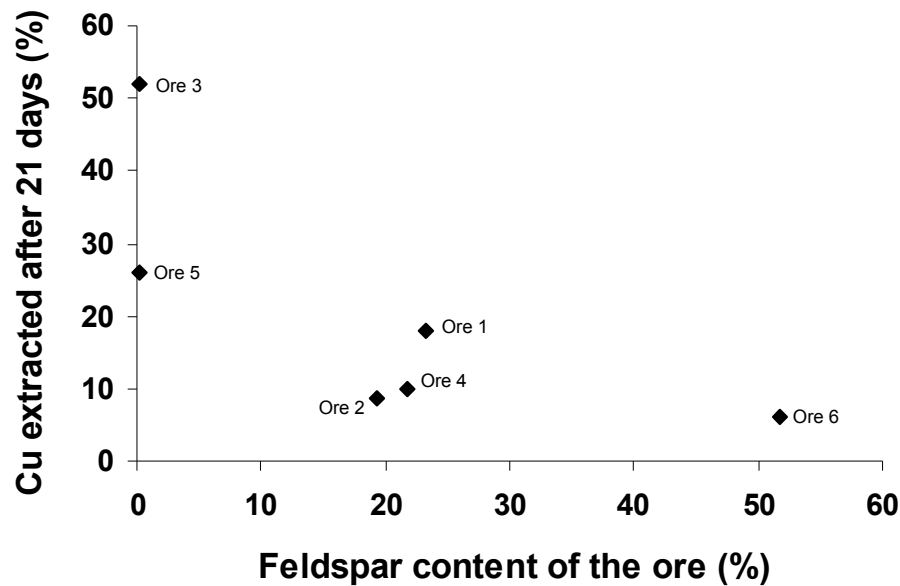


Figure 7.3. Final extraction of copper after 21 days of bioleaching at pH 2 in relation to the feldspar content of the ore.

7.3.4. Gangue mineral dissolution during (bio)leaching

Magnesium is a component in many of the gangue minerals that are contained in the ores used in this study. Magnesium is stable in solution over a wide range of pH conditions, and it does not precipitate in the presence of a large number of anions (including sulphide). For these reasons, magnesium has been viewed as a chemically-stable indicator of gangue mineral dissolution in this study. It would be expected that other nuisance elements that are associated with the gangue minerals (i.e. Al^{3+} , Na^+ , K^+ , etc.) would follow similar extraction trends.

Magnesium itself in high concentrations can cause problems in a bioleaching circuit. Magnesium-rich leaching solutions may be inhibitory to the bioleaching microorganisms and may cause downstream processing problems. The removal of magnesium from the pregnant

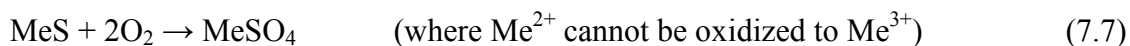
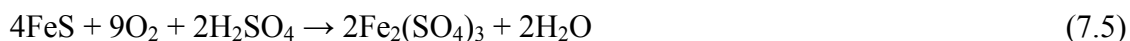
liquor and its disposal can represent a significant expense, as the market for magnesium salts is limited, and the price of magnesium metal does not allow for economical recovery. The relative importance of magnesium extraction varies significantly between the ores used in this study. With the Ontario ores that contain 3 to 4% magnesium, the extraction of that element may be of minor concern; however, Ore 3 contains 21% magnesium and only 0.3% nickel, making magnesium extraction a major concern.

The solution pH had a significant effect on the extraction of magnesium; with each ore, operating at pH 3 produced a statistically significant decrease in the extraction of magnesium compared to pH 2. Many nickel sulphide ores contain high levels of magnesium-rich silicate gangue minerals, particularly ultramafic-dominated ores such as Ore 3. Therefore, it may be advantageous to operate at higher pH levels (≥ 3) during bioleaching with high-magnesium nickel sulphide ores. There may be potential cost savings related to solution management and waste disposal.

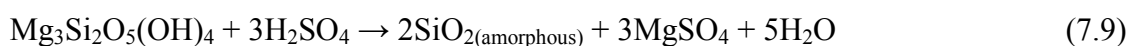
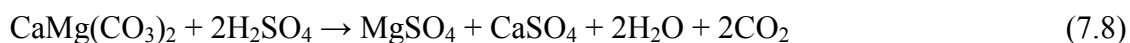
7.3.5. Acid consumption during bioleaching

Numerous chemical and biologically-mediated reactions are involved in the overall acid balance during bioleaching. The simplified stoichiometric equations describing the oxidative dissolution of pentlandite, pyrrhotite, pyrite, as well as a simple generic bivalent-metal sulphide are given in Equations 7.4 to 7.7 respectively. For simplicity, the formula for pyrrhotite has been written as FeS; although it is actually characterized by a iron-deficiency crystal lattice (Fe_{1-x}S). These equations include the biological oxidation of elemental sulphur and ferrous ion. In the absence of ferric precipitation, the complete oxidative dissolution of all sulphide minerals except disulphides is either acid consuming or acid neutral.

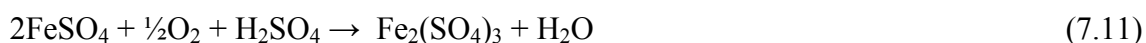




Many gangue minerals such as dolomite and lizardite dissolve under acidic conditions and consume acid (Equations 7.8 and 7.9 respectively).



Biologically-mediated reactions include the oxidation of elemental sulphur, which produces acidity, and the oxidation of ferrous ion, which consumes acidity (Equations 7.10 and 7.11 respectively). In addition, many microorganisms have the ability to oxidize reduced-sulphur compounds such as thiosulphate, which can be intermediates in the degradation of sulphide minerals (Schipper and Sand, 1999).



The precipitation of secondary ferric phases such as ferric hydroxide (Equation 7.12) and jarosite (Equation 17.3) produce acidity. It is interesting to note that the precipitation of ferric hydroxide-type compounds produces more acidity on a per mole of ferric ion basis than does jarosite.



Typically, the initial phase of a batch bioleaching process is acid consuming as the gangue minerals and the component sulphide minerals dissolve, while the later phase may be acid generating as secondary ferric compounds precipitate and reduced-sulphur by-products are oxidized by the bacteria. This cycle was most evident during bioleaching experiments at pH 3 in this study. At pH 3 with all the ores other than Ore 3, there was an initial period of acid consumption that coincided with a spike in the concentration of dissolved iron, followed by a period of acid production and a decrease in dissolved iron.

Acid consumption can be a major processing cost to a heap bioleaching operation (Watling, 2006). The mineralogy of the ore being leached is the principal factor in determining acid consumption; as such, one would expect acid consumption to follow a similar trend as the dissolution of the gangue minerals (i.e. extraction of magnesium in this study). With regard to the effect of pH, the consumption of sulphuric acid does follow the same trend as the extraction of magnesium. With each ore, operating at pH 3 produced a statistically significant decrease in the consumption of sulphuric acid compared to pH 2. This is most likely a result of less acid consumption by the gangue minerals in addition to acid generated by the precipitation of ferric-containing phases. On average, the total dissolved iron concentrations in the bioleaching experiments conducted at pH 2 were 1 to 2 orders of magnitude greater than the total dissolved iron concentrations in the bioleaching experiments at pH 3.

A large range of acid consumption was observed in this study at the three pH levels tested (Table 7.4). In general, the presence of the bacteria resulted in a statistically significant decrease in acid consumption; although some of the differences were not statistically significant at pH 2. As with the extraction of nickel, the most obvious point of comparison

with regard to the consumption of acid is between Ore 3 and the other five ores. The bioleaching of Ore 3 consumed acid at all pH levels tested, whereas with the other five ores, the bioleaching experiments were net acid consuming at pH 2 and net acid generating at pH 3 and 5. The net acid production with Ore 5 at pH 3 is somewhat unexpected, as it contains ~ 40% serpentine, which was determined to be the major acid-consuming phase during bioleaching of Ore 3 (Cameron *et al.*, 2009a).

7.4. Conclusions

A bioleaching study was conducted with six different nickel sulphide ores from different geographical locations across Canada. The primary sulphide minerals were generally the same, whereas there were large differences in the gangue minerals. The ores contained 0.3 to 1% nickel, which was present primarily in pentlandite and secondarily in pyrrhotite. The primary objective of this study was to assess the amenability of the ores to bioleaching and to identify broad trends with respect to mineralogical content on bioleaching. Stirred-tank experiments with finely ground ore were conducted to assess the effect of pH (2 to 5) and the impact of the bacteria.

All six ores showed a similar response to an increase in pH from 2 to 3; an increase in pH from 2 to 3 resulted in approximately the same extraction of both nickel and cobalt (within statistical error), and a statistically significant reduction in sulphuric acid consumption, magnesium extraction, and dissolved iron. Nickel extraction at pH 2 and 3 was generally good (49 to 86% after three weeks). Nickel extraction at pH 5 was generally poor, except with the low-pyrrhotite ultramafic-dominated ore from Manitoba (Ore 3), with which the initial nickel extraction was greater at pH 5, compared to both pH 2 and 3. Small column experiments are needed in order to determine if these trends scale up. In light of the results

obtained in this study, it is recommended that bioleaching studies with nickel sulphide ores and concentrates consider a wider pH range than what is generally considered optimum, particularly if a low-discharge process is desirable.

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Chapter 8 – Conclusions and Future Research Ideas

8.1. Conclusions and Significance of the Findings

The primary focus of this thesis was assessing the technical feasibility of applying elevated-pH bioleaching to a metamorphosed ultramafic-dominated low-grade nickel sulphide ore from Manitoba, Canada (Ore 3). The ore has a particularly challenging mineralogy that is not suited to either conventional concentration and smelting technologies or conventional bioleaching technologies. The research conducted in this thesis has arisen as a direct result of an industrial commercialization problem; consequently, it has made a direct contribution to the body of knowledge on bioleaching and has both academic and industrial implications.

Many stirred-tank experiments ranging from 10 days to 12 weeks in duration were used to define the dominant chemical, microbiological, and geochemical mechanisms in this system over a wide range of temperature and pH conditions. Experiments were designed to study the effect of nutrient levels, the presence of the bacteria, dissolved oxygen, pH, and temperature. A number of mechanisms were identified that are responsible for governing the dissolution of pentlandite and the subsequent release of nickel in the leachate, and the removal of nickel from the leachate during bioleaching.

This thesis contains a number of novel contributions to the field of bioleaching, and it is anticipated the results of this thesis will be used by other researchers. Furthermore, it is believed that a number of novel research ideas have been generated as a result of the work conducted that can be used as a starting-point for future academic and industrial research projects; several of which have been briefly detailed in Section 8.2. (Bio)leaching of nickel

sulphides at $\text{pH} > 3$ has not been documented in the technical literature, with the exception of Corrans and Scholtz (1976), which reported on the ferric sulphate leaching of a pentlandite concentrated at pH levels as high as 4. An extensive study with Ore 3 at pH 3 to 5 revealed that the rates of nickel bioleaching were statistically higher at pH 5 compared to both pH 3 and 4, over a wide range of temperature (5 to 45 °C). This trend was evident in the absence of the bacteria; although it was more pronounced with the bacteria. This observation is in contradiction to conventional bioleaching wisdom, which dictates that a low-pH environment is favourable to the (bio)leaching of sulphide minerals.

In light of the results obtained with Ore 3 in this study, it is recommended that bioleaching studies with nickel sulphide ores and concentrates consider a wider pH range than what is generally considered optimum. It is recognized that bioleaching at $\text{pH} \geq 3$ is not appropriate for sulphide ores that contain a substantial amount of copper, as extensive laboratory, pilot-scale, and full-scale studies by other authors have demonstrated. Copper is generally associated with nickel sulphide deposits but its relative importance varies significantly between ores.

During bioleaching of Ore 3, the appearance of nickel in the leachate is the product of several complementary and competing mechanisms: the acid, oxidative, and bacterial dissolution of pentlandite, which release nickel into solution; and the capture of nickel by lizardite, which removes nickel from solution. Comparisons between the biotic and abiotic experiments were used to quantify the bacterial contribution relative to the acid/oxidative contribution over a wide range of temperature and pH conditions. Further experimentation determined that the dominant mechanism responsible for dissolution of nickel at pH 5 and 30 °C appears to be oxidative in nature.

Comprehensive mineralogical characterization of the post-leach residues with XRD, SEM, and EPMA revealed substantial changes in the chemistry and structure of the dominant gangue minerals during bioleaching of Ore 3. The capture of nickel by the lizardite during bioleaching has not been documented in the technical literature. It potentially has significant implications for the (bio)leaching of high-serpentine ores, including ultramafic-dominated sulphide ores and ultramafic-dominated laterite ores. The definitive ‘capture’ mechanism is unknown at this time; however, evidence currently available suggests nickel is substituting for another cation in the lizardite matrix. If additional work determines this nickel-capture mechanism to be a result of a $\text{Ni}^{2+}/\text{Mg}^{2+}$ lattice substitution, it may provide some insight into the formation of the nickel-rich garnierite layers, which is typically found in many nickel laterite deposits.

The effect of pH was most dramatic on the behaviour of the most abundant phase lizardite. The dissolution of lizardite as a function of pH has implications for both bioleaching with similar ores and the understanding of neutralization potential during the formation of acidic mine drainage. In ultramafic-dominated ores, serpentine and olivine are a primary source of neutralization potential. The results of this thesis demonstrate that the lizardite contained in Ore 3 reacts to consume acidity at near-neutral pH levels.

A combination of classical microbiological and molecular biological techniques was used to identify and enumerate the microorganisms present during the bioleaching of Ore 3 as a function of time over a range of temperatures and pH conditions. DGGE analysis revealed the presence of at least 16 distinct 16S rRNA gene sequences, two of which are not closely related to existing GenBank sequences, and may possibly be from novel species. DGGE analysis revealed large changes in the bacterial community structure as a function of

temperature, pH, and time, strongly implying that the original microbial consortium was quite diverse and robust. Of particular interest was that a difference of two orders of magnitude in proton activity (i.e. pH 3 to 5) had an insignificant effect on the rate of growth for both iron- and sulphur-oxidizing bacteria at 5 °C. This finding may have implications for both bioleaching at sub-optimum temperatures and the formation of acidic mine drainage at low temperatures.

It is believed that one of the major obstacles preventing the adaptation of heap bioleaching practices in northern climates (such as Canada) is the lack of technical data demonstrating robust bioleaching performance under harsh climate conditions. This is of particular concern with Ore 3 as the ore body is located in a cool climatic region in Manitoba and contains a low content of sulphide minerals (< 3% wt/wt). Experiments conducted at 5 °C demonstrate robust growth of the microorganisms over a wide range of pH conditions and reasonable nickel extraction kinetics.

In a separate study conducted as part of this thesis, additional stirred-tank bioleaching experiments were conducted with six different nickel sulphide ores (Ore 3 plus five other ores) from different geographical locations across Canada. The primary sulphide minerals were generally the same, whereas there were large differences in the gangue mineral assemblages. The primary objective of this study was to assess the amenability of the ores to bioleaching and to identify broad trends with respect to the effect of mineralogical content on bioleaching. The ores were subjected to the same crushing and grinding procedure, and subjected to the same stirred-tank bioleaching tests with bacteria that were enriched from the same source. This is the only known study that has examined the bioleaching of several

different ores under identical experimental conditions, thus enabling comparisons to be made on the basis of chemical and mineralogical composition.

Nickel extraction at pH 2 and 3 was generally good (49 to 86% after three weeks). Nickel extraction at pH 5 was generally poor, except with Ore 3. All six ores showed a similar response to an increase in pH from 2 to 3; an increase in pH from 2 to 3 resulted in approximately the same extraction of both nickel and cobalt (within statistical error), and a statistically significant reduction in sulphuric acid consumption, magnesium extraction, and dissolved iron. The results of this study suggest that operating at pH levels higher than what is generally considered to be optimum might have the potential to result in cost savings during bioleaching of some nickel sulphide ores, particularly if a zero-discharge process is desirable.

8.2. Future Research Ideas

1. Bioleach Ore 3 at elevated-pH in small column experiments to see if the results scale-up. This is currently in progress at CANMET-MMSL by the author.
2. Further investigate the oxidative abiotic leaching of Ore 3 at pH 5. Perform a series of experiments at different levels of dissolved oxygen to determine a rate law for the bioleaching of Ore 3 by molecular oxygen.
3. Further investigate the capture of nickel by the lizardite in Ore 3. This is interesting from the perspective of both elevated-pH bioleaching and investigating the formation of garnierite.
4. Further investigate the fate of cobalt under elevated-pH conditions. Determine why cobalt extraction was found to lag nickel extraction when both metals originate from the same mineral.

5. Develop a process for utilizing pyrrhotite waste. Pyrrhotite was observed to be bioleached to near completion over a range of pH conditions. In a conventional concentration and smelting operation, pyrrhotite is removed during beneficiation and is placed directly in the tailings. These finely-ground pyrrhotite waste piles contain massive quantities of nickel that could potentially be recovered by bioleaching.
6. Investigate the addition of serpentine-rich waste rock to potentially acid-producing material, such as pyrrhotite tailings. This has been suggested; however, few studies exist that test this hypothesis in the laboratory.
7. Study the galvanic interactions between pentlandite and pyrrhotite, and determine the extent to which the presence of pyrrhotite suppresses the dissolution of pentlandite.

Pentlandite is the most economically important nickel sulphide mineral and pyrrhotite is the most abundant sulphide minerals in most nickel sulphide ores. Generally, they are intimately associated, and pentlandite is frequently found as inclusions within larger pyrrhotite grains. The presence of pyrrhotite is often assumed to suppress the dissolution of pentlandite; however, there is not a single study that has offered conclusive proof that galvanic interactions are involved.

Appendix A – Response Surface Methodology

Response surface modeling of the three by five experimental matrix was used to create the three dimensional graphs presented in this chapter. Minitab release 14 was used to analyze the impact of pH and temperature on five different response variables using a quadratic function of pH and temperature, with all interaction terms. The general formula for a full second degree polynomial model is given in Equation A.1, where $\beta_0, \beta_1, \beta_2, \beta_{11}, \beta_{22}$ and β_{12} are the regression constants, and Y is the predicted response.

$$Y = \beta_0 + \beta_1 T + \beta_2 pH + \beta_{11} T^2 + \beta_{22} pH^2 + \beta_{12} TpH \quad (A.1)$$

Nickel extraction, magnesium extraction, sulphuric acid consumption, the %Ni:%Mg ratio, cobalt extraction, and the %Ni:%Co ratio as a f(pH,T) after 2, 7, 14, 21, 28, and 35 days of bioleaching is presented in Figures A.1 through A.6 respectively, while the regression constants and the lack-of-fit p-values are given in Tables A.2 through A.6 respectively. The data was analyzed in coded units; however, the regression constants given in Tables A.2 through A.6 are given for uncoded units (i.e. °C and numerical pH units). The correlation between the coded units and the uncoded units is given in Table A.1.

Table A.1. Unit coding used for surface response analysis.

Temperature		pH	
Uncoded	Coded	Uncoded	Coded
5	-1	3	-1
15	-0.5	4	0
22.5	-0.125	5	1
30	0.25		
45	1		

Table A.2. Regression constants and the Lack-of-fit P-values for the response surfaces describing the extraction of nickel from Ore 3 as a function of temperature and pH.

Figure	Days leaching	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	Lack-of-fit (P)
A.C.1a.	2	32.59	-0.1082	-16.67	-0.004746	2.030	0.1845	0.035
A.C.1b.	7	-11.78	2.720	-5.190	-0.01569	1.714	-0.1963	0.167
A.C.1c.	14	-74.04	5.796	18.41	-0.04258	-0.4609	-0.5413	0.206
A.C.1d.	21	-100.2	7.233	31.06	-0.05092	-1.554	-0.8078	0.125
A.C.1e.	28	-108.9	8.054	36.04	-0.05805	-1.884	-0.9588	0.071
A.C.1f.	35	-110.7	8.089	40.02	-0.05621	-2.241	-1.023	0.048

Regression constants are given in uncoded units (°C and numerical pH units).

Table A.3. Regression constants and the Lack-of-fit P-values for the response surfaces describing the extraction of magnesium from Ore 3 as a function of temperature and pH.

Figure	Days leaching	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	Lack-of-fit (P)
A.C.2a.	2	14.65	0.8186	-5.148	0.004510	0.6806	-0.1945	0.054
A.C.2b.	7	13.36	1.076	-3.085	0.005271	0.3400	-0.2447	0.093
A.C.2c.	14	8.869	1.106	0.8669	0.005831	-0.2642	-0.2513	0.000
A.C.2d.	21	1.698	1.116	5.748	0.0073291	-0.9301	-0.2650	0.000
A.C.2e.	28	-2.167	1.178	8.269	0.007599	-1.269	-0.2796	0.000
A.C.2f.	35	-7.401	1.263	11.14	0.008275	-1.619	-0.3019	0.000

Regression constants are given in uncoded units (°C and numerical pH units).

Table A.4. Regression constants and the Lack-of-fit P-values for the response surfaces describing the consumption of sulphuric acid (g acid/ kg ore) during bioleaching of Ore 3 as a function of temperature and pH.

Figure	Days leaching	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	Lack-of-fit (P)
A.C.3a.	2	123.5	8.153	-36.97	0.04114	4.875	-1.950	0.002
A.C.3b.	7	61.77	10.61	9.463	0.04108	-1.834	-2.383	0.000
A.C.3c.	14	33.36	10.53	41.66	0.04460	-7.059	-2.369	0.000
A.C.3d.	21	-21.82	10.76	77.28	0.05152	-11.89	-2.457	0.000
A.C.3e.	28	-63.27	11.31	102.1	0.05882	-15.05	-2.628	0.000
A.C.3f.	35	-100.6	12.18	122.7	0.06690	-17.48	-2.883	0.000

Regression constants are given in uncoded units (°C and numerical pH units).

Table A.5. Regression constants and the Lack-of-fit P-values for the response surfaces describing the %Ni: %Mg ratio from Ore 3 as a function of temperature and pH.

Figure	Days leaching	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	Lack-of-fit (P)
A.C.4a.	2	8.091	-0.06045	-4.307	-0.001057	0.5633	0.03557	0.116
A.C.4b.	7	9.284	0.1254	-6.232	-0.003181	0.9489	0.02106	0.449
A.C.4c.	14	7.790	0.3119	-6.405	-0.005442	1.084	0.001812	0.230
A.C.4d.	21	7.587	0.3934	-6.592	-0.005579	1.199	-0.02303	0.374
A.C.4e.	28	7.824	0.4353	-6.863	-0.005345	1.303	-0.04183	0.424
A.C.4f.	35	8.843	0.4256	-7.295	-0.004490	1.409	-0.05541	0.167

Regression constants are given in uncoded units (°C and numerical pH units).

Table A.6. Regression constants and the Lack-of-fit P-values for the response surfaces describing the extraction of cobalt from Ore 3 as a function of temperature and pH.

Figure	Days leaching	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	Lack-of-fit (P)
A.C.5a.	2	-22.43	0.5305	11.18	-0.005533	-1.364	-0.02254	0.051
A.C.5b.	7	-102.5	3.119	45.58	-0.01135	-4.960	-0.4743	0.082
A.C.5c.	14	-135.6	5.724	58.52	-0.02741	-6.296	-0.8501	0.126
A.C.5d.	21	-134.0	6.792	58.91	-0.03374	-6.384	-1.030	0.051
A.C.5e.	28	-118.1	7.124	54.13	-0.03766	-5.946	-1.086	0.011
A.C.5f.	35	-123.8	6.988	61.47	-0.03436	-6.991	-1.115	0.008

Regression constants are given in uncoded units (°C and numerical pH units).

Table A.7. Regression constants and the Lack-of-fit P-values for the response surfaces describing the %Ni: %Co ratio from Ore 3 as a function of temperature and pH.

Figure	Days leaching	β_0	β_1	β_2	β_{11}	β_{22}	β_{12}	Lack-of-fit (P)
A.C.5a.	2	11.66	-0.09526	-5.390	0.0004256	0.6958	0.02275	0.482
A.C.5b.	7	14.55	-0.1238	-6.972	9.930×10^{-5}	0.8842	0.03612	0.181
A.C.5c.	14	15.36	-0.1478	-7.409	2.786×10^{-4}	0.9506	0.03983	0.062
A.C.5d.	21	18.63	-0.1983	-8.826	4.818×10^{-4}	1.117	0.04869	0.057
A.C.5e.	28	18.48	-0.1729	-8.980	1.457×10^{-4}	1.149	0.04746	0.149
A.C.5f.	35	19.07	-0.1479	-9.513	-2.489×10^{-4}	1.230	0.04673	0.349

Regression constants are given in uncoded units (°C and numerical pH units).

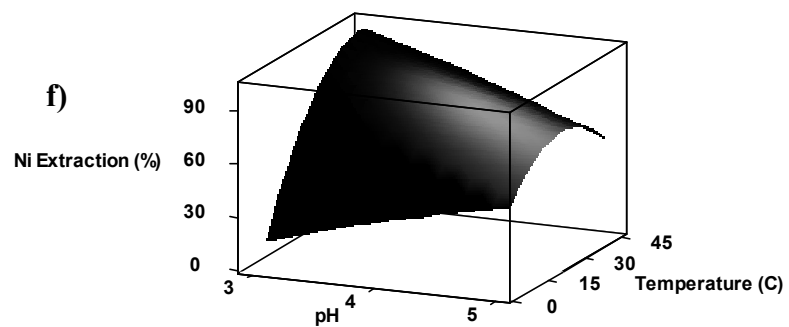
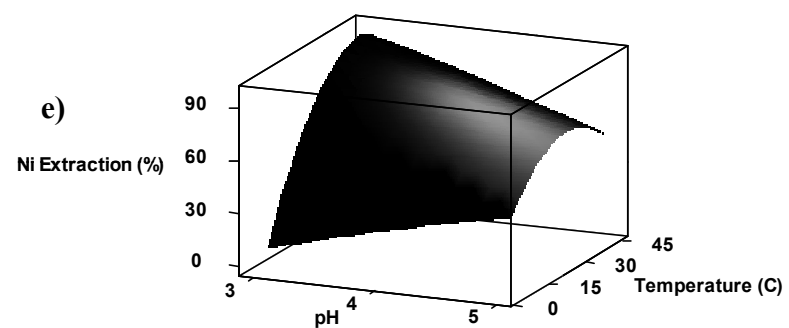
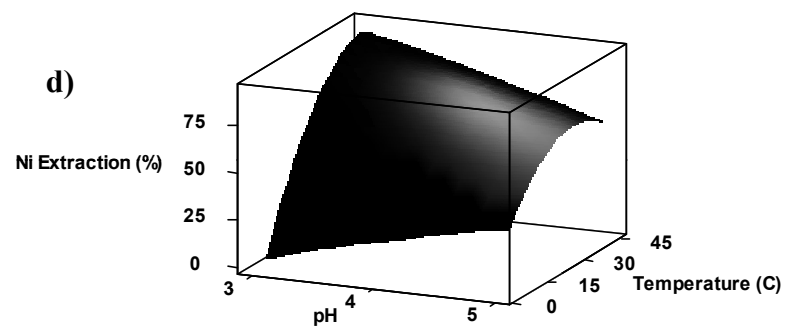
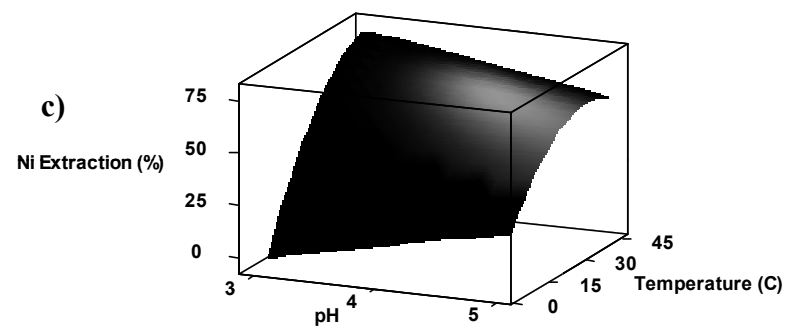
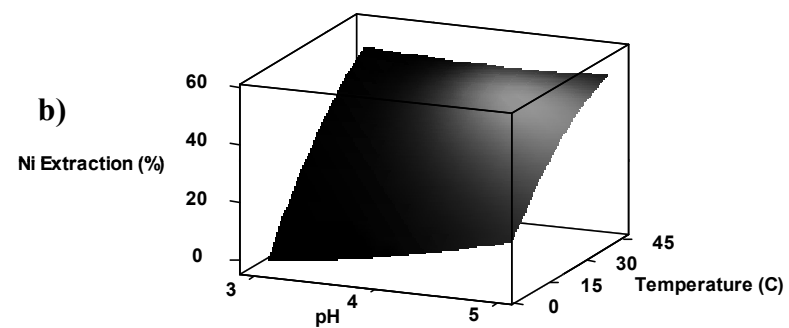
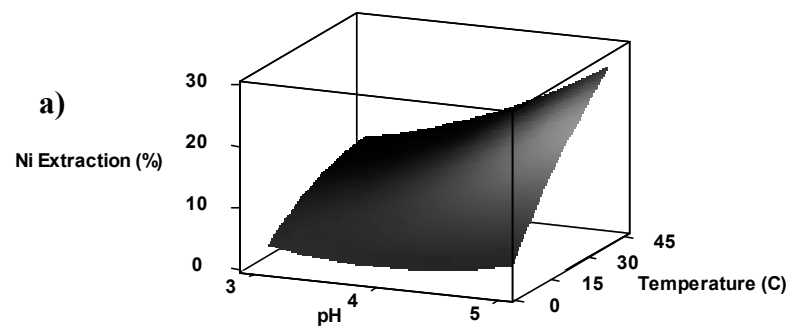


Figure A.1. Nickel extraction during bioleaching of Ore 3 as a function of pH and temperature after a) 2 days, b) 7 days, c) 14 days, d) 21 days, e) 28 days, and f) 35 days.

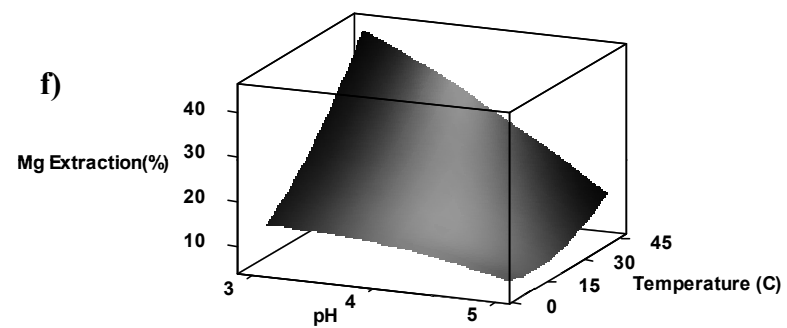
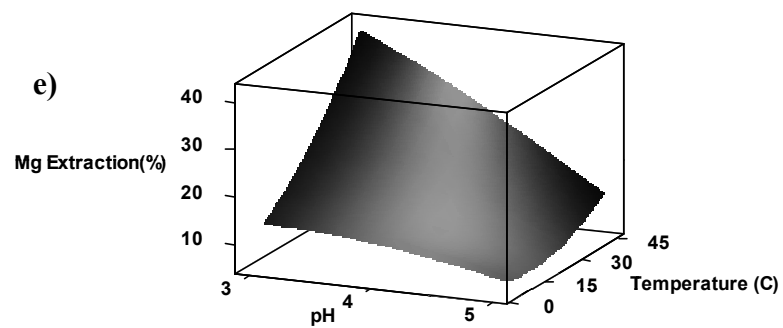
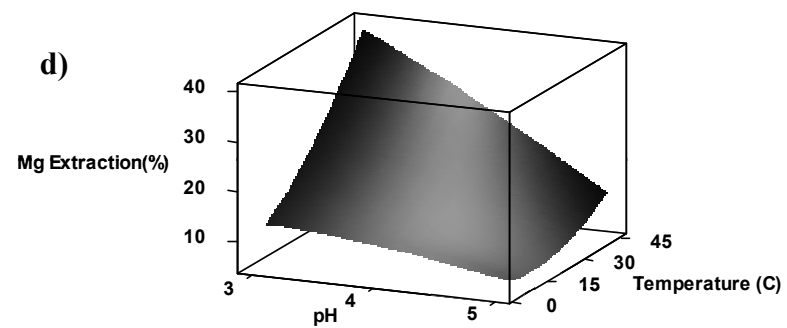
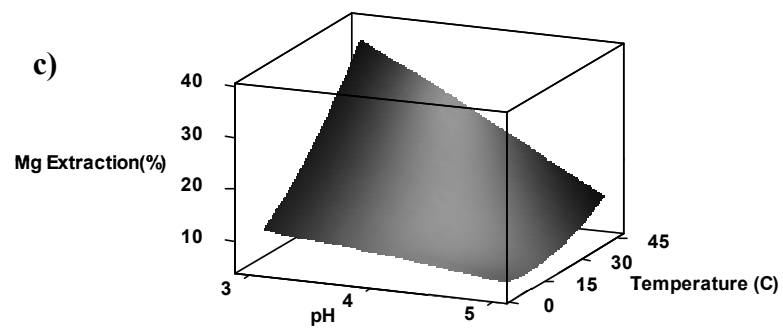
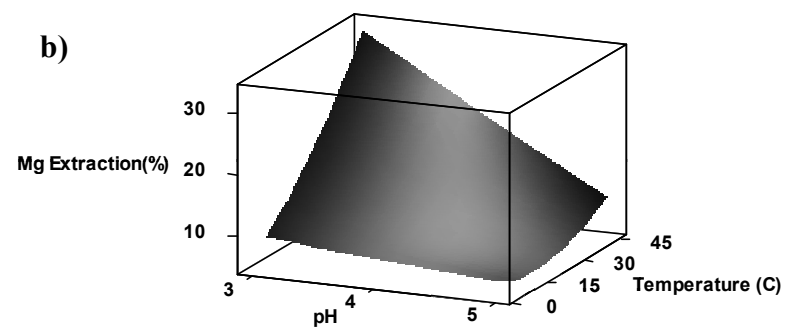
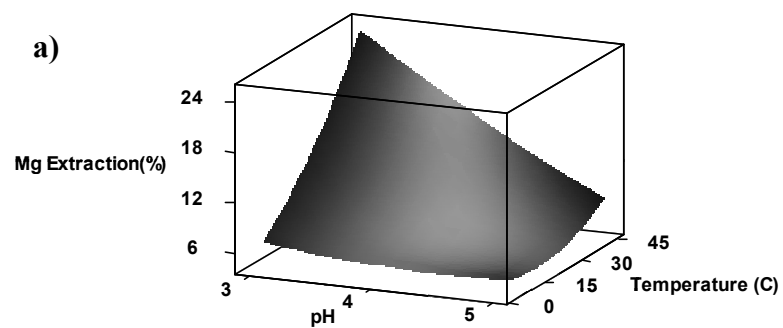


Figure A.2. Magnesium extraction during bioleaching of Ore 3 as a function of pH and temperature after a) 2 days, b) 7 days, c) 14 days, d) 21 days, e) 28 days, and f) 35 days.

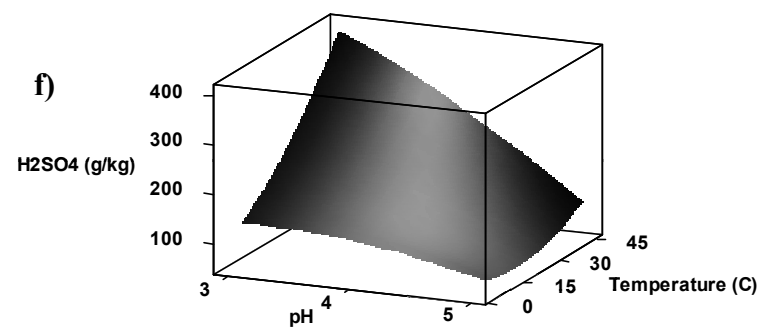
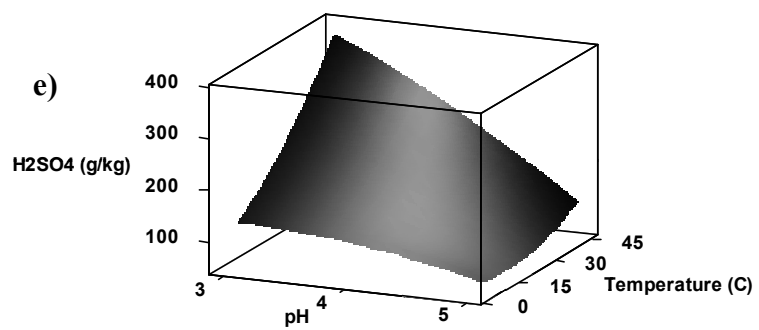
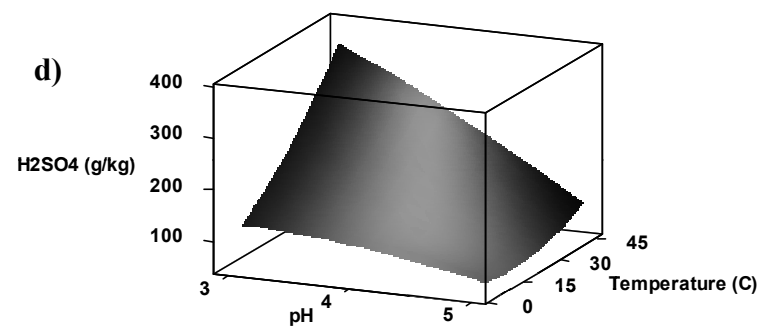
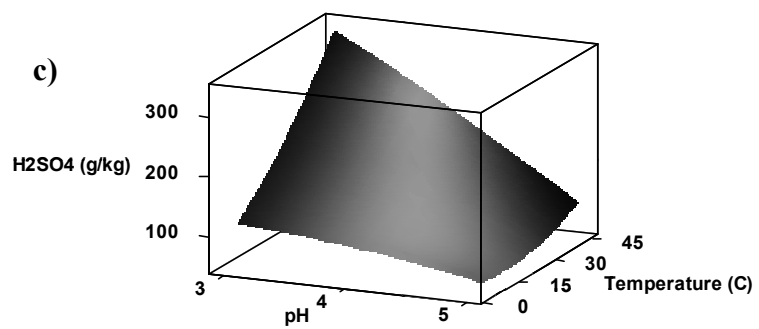
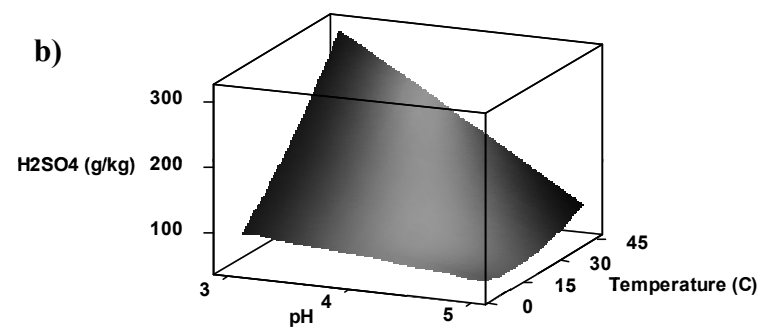
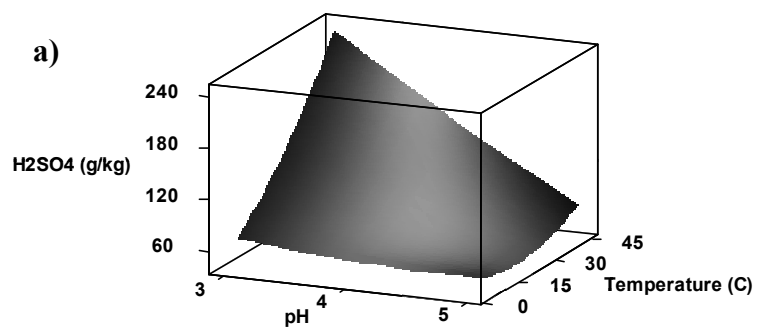


Figure A.3. Sulphuric acid consumption during bioleaching of Ore 3 as a function of pH and temperature after a) 2 days, b) 7 days, c) 14 days, d) 21 days, e) 28 days, and f) 35 days.

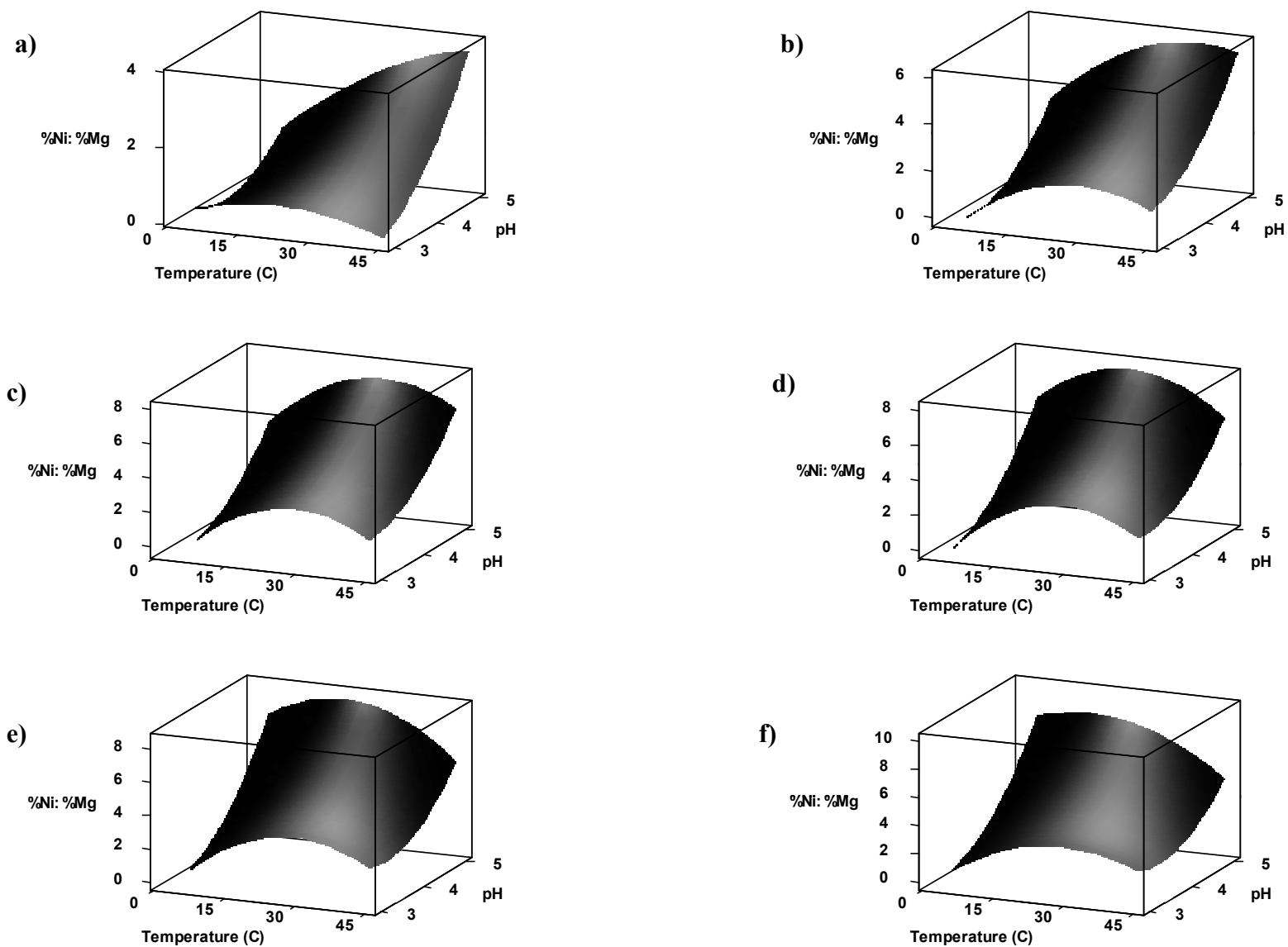


Figure A.4. The percent nickel extracted to % magnesium extracted ratio during bioleaching of Ore 3 as a function of pH and temperature after a) 2 days, b) 7 days, c) 14 days, d) 21 days, e) 28 days, and f) 35 days.

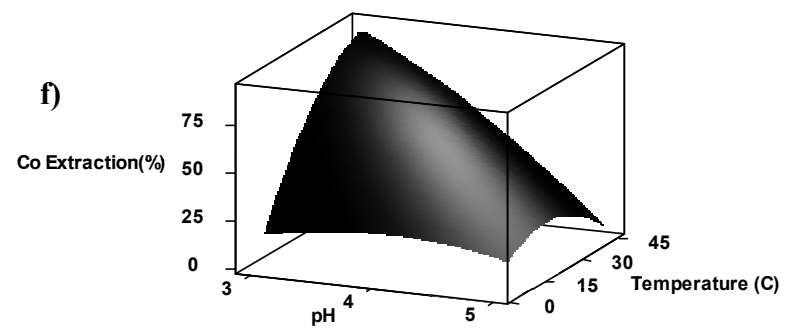
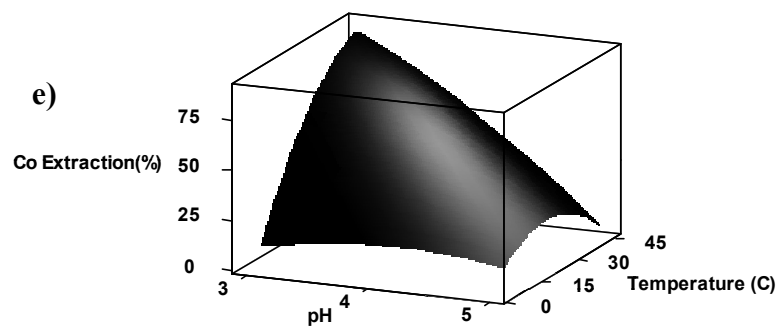
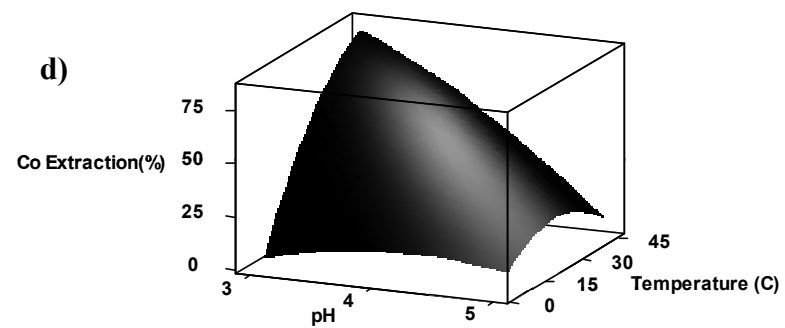
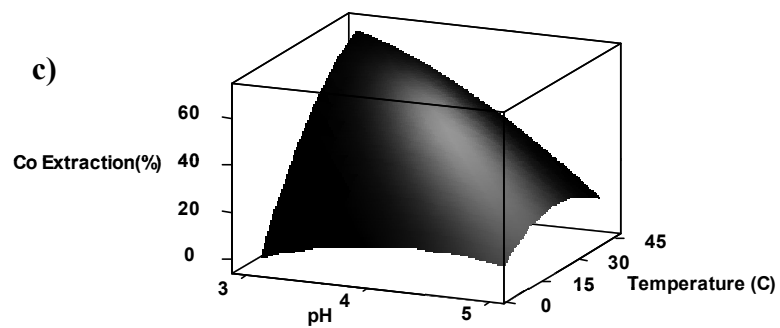
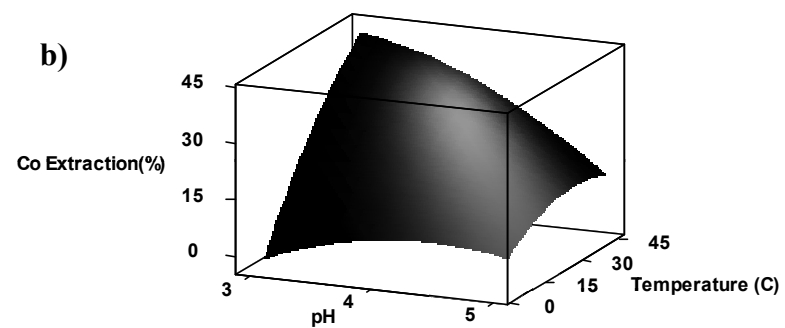
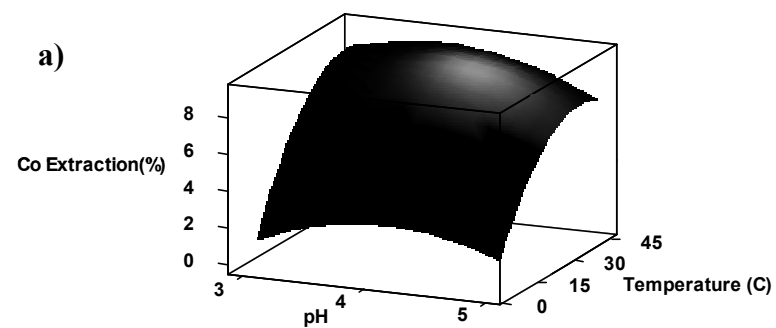


Figure A.5. Cobalt extraction during bioleaching of Ore 3 as a function of pH and temperature after a) 2 days, b) 7 days, c) 14 days, d) 21 days, e) 28 days, and f) 35 days.

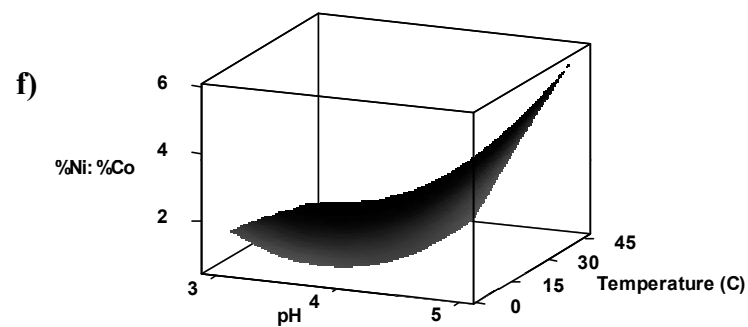
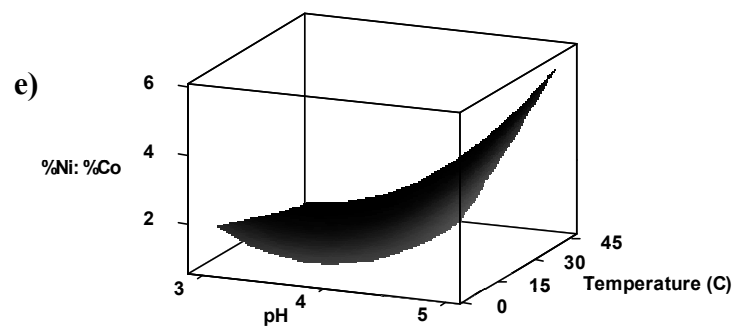
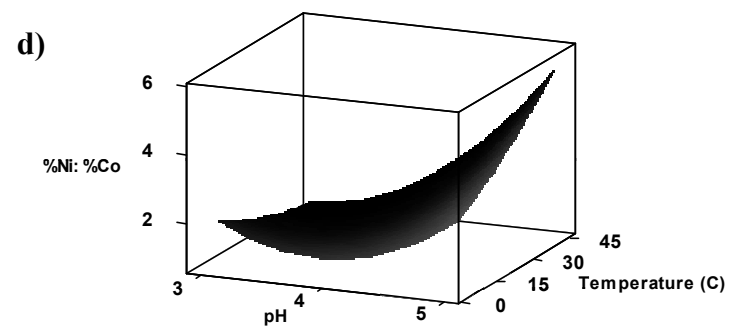
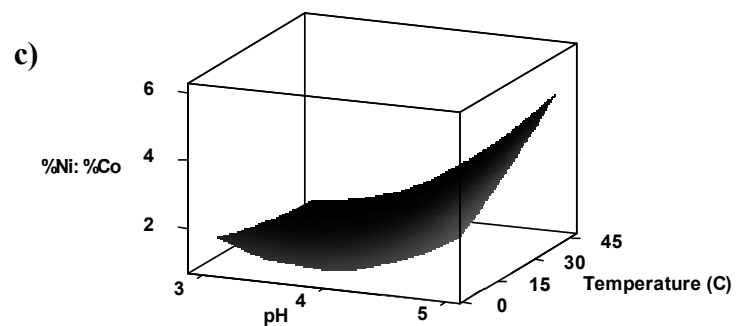
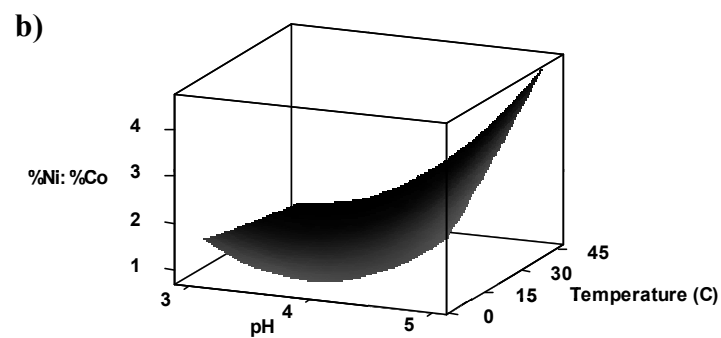
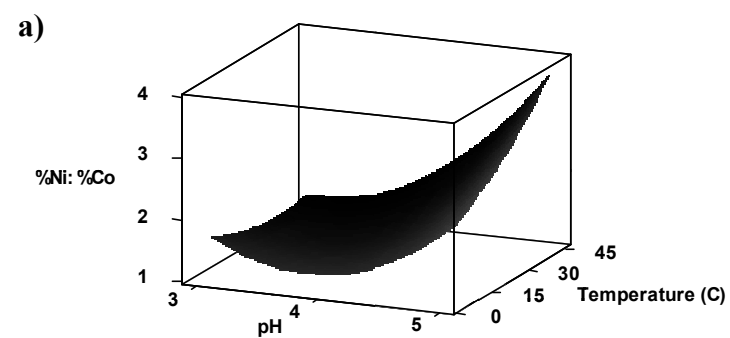


Figure A.6. The percent nickel extracted to percent cobalt extracted during bioleaching of Ore 3 as a function of pH and temperature after a) 2 days, b) 7 days, c) 14 days, d) 21 days, e) 28 days, and f) 35 days.

Appendix B – List of Stirred-tank Reactor Experiments

Completed

Table B.1. Comprehensive list of stirred-tank reactor experiments conducted.

Exp #	Ore	Experimental conditions			Primary Experimental Objective
		Temp (° C)	Media	pH	
STR 1	Ore 3	30	McCready	2	Preliminary
STR 2	Ore 3	30	McCready	3	Preliminary
STR 3	Ore 3	30	McCready	9 g S ₈	Establish a pH range
STR 4	Ore 3	30	mTK	3	Examine effect of media type
STR 5	Ore 3	30	McCready	4	Evaluate bioleaching
STR 6	Ore 3	30	McCready	5	Evaluate bioleaching
STR 7	Ore 3	30	McCready	6	Evaluate bioleaching
STR 8	Ore 3	30	McCready	2	Evaluate bioleaching
STR 9	Ore 3	30	McCready	3	Evaluate bioleaching
STR 10	Ore 3	30	McCready	4	Evaluate bioleaching
STR 11	Ore 3	30	McCready with thymol & methanol	3	Chemical control
STR 12	Ore 3	30	McCready with thymol & methanol	4	Chemical control
STR 13	Ore 3	30	McCready	6	Evaluate bioleaching
STR 14	Ore 3	30	McCready	3	Evaluate bioleaching
STR 15	Ore 3	30	McCready	5	Evaluate bioleaching
STR 16	Ore 3	30	McCready with thymol & methanol	4	Chemical control
STR 17	Ore 3	30	McCready with thymol & methanol	3	Chemical control
STR 18	Ore 3	45	McCready	3	Evaluate bioleaching
STR 19	Ore 3	45	McCready	4	Evaluate bioleaching
STR 20	Ore 3	45	McCready	5	Evaluate bioleaching
STR 21	Ore 3	15	McCready	3	Evaluate bioleaching
STR 22	Ore 3	15	McCready	4	Evaluate bioleaching
STR 23	Ore 3	15	McCready	5	Evaluate bioleaching
STR 24	Ore 3	15	McCready	3	Evaluate bioleaching
STR 25	Ore 3	15	McCready	4	Evaluate bioleaching
STR 26	Ore 3	15	McCready	5	Evaluate bioleaching
STR 27	Ore 3	30	McCready	3	Evaluate bioleaching
STR 28	Ore 3	30	McCready	4	Evaluate bioleaching
STR 29	Ore 3	45	McCready	3	Evaluate bioleaching
STR 30	Ore 3	45	McCready	4	Evaluate bioleaching

STR 31	Ore 3	45	McCready	5	Evaluate bioleaching
STR 32	Ore 3	30	McCready	3	35-day growth curve
STR 33	Ore 3	30	McCready	5	35-day growth curve
STR 34	Ore 3	30	McCready with thymol & methanol	3	Chemical control
STR 35	Ore 3	30	McCready with thymol & methanol	5	Chemical control
STR 36	Ore 3	45	McCready with thymol & methanol	3	Chemical control
STR 37	Ore 3	45	McCready with thymol & methanol	5	Chemical control
STR 38	Ore 3	15	McCready with thymol & methanol	3	Chemical control
STR 39	Ore 3	15	McCready with thymol & methanol	5	Chemical control
STR 40	Ore 3	30	McCready	3	10-day growth curve
STR 41	Ore 3	30	McCready	5	10-day growth curve
STR 42	Ore 1	30	McCready	3	Bioleaching
STR 43	Ore 1	30	McCready	5	Evaluate bioleaching
STR 44	Ore 2	30	McCready	3	Evaluate bioleaching
STR 45	Ore 2	30	McCready	5	Evaluate bioleaching
STR 46	Ore 3	22.5	McCready	3	Evaluate bioleaching
STR 47	Ore 3	22.5	McCready	5	Evaluate bioleaching
STR 48	Ore 3	22.5	McCready	3	Evaluate bioleaching
STR 49	Ore 3	22.5	McCready	5	Evaluate bioleaching
STR 50	Ore 2	30	McCready	3	Evaluate bioleaching
STR 51	Ore 2	30	McCready	5	Evaluate bioleaching
STR 52	Ore 1	30	McCready	3	Evaluate bioleaching
STR 53	Ore 1	30	McCready	5	Evaluate bioleaching
STR 54	Ore 4	30	McCready	3	Evaluate bioleaching
STR 55	Ore 4	30	McCready	5	Evaluate bioleaching
STR 56	Ore 4	30	McCready	3	Evaluate bioleaching
STR 57	Ore 4	30	McCready	5	Evaluate bioleaching
STR 58	Ore 3	5	McCready	3	Evaluate bioleaching
STR 59	Ore 3	5	McCready	4	Evaluate bioleaching
STR 60	Ore 3	5	McCready	5	Evaluate bioleaching
STR 61	Ore 3	5	McCready	3	Evaluate bioleaching
STR 62	Ore 3	5	McCready	4	Evaluate bioleaching
STR 63	Ore 3	5	McCready	5	Evaluate bioleaching
STR 64	Ore 3	22.5	McCready	4	Evaluate bioleaching
STR 65	Ore 3	22.5	McCready	4	Evaluate bioleaching
STR 66	Ore 3	5	McCready with thymol & methanol	3	Chemical control
STR 67	Ore 3	5	McCready with thymol & methanol	5	Chemical control

STR 68	Ore 3	30	McCready with thymol & methanol	5	Baseline control for STR 67
STR 69	Ore 3	30	McCready with thymol, methanol, and N ₂	5	Determine primary oxidant sparged with N ₂
STR 70	Ore 1	30	McCready with thymol & methanol	2	Chemical control
STR 71	Ore 2	30	McCready with thymol & methanol	2	Chemical control
STR 72	Ore 2	30	McCready	2	Evaluate bioleaching
STR 73	Ore 2	30	McCready	2	Evaluate bioleaching
STR 74	Ore 4	30	McCready with thymol & methanol	2	Chemical control
STR 75	Ore 5	30	McCready with thymol & methanol	2	Chemical control
STR 76	Ore 1	30	McCready with thymol & methanol	3	Chemical control
STR 77	Ore 2	30	McCready with thymol & methanol	3	Chemical control
STR 78	Ore 1	30	McCready	2	Evaluate bioleaching
STR 79	Ore 1	30	McCready	2	Evaluate bioleaching
STR 80	Ore 4	30	McCready	2	Evaluate bioleaching
STR 81	Ore 4	30	McCready	2	Evaluate bioleaching
STR 82	Ore 4	30	McCready with thymol & methanol	3	Chemical control
STR 83	Ore 5	30	McCready with thymol & methanol	3	Chemical control
STR 84	Ore 5	30	McCready	2	Evaluate bioleaching
STR 85	Ore 5	30	McCready	2	Evaluate bioleaching
STR 86	Ore 5	30	McCready	3	Evaluate bioleaching
STR 87	Ore 5	30	McCready	3	Evaluate bioleaching
STR 88	Ore 5	30	McCready	5	Evaluate bioleaching
STR 89	Ore 5	30	McCready	5	Evaluate bioleaching
STR 90	Ore 5	30	mTK	2	Evaluate bioleaching
STR 91	Ore 5	30	McCready	2	Evaluate bioleaching
STR 92	Ore 5	30	McCready	3	Evaluate bioleaching
STR 93	Ore 5	30	McCready	5	Evaluate bioleaching
STR 94	Ore 3	5	McCready	3	Bacterial identification
STR 95	Ore 3	5	McCready	5	Bacterial identification
STR 96	Ore 3	15	McCready	3	Bacterial identification
STR 97	Ore 3	30	McCready	3	Bacterial identification
STR 98	Ore 3	30	McCready	5	Bacterial identification
STR 99	Ore 3	45	McCready	3	Bacterial identification
STR 100	Ore 6	30	McCready	2	Evaluate bioleaching
STR 101	Ore 6	30	McCready	3	Evaluate bioleaching
STR 102	Ore 6	30	McCready	2	Evaluate bioleaching
STR 103	Ore 6	30	McCready	3	Evaluate bioleaching
STR 104	Ore 6	30	McCready with thymol & methanol	2	Chemical control

STR 105	Ore 6	30	McCready with thymol & methanol	3	Chemical control
STR 106	Ore 6	30	McCready	5	Evaluate bioleaching
STR 107	Ore 6	30	McCready	5	Evaluate bioleaching

Appendix C – Representative Sample Calculations

Experiments conducted with heterogeneous solid materials require a rigorous sampling protocol to ensure repeatability. Prior to sample preparation, a minimum sample size was determined for each step in the crushing/grinding process based on a maximum fundamental error of 5% per step. A liberation diameter of 100 μm was estimated based on similar ores, which turned out to be a conservative estimate, as liberation analysis determined the actual liberation diameter of pentlandite in all the ores to be less than 100 μm (Lastra *et al.* (2007a; 2007b; 2008; 2009a; 2009b; 2010)).

Gy's sampling theory (Pitard, 1993) was used to determine the minimum sample size as a function of the nominal crush size (d_N) and the acceptable fundamental error (σ_{FSE}) (Equation C.1). M_S and M_L are the sample and lot mass respectively, and f is the shape factor. The parameters c , g , and l may be calculated according to Equations C.2 to C.4, where α is the fraction of the mineral of interest; ρ_M , ρ_G , and ρ are the densities of the minerals of interest, the gangue, and the ore respectively; v_{bar} is the average particle volume; v_N is the volume of the largest particle (nominal); and d_l is the liberation diameter. For calculations, the shape factor was chosen to be 0.5 (spherical), and g was estimated to be 0.25 (typical of non-calibrated material).

$$\sigma_{FSE}^2 = clfgd_N^3 \left(\frac{1}{M_S} - \frac{1}{M_L} \right) \quad (\text{C.1})$$

where,

$$c = \frac{(1 - \alpha) / \alpha}{\rho_M \rho_G / \rho}, \quad (\text{C.2})$$

$$g = \frac{\bar{v}}{v_N}, \text{ and} \quad (\text{C.3})$$

$$l = \left(\frac{d_l}{d_N} \right)^{0.5} \quad (\text{C.4})$$

The different ores were received in bulk samples ranging in size from 100 to 2000 kg, and each was subjected to the same crushing and grinding protocol. Each bulk sample was crushed to -12.7 mm, and after thorough mixing, a sub-sample of 5 to 10 kg was crushed to -6.35 mm. From the sub-sample, a portion was used for mineralogical characterization, and a portion was pulverized to -147 μm (100 Tyler mesh) and used for stirred-tank (bio)leaching experiments and bacterial culture maintenance. Large sub-samples were obtained with a Jones splitter, while small sub-samples for the individual reactions were obtained by coning and quartering or by using a rotary splitter. Table C.1 gives examples of the minimum sample size for the sampling protocol described above. For each step in the crushing and grinding procedure, the fundamental error was between 1 and 5%.

Table C.1. Minimum sample size calculation examples.

Lot size (kg)	Nominal diameter	Fundamental error (%)	Minimum sample size
600	12.7 mm ($\frac{1}{2}$ ")	5.0 1.0	3.4 kg 75 kg
10	6.35 mm ($\frac{1}{4}$ ")	5.0 1.0	570 g 6.0 kg
2.5	147 μm	5.0 1.0	0.05 g 1.2 g

References:

- Lastra, R., Wilson, J., Smith, D., Thibault, Y., 2007a. Mineralogical characterization of ore 1 for the project on the bioleaching of low-grade nickel sulphide ores. Natural Resources Canada, Mining and Mineral Sciences Laboratories, Report MMSL 07-118(TR).
- Lastra, R., Wilson, J., Smith, D., Thibault, Y., 2007b. Mineralogical characterization of ore 2 for the project on the bioleaching of low-grade nickel sulphide ores. Natural Resources Canada, Mining and Mineral Sciences Laboratories, Report MMSL 07-117(TR).

- Lastra, R., Wilson, J., Smith, D., Thibault, Y., 2008. Mineralogical characterization of ore 3 for the project on the bioleaching of low-grade nickel sulphide ores. Natural Resources Canada, Mining and Mineral Sciences Laboratories, Report MMSL 08-012(TR).
- Lastra, R., Thibault, Y., Smith, D., 2009a. Mineralogical characterization of ore 4 for the project on the bioleaching of low-grade nickel sulphide ores. Natural Resources Canada, Mining and Mineral Sciences Laboratories, Report MMSL 09-048(TR).
- Lastra, R., Thibault, Y., Smith, D., 2009b. Mineralogical characterization of ore 5 for the project on the bioleaching of low-grade nickel sulphide ores. Natural Resources Canada, Mining and Mineral Sciences Laboratories, Report MMSL 09-093(TR).
- Lastra, R., Thibault, Y., Smith, D., 2010. Mineralogical characterization of ore 6 for the project on the bioleaching of low-grade nickel sulphide ores. Natural Resources Canada, Mining and Mineral Sciences Laboratories, in progress.
- Pitard, F.F., 1993. Sampling Theory and Sampling Practice- Heterogeneity, Sampling Correctness, and Statistical Process Control, second edition, CRC Press.

Appendix D – Metal Analyses and Measured Parameters during the STR Experiments

Appendix D contains 108 tables of metal analyses and measured parameters from the stirred-tank experiments that were conducted for this thesis. Two examples of those tables are provided below. The remainder of the data is available from the author upon request. Acid consumption was measured by volume but it has been converted to grams of concentrated sulphuric acid per kg ore. In addition, Table D.108 contains the weight, and the metal and sulphur analyses of the post-leach residues.

Table D.1. Metal concentrations, ORP, and sulphuric acid consumption as f(t) during bioleaching experiment (STR 1) with Ore 3 at 30 °C pH 2 with McCready media.

Day	Co (ppm)	Cu (ppm)	Fe (ppm)	Mg (ppm)	Ni (ppm)	ORP (mV)	Acid consumed (g acid/Kg ore)
0	0.47	3.62	72.6	833.8	10.2	476.2	9.8
0.042	0.51	4.16	97.36	1564	10.7	425.7	89.5
0.13	0.87	3.72	120.4	2030	11.1	404.5	115.3
0.21	1.15	3.59	139.3	2265	11.6	405.8	132.5
1.13	0.93	3.61	82.45	3705	24	503.6	196.3
5.13	4.26	4.31	393.1	6326	114.2	597.7	309.1
8.13	6.07	4.92	520.7	7031	155.2	646.5	350.8
12.13	7.12	5.31	687.3	7885	201.3	652.3	387.6
20.13	9.65	5.81	692	8108	230	669.3	430.6
28.13	9.4	6.86	765.3	9095	248	657.8	463.7
34.13	9.7	8.06	755.7	9902	261.8	663.3	490.7
41.13	11.29	8.73	1006	10881	278.7	663.4	525.0
62.13	10.44	8.49	1082	11802	275	629.6	586.3

Table D.2. Metal concentrations, ORP, and sulphuric acid consumption as f(t) during bioleaching experiment (STR 2) with Ore 3 at 30 °C pH 3 with McCready media.

Day	Co (ppm)	Cu (ppm)	Fe (ppm)	Mg (ppm)	Ni (ppm)	ORP (mV)	Acid consumed (g acid/Kg ore)
0	0.35	0.13	6.1	581	7.1	303.5	9.8
0.042	0.42	1.08	34.3	1117	10.1	381.7	46.6
0.13	0.47	1.02	38.5	1475	11.8	403.8	66.9
0.21	0.44	1.03	33.5	1666	12.7	390.6	77.3
1.13	0.97	1.08	9.4	2556	25.3	460.5	118.4
5.13	3.86	0.925	5.68	4514	103.6	536.3	192.6
8.13	5.02	1.1	7.5	4572	136.4	563.1	204.2
12.13	7.39	2.13	45.05	5140	205.6	595.7	222.0
20.13	10.41	2.1	13.34	5493	262.2	593.2	231.2
28.13	10.51	6.03	38.22	5653	274.7	586.8	239.2
34.13	10.35	4.07	34.05	5882	284.1	588.4	246.6
41.13	10.95	3.27	44.11	6013	284.4	602.5	253.9
62.13	10.22	2.75	13.09	5945	277.9	524.7	265.6

Appendix E – Sample Calculations

E.1. Apparent activation energy calculation:

Chemical leaching follows Arrhenius' law, which describes the reaction rate constant (k) as a function of temperature (T) and activation energy (E) according to Equation E.1, where: A is a frequency factor, and R is the ideal gas constant.

$$k = A * e^{\frac{-E}{RT}} \quad (\text{E.1})$$

Equation E.1 rearranges to:

$$\ln(k) = -\frac{E}{RT} - \ln(A) \quad (\text{E.2})$$

Therefore a plot of $\ln(k)$ versus $1/T$ will should yield a straight line with slope $-E/R$ if the reaction is conducted under chemical reaction control. In this study, the slope of the nickel extraction curve at $t = 0$ has been used to estimate the initial reaction rate k' (which is proportional to the reaction rate constant k). The leaching curves were fitted to a second order polynomial and the initial extraction rates were calculated from slopes of the polynomials evaluated at $t = 0$.

An example of the application of this procedure is given below. Figure E.1 shows the nickel extraction curves during abiotic leaching of Ore 3 at 5, 15, 30, and 45 °C, where α is the fraction of nickel in solution ($0 \leq \alpha \leq 1$). Excel has been used to fit a second order polynomial to each curve, and the equation of the fitted curve with its corresponding R^2

value has been given on the right. The initial reaction rate (day^{-1}) has been estimated by the slope of each line at $t = 0$. For example, at pH 3 45 °C:

$$k'_{45^\circ\text{C}} = -0.0008t^2 + 0.0525t - 0.0049 \quad (\text{E.3})$$

$$\therefore \left[\frac{dk'_{45^\circ\text{C}}}{dt} \right]_{t=0} = 0.0525 \frac{1}{\text{day}} \quad (\text{E.4})$$

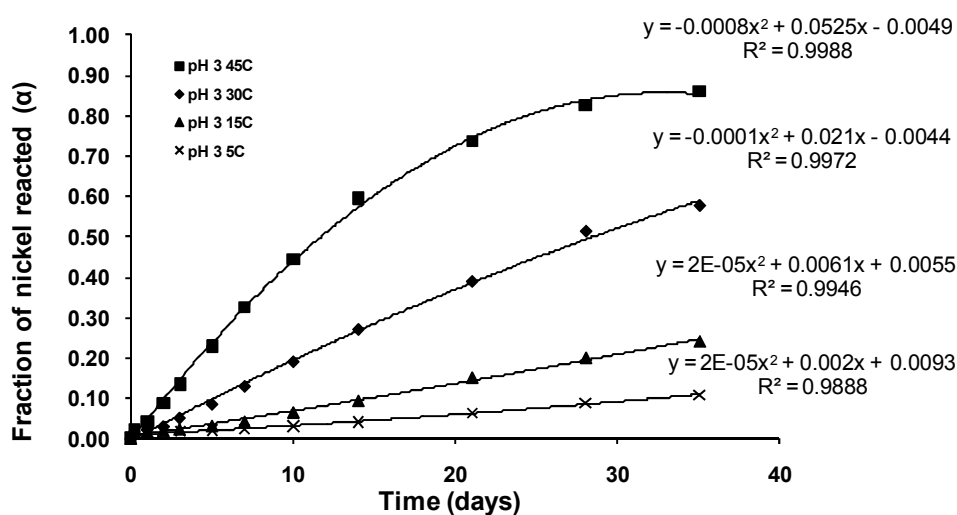


Figure E.1. The extraction of nickel during abiotic leaching of Ore 3 at pH 3 and different temperatures.

The slope of the fitted curve at $t = 0$ was determined for each temperature, and the linear regression function in Excel was used to fit a straight line through a plot of the natural logarithm of the initial reaction rate versus $1000/T$ (K^{-1}) at the different temperatures (referred to as an Arrhenius plot), shown in Figure E.2.

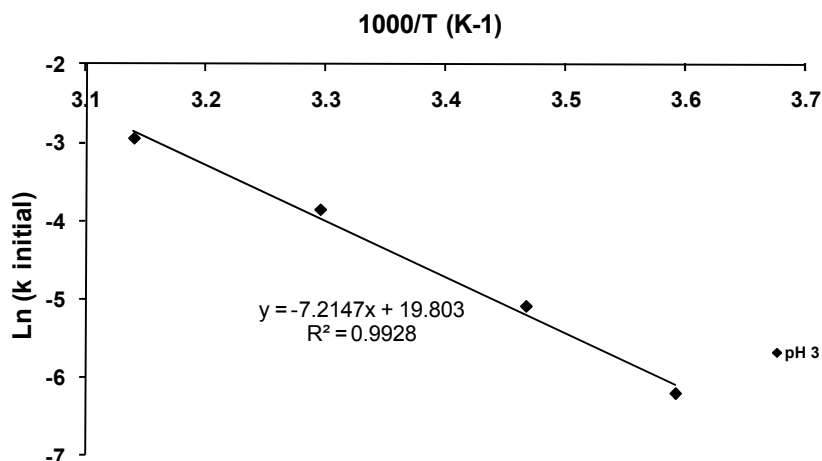


Figure E.2. Arrhenius plots for the leaching of pentlandite from Ore 3 at pH 3.

The activation energy was determined from the fitted linear equation, according to:

$$\text{slope} = -7.2147 = -\frac{E}{R} \quad (\text{E.5})$$

$$\therefore E = 7.2147 \frac{1000K}{1} \times 8.314 \frac{J}{mol \cdot K} \times \frac{KJ}{1000J} = 59.98 \frac{KJ}{mol} \quad (\text{E.6})$$

The 80% confidence intervals of the slope was determined from the “upper 80%” and “lower 80%” in the regression output (bold and yellow in Figure E.3), and this error was propagated to the estimate of E (Equation E.7).

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.99637015							
R Square	0.992753476							
Adjusted R Square	0.989130214							
Standard Error	0.148823492							
Observations	4							
ANOVA								
	<i>df</i>	<i>SS</i>	<i>MS</i>	<i>F</i>	<i>Significance F</i>			
Regression	1	6.068545899	6.068545899	273.9943814	0.00362985			
Residual	2	0.044296864	0.022148432					
Total	3	6.112842763						
	<i>Coefficients</i>	<i>Standard Error</i>	<i>t Stat</i>	<i>P-value</i>	<i>Lower 95%</i>	<i>Upper 95%</i>	<i>Lower 80.0%</i>	<i>Upper 80.0%</i>
Intercept	19.80320504	1.471983966	13.45341084	0.005479666	13.46976921	26.13664086	17.02760546	22.57880462
X Variable 1	-7.214707064	0.435860862	-16.55277564	0.00362985	-9.090064992	-5.339349135	-8.036574187	-6.39283994

Figure E.3. The summary output from a linear regression in Excel.

$$E = \left[59.98 \frac{\text{KJ}}{\text{mol}} \pm \frac{(8.0365 - 6.3928)}{2} \right] \times 8.314 = 60 \pm 7 \frac{\text{KJ}}{\text{mol}} \quad (\text{E.7})$$

E.2. Calculation of bacterial doubling time:

Bacterial doubling times can be calculated from the growth curves during exponential growth. A plot of the natural logarithm of the bacterial densities during exponential growth versus time produces a straight line with a slope equal to the specific growth rate μ (time^{-1}), which is related to the doubling time T_2 (time) by equation E.8.

$$T_2 = \frac{\ln 2}{\mu} \quad (\text{E.8})$$

For example, the concentration of iron-oxidizing bacteria at 5 °C pH3 (STR is given in Figure E.4. The natural logarithm of the concentration of bacteria versus time is plotted in Figure E.5.

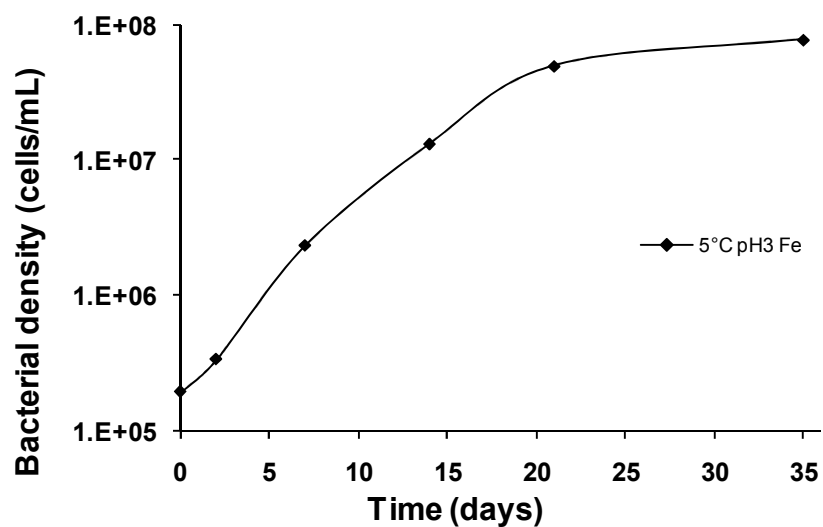


Figure E.4. The concentration of iron-oxidizing bacteria (cell/mL) as a function of time at 5 °C pH 3.

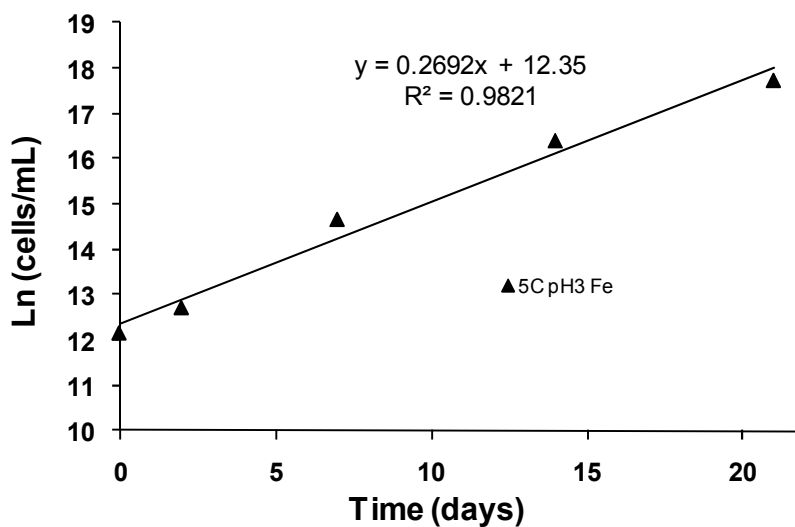


Figure E.5. The natural logarithm of the concentration of iron-oxidizing bacteria (cell/mL) as a function of time at 5 °C pH 3.

It should be noted that only the points that lie along a straight line were used in calculating the slope. In this example, only the first 21 days were used, although the experiment lasted 35 days. The doubling time during exponential growth was calculated according to Equation E.8. The 80% confidence interval in μ was calculated from the regression output in Excel (Figure E.6).

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.9910095							
R Square	0.982099829							
Adjusted R Square	0.976133106							
Standard Error	0.365074556							
Observations	5							
ANOVA								
	df	SS	MS	F	Significance F			
Regression	1	21.93728354	21.93728354	164.5961665	0.001021935			
Residual	3	0.399838295	0.133279432					
Total	4	22.33712183						
	Coefficients	Standard Error	t Stat	P-value	Lower 95%	Upper 95%	Lower 80.0%	Upper 80.0%
Intercept	12.35025797	0.246458144	50.11097527	1.75005E-05	11.56591816	13.13459778	11.94662254	12.7538934
X Variable 1	0.26916184	0.02097991	12.82950375	0.001021935	0.202394402	0.335929279	0.23480211	0.30352157

Figure E.6. The summary output from a linear regression in Excel.

The upper 80% and lower 80% doubling times were calculated from the “upper 80%” and “lower 80%” in the regression output (bold and yellow in Figure E.6), shown in Equations E.9 and E.10 respectively. Equation E.11 shows the calculated bacterial doubling time with the 80% confidence interval.

$$\text{upper 80\% doubling time} = \frac{\ln 2}{0.3035 \text{ day}^{-1}} \times \frac{24 \text{ hours}}{1 \text{ day}} = 54.8 \text{ hours} \quad (\text{E.9})$$

$$\text{lower 80\% doubling time} = \frac{\ln 2}{0.2348 \text{ day}^{-1}} \times \frac{24 \text{ hours}}{1 \text{ day}} = 70.8 \text{ hours} \quad (\text{E.10})$$

$$\therefore T_2 = \frac{\ln 2}{0.2691 \text{ day}^{-1}} \times \frac{24 \text{ hours}}{1 \text{ day}} \pm \frac{(70.8 - 54.8)}{2} = 62 \pm 8 \text{ hours} \quad (\text{E.11})$$

E.3. Significance testing of the data:

Significance testing was performed by analyzing the data using the one-way analysis of variance (ANOVA) function in Minitab release 14. Figure E.7 displays an example of the analysis used to identify significant differences in nickel extraction after 35 days of bioleaching with pH as the factor. In this example, a 95% confidence interval was used. Significant differences were identified by visual inspection of the figure (i.e. overlapping error bars indicates a difference that is not significant).

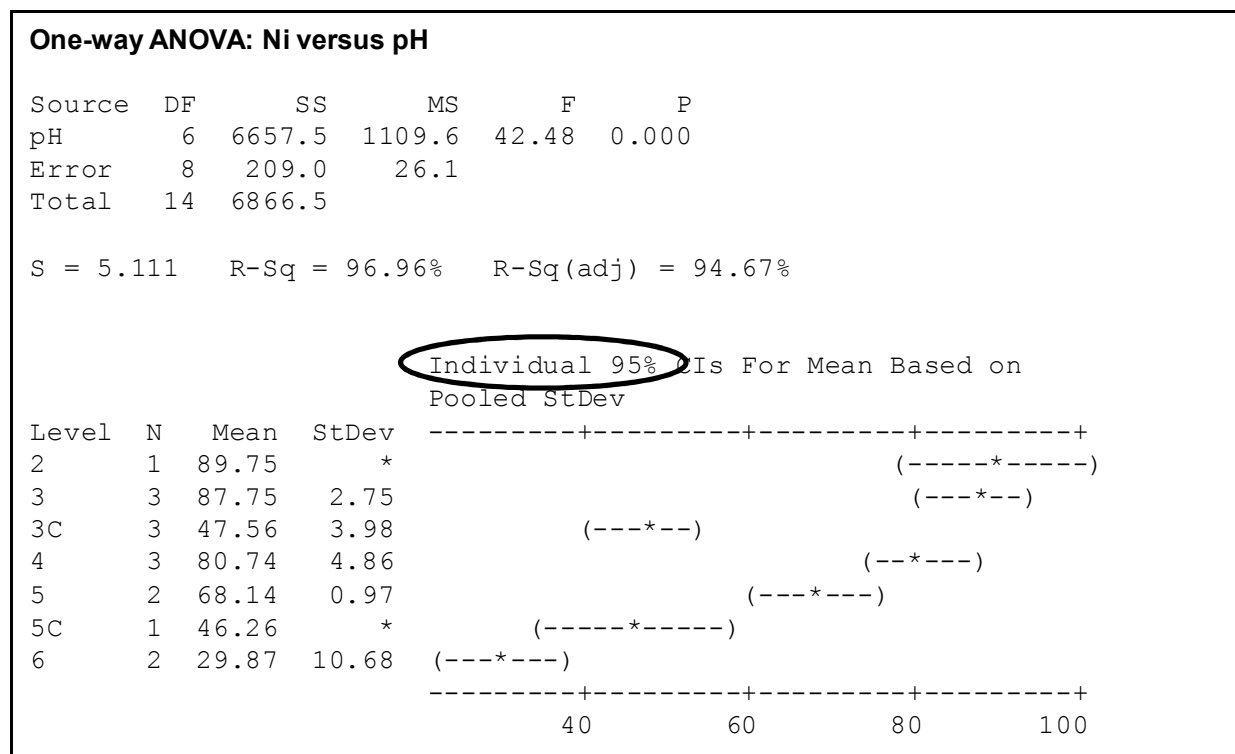


Figure E.7. One-way ANOVA analysis report from Minitab release 14.

E.4. Metal extraction calculations:

A mass balance approach has been used to calculate metal extraction curves that have been modified by the appropriate dilution factors to account for sample removal. The nickel determinations from STR 5 (30 °C pH 4 with Ore 3) have been used as an example (Figure E.8).

	A	B	C	D	E	F	G
	Days from t=0	Sample Volume (ml)	Ni (ppm)	Total Ni lost due to sampling (ppm)	Cumulative Ni loss due to sampling (ppm)	Adjusted [Ni] in solution (ppm)	Ni Recovery (%)
1					0		
2	0	5	4.680	0.016	0.016	4.680	1.53%
3	2	5	53.210	0.177	0.193	53.226	17.44%
4	7	5	145.000	0.483	0.676	145.193	47.57%
5	14	5	269.100	0.897	1.573	269.776	88.38%
6	21	5	280.200	0.934	2.507	281.773	92.31%
7	28	5	273.700	0.912	3.420	276.207	90.49%
8	35	5	285.900	0.953	4.373	289.320	94.78%

Figure E.8. Nickel determinations during bioleaching experiment STR 5 with Ore 3.

This example refers to the calculation of nickel extraction after 7 days of bioleaching (i.e. cell G4). In accordance with Figure E.8, the following cells are defined: A4 - day on which the sample was taken (day 7); B3 - volume of leachate that was removed during the previous sampling day (day 2 in this case); and C3 & C4 - metal concentrations in the leachate on days 2 and 7 respectively. It is important to keep in mind that the total volume used during the STR experiments was 1500 mL, and 100% nickel extraction would result in 305.3 ppm nickel in the leachate. The following cell calculations are made:

D3 - nickel lost due to sampling on day 2 = $C3 \cdot (B3/1500) = 0.177$ ppm;

E3 - cumulative nickel loss on all days prior to day 7 = $D3 + E2 = 0.193$ ppm;

F4 - adjusted nickel in solution after 7 days = $C4 + E3 = 145.19$ ppm; and

G4 - The calculated nickel extraction after 7 days = $(F4/305.3) \cdot 100\% = 47.5\%$.