CHAPTER 1

INTRODUCTION

As global technologies and civilisation have advanced, the utilization of diverse mineral products has increased, resulting in the progressive depletion of high-grade mineral deposits (Jian and Sharma, 2004). Consequently, metal production has to rely more on the use of lower-grade or complex ores, as well as metal extraction from mining and industrial wastes (Torma, 1986; Ehrlich, 1999; Costa *et al*., 2003). Since 1986, depletion of the richer iron ore deposits (>60% Fe; <0.24% K) worldwide necessitated the processing of lower-quality iron ore (<60% Fe; >0.24% K) (Personal communication[*]). Impurities, such as phosphorous (P) and potassium (K) contained within the lower-quality iron ore have a detrimental effect on the steel-making process, and therefore, steel-making plants charge penalties when purchasing iron ore with P and K levels exceeding 0.24%.

Smelting in blast furnaces is adversely affected by alkali's, such as K (Yusfin *et al*., 1999). Compounds of alkali metals are deposited on the surface of the coke, where they act as a catalyst in the gasification of carbon in the presence of CO_2 (Yusfin *et al*., 1999). The coke strength is reduced as a result of the gasification reaction occurring at lower temperatures (Yusfin *et al*., 1999). In addition to speeding up the gasification reaction, K present in the pores and cracks of the coke leads to the formation of $K_2O.SiO_2$ and $K_2O.Al_2O_3.2SiO_2$, leading to an increase in the volume of the coke and its subsequent fracture (Yusfin *et al*., 1999). K compounds, in particular, are the major cause of the destruction of the refractory lining of blast furnaces in the lower part of the stack, the bosh, and in some cases the tuyere zone of the hearth (Yusfin *et al*., 1999). K penetrates the monolithic alumosilicate lining in these regions, resulting in the formation of new minerals, such as silicide ($K_2O.Al_2O_3.6SiO_2$) or leucite ($K_2O.Al_2O_3.4SiO_2$) and the

[*] Mr Corne Taljaard, Kumba Iron Ore, Ltd., Lakefield Office Park, Building D, Lenchen North, Centurion, South Africa

rearrangement of the crystalline lattice of the refractories, which is accompanied by a reduction in their softening point and mechanical strength (Yusfin *et al*., 1999). The result is the creation of stresses that cause cracks to form in the refractory lining, followed by the subsequent destruction of the lining (Yusfin *et al*., 1999). Therefore, most steel-making companies in the leading industrial nations have established limits on the concentration of alkali's (such as K_2O) in the charge material without changing the smelting practice (Yusfin *et al*., 1999). These limits on the alkali levels range from 0.25 % mass in Japan to 0.55 % mass in Switzerland (Yusfin *et al*., 1999).

In the past, the lower quality ore (<60% Fe; >0.24% K) has been blended with high quality ore (>60% Fe; <0.24% K) to "dilute" the P and K in the final iron ore product, which is exported to the steel-making plants (Personal communication[*]). Similar practices have been reported from other parts of the world, such as the Hamersley Province in Australia, where low-phosphorous ore (0.05% P) is blended with high-phosphorous ore (0.10% P), the former being the major component of the blend (Dukino *et al*., 2000). To date, blending of different quality iron ores has minimised the penalties charged by steel-making companies. However, the ratio of low-quality ore (<60% Fe; >0.24% K) to high-quality ore (>60% Fe; <0.24% K) is on the increase, and thus it is becoming an escalating problem within the economic functioning of the Sishen Iron Ore Mine.

A population study of the process- and ground water, as well as the iron ore of the mine would give insight into the microorganisms present in the mine environment. It might be possible to utilise existing microorganisms present in the environment to design and optimise an economically viable biotechnology process for the removal of P and K from the iron ore. Several microorganisms are capable of converting nutrients available in the environment to products, such as inorganic and organic acids, that can be used in industrial processes (Gupta and Sharma, 2002; Lesniak *et al*., 2002). Microorganisms can produce organic and inorganic acids, i.e. certain *Acidithiobacillus* spp. are capable of producing sulphuric acid (H_2SO_4), and A*spergillus niger* and certain *Penicillium* spp. are

[*] Mr Danie Krige, Kumba Iron Ore, Ltd., Rust Building, Frikkie Meyer Road, Pretoria West, South Africa

able to produce citric acid (Cunningham and Kuiack, 1992; Krebs *et al*., 1997; Alvarez-Vasquez *et al*., 2000; Gupta and Sharma, 2002; Lesniak *et al*., 2002; El-Holi and Al-Delaimy, 2003). These microbially produced acids may be valuable in a leaching process to reduce the P and K content of the iron ore.

During a previous study whereby a range of inorganic- and organic acids were tested for the removal of P and K from the iron ore of the Sishen Iron Ore Mine, it was discovered that citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) proved to be the best leaching agent for the removal of P and K without a major reduction in the iron content (0.46%) of the iron ore concentrate. Citric acid is an intermediate in the tricarboxylic acid (TCA) cycle, which is widely used in the food, beverage, pharmaceutical and cosmetic industries, but also has other applications in the textile, electroplating and bioremediation industries (Wang and Liu, 1996; Tran *et al*., 1998; Ates *et al*., 2002). The most popular microorganism for the large-scale production of citric acid is the white-rot fungus *Aspergillus niger*, due to its high citric acid productivity at low pH without the secretion of toxic metabolites (Kim, 2004). A possible process for the removal of P and K from the iron ore concentrate, may therefore, involve the fungal production of citric acid that can subsequently be used as a chemical leaching agent for the removal of P and K. In addition, it may be possible to use heap leaching technology, whereby the iron ore concentrate is directly inoculated with *A. niger*. The P and K contained within the iron ore concentrate may act as the sole source of these limiting growth factors required for fungal growth, which may consequently result in the selective removal of P and K from the iron ore concentrate by the fungus.

It has become important to develop an economically viable and environmentally friendly process to reduce the high P and K levels contained in the ore to improve the quality of iron ore that is being exported from the Sishen Iron Ore Mine. Currently such an economically viable biotechnological process for the reduction of P and K present in iron ore does not exist, and would therefore give Sishen Iron Ore Mine a competitive advantage in the international iron ore arena.

Introduction

The objectives of this study were to:

1. Investigate the microbial community of the water from the process dam, water flowing into the slime dam, water flowing from the slime dam and the ground water of the Sishen Iron Ore Mine.
2. Investigate the microbial community associated with the iron ore concentrate of the Sishen Iron Ore Mine.
3. Conduct a chemical analysis of the water from the process dam, water flowing into the slime dam, water flowing from the slime dam and the ground water of the mine, as well as chemically characterise the final iron ore concentrate from the Sishen Iron Ore Mine.
4. Evaluate a range of commercially available acids and oxidative chemicals for their chemical leaching ability.
5. Compare solid substrate- and submerged fermentation for the production of citric acid by *A. niger*.
6. Conduct chemical leaching of P and K from the iron ore concentrate using the citric acid produced by *A. niger*.
7. Conduct "heap leaching" of the iron ore concentrate using *A. niger*.
8. Propose an economically viable biotechnology process for the removal of P and K from the iron ore concentrate of Sishen Iron Ore Mine.

References:

ALVAREZ-VASQUEZ F, GONZALEZ-ALCON C and TORRES NV (2000) Metabolism of citric acid production by *Aspergillus niger*: Model definition, steady-state analysis and constrained optimisation of citric acid production rate. *Biotechnol Bioeng* **70 (1):** 82-108.

ATES S, DINGIL N, BAYRAKTAR E and MEHMETOGLU U (2002) Enhancement of citric acid production by immobilized and freely suspended *Aspergillus niger* using silicone oil. *Proc Biochem* **38:** 433-436.

COSTA MC, CARVALHO N, IGLESIAS N and PALENCIA I (2003) Bacterial leaching studies of a Portuguese flotation tailing. *Proc IBS* Athens-Hellas (Greece) p. 115.

CUNNINGHAM JE and KUIACK C (1992) Production of citric and oxalic acids and solubilization of calcium phosphate by *Penicillium bilaii*. *Appl Environ Microbiol* **58(5):** 1451-1458.

DUKINO RD, ENGLAND BM and KNEESHAW M (2000) Phosphorous distribution in BIF-derived iron ores of Hamersley Province, Western Australia. *Trans Instn Min Metall* (*Sect B: Appl Earth Sci*) **109:** B168-B176.

EL-HOLI MA and AL-DELAIMY KS (2003) Citric acid production from whey with sugars and additives by *Aspergillus niger*. *African Journal of Biotechnology* **2(10):** 356-359.

EHRLICH HL (1999) Past, present and future of Biohydrometallurgy. *Proc IBS* Madrid (Spain) pp. 3-12.

GUPTA S and SHARMA CB (2002) Biochemical studies of citric acid production and accumulation by Aspergillus niger *mutants.* W J Microbiol Biotechnol ***18:*** *379-383.*

JAIN N and SHARMA DK (2004). Biohydrometallurgy for non-sulphidic minerals-a review. Geomicrobiol J ***21:*** *135-144.*

KIM J (2004) Optimisation of citric acid production by Aspergillus niger NRRL 567 in various fermentation systems. PhD Thesis. Department of Biosystems Engineering, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada.

KREBS W, BROMBACHER C, BOSSHARD PP, BACHOFEN R and BRANDL H (1997) Microbial recovery of metals from solids. *FEMS Microbiol Rev* **20:** 605-617.

LESNIAK W, PIETKIEWICZ J and PODGORSKI W (2002) Citric acid fermentation from starch and dextrose syrups by a trace metal resistant mutant of Aspergillus niger. Biotechnol Lett **24:** 1065-1067.

TORMA AE (1986) Biohydrometallurgy as an emerging technology. *Biotechnol Bioeng Symp* **16:** 49-63.

TRAN CT, SLY LI and MITCHELL DA (1998) Selection of a strain of *Aspergillus* for the production of citric acid from pineapple waste in solid-state fermentation. *W J Microbiol Biotechnol* **14:** 399-404.

WANG J and LIU P (1996) Comparison of citric acid production by Aspergillus niger immobilized in gels and cryogels of polyacrylamide. J Ind Microbiol ***16:*** *351-353.*

YUSFIN YS, CHERNOUSOV PI, GARTEN V, KARPOV YA and PETELIN AL (1999) The role of alkali's and conserving resources in blast-furnace smelting. *Metallurgist* **43:** 54-58.

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Sishen Iron Ore Mine is situated in the Northern Province of South Africa approximately 280km north-west of Kimberley. This mine is one of the largest open cast mines in the world, yielding 25 million tonnes of high-grade hematite iron ore per annum. The iron ore concentrate that is exported to various steel making companies worldwide, contains phosphorous (P) and (K), which adversely affects the smelting process in blast furnaces. Therefore, the steel making companies charge penalties when purchasing iron ore concentrates with a P and K content above certain levels. The limit on the allowable amount of P and K is determined by the steel making companies and range from 0.25% mass in Japan to 0.55% mass in Switzerland.

In the past, lower quality iron ore has been blended with high quality iron ore in an attempt to "dilute" the P and K, however, the low quality iron ore stockpiles are increasing, and thus, becoming an escalating problem within the economical functioning of the mine. Therefore, it has become important to develop an economically viable process to reduce the P and K levels in the iron ore concentrate, in order to minimize the penalties charged by the international steel making companies. Bioleaching may provide a low-cost, environmentally friendly process technology for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine.

Conventional bioleaching/biooxidation refers to the microbial conversion of insoluble metals into soluble forms. Natural bioleaching has been known since A.D. 162, while the commercial application of bacterial leaching began in the 1950's at the Kennecott Utah Copper Company's Bingham Canyon Mine near Salt Lake City, Utah, USA. Various bioleaching technologies have since been developed, using microbes to catalyse the oxidation of sulphide minerals from ores that are otherwise not

processable by conventional pyrometallurgical techniques. Various metals, such as cobalt, copper, nickel, uranium and zinc, are extracted using bioleaching technology.

Certain substances in non-sulphide minerals, such as the P and K in the iron ore concentrate of the Sishen Iron Ore Mine, may be solubilised by a process of complexation using organic acids, such as citric and oxalic acid. Citric acid contains several carboxyl groups, which tend to donate protons (H^+), resulting in negatively charged carboxyl groups that are capable of forming stable complexes with several cations. Therefore, it would be possible that these negatively charged carboxyl groups might form stable complexes with the positively charged K cations present, and at the same time the release of H^+ ions may result in a hydrolysis reaction involving the P contained in the iron ore, resulting in the subsequent P and K removal from the iron ore concentrate.

Citric acid is an intermediate in the tricarboxylic acid (TCA) cycle, and can be produced by solid substrate- or submerged fermentation, the latter being used for the majority of the worldwide citric acid production. *Aspergillus niger* is the most popular microorganism for the large-scale production of citric acid due to its high citric acid productivity at low pH without the secretion of toxic metabolites. Citric acid production involves the catabolic production of pyruvate and Acetyl-coenzyme A (Acetyl-CoA) from hexoses by glycolysis, followed by the formation of citric acid by the TCA cycle.

The production of citric acid by *A. niger*, coupled with the chemical leaching of the iron ore concentrate using the produced acid, may be a suitable process for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine.

2.2 Iron Ore Deposits in the Northern Cape Province of South Africa

The largest known resources of high-grade hematite ore on the Southern African sub-continent can be found at the Palaeo-Preterozoic Transvaal Supergroup in the Northern Cape Province of South Africa (Carney and Mienie, 2003). The presence of excavations in the Northern Cape has been recorded from as far back as 1804 (Cairncross and Dixon, 1995), while mining activities in the Postmasburg area date

back to 2000 BC (Carney and Mienie, 2003). In 1945 the first commercial-scale exploitation of hematite ore commenced after the potential of the iron resources in this region was recognised (Carney and Mienie, 2003). Kumba Iron Ore, Ltd extracts hematite ore at the Sishen Iron Ore Mine for local and international markets (Figure 2.1) (Carney and Mienie, 2003).

2.2.1 Regional Geology

Superior-type banded iron formations (BIF's) of the Transvaal Supergroup crop out along the western margin of the Kaapvaal craton in the Northern Cape Province (Carney and Mienie, 2003). These BIF's consist of a range of distinctive hills, stretching for 400 km from Prieska in the south to Pomfret in the north (Carney and Mienie, 2003). The Postmasburg and Sishen areas are host to the bulk of the high-grade hematite ore (Carney and Mienie, 2003). The iron ore and associated lithologies of the Transvaal and Olifantshoek Supergroups crop out along an acruate belt for approximately 60 km, defining a regional anticlinal structure known as the Maremane anticline (Carney and Mienie, 2003). The Sishen Iron Ore Mine is located at the northern end of the Maremane anticline where the bulk of the hematite ores lie buried beneath younger cover lithologies, which strike north-south and plunge away from the centre of the anticline (Carney and Mienie, 2003). Laminated and massive ores constitute the bulk of the resource at the Sishen deposit, which are non-uniformly overlain by conglomerates, shales, flagstone and quartzite, termed the Gamagara Subgroup (South African Committee for Stratigraphy, 1995). Diamictite of the Makganyene Formation and lavas of the Ongeluk Formation have been thrust over the sedimentary rocks of the Gamagara Subgroup, followed by erosion by later events (Carney and Mienie, 2003). The erosional unconformities have been covered by tillite of the Dywka Group and clay and calcrete of the Kalahari Group (Carney and Mienie, 2003).

FIGURE 2.1

Location of the Sishen Iron Ore Mine in the Northern Cape Province of South Africa (Map modified from www.places.co.za)

2.2.2 The Sishen Iron Ore Mine

Sishen Iron Ore Mine (Figure 2.2) was established in 1953 and is situated approximately 280km north-west of Kimberley (Figure 2.1), and is one of the seven largest open cast mines in the world, with an open pit of approximately 11km long, 1.5 km wide and 400m deep (Personal communication*). The crushing and sorting plant is capable of processing in excess of 30 million tonnes of raw iron ore per

* Mr Danie Krige, Kumba Iron Ore, Ltd., Rust Building, Frikkie Meyer Road, Pretoria West, South Africa

annum that in turn yields 25 million tons of high-grade hematite iron ore, which is exported to various parts of the world via Saldanha Bay on the South African west coast. China is currently the largest market for the Sishen iron ore concentrate, although British Steel is the largest single customer (Personal communication*).

FIGURE 2.2

The Sishen Iron Ore Mine Situated in the Northern Cape Province of South Africa

The bulk of the iron ore resource of the Sishen Iron Ore Mine comprises of laminated and massive ores belonging to the Asbestos Hills Subgroup (Carney and Mienie, 2003). These orebodies are intensely folded and faulted, with a regional dip of 11º in a north-westerly direction (Carney and Mienie, 2003). The mine consists of a single elongated pit of ~10 km long and ~1.5 km wide with a mineable reserve of 895 metric tonnes (Mt) (Carney and Mienie, 2003). This deposit is capable of producing high-grade ore with an average iron content of 64.83%, P content of 0.073% and K content of 0.232% (Carney and Mienie, 2003).

* Mr Danie Krige, Kumba Iron Ore, Ltd., Rust Building, Frikkie Meyer Road, Pretoria West, South Africa

2.2.3 Potassium and Phosphorous Bearing Ores of the Sishen Iron Ore Mine

To date, muscovite remains the only K-containing mineral in the iron ore of the Sishen Iron Ore Mine, while the P bearing minerals are apatite, goyazite and woodhouseite (Personal communication*).

2.2.3.1 Muscovite

Muscovite (Table 2.1), also known as potash mica, is frequently found in igneous, metamorphic and detrital sedimentary rocks, and has a layered structure of aluminium silicate sheets, which are weekly bonded together by K^+ ions (Amethyst Galleries' Mineral Gallery, 1996; Wikipedia, 2006d). The K^+ ions are responsible for the perfect cleavage of muscovite, producing thin sheets or flakes that are highly flexible and elastic (Figure 2.3) (Amethyst Galleries' Mineral Gallery, 1996).

TABLE 2.1

Classification of Muscovite

Chemical Name	Potassium Aluminium Silicate (Hydroxide, Fluoride)
Chemical Formula	$KAl_2(AlSi_3O_{10})(F, OH)_2$
Class	Silicates
Subclass	Phyllosilicates
Group	Micas
Mohs Hardness	2 - 2.5
Specific Gravity	2.76 – 3

Various colours of muscovite exist, ranging from colourless to shades of grey, brown, green, yellow, or rarely violet or red (Amethyst Galleries' Mineral Gallery, 1996; Wikipedia, 2006d). Due to the colour variations, as well as often being translucent, muscovite crystals accompany various valuable minerals such as tourmaline, topaz, beryl and almandine, although muscovite is not often valuable as a mineral specimen (Amethyst Galleries' Mineral Gallery, 1996). Two valuable varieties of muscovite include the rare yellow five pointed stars, known as Star Muscovite (Figure 2.4), and

* Mr Japie Oberholzer, Kumba Iron Ore, Ltd., Lakefield Office Park, Building D, Lenchen North, Centurion, South Africa

a deep green variety due to colouration by chromium impurities, known as fuchsite (Figure 2.4) (Amethyst Galleries' Mineral Gallery, 1996).

FIGURE 2.3

Flexible and Elastic Sheet of Muscovite (Brazilian Rockhounds, 2005)

Muscovite is of importance in the manufacturing of fireproofing, insulating materials, lubricants, as well as electrical components due to the high heat and electrical insulating properties it has (Amethyst Galleries' Mineral Gallery, 1996; Wikipedia, 2006d). The name of muscovite is derived from Muscovy-glass, which is the name formerly used for the mineral due to its use for windows in Russia, and it was also used for the manufacturing of kitchen oven windows before synthetic materials replaced them (Amethyst Galleries' Mineral Gallery, 1996; Wikipedia, 2006d).

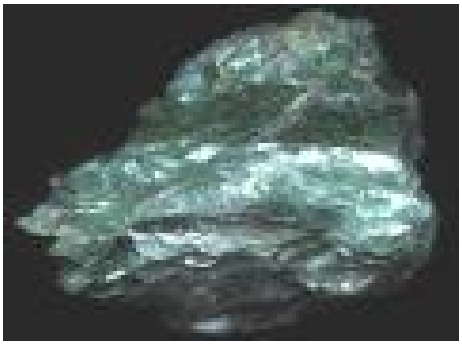

FIGURE 2.4

Yellow five pointed stars, known as Star Muscovite (left) (Wright's Rock Shop, 2005), and Green Muscovite, known as Fuchsite (Copyright [2005] by Andrew Alden, reproduced under educational fair use, http://geology.about.com)

2.2.3.2 Apatite

The name Apatite is derived from a Greek word meaning "to deceive", as it is very similar to other more valuable minerals such as olivine, peridot and beryl (Amethyst Galleries' Mineral Gallery, 1996). Apatite (Table 2.2) is a group of phosphate minerals, and can be classified as three different minerals, namely hydroxylapatite, fluorapatite and chlorapatite (Amethyst Galleries' Mineral Gallery, 1996; Wikipedia, 2006a). Fluorine, chlorine and hydroxyl ions are able to freely substitute one another within the crystal lattice, as all three elements are usually present in each specimen of apatite (Amethyst Galleries' Mineral Gallery, 1996). Apatite usually exists as small cryptocrystalline fragments and is widely distributed in igneous-, sedimentary- and metamorphic rocks, however, it can also exist as large crystals in certain contact metamorphic rocks, which can be cut as gems (Figure 2.5) (Amethyst Galleries' Mineral Gallery, 1996; Scandinavian Mineral Gallery, 1998).

Apatite also exists and is produced by biological systems. Hydroxylapatite is a major constituent of tooth enamel, as well as bone material (Amethyst Galleries' Mineral Gallery, 1996). When fluoridated water is ingested, hydroxyl ions are exchanged with fluoride ions, resulting in the formation of fluorapatite, which is stringer than hydroxylapatite, thus strengthening teeth and bone material (Amethyst Galleries' Mineral Gallery, 1996).

TABLE 2.2 **Classification of Apatite**	
Chemical Name	Calcium (Fluoro, Chloro, Hydroxyl) Phosphate
Chemical Formula	$Ca_5(PO_4)_3(OH, F, Cl)$
Class	Phosphates
Group	Apatite
Mohs Hardness	5
Specific Gravity	3.17 – 3.23

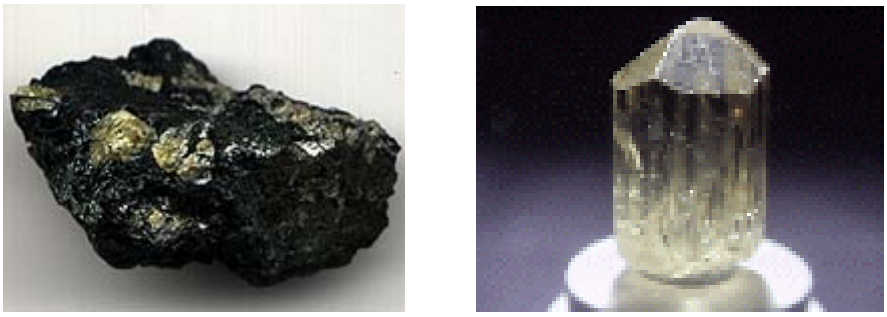

FIGURE 2.5

Chlorapatite from Altermark, Norway (left), and an apatite gemstone (right) (Amethyst Galleries' Mineral Gallery, 1996; Scandinavian Mineral Gallery, 1998)

2.2.3.3 Woodhouseite

Woodhouseite (Table 2.3) is a rare mineral, which forms flesh-coloured to colourless psuedocubic rhombohedrons (Figure 2.6) (Amethyst Galleries' Mineral Gallery, 1999). Woodhouseite is formed in quartz veins where it is frequently associated with topaz, tourmaline, andalusite and svanbergite, and is difficult to classify because it has both a sulphate anion group and a phosphate anion group (Amethyst Galleries' Mineral Gallery, 1999).

TABLE 2.3 Classification of Woodhouseite	
Chemical Name	Calcium Aluminium Phosphate Sulphate Hydroxide
Chemical Formula	$CaAl_3PO_4SO_4(OH)_6$
Class	Sulphates (sometimes Phosphates)
Group	Beudantite
Mohs Hardness	4.5
Specific Gravity	3

FIGURE 2.6

A cluster of quartz crystals coated with a honey-coloured dusting of woodhouseite crystals (Amethyst Galleries' Mineral Gallery, 1999)

2.2.3.4 Goyazite

Rhombohedral goyazite crystals occur as distinct particles dispersed in a matrix (Mineralogy Database, 2005). Goyazite crystals are transparent and range in colour from lemon yellow and pink to colourless (Figure 2.7) (Mineralogy Database, 2005). This mineral, named after the province of Goyaz, Brazil, is also known as bowmanite, hamlinite or lusungite (Table 2.4) (Mineralogy Database, 2005).

TABLE 2.4 Classification of Goyazite	
Chemical Formula	$SrAl_3(PO_4)_2(OH)_5.H_2O$
Subclass	Anhydrous Phosphates, Arseniates, Vanadates
Group	Crandallite
Mohs Hardness	4.5 - 5 (Flourite-Apatite)
Specific Gravity	3.22

FIGURE 2.7

Flat, tabular crystals of goyazite on dolomite (Paulin, 2005)

2.3 Blast Furnace Technology

The research into the effect of alkalis on blast furnace smelting peaked during the 1970's (Yusfin *et al*., 1999). This period is characterised by the construction of powerful blast furnaces, as well as the trend to use large quantities of iron ore pellets in the charge (Yusfin *et al*., 1999). At the same time, the problems related to the alkali activity during smelting became so sensitive, that a commission was organised by the "Ernkontorens" Metallurgical Society in Switzerland in 1972 to investigate the problem (Yusfin *et al*., 1999).

2.3.1 The Basic Functioning of a Blast Furnace

A diagrammatic representation of the functioning of a blast furnace is shown in Figure 2.8. Blast furnaces are used during the steel making process to chemically

reduce and physically convert iron oxides into liquid iron, known as "hot metal" (Ricketts, 2005). The blast furnace is built in the form of a tall chimney-like structure lined with refractory brick, where iron ore (iron oxide), coke (carbonaceous material), and limestone flux are dumped into the top of the furnace, while preheated air is blown into the bottom of the furnace (Ricketts, 2005). The raw materials require 6 to 8 hours to descend to the bottom of the furnace where they become the final product of liquid slag and liquid iron, known as "pig" iron, which are drained from the furnace at regular intervals (Ricketts, 2005). The preheated air, which was blown into the bottom of the furnace, ascends to the top in 6 to 8 seconds after going through several chemical reactions (Ricketts, 2005).

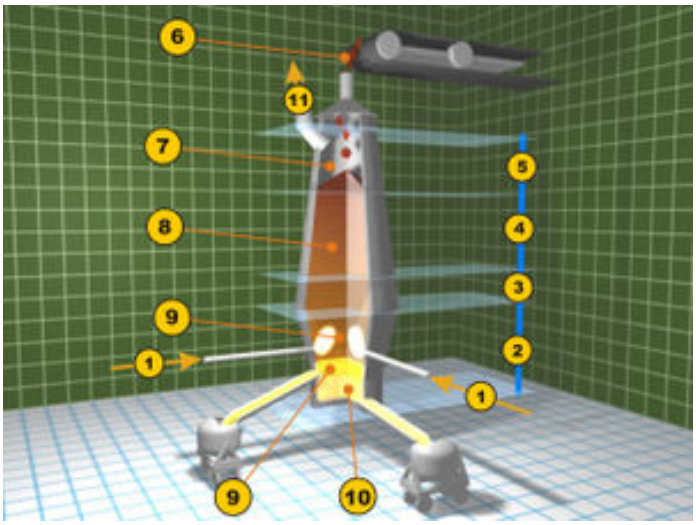

FIGURE 2.8

Diagrammatic representation of the functioning of a blast furnace (http://en.wikipedia.org/wiki/Blast_furnace#Chemistry, 2007)

1) Hot blast from Cowper stoves (Tuyere zone); 2) Melting zone (bosh); 3) Reduction zone of ferrous oxide (barrel); 4) Reduction zone of ferric oxide (stack); 5) pre-heating zone (throat); 6) Feed of ore, limestone and coke; 7) Exhaust gasses; 8) Column of ore, limestone and coke; 9) Removal of slag; 10) Tapping of molten pig ore; 11) Collection of waste gasses

In a blast furnace there are numerous chemical reactions that produce the desired final molten iron, which can be summarised as follows (Ricketts, 2005):

1) $3Fe_2O_3 + CO \rightarrow CO_2 + 2Fe_3O_4$ (Begins at 455ºC)
2) $Fe_3O_4 + CO \rightarrow CO_2 + 3FeO$ (Begins at 590ºC)
3) $FeO + CO \rightarrow CO_2 + Fe$ (Begins at 700ºC)

As the iron oxides undergo these chemical reactions, they begin to melt, resulting in the liquid iron trickling through the coke to the bottom of the furnace (Ricketts, 2005). The coke descends to level where the preheated air or hot blast enters the blast furnace, where it is ignited to generate heat by the following reaction (Ricketts, 2005):

$C + O_2 \rightarrow CO_2 + Heat$

The abovementioned reaction takes place in the presence of excess carbon at a high temperature, resulting in the CO_2 being reduced to carbon monoxide (required for the reduction of iron oxides) as follows (Ricketts, 2005):

$CO_2 + C \rightarrow 2CO$

The decomposition of the limestone flux results in the production of calcium oxide that removes sulphur from the iron ore, as well as silica (SiO_2), aluminium (Al_2O_3), magnesium (MgO), and calcium (CaO), which becomes part of the slag (Ricketts, 2005). Hot dirty gas is produced in the furnace during the iron making process, which exit at the top of the furnace where particulate matter is removed and the gas cooled (Ricketts, 2005). The gas is used as a fuel in the "hot blast stoves" used to preheat the air entering the blast furnace, while any excess gas is moved to the boiler house where it is used to generate steam (Ricketts, 2005).

Although the efficiency of blast furnaces is constantly evolving, the chemical and physical reactions remain the same (Ricketts, 2005). According to the American Iron and Steel Institute, "Blast furnaces will survive into the next millennium because the larger, efficient furnaces can produce hot metal at costs competitive with other iron making technologies" (Ricketts, 2005). However, one of the biggest drawbacks of the

blast furnaces is the inevitable CO_2 production as the iron is reduced from the iron oxides by carbon (Ricketts, 2005).

2.3.2 The Effect of Alkalis on the Functioning of the Blast Furnace

Smelting in blast furnaces is adversely affected by alkalis, in particular by K (Yusfin *et al*., 1999). These alkalis enter the blast furnace with the charge materials (Yusfin *et al*., 1999). The main sources of alkalis are not usually the iron ore or their concentrates, but instead the fluxes and strengthening additions used for making the pellets for the blast furnace charge (Yusfin *et al*., 1999). The alkali content of the limestone flux may reach 0.2 – 0.6%, while that of the coke may fluctuate from 0.04 – 0.25% (Yusfin *et al*., 1999). These alkalis are deposited on the surface of the coke, where they act as a catalyst and intensify the gasification of carbon in the presence of CO_2 (Yusfin *et al*., 1999). The gasification reaction is shifted toward lower temperatures resulting in the reduction in the strength of the coke (Yusfin *et al*., 1999). In addition to accelerating the gasification process, K present in the pores and cracks of the coke leads to the formation of compounds such as potassium silicate ($K_2O.SiO_2$) and potassium alumosilicate ($K_2O.Al_2O_3.2SiO_2$), resulting in an increase in the volume of the coke and its subsequent fracture (Yusfin *et al*., 1999).

The action of K is the major reason for the destruction of the refractory lining of blast furnaces in the lower part of the stack, the bosh, and in some cases the tuyere zone of the hearth (Figure 2.8) (Yusfin *et al*., 1999). Within these regions, K actively penetrates the monolithic alumosilicate lining, which leads to the formation of new minerals such as silicide ($K_2O.Al_2O_3.6SiO_2$) or leucite ($K_2O.Al_2O_3.4SiO_2$) (Yusfin *et al*., 1999). The rearrangement of the crystalline lattice of the refractories takes place, which is accompanied by a reduction in their softening point and mechanical strength, resulting in the creation of stresses that cause cracks to form (Yusfin *et al*., 1999). In the tuyere zone, the situation is aggravated by the formation of a condensed phase based on KCN (Yusfin *et al*., 1999). Condensation of alkali compounds on the surface of iron oxide pellets leads to their fracture and a deterioration in the gas permeability of the stock, as well as the rapid disintegration of the iron oxide pellets (Yusfin *et al*., 1999). In addition, the viscosity of the basic blast furnace slags are adversely affected by the presence of alkalis, which promotes the further formation of

alkaline alumosilicate groups such as $K_2O.Al_2O_3.2SiO_2$ in the slag melt (Yusfin *et al.*, 1999).

Most steel making companies in the leading industrial nations have established a limiting alkali load for blast furnaces, i.e. have set limits on the amounts of alkalis that may be present in the charge materials without changing the smelting practice (Table 2.5) (Yusfin *et al.*, 1999). Therefore, it is becoming increasingly important to remove these alkalis from the charge materials before they enter the blast furnace. The main methods that can be used to reduce the alkali content in blast furnaces are:

* Reducing the content of alkalis in the charge materials;
* Removing top dust and blast furnace slag from the sintering-machine charge;
* Operating the furnace on acid slags; and
* Increasing the volume of slag, especially in furnaces in which the slag volume is already low.

TABLE 2.5

Allowable Alkali load on the Blast Furnaces of Companies in Several of the Leading Industrial Nations (Yusfin *et al.*, 1999)

Nation	Company	Allowable Alkali Load kg.tonne^{-1} Pig Iron
Canada	STIKLO, Dofasko	3.0
Japan	Kawasaki, Seitetsu, Kobe Seikose, Sin Nippon Seitetsu, Nippon Kokan	2.5 – 3.1
United States of America	Ellenwood Steel, Jones and Laughlin Steel, United Sates Steel	3.2 – 4.5
England	British Steel	3.5
Germany	August Thyssen-Hütte	4.0
Switzerland	Grenges	5.5

Removing the P and K from the iron ore concentrate would, therefore, reduce the content of alkalis in the charge material entering the blast furnaces of the steel making companies to whom Kumba Iron Ore, Ltd. export iron ore concentrate. Bioleaching

may provide a low-cost, environmentally friendly processing technology for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine.

2.4 Conventional Bioleaching

Various technologies developed in the last couple of decades include microbial use for the extraction of different valuable metals, which is commonly known as bioleaching (Gilbertson, 2000; Rohwerder *et al*., 2002). Bioleaching refers to the microbial conversion of insoluble metals (e.g. metal sulphides such as CuS, NiS and ZnS) into soluble forms (usually metal sulphates such as $CuSO_4$, $NiSO_4$ and $ZnSO_4$). Due to the many oxidation reactions that occur during bioleaching, it can also be referred to as biooxidation (Sand, 2001; Rawlings, 2002, Roberto, 2002).

2.4.1 Historical Overview of Bioleaching

The phenomenon of natural bioleaching has been known since ancient times. Galen, a naturalist and physician from Pergamum, reported the utilisation of *in-situ* copper leaching in A.D. 162 from the island of Cyprus, making this one of the earliest records of the effects of bioleaching (Constantinou, 1992). Cupriferous solutions were collected from the mine water of the Skouriotissa mines, followed by concentration by evaporation, leaving behind crystallised copper sulphate (Constantinou, 1992). Pliny the Elder (A.D. 23-73) discussed the *vitreolus quasi vitrum*, a glass-like substance discovered on rocks, in his treatise on natural history (Dresher, 2004). Recent findings have revealed evidence, which predates these accounts of historical bioleaching practices (Dresher, 2004).

The natural leaching of copper and the formation of ‘gall springs’ during the East Han Dynasty (B.C. 206-A.D. 220) in China have been recorded (Dresher, 2004). This process was also recorded as being used during the Song Dynasty (A.D. 960-1271) (Dresher, 2004). During this process, also known as cementation, copper was precipitated from solution by dipping iron into the solution, a process that was used as far back as B.C. 150 in China (Dicinoski *et* al., 1998; Dresher, 2004).

Iron-rich acidic water draining from abandoned coal and metal mines, as well as unmined mineralised areas provide more evidence of natural bioleaching (Dresher, 2004). The production of acid mine drainage due to mining activities have in the past resulted in the naming of rivers such as Tinto, Tintillo and Aguas Teñidas, referring to the characteristic colouration of the rivers (Dresher, 2004). Industrial scale heap leaching, involving roasting of copper and iron sulphides, was conducted at the Rio Tinto mine (Spain) in the late 1700's, but was prohibited by law in 1888 due to the serious environmental damage caused by the clouds of sulphur dioxide, resulting in acid mine drainage (Gilbertson, 2000; Dresher, 2004). Heap leaching of copper, without the prohibited roasting of copper and iron sulphides, was continued with success until the 1970's (Gilbertson, 2000; Dresher, 2004). The success of the heap leaching of copper was attributed in 1947 to the presence of bacteria (Colmer and Hinkle, 1947).

The commercial application of bacterial leaching began in the 1950's at the Kennecott Utah Copper Company's Bingham Canyon Mine near Salt Lake City, Utah, USA. This process started after it became evident that blue copper-containing solutions were running out of waste piles containing copper sulphate minerals (Dresher, 2004). Due to the absence of powerful oxidising agents and acid, investigations were started, which revealed that the natural occurring bacteria were responsible for the oxidation of the iron sulphides in the piles (Malouf and Prater, 1961). The bacteria were given the names *Thiobacillus ferrooxidans* for their ability to oxidise iron sulphides, and *Thiobacillus thiooxidans* for their ability to oxidise sulphur to yield sulphuric acid (Malouf and Prater, 1961).

2.4.2 Development of the Bioleaching Industry

Bioleaching occurs as a natural process whereby microorganisms assist in the slow weathering of sulphidic orebodies, and thus, by using this concept for the extraction of metals from ores is simply the attachment of a natural process to commercial purposes (Gilbertson, 2000; Rawlings, 2002; Rohwerder *et al*., 2002). Various technologies developed in the last couple of decades include the microbial ability to catalyse the oxidation of sulphide minerals (Table 2.6) for the subsequent extraction of different valuable metals from ores that are otherwise not processable by

pyrometallurgical techniques, as well as for the extraction of metals from low-grade ores (Rawlings, 2002; Rohwerder *et al*., 2002; Dresher, 2004). This phenomenon, known as bioleaching, is distinguished from conventional acid leaching wherein only oxidised minerals are leached (Rohwerder *et* al., 2002; Dresher, 2004). During bioleaching, insoluble metals (e.g. metal sulphides such as CuS, NiS and ZnS) are converted into soluble forms (usually metal sulphates such as $CuSO_4$, $NiSO_4$ and $ZnSO_4$) due to the oxidation catalysed by microorganisms (Sand, 2001; Rawlings, 2002, Roberto, 2002). Previously only mesophilic bacteria were considered to be important during bioleaching, but the use of moderately and extremely thermophilic bacteria have become attractive during bioleaching (Johnson, 1998; Norris *et al*., 2000).

TABLE 2.6

Major Mineral Sulphides Present in Nature (Glazer and Nikaido, 1995)

Mineral	Chemical Formula	Name
Arsenic (As)	AsFeS	Arsenopyrite
Cobalt (Co)	$CuCo_2S_4$	Carrolite
Copper (Cu)	$CuFeS_2$	Chalcopyrite
	Cu_2S	Chalcocite
	CuS	Covellite
	Cu_5FeS_4	Bornite
Iron (Fe)	FeS_2	Pyrite, Marcasite
Lead (Pb)	PbS	Galena
Molybdenum (Mo)	MoS_2	Molybdenite
Nickel (Ni)	NiS	Millerite
	FeNiS	Pentlandite
Silver (Ag)	AgS	Argenite
Zinc (Zn)	ZnS	Sphalerite

Various metals are extracted in this manner, which include cobalt, copper, nickel, uranium and zinc (Olson *et al*., 2003; Rawlings *et al*., 2003). The recovery of gold and silver, however, applies the microbial activity for the removal of interfering metal sulphides from the ore prior to cyanidation treatment (Rohwerder *et al*., 2003). In this

case the term biooxidation should be used due to the fact that the bioleached metals are not recovered during the process (Rohwerder *et al*., 2003). Both bioleaching and biooxidation processes could be referred to in general as biomining (Bosecker, 1997; Rawlings, 2002; Rohwerder *et al*., 2002; Olson *et al*., 2003).

The type of resource to be processed determines which bioleaching process is to be used (Dresher, 2004). Currently three different types of bioleaching processes exist, depending on the raw material to be processed. A widely used process is dump leaching, whereby waste rock, low-grade ore or concentrator tailings are leached at the site of disposal (Dresher, 2004). Another process that is increasingly being used is heap leaching, which involves the leaching of newly mined material (intermediate grade ore, oxides and secondary sulphides) by depositing the material on an impervious natural surface or pad (Dresher, 2004). The material may be leached as mined or alternatively may be partially crushed and mixed with an acid prior to the depositing on the heap. The third process, agitated leaching, involves placing intermediate- to high-grade chalcopyrite concentrates in a tank, where it is leached using mechanical agitation (Dresher, 2004). Agitated leaching, however, is only in the experimental stage and is not commercially used.

Microorganisms are used during bioleaching to catalyse the oxidation reaction of iron sulphides to create ferric sulphate and sulphuric acid (Dresher, 2004). During copper extraction, ferric sulphate is responsible for the oxidation of copper sulphide minerals, followed by the leaching of the copper by the sulphuric acid (Dresher, 2004). In the case of uranium extraction, tetravalent uranium oxide is oxidised by ferric sulphate, rendering hexavalent uranium oxide, which is leached by the sulphuric acid (Dresher, 2004). The biooxidation of refractory gold ores involve bacteria that are able to oxidise a iron sulphide matrix in which the gold particles are embedded, resulting in the availability of the gold particles for cyanide leaching (Dresher, 2004). The desulphurisation of coal also involves bacteria for the oxidation of the pyrite contaminant present in coal, resulting in the solubilization of sulphur as ferric sulphate (Dresher, 2004).

Bioleaching offers several advantages for the extraction of base metals, such as:

1. The use of naturally occurring components (microorganisms, water and air).
2. Extendibility with a single reactor or a series of reactors.
3. Stirred tanks are simple to operate and maintain.
4. Relatively low pressures and temperatures prevail in the system.
5. No dust and SO_2 production.
6. Arsenic can be handled and disposed of in a stable form.
7. Capital costs are generally lower than conventional smelting and refining processes.
8. In the case of copper it is a compatible technology with current existing solvent extraction-electrowinning (SX-EW) plants.
9. Quick start-ups are possible, such as the BIOX® refractory gold plants.
10. The ability to economically process run-of-the-mine low-grade sulphide ores.
11. The ability to process ores that may not be feasible to be smelted due to the negative impact it may present on the environment (Gilbertson, 2000; Dresher, 2004).

However, bioleaching will not completely replace smelting for the following reasons:

1. Bioleaching does not recover precious metals in the ore, which are often an important component in the profitability of the operation.
2. The smelter requires the production of acid for conventional leaching, as well as to supplement bioleaching in the case that the ore body is high in acid consuming minerals. Certain acids, such as sulphuric acid are in short supply and expensive to deliver to remote locations where these operations normally exist.
3. Some ore bodies are not sufficiently high in acid consuming minerals, causing residual acid that is generated to have to be neutralised during the leaching process. In such a case smelting remains the only economically viable processing means (Dresher, 2004).

The use of microbes during ore processing has several advantages over conventional physicochemical methods, such as the fact that it is more environmentally friendly,

less energy is required and sulphur dioxide and other environmentally harmful gasses are not emitted (Rawlings, 2002). Furthermore, the wastes created by physicochemical mining procedures may lead to unfavourable acid and metal pollution (acid rock/mine drainage) when exposed to water and air through biological leaching (Colmer and Hinkle, 1947; Rawlings, 2002).

2.4.3 Microbiology of Bioleaching

Due to the substrate limitations that exist in mining environments, it was previously thought that these environments would have a low diversity of microbial flora (Rohwerder *et al*., 2003). Through extensive research conducted by Johnson (1998), as well as Hallberg and Johnson (2001), it has become evident that this is in fact not the case. At least 11 recognized prokaryotic divisions have shown to exist at acid mine drainage sites (Baker and Banfield, 2003). A variety of bacteria found in mining environments are able to play a role in bioleaching of metal sulphides, which include thermophilic microorganisms, heterotrophic bacteria as well as members of the genera *Leptospirillum* and *Acidithiobacillus* (Glazer and Nikaido, 1995).

The biology of microorganisms involved in bioleaching is becoming more complex due to increasing data on 16S rDNA gene sequences, enabling the description of new species of leaching bacteria and the reclassification of known species (Rohwerder *et al*., 2003). In the past only mesophilic bacteria were considered to be important for bioleaching, but nowadays genera of moderately and extremely thermophilic bacteria have also become attractive (Johnson, 1998; Norris *et al*., 2000). Furthermore, the direct enzymatic oxidation of the sulphur moiety of heavy metal sulphides, as described by Sand *et al*. (1995), does not exist (Rohwerder *et al*., 2003). Instead, the "indirect mechanism", i.e., non-enzymatic metal sulphide oxidation by iron(III) ions, combined with enzymatic re-oxidation of the resulting iron (II) ions, remains and now comprises two sub-mechanisms, namely contact and non-contact mechanisms (Sand *et al*., 2001; Rawlings, 2002). The contact mechanism involves the attachment of bacterial cells to the surface of the sulphide minerals, leading to the dissolution of the sulphide minerals as a result of the electrochemical processes, which takes place at the interface between the bacterial cell wall and the mineral sulphide surface (Sand *et al*., 2001; Rawlings, 2002). The non-contact mechanism is exerted by planktonic

bacteria, which are responsible for the oxidation of iron(II) ions in solution (Sand *et al*., 2001; Rawlings, 2002). The resulting iron(III) ions come into contact with the sulphide mineral surfaces where oxidation takes place (Sand *et al*., 2001; Takai *et al*., 2001; Rawlings, 2002). Both contact- and non-contact mechanisms involve bacteria, which contribute to mineral dissolution by generation of the oxidizing agent, iron(III) ions, and by the subsequent oxidizing of the sulphur compounds resulting from the dissolution (Sand *et al*., 2001; Rawlings, 2002).

2.4.3.1 The Diversity Among Leaching Bacteria

The predominant bioleaching microorganisms are extremely acidophilic bacteria, which are able to oxidise either inorganic sulphur compounds and/or iron(II) ions (Rohwerder *et al*., 2003). The classical leaching bacteria belong to the genus *Acidithiobacillus* (formerly *Thiobacillus*) (Kelly and Wood, 2000). The mesophilic *At. ferrooxidans* and *At. thiooxidans* are the first isolates of extremely acidophilic sulphur and/or iron(II)-oxidizing bacteria, and together with the moderately thermophilic *At. caldus* they belong to the Gram-negative γ-proteobacteria (Kelly and Wood, 2000). Other proteobacteria used for leaching include species of the genus *Acidiphilium*, such as *Ac. acidophilum* (Hiraishi *et al*., 1998), as well as members of the genus *Leptospirillum*, which belong to a new bacterial division (Hippe, 2000; Coram and Rawlings, 2002). In addition, some Gram-positive leaching bacteria have also been described, which include moderately thermophilic members of the genera *Acidimicrobium*, *Ferromicrobium* and *Sulfobacillus* (Clark and Norris, 1996; Norris *et al*., 1996; Johnson and Roberto, 1997). *Sulfolobales*, a group of extremely thermophilic, sulphur- and iron(II)-oxidizing archaebacteria, have also been implicated in leaching processes and include genera such as *Sulfolobus*, *Acidianus*, *Metallosphaera* and S*ulfurisphaera* (Fuchs *et al*., 1995; Fuchs *et al*., 1996; Kurosawa *et al*., 1998; Norris *et al*., 2000). Two species of mesoacidophilic iron(II) oxidizing archaebacteria have also been reported to play a role in bioleaching, namely *Ferroplasma acidiphilum* and *F. acidarmanus* (Edwards *et al*., 2000; Golyshina *et al*., 2000).

Bioleaching bacteria include species with an extremely limited substrate spectrum, in particular *L. ferrooxidans* and *L. ferriphilum*, which are only able to grow by

aerobically oxidizing iron(II) ions (Rohwerder *et al*., 2003). In contrast, *At. ferrooxidans* has a broad metabolic capacity, allowing this bacterium to live on the oxidation of reduced sulphur compounds, as well as the oxidation of molecular hydrogen, formic acid and iron(II) ions (Rohwerder *et al*., 2003). In addition, *At. ferrooxidans* is able to grow anaerobically by oxidation of sulphur compounds or hydrogen coupled with iron(III) ion reduction (Das *et al*., 1992; Pronk *et al*., 1992; Ohmura *et al*., 2002). At least 11 different cytochromes of the *c* type have been identified in the genome of *At. ferrooxidans*, suggesting the application of electron acceptors other than oxygen (Yarzábal *et al*., 2002).

Further metabolic diversity among leaching bacteria has been found with respect to their carbon assimilation pathways (Rohwerder *et al*., 2003). *Acidithiobacillus* spp. and *Leptospirillum* spp. are only able to grow chemolithoautotrophically, whereas *Acidiphilium acidophilum* and *Acidimicrobium ferrooxidans* are able to grow autotrophically with sulphur and iron(II) compounds, heterotrophically with glucose or yeast extract, and mixotrophically with all of these substrates (Clark and Norris, 1996; Hiraishi *et al*., 1998). An obligate mixotrophic iron(II)-oxidizing bacterium is *Ferromicrobium acidophilus* (Johnson and Roberto, 1997), while some *Sulfobacillus* spp. show poor chemolithotrophic growth, as do many thermophilic *Sulfolobales* (Johnson, 1998). Several *Acidiphilium* spp. and *Acidisphaera rubrifaciens* contain pigments, which may indicate the ability for photosynthetic activity (Hiraishi *et al*., 1998; Hiraishi *et al*., 2000; Hiraishi and Shimada, 2001).

2.4.3.2 Attachment of Leaching Bacteria to Sulphide Minerals

The attachment of leaching bacteria to the sulphidic energy source and the subsequent biofilm formation are prerequisites for mineral dissolution in natural environments, as well as industrial operations (Ruiz *et al*., 2007). These processes are mediated by extracellular polymeric substances (EPS), comprising polysaccharides, proteins and DNA (Vandevivere and Kirchman, 1993; Gehrke *et al*., 1998; Sand and Gehrke, 2006). The EPS mediates the attachment to a metal sulphide surface where it concentrates iron(III) ions by complexation through uronic acids or other residues at the mineral surface, thus, allowing an oxidative attack on the sulphide mineral (Sand and Gehrke, 2006). In the case of *At. ferrooxidans* R1 and pyrite, it was demonstrated

that produced EPS consisted of glucose, rhamnose, fucose, xylose, mannose, C_{12}-C_{20} saturated fatty acids, glucuronic acid and iron(III) ions (Gehrke *et al*., 1998; Gehrke *et al*., 2001). The attachment occurs due to mainly electrostatic interaction of the positively charged cells with negatively charged pyrite (Solari *et al*., 1992; Blake *et al*., 1994). In contrast, hydrophobic interactions do not play a role in the attachment of the bacteria to metal sulphide surfaces (Gehrke *et al*., 1998; Sampson *et al*., 2000). Cells that are grown on elemental sulphur do not grow on pyrite due to a considerably modified EPS composition (Rohwerder *et al*., 2003). Sulphur-grown EPS contains no complexed iron(III) ions or other positively charged groups, and less sugars and uronic acids, while it contains more fatty acids than pyrite-grown EPS (Rohwerder *et al*., 2003). Therefore, hydrophobic interactions play a role during the attachment of *At. ferrooxidans* to sulphur (Gehrke *et al*., 1998). This suggests that the leaching bacteria are able to adapt the composition and amount of their EPS according to the growth substrate (Rohwerder *et al*., 2003). In addition, the EPS serves as the reaction space where the biooxidation reactions take place (Sand *et al*., 1995; Gehrke *et al*., 1998; Tributsch, 2001; Rohwerder *et al*., 2003).

2.4.3.3 The Effect of Temperature on Bioleaching

Bioleaching processes have been operated at a range of temperatures from ambient to 80ºC (Rawlings *et al*., 2003). The types of iron- and sulphur oxidizing bacteria used for bioleaching practices differ depending on the temperature range of the process (Rawlings, 2005). The types of leaching bacteria found in processes operating from ambient to 40ºC tend to be similar irrespective of the mineral being treated, as are those within the temperature ranges 45-55ºC and 75-80ºC (Rawlings, 2005). Mineral solubilization processes are exothermic, and therefore, during some bioleaching processes cooling is required to keep the process at the optimum leaching temperature (Rawlings, 2005). Higher leaching temperatures generally lead to a much higher rates of chemical reactions, and in the case of minerals such as chalcopyrite, temperatures of 75-80ºC are required for copper extraction to take place at an economically viable rate (Rawlings, 2005).

2.4.4 Industrial Bioleaching Processes

Many industrial bioleaching operations, such as dump leaching and heap leaching, are currently in use worldwide. Dump leaching and heap leaching are almost identical processes, however, in the case of dump leaching, the ore is taken directly from the mine and stacked on a leach pad, whereas in the case of heap leaching, the ore is crushed into finer particles before it is heaped on a leach pad (Wikipedia, 2007a; Wikipedia, 2007b). During both processes the stacked ore is irrigated with leaching solution, which percolates through the heap and leaches out the valuable metals (Wikipedia, 2007a; Wikipedia, 2007b).

The early development and application of the concept of bioleaching was conducted in the United States, but since many other countries, such as Chile, have joined this revolutionising concept in the mining industry (Dresher, 2004).

2.4.4.1 The BIOX® Process

The Biological Oxidation (BIOX®) Process for the pre-treatment of refractory sulphide ores was commercialised in 1988, following extensive research conducted at Billiton Process Research (formerly GENCOR Process Research) in the 1970's and 1980's (Van Aswegen and Marais, 1998). This process offers many advantages over other conventional refractory treatment processes, such as roasting and pressure oxidation, which includes the following:

1. the bacterial cultures are able to withstand the fluctuations experienced on an operating plant,
2. the process is suited for operation in remote areas due to its simplicity,
3. the scale-up capability of the process has been demonstrated, and thus the process may be applied to large refractory ore bodies, and
4. due to the neutralisation of the plant effluents, precipitates are produced which comply with the most stringent environmental regulations.

In 1988, when it was decided to commercialise the technology on a licensing basis, the only operating BIOX® plant was the 10 tonne per day demonstration plant located

at Fairview Mine in South Africa (Van Aswegen and Marais, 1998). Commercial BIOX® plants which have been, or in the process of being commissioned are listed in Table 2.7.

The BIOX® process involves a mixed population of bacteria, namely *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, which are able to break down the sulphide mineral matrix in the ore, resulting in the liberation of the occluded gold for subsequent cyanidation (Van Aswegen and Marais, 1998). The thickened flotation concentrate is continuously fed to a series of aerated stirred tank reactors (Van Aswegen and Marais, 1998). Parameters such as pH and temperature are controlled within narrow ranges to maintain the right balance of bacteria in order to achieve the optimum rate of oxidation (Van Aswegen and Marais, 1998). The optimum operating parameters of the BIOX® process are listed in Table 2.8.

TABLE 2.7

Commercial BIOX® Plants (Van Aswegen and Marais, 1998)

Location	Construction date	Capacity (t/day)
Fairview, South Africa	1984	55
Sao Bento, Brazil	1990/1991	150
Harbour Lights, Australia*	1992	40
Wiluna Mine, Australia	1993	158
Ashanti's Sansu, Ghana	1994	960
Tamboraque, Peru**	1997	60
Amantaytau, Uzbekistan***	?	2 055
Olympias, Greece***	?	668
Fosterville, Australia***	?	120

*Not in operation since 1994 due to the closing of the mine

**Decommissioned in 2000, but currently being recommissioned

***Future Developments

TABLE 2.8 Optimal Operating Parameters of the BIOX® Process	
Temperature	40-45ºC
pH	1.2-1.6
Percentage Solids in Feed	20%
Dissolved Oxygen	>2ppm
Retention Time	4-6 days
Nutrients	Fertilizer type ammonium, potassium and phosphorous salts

The bacteria attach to the metal sulphide surfaces in the ore, resulting in the increased oxidation of the sulphide minerals (Van Aswegen and Marais, 1998). The oxidation reactions that occur during the process are exothermic, and thus, circulating cooling water and removing the excess heat by cooling towers, cool the reactors (Van Aswegen and Marais, 1998). Limestone or sulphuric acid is added to keep the pH of the slurry within the optimum range, while large volumes of air is injected and dispersed in the slurry (Van Aswegen and Marais, 1998). The air contains sufficient oxygen required for sulphide mineral oxidation, as well as carbon (in the form of carbon dioxide) required to maintain microbial growth (Van Aswegen and Marais, 1998). In addition to the carbon dioxide introduced by injecting air into the slurry, carbonate minerals or limestone can be added to maintain sufficient levels of carbon necessary for microbial growth (Van Aswegen and Marais, 1998).

The overall residence time in the biooxidation reactors ranges between 4 and 6 days depending on the type of ore that is being processed, i.e. a shorter residence time can be expected when arsenopyrite is processed as compared to pyrite, because the oxidation rate of the former is faster than that of pyrite (Van Aswegen and Marais, 1998). Following the oxidation process, the BIOX® product is washed in a counter-current decantation circuit, followed by neutralisation of the solution by adding limestone and/or lime (Van Aswegen and Marais, 1998). Gold is eventually recovered from the washed BIOX® in a conventional cyanidation plant (Van Aswegen and Marais, 1998).

2.4.4.2 The GEOCOAT™ Process

Copper has been extracted from chalcopyrite for centuries using conventional pyrometallurgical methods (Harvey *et al*., 2002). Although these methods are relatively simple, they have certain disadvantages, such as high capital investments, operating costs and a negative impact on the environment (Harvey *et al*., 2002). In addition, these methods are inflexible when it comes to treating complex metal sulphides (Harvey *et al*., 2002). Therefore, dump- and stirred tank leaching using bacteria in a thermophilic system was developed (Brierley, 2000; Harvey *et al*., 2002). Commercial bioheap operations currently in operation are listed in Table 2.9.

The GEOCOAT™ process was developed by GeoBiotics, Inc. and involves heap leaching for the extraction of copper concentrates from chalcopyrite ores (Harvey *et al*., 2002). With the advantages of stirred tank systems and the simplicity of conventional heap leaching, this process provides an economical solution to the bioleaching of chalcopyrite ores (Harvey *et al*., 2002).

TABLE 2.9

Commercial Bioheap Leach Operations Currently in Operation (Harvey *et al*., 2002)

Location	Capacity (t/day)	Years in Operation
Lo Aguirre, Chile	16 000	1980-Present
Cerro Colorado, Chile	16 000	1993-Present
Girrilambone, Australia	2 000	1993-Present
Ivan, Chile	1 500	1994-Present
Quebrada Blanca, Chile	17 300	1994-Present
Andacollo, Chile	16 000	1996-Present
Dos Amigos, Chile	3 000	1996-Present
Cerro Verde, Peru	32 000	1996-Present
Zaldivar, Chile	~20 000	1998-Present
S&K Copper, Myanmar	15 000	1998-Present

Copper-bearing sulphide minerals are concentrated by flotation and thickened, resulting in a concentrate slurry (Harvey *et al*., 2002). A solid substrate, usually crushed, screened support rock, is thinly coated with the concentrate slurry, followed by stacking on a lined pad, where it is allowed to biooxidise (Johansson *et* al., 1999; Harvey *et al*., 2002). The biooxidation heap is inoculated with naturally occurring sulphide-oxidising bacteria, depending on the desired operation temperature (Johansson *et* al., 1999; Harvey *et al*., 2002). Moderate thermophiles, such as *Acidithiobacillus caldus* and *Sulfobacillus thermosulfidooxidans*, as well as extreme thermophiles, such as *Acidianus brierleyi*, *Acidianus infernus*, *Metallosphaera sedula*, *Sulfolobus acidocaldarius*, *Sulfolobus shibatae* and *Sulfolobus metallicus*, are frequently used for the GEOCOAT™ process (Harvey *et al*., 2002). The biooxidation heap is irrigated with leaching solution consisting of sulphuric acid, ferric iron and nutrients, while the heap is aerated by low-pressure blowers through a perforated pipe network at the base of the heap (Johansson *et* al., 1999; Harvey *et al*., 2002). As the biooxidation progresses, the sulphides are oxidized and the resulting soluble copper, iron arsenic and sulphate are removed from the heap by the recirculating solution, followed by neutralisation and conventional recovery methods (Harvey *et al*., 2002).

2.4.4.3 The BioCOP™ Process

The BioCOP™ process was developed by BHP Billiton, Ltd. and is conducted in an aerated stirred reactor containing dilute sulphuric acid and hyperthermophilic microorganisms, which are able to metabolise at temperatures between 60ºC and 90ºC. Limestone is added to the process to maintain the pH of the solution, as well as to provide the microorganisms with carbon dioxide needed for bacterial growth. The copper concentrate is added to the reactor and the leaching of chalcopyrite concentrates is complete within 10 days (Batty and Rorke, 2006).

2.4.4.4 The BacTech/Mintek Process

The BacTech/Mintek process involves a series of countercurrent reactors, the Circox™ bioreactor and the BAR™ (BacTech Aerated Reactor). The two proprietary bioreactors are in the experimental stage in Mexico. The Circox™ was originally developed for the bioremediation of municipal sewage and industrial wastewater.

Paques Bio Systems B.V. of the Netherlands holds the licence for the Circox™ bioreactor. The Circox™ bioreactor uses an airlift to circulate the solids within the reactor. Thermophilic microorganisms are used in the process, which operate between 25ºC and 55ºC and a pH of 0.5 to 2.5 is maintained within the reactor. Ambient air used to airlift the suspended solids within the reactor, is also responsible for adding carbon dioxide to the process. The nutrients for the microorganisms are added to the leaching liquor and the retention time is in the order of 30 days (Van Staden *et al*., 2003).

2.5 Complexation of Non-Sulphide Minerals

Metals in certain non-sulphide minerals, such as the iron ore concentrate of the Sishen Iron Ore Mine, may be solubilised by a process of complexation using organic acids, such as citric and oxalic acid (Rawlings, 2005). These organic acids are typically produced by certain types of fungi, such as *A. niger* (Jianlong, 2000; Vandenberghe *et al*., 2000; Rawlings, 2005). Citric acid contains several carboxyl groups, which tend to donate protons (H^+), resulting in negatively charged carboxyl groups that are capable of forming stable complexes with several cations (Sayer and Gadd, 2001). Therefore, it would be possible that these negatively charged carboxyl groups might form stable complexes with the positively charged K cations present. The H^+ ions released by the carboxyl groups on the other hand may be involved in a hydrolysis reaction, whereby they may react with the PO_4^{3-} ions, resulting in the subsequent P and K removal from the iron ore concentrate.

In contrast to bioleaching of sulphidic minerals using chemolithoautotrophic bacteria, which is the most studied and commercially exploitable aspect of mineral biotechnology today, there is a scarcity of literature on the dissolution of non-sulphidic minerals, such as oxides, silicate, carbonate and hydroxide minerals (Jain and Sharma, 2004). Complexation of non-sulphidic minerals involves the use of heterotrophic microorganisms, which require an organic carbon source as a source of energy and carbon for their growth (Jain and Sharma, 2004). As non-sulphidic ores generally contain no energy source for these microorganisms to utilize, an energy source must be added to the system (Jain and Sharma, 2004).

Heterotrophic microorganisms produce metabolic by-products that may interact with a mineral surface (Jain and Sharma, 2004). In addition to forming several organic acids such as acetic, citric, oxalic and keto-gluconic acid (Castro *et al*., 2000; Natarajan and Deo, 2001), heterotrophic microorganisms also produce exopolysaccharides, amino acids and proteins which are also able to solubilise metals via a variety of mechanisms (Welch and Vandevivere, 1995; Welch *et al*., 1999). However, organic acids have the advantage of producing both protons and a metal complexing organic acid anion (Gadd, 1999). Heterotrophic microorganisms also have other mechanisms that enable them to effectively leach non-sulphidic minerals. These mechanisms include bio-reduction, acidification, complexolysis and alkalinisation.

Bio-reduction involves microorganisms which are able to solubilise minerals, such as limonite, goethite or hematite, by reduction (Ehrlich, 1986; Ferris *et al*., 1989; Jain and Sharma, 2004). Ghiorse (1988) proposed that the production of oxalic acid by a fungus can affect the reduction of Fe (III) to Fe (II), thus increasing iron solubility. A process for the biological reduction of iron ore using *Pseudomonas* sp. has been developed (Hoffman *et al*., 1989), while Rusin *et al*. (1994) suggested a process for bioremediation of heavy metal contaminated soil using an iron-reducing *Bacillus* strain.

Acidification may result either from the formation of an acidic metabolite or from a preferential utilization of alkaline substrate (Jain and Sharma, 2004). It has been found that lowering the pH to less than 5 resulted in an increased dissolution rate of many silicate and aluminium silicate minerals (Welch and Ullman, 1996). Among the organic acids, 2-ketogluconic acid produced by some bacteria and citric acid and oxalic acid produced by some fungi have been shown to be effective in the dissolution of silicates by furnishing protons that help in breaking Si-O and Al-O bonds through protonation and catalysis (Welch and Ullman, 1996; Vandevivere *et al*., 1994; Drever and Stillings, 1997).

Complexolysis is a process that utilizes microbially formed complexing and chelating agents that mobilize mineral constituents (Fe, Al, Cu, Zn, Ni, Mn, Ca, Mg, etc.) (Jain and Sharma, 2004). Microorganisms are able to produce and excrete organic ligands

as a result of fermentation and degradation of organic macromolecules (Tzeferis and Agatzini-Leonardou, 1994; Paris *et al.,* 1996; Gadd, 1999). These organic ligands are able to increase the rates of mineral weathering by forming stable soluble metal-organic complexes in solution, resulting in increased mineral solubility (Amerhein and Surez, 1988; Bennett *et al*., 1988; Wieland *et al*., 1988). In addition, microbes are also able to produce extracellular polysaccharides, which are able to enhance mineral dissolution by complexing with ions in solution, or they can inhibit dissolution by irreversibly binding to reactive sites on the mineral surface (Welch and Vandevivere, 1995; Welch and Ullman, 1999; Welch *et al*., 1999). A further mechanism of metal solubilisation is the production of iron-chelating siderophores that specifically solubilise Fe (III) (Liermann *et al*., 2000).

Bio-solubilisation of silicates is also possible through alkalinisation of the media. Avakyan (1985) demonstrated the release of silicon from nepheline, plagioclase or quartz by using the bacteria *Sarcina ureae*. *S. ureae* produces ammonia, which results in the high alkalinisation of the medium. Under these conditions the Si-O bond is disrupted, resulting in the solubilisation of the mineral (Jain and Sharma, 2004).

Factors that affect the bioleaching process include the microbial population characteristics as well as various physicochemical parameters and the properties of the mineral to be leached (Brandl, 2001). Heterotrophic bioleaching is affected by the size of the microbial population, its metal tolerance and adaptation abilities to the mineral environment (Jain and Sharma, 2004). As an example, enhanced nickel extraction with *Aspergillus niger* and better cobalt extraction with *Penicillium funiculosum* was achieved from low-grade laterite ores (Valix *et al*., 2001b). Inoculum density has also been found to affect the bioleaching rate of minerals. A 50% enhancement in the rate of zinc extraction from filter dust was achieved on doubling the size of the inoculums of *P. simplicissimum* (Burgstaller *et al*., 1992). The rate of leaching is affected by the toxicity of certain metals, and therefore, the use of metal tolerant species enhances the rate of leaching (Tzeferis *et al*., 1994; Valix *et al*., 2001a). Physicochemical parameters, such as temperature, pH, oxygen supply, stirring rate and nutritional composition of the medium have a direct influence on the leaching efficiency of microbes (Jain and Sharma, 2004). The properties of the mineral to be leached also affect the leaching process. Pulp density of the solid to be

leached, particle size, mineralogical composition, effect of pre-treatment, surface area and hydrophobicity of the solids are major factors in determining the rate and extent of leaching (Jain and Sharma, 2004).

In addition to the extraction of metals from non-sulphidic ores and industrial residues, bioleaching with heterotrophic microorganisms can also be used to remove undesirable metal impurities from ores (biobeneficiation), as well as to detoxify soil, sediment and waste material polluted with heavy metals (Jain and Sharma, 2004).

There are both advantages and disadvantages of using heterotrophic microorganisms for bioleaching. Leaching by heterotrophs to solubilise metals from minerals is possible at high pH, while metal leaching by most of the autotrophic bacteria is possible only in acidic conditions (Burgstaller and Schinner, 1993; Krebs *et al*., 1997). The more neutral pH range at which heterotrophic microorganisms grow allows for easier microbial contamination to occur, and process sterilisation is costly and also presents technical problems for large scale operations (Jain and Sharma, 2004). Another cost consideration is the need for an organic carbon source for the growth of the heterotrophic microorganisms and the subsequent production of leaching agents. If cheap organic wastes generated in agriculture, in the food industry or in biotechnological processes can be used as growth substrates, leaching with fungi may be economic on an industrial scale (Jain and Sharma, 2004).

The identification, characterisation, selection, and development of bioprocesses for industrial and commercial applications require interdisciplinary cooperation between microbiologists, chemists, metallurgists and engineers. Heterotrophic leaching is extremely promising for the development of extraction technologies for non-sulphidic ores (Jian and Sharma, 2004).

2.5.1 Citric Acid Production by *Aspergillus niger*

Citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) (Figure 2.9) is an intermediate in the TCA cycle (also known as the citric acid cycle or Krebs cycle) (Figure 2.10), and is an important commercial product with global production reaching the 1.4 million ton/yr range (Graff, 2006). The majority of citric acid is produced by

submerged fermentation using the white rot fungus, *A. niger* (Jianlong, 2000; Vandenberghe *et al*., 2000). Citric acid is widely used in the food, beverage, pharmaceutical, chemical and cosmetic industries, and finds other applications in textiles, electroplating and bioremediation (Wang and Liu, 1996; Tran *et al*., 1998; Ates *et al*., 2002). In the United Kingdom, citric acid has been used as a buffer to increase the solubility of brown heroin. Single-use citric acid sachets have been used as an inducement in order to get heroin users to exchange their dirty needles for clean needles in an attempt to decrease the spread of HIV and hepatitis (Garden *et al*., 2003). Due to all the different applications, the volume of citric acid production by fermentation is continually increasing to keep up with the demand (Jianlong and Ping, 1998).

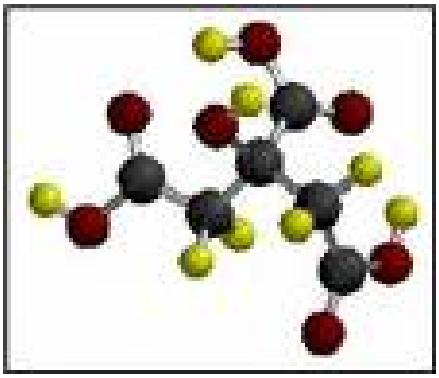

FIGURE 2.9

Molecular structure of citric acid (2-hydroxypropane-1,2,3-tricarboxylic acid) (Wikipedia 2006b)

Aspergillus niger is the most popular microorganism for the large-scale production of citric acid due to its high citric acid productivity at low pH without the secretion of toxic metabolites (Kim, 2004; Legiša and Mattey, 2007). In nature, *A. niger* grows on natural plant debris, such as leaves and on fruit (Legiša and Mattey, 2007). Most plant materials contain low levels of free carbohydrates (less than 100 µmol), whereas fruits may contain up to 500 mmol of sugars (Wrolstad and Shallenberger, 1981), and therefore, similar high levels of carbohydrates are implemented in the citric acid fermentation process (Legiša and Mattey, 2007).

Citric acid production by *A. niger* involves two main metabolic pathways, namely: 1) the catabolic pathway of hexoses to pyruvate and Acetyl-CoA by glycolysis, and 2) citric acid formation by the TCA cycle (Alvares-Vasquez *et al*., 2000).

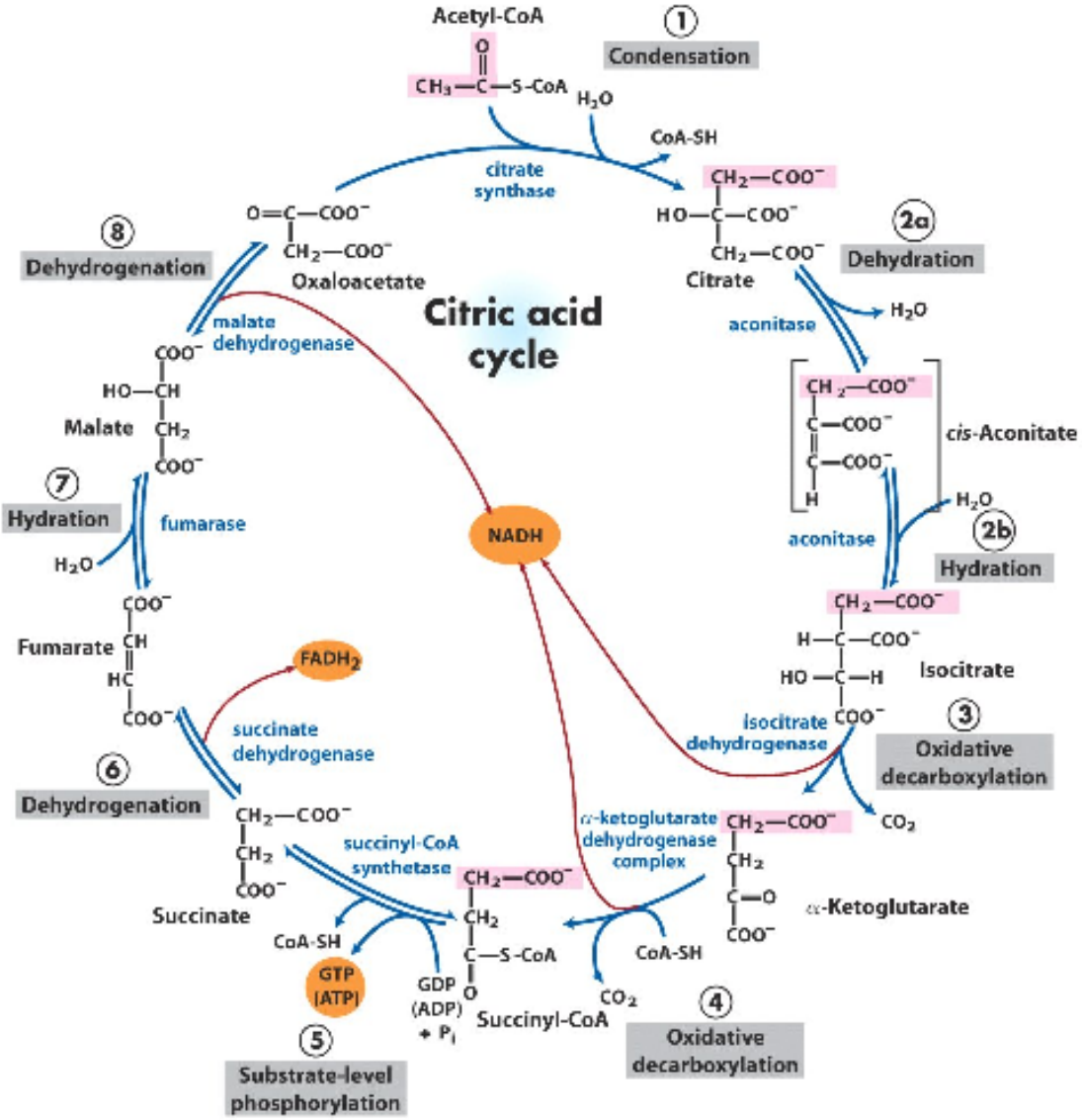

FIGURE 2.10

Major Intermediates of the Tricarboxylic Acid Cycle (Wikipedia, 2006c)

Glucose plays an important role in the production rate of citric acid, as its initial supply limits the glucolytic reactions of *A. niger* (Torres, 1994a; Torres, 1994b). Citric acid is excreted from *A. niger* cells in response to unfavourable intracellular conditions (Legiša and Mattey, 2007). This results in increased levels of TCA, such as citric acid and oxalic acid, during the growth of the fungus in high glucose

concentration media (Legiša and Mattey, 2007). It is documented that there are three main metabolic events that replenish TCA intermediates and predispose the fungal cells to product formation:

1. Fast uptake of glucose through simple diffusion,
2. Unrestricted metabolic flow through glycolysis, resulting in the availability of precursors for TCA intermediate synthesis, and
3. Uncoupled NADH re-oxidation resulting in lower levels of ATP and thus decreased anabolic reactions (Legiša and Mattey, 2007).

2.5.2 Uptake of Glucose Based on Passive Diffusion

Hexoses are commonly used in industry as the carbon source of choice for citric acid fermentation, since *A. niger* grow well on glucose and fructose (Hondmann and Visser, 1994). The hexoses are hydrolysed to monomers by invertase during the germination of the fungal spores (Legiša and Mattey, 2007). Although *A. niger* contains both high and low affinity carriers, which facilitate the uptake of glucose into the cell, it is documented that the glucose uptake rate and glucose concentration in citric acid producing systems is due to a simple diffusion model, rather than facilitated diffusion or active transport (Wayman and Mattey, 2000). This could be expected as both the high affinity carrier (encoded for by the sugar transporter gene, *mst*A) and low affinity carrier (presumed to be encoded for by the expression of the *mst*E gene) are inhibited at low pH and in the presence of citrate (VanKuyk *et al*., 2004; Forment *et al*., 2006; Legiša and Mattey, 2007). In contrast, citric acid producing systems showed no response to citric acid levels (Wayman and Mattey, 2000).

2.5.3 Regulation of Glycolysis

Physiological studies of *A. niger* during citric acid accumulation have indicated that no citric acid could be detected in the medium up to 24 h of growth in a batch system (Legiša and Mattey, 1986a). During the second day of fermentation, however, a relatively slow excretion rate was recorded, which was followed by a sudden increase in productivity thereafter (Ruijter *et al*., 1997; Papagianni *et al*., 2005). Abnormal spore germination in the form of bulbous cells can be observed during the early stages

of fungal growth, followed by the formation of highly branched filamentous hyphae after approximately 24 h (Legiša and Mattey, 2007). Legiša and colleagues (1981) showed that the filamentous hyphae, representing the major part of the biomass, are responsible for the citric acid excretion into the fermentation media.

The pentose phosphate pathway is predominant during the germination of the fungal spores, followed by a switch to glycolysis before citric acid is excreted (Legiša and Mattey, 1986b; Röhr *et al*., 1987). During these initial phases of fungal growth polyols are formed in addition to the accumulation of glucosamine (Röhr *et al*., 1987; Papagianni *et al*., 2005). As a result of this transient glucosamine accumulation in the medium, as well as the intermediate accumulation and later partial re-consumption of various polyols (glycerol, arabitol, erythritol and mannitol), quantitative balances indicate that more hexoses are taken up by the fungus than can be accounted for by the production of biomass, CO_2 and citrate during the first stage of fermentation, while more citrate is excreted during the later phase than sugar uptake would theoretically allow (Röhr *et al*., 1987; Papagianni *et al*., 2005). Polyols, mostly synthesised by the intermediates of the pentose phosphate pathway, may play an important role as osmoregulators in *A. niger* cells during growth in high sugar concentration media (Legiša and Mattey, 1986b; Röhr *et al*., 1987; Hondmann and Visser, 1994; Legiša and Kidrič, 1989).

The production phase of citric acid accumulation is initiated after approximately 24 h and accelerates after 40-50 h of growth in a batch system (Papagianni *et al*., 2005). During this phase the direct conversion of hexoses to pyruvate via glycolysis becomes predominant (Legiša and Mattey, 1986b). The mechanism causing the shift of glucose degradation from the pentose phosphate pathway to glycolysis is not yet fully understood (Legiša and Mattey, 2007). The central part of hexose metabolism is regulated at several levels:

1. At the transcription level,
2. By regulating the activity of allosteric enzymes by specific effectors, and
3. By post-translational modification (Mesojednik and Legiša, 2005; Mlakar and Legiša, 2006; Legiša and Mattey, 2007).

Panneman and colleagues (1998) showed that the transfer of the fungal mycelia to media with various carbon sources stimulated the synthesis of hexokinase, glucokinase and pyruvate kinase. The most pronounced effect on the initiation of transcription was observed in the case of glucose and fructose. In addition, it was found that the presence of a metabolisable carbon source was not sufficient for the expression of hexokinase and glucokinase genes in *A. niger*, but that active carbon metabolism was also required (Panneman *et al*., 1998).

2.5.3.1 Hexokinase and Glucokinase

The genes for hexokinase and glucokinase have been isolated from *A. niger* and the kinetics parameters have been determined (Panneman *et al*., 1996; Panneman *et al*., 1998). The proteins encoded for by the *A. niger* glucokinase gene *glk*A and hexokinase gene *hxk*A, respectively, both show similarity to other eukaryotic glucokinase and hexokinase proteins, in particular to the *Saccharomyces cerevisiae* glucokinase protein and the hexokinase proteins of budding yeasts (Panneman *et al*., 1996; Panneman *et al*., 1998). The hexokinase and glucokinase enzymes of *A. niger* are responsible for catalysing the hexose phosphorylation step (Legiša and Mattey, 2007). The hexokinase and glucokinase contribution towards the glucose phosphorylation was discovered to be dependant on the intracellular pH, as well as the glucose concentration in the medium (Panneman *et al*., 1998). At a pH of 7.5 it was found that the glucokinase activity was predominant, while at a pH of 6.5 and a glucose concentration above 0.5 mM the hexokinase activity became predominant (Panneman *et al*., 1998). The glucokinase and hexokinase genes are both expressed constitutively during active carbon metabolism, however, wider substrate specificity was observed with hexokinase than with glucokinase (Panneman *et al*., 1996; Panneman *et al*., 1998).

2.5.3.2 6-Phosphofructo-1-kinase

ATP-dependent 6-phosphofructose-1-kinase (*Pfk*1) is the second allosteric enzyme of the glycolytic pathway, which plays a crucial role in controlling metabolic flux in eukaryotes by catalysing the second essentially irreversible reaction of glycolysis, the

phosphorylation of fructose 6-phosphate using Mg-ATP to form fructose 1,6-biphosphate and releasing Mg-ADP (Legiša and Mattey, 2007).

2.5.4 Fermentation Conditions Affecting Citric Acid Production

Medium composition, such as carbon, nitrogen, P and K, plays an important role in the growth and metabolism of microorganisms, and therefore, optimising the medium composition may enhance the production of citric acid by *A. niger*.

Sucrose, fructose and glucose are the carbon sources of choice for the production of citric acid by *A. niger* (Sassi *et al*., 1991). Apple peels and pomace, grape pomace, banana extract, sugar cane bagasse and sugar beet molasses have been used in the past for the production of citric acid (Ngadi and Correia, 1992; Wang, 1998; Gutierrez-Correa *et al*., 1999). Of these carbon substrates, glucose is readily used by *A. niger*, as it does not need any modification to be metabolised (Kim, 2004). An increase in the glucose flux through glycolysis causes the over-production of citric acid during solid substrate fermentation, however, low concentrations of glucose may lead to the production of oxalic acid (Habison *et al*., 1983; Röhr and Kubicek, 1981; Alvarez-Vasquez *et al*., 2000; Leangon *et al*., 2000).

The effect of nitrogen on citric acid production has been studied extensively (Papagianni *et al*., 2005). Protein catabolism, as a result of manganese deficiency, leads to a high intracellular ammonium (NH_4^+) concentration, causing the inhibition of the enzyme phosphofructokinase (Röhr and Kubicek, 1981; Habison *et al*., 1983). Phosphofructokinase is an essential enzyme in the conversion of glucose and fructose to pyruvate, and therefore, its inhibition leads to a flux through glycolysis and the formation of citric acid (Röhr and Kubicek, 1981; Habison *et al*., 1983). Nitrogen and phosphate concentrations are low in media designed for citric acid fermentation by *A. niger* (Papagianni *et al*., 2005). In defined media, nitrogen can be supplied as ammonium sulphate and ammonium nitrate, resulting in a decrease in pH, which is a prerequisite of citric acid fermentation (Papagianni *et al*., 2005). Exogenous addition of NH_4^+ during citric acid fermentation was found to stimulate the rate of citrate production (Choe and Yoo, 1991; Yigitoglu and McNeil, 1992). The exogenous P concentration, like in the case of nitrogen, plays an important role in citric acid

fermentation (Mirminachi *et al*., 2002). The limiting concentration of P can be supplied as KH_2PO_4 or K_2HPO_4, inducing higher citric acid production and yield (Mirminachi *et al*., 2002).

Citric acid production is influenced by a number of culture conditions, and therefore, it is important to study the influence that the physical and chemical environments have on citric acid fermentation (Jianlong and Ping, 1998). Fungal cells show signs of adverse growth and metabolic production when cultivated under unfavourable temperatures (Ellaiah *et al*., 2003). Under higher temperatures than optimal, enzyme denaturation and inhibition, excess moisture losses and the ceasing of growth occurs, while at lower temperatures metabolic activity decreases (Adinarayana *et al*., 2003). The optimal temperature for the production of citric acid by *A. niger* ranges from 28 to 40ºC, depending on the fungal strain used (Roukas, 2000; Papagianni *et al*., 2005; Papagianni and Mattey, 2006).

Most filamentous fungi grow well under slightly acidic conditions ranging from pH 3 to 6, while some are able to grow at a pH below 2 in order to compete more efficiently with bacteria (Fawole and Odunfa, 2003). For citric acid production by *A. niger*, an initial pH range from 2 to 6 is required for solid substrate and submerged fermentation (Watanabe *et al*., 1998; Adham, 2002; Lesniak *et al*., 2002).

Inoculum density plays an important role during citric acid fermentation. A high inoculum density leads to population over-crowding, higher nutrient competition and rapid exhaustion of nutrients, while a lower inoculum density leads to a decrease in metabolite production, as well as an increase in contamination risk as a result of an insufficient cell population (Kota and Sridhar, 1999). For citric acid fermentation by *A. niger*, an inoculum density between 1×10^4 to 1×10^9 spores.ml^{-1} was found suitable (Favela-Torres *et al*., 1998; Ruijter *et al*., 2000; Adham, 2002; Papagianni *et al*., 2005; Papagianni and Mattey, 2006).

2.6 Recommendations

Bioleaching offers several advantages over conventional physicochemical methods for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine.

Bioleaching offers more environmentally friendly processes, less energy is required for operation and sulphur dioxide and other environmentally harmful gasses are not emitted (Rawlings, 2002). The P and K contained within the iron ore concentrate are both non-sulphidic minerals, and therefore, conventional bioleaching may not be a suitable candidate for their removal from the ore. However, it is known that non-sulphidic minerals, such as the P and K contained within the iron ore concentrate, may be solubilised by complexation using organic acids, such as citric acid (Rawlings, 2005). Citric acid contains several carboxyl groups, which tend to donate protons (H^+), resulting in negatively charged carboxyl groups that are capable of forming stable complexes with several cations, such as K present in the iron ore, and at the same time the release of H^+ ions may result in a hydrolysis reaction involving the P contained in the iron ore, resulting in the subsequent P and K removal from the iron ore concentrate of the Sishen Iron Ore Mine.

Therefore, the following recommendations are suggested for the removal of P and K from the iron ore concentrate of the Sishen Iron Ore Mine:

1. A microbial community study of the water resources and iron ore concentrate must be conducted to investigate the possibility of using microorganisms already present, and thus adapted to the extreme environment of the Sishen Iron Ore Mine, for the development of a viable process to remove the P and K from the iron ore concentrate,
2. A range of inorganic- and organic acids must be evaluated for their potential to chemically remove P and K from the iron ore concentrate,
3. The production of citric acid by *A. niger* must be evaluated to determine the most economically viable acid fermentation process,
4. The chemical leachability of the P and K from the iron ore concentrate using different citric acid concentrations and leaching temperatures must be determined.
5. The results of the abovementioned recommendations must be taken into account to propose an economically viable biotechnology process for the removal of the P and K from the iron ore concentrate of the Sishen Iron Mine.

2.7 References

ADHAM NZ (2002) Attempts at improving citric acid fermentation by *Aspergillus niger* in beet-molasses medium. *Biores Technol* **84:** 97-100.

ADINARAYANA K, PRABHAKAR T, SRINIVASULU V, ANITHA RM, JHANSI LP and ELLAIAH P (2003) Optimisation of process parameters cephalosporin C production under solid state fermentation from *Acremonium chrysogenum*. *Proc Biochem* **39:** 171-177.

ALVARES-VASQUEZ F, GONZALEZ-ALCO C and TORRES NV (2000) Metabolism of citric acid production by *Aspergillus niger*: model definition, steady-state analysis and constrained optimisation of citric acid production rate. *Biotechnol Bioeng* **70:** 82-108.

AMERHEIN C and SUREZ DL (1988) The use of a surface complexation model to describe the kinetics of ligand promoted dissolution of anorthite. *Geochim Cosmochim Acta* **52:** 2785-2793.

AMETHYST GALLERIES' MINERAL GALLERY (1996) http://mineral.galleries.com.

AMETHYST GALLERIES' MINERAL GALLERY (1999) http://mineral.galleries.com.

ATES S, DINGIL N, BAYRAKTAR E and MEHMETOGLU U (2002) Enhancement of citric acid production by immobilized and freely suspended *Aspergillus niger* using silicone oil. *Process Biochem* **38:** 433-436.

AVAKYAN ZA (1985) Microflora of rock and its role in the leaching of silicate minerals. In: KARAVAIKO GI and GROUDEV SN (Editors) Biogeotechnology of metals. Moscow: Centre of International Projects, GKNT. p. 175-194.

BAKER BJ and BANFIELD JF (2003) Microbial communities in acid mine drainage. *FEMS Microbiol Ecol* **44:** 139-152.

BATTY JD and RORKE GV (2006) Development and commercial demonstration of the BioCOP™ thermophile process. *Hydrometallurgy* **83:** 83-89.

BENNETT PC, MELCER ME, SIEGEL DI and HASSETT JP (1988) The dissolution of quartz in dilute aqueous solutions of organic acids at 25°C. *Geochim Cosmochim Acta* **52:** 1521-1530.

BLAKE RC II, SHUTE EA and HOWARD GT (1994) Solubilization of minerals by bacteria: electrophoretic mobility of *Thiobacillus ferrooxidans* in the presence of iron, pyrite, and sulfur. *Appl Environ Microbiol* **60:** 3348-3357.

BOSECKER K (1997) Bioleaching: Metal solubilization by microorganisms. *FEMS Microbiol Rev* **20:** 591-604.

BRANDL H (2001) Microbial leaching of metals. In: REHM H-J (Editor) Biotechnology, 2nd Edition (Volume 10). Wiley-VCH, Germany. pp 191-224.

BRAZILIAN ROCKHOUNDS (2005) http://brhounds.com.

BRIERLEY CL (2000) Bacterial succession in bioheap leaching. *Hydrometallurgy* **59:** 249-255.

BURGSTALLER W and SCHINNER F (1993) Leaching of metals with fungi. *J Biotechnol* **27:** 91-116.

BURGSTALLER W, STRASSER H, WOBKING H and SCHINNER F (1992) Solubilisation of zinc oxide from filter dust with *Penicillium simplicissimum*: bioreactor leaching and stoichiometry. *Environ Sci Technol* **26:** 340-346.

CAIRNCROSS B and DIXON R (1995) Minerals of South Africa. Geological Society of South Africa, Johannesburg.

CARNEY MD and MIENIE PJ (2003) A geological comparison of the Sishen and Sishen South (Welgevonden) iron ore deposits, Northern Cape Province, South Africa. *Appl Earth Sci (Trans Inst Min Metall B)* **112:** B81-B88.

CASTRO IM, FIETTO JLR, VIEIRA RX, TROPIA MJM, CAMPOS LMM, PANIAGO EB and BRANDAO RL (2000) Bioleaching of zinc and nickel from silicate using *Aspergillus niger* culture. *Hydrometallurgy* **57:** 39-49.

CHOE J and YOO YJ (1991) Effect of ammonium ion concentration and application to fed-batch culture for over-production of citric acid. *J Ferm Bioeng* **72:** 106-109.

CLARK DA and NORRIS PR (1996) *Acidimicrobium ferrooxidans* gen. nov., sp. nov.: mixed-culture ferrous iron oxidation with *Sulfobacillus* species. *Microbiology* **142:** 785-790.

COLMER AR and HINKLE ME (1947) The role of microorganisms in acid mine drainage: a preliminary report. *Science* **106:** 253.

CONSTANTINOU G (1992) Ancient copper mining in Cyprus. In: Marangou, A. (Editor) Cyprus: Copper and the Sea. Publication accompanying the Cyprus exhibition at the 1992 Seville Universal Exhibition EXPO 92.

CORAM NJ and RAWLINGS DE (2002) Molecular relationships between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferriphilum* sp. nov. dominates South African commercial biooxidation tanks that operate at 40 degrees C. *Appl Environ Microbiol* **68:** 838-845.

DAS A, MISHRA AK and ROY P (1992) Anaerobic growth on elemental sulfur using dissimilar iron reduction by autotrophic *Thiobacillus ferrooxidans*. *FEMS Microbiol Lett* **97:** 167-172.

DICINOSKI WB, SCHLITT WJ and AMBALAVANER VJ (1998) A global engineer's perspective of copper leaching, solvent extraction and electrowinning. Paper presented at ALTA Copper Hydrometallurgy Forum, Brisbane, Australia.

DRESHER WH (2004) Innovations in copper: Mining and extraction: Producing copper nature's way: Bioleaching. www.copper.org/innovations/2004/May.

DREVER JI and STILLING LL (1997) The role of organic acids in mineral weathering. *Coll Surf* **120:** 167-181.

EDWARDS KJ, BOND PL, GIHRING TM and BANFIELD JF (2000) An archaeal iron-oxidizing extreme acidophile important in acid mine drainage. *Science* **279:** 1796-1799.

ELLAIAH P, SRINIVASULU B and ADINARAYANA K (2003) Optimisation studies on neomycin production by a mutant strain of *Streptomyces marinensis* in solid state fermentation. *Proc Biochem* **39:** 529-534.

EHRLICH HL (1986) What type of microorganisms are effective in bioleaching, bioaccumulation of metals; ore beneficiation and desulfurization of fossil fuels. *Biotechnol Bioeng Symp* **16:** 227-237.

FAVELA-TORRES E, CORDOVA-LOPEZ J, GARCIA-RIVERO M and GUTIERREZ-ROJAS M (1998) Kinetics of growth of *Aspergillus niger* during submerged, agar surface and solid state fermentations. *Proc Biochem* **33:** 103-107.

FAWOLE OB and ODUNFA SA (2003) Some factors affecting production of pectin enzymes by *Aspergillus niger*. *Int Biodet* **51:** 223-227.

FERRIS FG, SCHULTZE S, WITTEN TC, FYFE WS and BEVERIDGE TJ (1989) Metal interaction with microbial biofilms in acidic and neutral pH environments. *Appl Environ Microbiol* **55:** 1249-1257.

FORMENT JV, FLIPPHI M, RAMÓN D, VENTURA L and MacCABE AP (2006) Identification of the *mst*E gene encoding a glucose-inducible, low affinity glucose transporter in *Aspergillus nidulans*. *J Biol Chem* **281:** 8339-8346.

FUCHS T, HUBER H, TEINER K, BURGGRAF S and STETTER KO (1995) *Metallosphaera prunae*, sp. nov., a novel metal-mobilizing, thermoacidophilic archaeum, isolated from a uranium mine in Germany. *Syst Appl Microbiol* **18:** 560-566.

FUCHS T, HUBER H, BURGGRAF S and STETTER KO (1996) 16S rDNA-based phylogeny of the archaeal order *Sulfolobales* and reclassification of *Desulfurolobus ambivalens* as *Acidianus ambivalens* comb. nov. *Syst Appl Microbiol* **19:** 56-60.

GADD GM (1999) Fungal production of citric and oxalic acid: importance in metal speciation, physiology and biogeochemical processes. *Adv Microb Physiol* **41:** 47-92.

GARDEN J, ROBERTS K, TAYLOR A and ROBINSON D (2003) Evaluation of the provision of single use citric acid sachets to injecting drug users. *Scottish Centre for Infection and Environmental Health*. http://www.drugmisuse.isdscotland.org

GEHRKE T, TELEGDI D and SAND W (1998) Importance of extracellular polymeric substances from *Thiobacillus ferrooxidans* for bioleaching. *Appl Environ Microbiol* **64:** 2743-2747.

GEHRKE T, HALLMANN R, KINZLER K and SAND W (2001) The EPS of *Acidithiobacillus ferrooxidans* – a model for structure-function relationships of attached bacteria and their physiology. *Water Sci Technol* **43:** 159-167.

GILBERTSON BP (2000) Creating value through innovation: biotechnology in mining. *Trans Instn Min Metall* **109:** C61-C67.

GHIORSE WC (1988) Microbial reduction of manganese and iron. In: ZEHNDER AJB (Editor) Biology of anaerobic microorganisms. New York, Wiley and Sons, p. 178-210.

GLAZER AN and NIKAIDO H (1995) Application of biotechnology for mineral processing. In: Microbial Biotechnology: fundamentals of applied microbiology. Freeman, New York. pp. 268-287.

GOLYSHINA OV, PIVOVAROVA TA, KARAVAIKO GI, KONDRATEVA TF, MOORE ER, ABRAHAM WR, LUNSDORF H, TIMMIS KN, YAKIMOV MM and GOLYSHIN PN (2000) *Ferroplasma acidiphilum* gen. nov., sp. nov., and acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the *Archaea*. *Int J Syst Evol Microbiol* **50:** 997-1006.

GRAFF G (2006) Citric acid pricing pattern due for upward turn. Purchasing Magazine Online, Reed Elsevier, Inc. www.purchasing.com.

GUTIERREZ-CORREA M, PORTAL L, MORENO P and TENGERDY RP (1999) Mixed culture solid substrate fermentation of *Trichoderma reesei* with *Aspergillus niger* on sugar cane bagasse. *Biores Technol* **68:** 173-178.

HABISON A, KUBICEK CP and RÖHR M (1983) Partial purification and regulatory properties of phosphofructokinase from *Aspergillus niger*. *Biochem J* **209:** 669-676.

HALLBERG KB and JOHNSON DB (2001) Biodiversity of acidophilic prokaryotes. *Adv Appl Microbiol* **49:** 37-84.

HARVEY TJ, HOLDER N and STANEK T (2002) Thermophilic bioleaching of chalcopyrite concentrates with GEOCOAT® process. GeoBiotics, LLC, Lakewood, Colorado, USA. Presented at Alta 2002 Nickel/Cobalt 8-Copper 7 Conference, Perth, Australia.

HIPPE H (2000) *Leptospirillum* gen. nov. (ex Markosyan, 1972), nom. rev., including *Leptospirillum ferrooxidans* sp. nov. (ex Markosyan, 1972), nom. rev. and *Leptospirillum thermoferrooxidans* sp. nov. (Golovacheva *et al*., 1992). *Int J Syst Evol Microbiol* **50:** 501-503.

HIRAISHI A and SHIMADA K (2001) Aerobic anoxygenic photosynthetic bacteria with zinc-bacteriochlorophyll. *J Gen Appl Microbiol* **47:** 161-180.

HIRAISHI A, NAGASHIMA KVP, MATSUURA K, SHIMADA K, TAKAICHI S, WAKAO N and KATAYAMA Y (1998) Phylogeny and photosynthetic features of *Thiobacillus acidophilus* and related acidophilic bacteria: its transfer to the genus *Acidiphilium* as *Acidiphilium acidophilum* comb. nov. *Int J Syst Bacteriol* **48:** 1389-1398.

HOFFMANN MR, ARNOLD RG and STEPHANOPOULOS G (1989) Microbial reduction of iron ore. *US Patent 4,880,740*.

HONDMANN DHA and VISSER J (1994) Carbon Metabolism. In: MARTINELLI SD and KINGHORN JR (Editors) Aspergillus: 50 years on, progress in industrial microbiology, volume 29. Elsevier, Amsterdam, pp. 59-139.

JAIN N and SHARMA DK (2004) Biohydrometallurgy for non-sulfidic minerals-a review. *Geomicrobiol J* **21:** 135-144.

JIANLONG W (2000) Enhancement of citric acid production by *Aspergillus niger* using *n*-dodecane as an oxygen vector. *Proc Biochem* **35:** 1079-1083.

JIANLONG W and PING L (1998) Phytate as a stimulator of citric acid production by *Aspergillus niger*. *Process Biochem* **33:** 313-316.

JOHANSSON C, SHRADER V, SUISSA K, ADUTWUM K and KOHR W (1999) Use of the GEOCOAT™ process for the recovery of copper from chalcopyrite. GeoBiotics, LLC, Lakewood, Colorado, USA. Presented at IBS Biomin'99, Madrid, Spain.

JOHNSON DB (1998) Biodiversity and ecology of acidophilic microorganisms. *FEMS Microbiol Ecol* **27:** 307-317.

JOHNSON B and ROBERTO FF (1997) Heterotrophic acidophiles and their roles in bioleaching of sulphide minerals. In: Rawlings, D.E. (Editor) Biomining: theory, microbes and industrial processes. Springer, New York. pp. 259-279.

KELLY DP and WOOD AP (2000) Reclassification of some species of *Thiobacillus* to the newly designated genera *Acidothiobacillus* gen. nov., *Halothiobacillus* gen. nov. and *Thermithiobacillus* gen. nov. *Int J Syst Evol Microbiol* **50:** 511-516.

KIM J (2004) Optimisation of citric acid production by *Aspergillus niger* NRRL 567 in various fermentation systems. PhD Thesis. Department of Biosystems Engineering, McGill University, Ste-Anne-de-Bellevue, Quebec, Canada.

KOTA KP and SRIDHAR P (1999) Solid state cultivation of *Streptomyces clavuligerus* for cephamycin C production. *Proc Biochem* **34:** 325-328.

KREBS W, BROMBACHER C, BOSSHARD PP, BACHOFEN R and BRANDL H (1997) Microbial recovery of metals from solids. *FEMS Microbiol Rev* **20:** 605-617.

KUROSAWA N, ITOH YH, IWAI T, SUGAI A, UDA I, KIMURA N, HORIUCHI T and ITOH T (1998) *Sulfurisphaera ohwakuensis* gen. nov., sp. nov., a novel extremely thermophilic acidophile of the order *Sulfolobales*. *Int J Syst Bacteriol* **48:** 451-456.

LEANGON S, MADDOX IS and BROOKS JD (2000) A proposed biochemical mechanism for citric acid accumulation by *Aspergillus niger* Yang No. 2 growing in solid state fermentation. *W J Microbiol Biotechnol* **16:** 271-275.

LEGIŠA M and KIDRIČ J (1989) Initiation of citric acid accumulation in the early stages of *Aspergillus niger* growth. *Appl Microbiol Biotechnol* **31**: 453-457.

LEGIŠA M and MATTEY M (1986a) Glycerol as an initiator of citric acid accumulation in *Aspergillus niger*. *Enzyme Microb Technol* **8:** 258-259.

LEGIŠA M and MATTEY M (1986b) Glycerol synthesis by *Aspergillus niger* under citric acid accumulating conditions. *Enzyme Microb Technol* **8:** 607-609.

LEGIŠA M and MATTEY M (2007) Changes in primary metabolism leading to citric acid overflow in *Aspergillus niger*. *Biotechnol Lett* **29:** 181-190.

LEGIŠA M, CIMERMAN A and STERLE M (1981) Germination of *Aspergillus niger* in a high citric acid yielding medium. *FEMS Microbiol Lett* **11:** 149-152.

LESNIAK W, PIETKIEWICZ J and PODGORSKI W (2002) Citric acid fermentation from starch and dextrose syrups by a trace metal resistant mutant of *Aspergillus niger*. *Biotechnol Lett* **24:** 1065-1067.

LIERMANN LJ, KALINOWSKI BE and FERRY BJ (2000) Role of bacterial siderophores in dissolution of hornblende. *Geochim Cosmochim Acta* **64:** 587-602.

MALOUF EE and PRATER JD (1961) Role of bacteria in the alteration of sulphide. *J Metals* **13:** 353-356.

MESOJEDNIK S and LEGIŠA M (2005) Post-translational modification of 6-phosphofructo-1-kinase in *Aspergillus niger*. *Appl Environ Microbiol* **71:** 1425-1432.

MINERALOGY DATABASE (2005) http://webmineral.com.

MIRMINACHI F, ZHANG A and ROEHR M (2002) Citric acid fermentation and heavy metal ions: Effect of iron, manganese and copper. *Acta Biotechnol* **22:** 363-373.

MLAKAR T and LEGIŠA M (2006) Citrate inhibition resistant from of 6-phosphofructo-1-kinase from *Aspergillus niger*. *Appl Environ Microbiol* **72:** 4515-4521.

NATARAJAN KA and DEO N (2001) Role of bacterial interaction and bioreagents in iron ore flotation. *Int J Miner Process* **62:** 143-157.

NGADI MO and CORREIA LR (1992) Kinetic of solid-state fermentation from apple pomace. *J Food Eng* **17:** 97-116.

NORRIS PR, CLARK DA, OWEN JP and WATERHOUSE S (1996) Characteristics of *Sulfobacillus acidophilus* sp. nov. and other moderately thermophilic mineral-sulphide-oxidizing bacteria. *Microbiology* **142:** 775-783.

NORRIS PR, BURTON NP and FOULIS NAM (2000) Acidophiles in bioreactor mineral processing. *Extremophiles* **4:** 71-76.

OHMURA N, SASAKI K, MATSUMOTO N and SAIKI H (2002) Anaerobic respiration using Fe^{3+}, S^0, and H_2 in the chemolithoautotrophic bacterium *Acidithiobacillus ferrooxidans*. *J Bacteriol* **184:** 2081-2087.

OLSON GJ, BRIERLEY JA and BRIERLEY CL (2003) Progress in bioleaching: Applications of microbial processes by the minerals industries. *Appl Microbiol Biotechnol* **63:** 249-257.

PANNEMAN H, RUIJTER GJ, VAN DEN BROECK HC, DRIEVER ET and VISSER J (1996) Cloning and biochemical characterisation of an *Aspergillus niger* glucokinase. Evidence for the presence of separate glucokinase and hexokinase enzymes. *Eur J Biochem* **240:** 518-525.

PANNEMAN H, RUIJTER GJ, VAN DEN BROECK HC and VISSER J (1998) Cloning and biochemical characterisation of *Aspergillus niger* hexokinase – the enzyme is strongly inhibited by physiological concentrations of trehalose 6-phosphate. *Eur J Biochem* **258:** 223-232.

PAPAGIANNI M, WAYMAN F and MATTEY M (2005) Fate and role of ammonium ions during fermentation of citric acid by *Aspergillus niger*. *Appl Environ Microbiol* **71:** 7178-7186.

PAPAGIANNI M and MATTEY M (2006) Morphological development of *Aspergillus niger* in submerged citric acid fermentation as a function of the spore inoculum level. Application of neural network and cluster analysis for characterisation of mycelial morphology. *Microbial Cell Factories* **5:** 3.

PARIS F, BOTTON B and LAPEYRIE F (1996) In vitro weathering of phlogopite by ecto-mycorrhizal fungi. II. Effect of K^+ and Mg^{2+} deficiency and N sources on accumulation of oxalate and H^+. *Plant Soil* **179:** 141-150.

PAULIN J-Y (2005) La Mistique Des Pierres. http://lamistiquedespierres.free.fr.

PRONK JT, DE BRUYN JC, BOS P and KUENEN JG (1992) Anaerobic growth of *Thiobacillus ferrooxidans*. *Appl Environ Microbiol* **58:** 2227-2230.

RAWLINGS DE (2002) Heavy metal mining using microbes. *Annu Rev Microbiol* **56:** 65-91.

RAWLINGS DE (2005) Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. *Microbial Cell Fact* **4:** 13-27.

RAWLINGS DE, DEW D and DU PLESSIS C (2003) Biomineralization of metal-containing ores and concentrates. *Trends Biotechnol* **21:** 38-44.

RICKETTS JA (2005) How a blast furnace works. American Iron and Steel Institute. http://www.steel.org.

ROBERTO FF (2002) Bioleaching of metals. In: Bitton, G. (Editor) Environmental Microbiology. John Wiley & Sons, Inc. New York. p. 641.

RÖHR M and KUBICEK CP (1981) Regulatory aspects of citric acid fermentation by *Aspergillus niger*. *Proc Biochem* **16:** 34-37.

RÖHR M, KUBICEK CP, ZEHENTGRUBER O and ORTHOFER R (1987) Accumulation and partial re-consumption of polyols during citric acid fermentation by *Aspergillus niger*. *Appl Microbiol Biotechnol* **27:** 235-239.

ROHWERDER T, JOZSA P, GEHRKE T and SAND W (2002) Bioleaching. In: Bitton, G. (Editor) Encyclopaedia of Environmental Microbiology, Volume 2. John Wiley & Sons, Inc., New York. pp. 632-641.

ROHWERDER T, GEHRKE T, KINZLER K and SAND W (2003) Bioleaching review part A: Progress in bioleaching: Fundamentals and mechanisms of bacterial metal sulphide oxidation. *Appl Microbiol Biotechnol* **63:** 239-248.

ROUKAS T (2000) Citric and gluconic acid production from fig by *Aspergillus niger* using solid-state fermentation. *J Ind Microbiol Biotechnol* **25:** 298-304.

RUIJTER GJG, PANNEMAN H and VISSER J (1997) Overexpression of phosphofructokinase and pyruvate kinase in citric acid-producing *Aspergillus niger*. *Biochim Biophys Acta* **1334:** 317-326.

RUIJTER GJG, PANNEMAN H, XU DB and VISSER J (2000) Properties of *Aspergillus niger* citrate synthase and effect of *citA* overexpression on citric acid production. *FEMS Microbiol Lett* **184:** 35-40.

RUIZ LM, GONZALES A, FREZZA M, SOULERE L, QUENEAU Y, DOUTHEAU A, ROHWERDER T, SAND W, JEREZ CA and GUILIANI N (2007) Is the quorum sensing type AI-1 system of *Acidithiobacillus ferrooxidans* involved in its attachment to mineral surfaces. *Adv Mat Res* **20-21:** 345-349.

RUSIN PA, QUINTANA L, BRAINARD JR, STRIETELMEIER BA, TAIT CD, EKBERG SA, PALMER PD, NEWTON TW and CLARK DL (1994) Solubilisation of plutonium hydrous oxide by iron-reducing bacteria. *Environ Sci Technol* **28:** 1686-1690.

SAMPSON MI, PHILLIPS CV and BLAKE RC II (2000) Influence of the attachment of acidophilic bacteria during the oxidation of mineral sulfides. *Min Eng* **13:** 373-389.

SAND W (2001) Microbial corrosion and its inhibition. In: Rehm, H-J., Reed, G., Pühler, A. and Stadler, P. (Editors). Biotechnology. Wiley-VCH, Weinheim, Germany. pp. 265-316.

SAND W and GEHRKE T (2006) Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron(III) ions and acidophilic bacteria. *Res Microbiol* **157:** 49-56.

SAND W, GEHRKE T, HALLMANN R and SCHIPPERS A (1995) Sulfur chemistry, biofilm, and the (in)direct attack mechanism – critical evaluation of bacterial leaching. *Appl Microbiol Biotechnol* **43:** 961-966.

SAND W, GEHRKE T, JOZSA PG and SCHIPPERS A (2001) (Bio)chemistry of bacterial leaching – direct vs indirect bioleaching. *Hydrometallurgy* **59:** 159-175.

SASSI G, RUGGERI B, SPECCHIA V and GIANETTO A (1991) Citric acid production by *Aspergillus niger* with banana extract. *Biores Technol* **37:** 259-269.

SAYER JA and GADD GM (2001) Binding of cobalt and zinc by organic acids and culture filtrates of *Aspergillus niger* grown in the absence or presence of insoluble cobalt or zinc phosphate. *Mycol Res* **105:** 1261-1267.

SCANDINAVIAN MINERAL GALLERY (2002) http://geology.neab.net.

SOLARI JA, HUERTA G, ESCOBAR B, VARGAS T, BADILLA-OHLBAUM R and RUBIO J (1992) Interfacial phenomena affecting the adhesion of *Thiobacillus ferrooxidans* to sulphide mineral surfaces. *Colloid Surf* **69:** 159-166.

SOUTH AFRICAN COMMITTEE FOR STRATIGRAPHY (1995) Developments in South African Stratigraphy: 1980-1995. *Geobulletin* **38:** 4-6.

TAKAI M, KAMIMURA K and SUGIO T (2001) A new iron oxidase from a moderately thermophilic iron oxidizing bacterium strain T1-1. *Eur J Biochem* **68:** 1653-1658.

TORRES NV (1994a) Modelling approach to control of carbohydrate metabolism during citric acid accumulation by *Aspergillus niger*: I. Model definition and stability of steady state. *Biotechnol Bioeng* **44:** 104-111.

TORRES NV (1994b) Modelling approach to control of carbohydrate metabolism during citric acid accumulation by *Aspergillus niger*: II. Sensitivity analysis. *Biotechnol Bioeng* **44:** 112-118.

TRAN CT, SLY LI and MITCHELL DA (1998) Selection of a strain of *Aspergillus* for the production of citric acid from pineapple waste in solid-state fermentation. *W J Microbiol Biotechnol* **14:** 399-404.

TRIBUTSCH H (2001) Direct vs indirect bioleaching. *Hydrometallurgy* 59: 177-185.

TZEFERIS PG and AGATZINI-LEONARDOU S (1994) Leaching of nickel and iron from Greek nonsulfide nickeliferous ores by organic acids. *Hydrometallurgy* **36:** 345-360.

TZEFERIS PG, AGATZINI S and NERANTZIS ET (1994) Mineral leaching of non-sulfide nickel ores using heterotrophic micro-organisms. *Lett Appl Microbiol* **18:** 209-213.

VALIX M, TANG JY and MALIK R (2001a) Heavy metal tolerance of fungi. *Miner Eng* **14:** 499-505.

VALIX M, USAI F and MALIK R (2001b) Fungal bioleaching of low-grade laterite ores. *Miner Eng* **14:** 197-203.

VAN ASWEGEN PC and MARAIS HJ (1998) Advances in the biological oxidation of refractory gold ores. Gold Fields Limited.

VANDENBERGHE LPS, SOCCOL CR, PANDEY A and LEBEAULT JM (2000) Solid-state fermentation for the synthesis of citric acid by *Aspergillus niger*. *Biores Technol* **74:** 175-178.

VANDEVIVERE P and KIRCHMAN DL (1993) Attachment stimulates exopolysaccharide synthesis by a bacterium. *Appl Environ Microbiol* **59:** 3280-3286.

VANDEVIVERE P, WELCH SA, ULLMAN WJ and KIRCHMAN DL (1994) Enhanced dissolution of silicate minerals by bacteria at near neutral pH. *Microb Ecol* **27:** 241-251.

VANKUYK PA, DIDERICH JA, MacCABE AP, HERERRO O, RUIJTER GJG and VISSER J (2004) *Aspergillus niger mst*A encodes a high-affinity sugar/H^+ symporter which is regulated in response to extracellular pH. *Biochem J* **379:** 375-383.

VAN STADEN P, RHODES M and MARTINEZ T (2003) Piloting and feasibility study of a bioleach process for the treatment of polymetallic base metal concentrate. Abstract of the Annual Meeting of the Society for Mining. Metallurgy and Exploration. 24-26 February 2003, Cincinnati, Ohio, USA.

WANG J (1998) Improvement of citric acid production by *Aspergillus niger* with addition of phytate to beet molasses. *Biores Technol* **65:** 243-245.

WANG J and LIU P (1996) Comparison of citric acid production by *Aspergillus niger* immobilized in gels and cryogels of polyacrylamide. *J Ind Microbiol* **16:** 351-353.

WATANABE T, SUZUKI A, NAKAGAWA H, KIRIMURA K and USAMI S (1998) Citric acid production from cellulose hydrolysate by 2-deoxyglucose resistant mutant strain of *Aspergillus niger*. *Biores Technol* **66:** 271-274.

WAYMAN FM and MATTEY M (2000) Simple diffusion is the primary mechanism for glucose uptake during the production phase of the *Aspergillus niger* citric acid process. *Biotechnol Bioeng* **67:** 451-456.

WELCH SA and ULLMAN WJ (1993) The effect of organic acids on plagioclase dissolution rates and stoichiometry. *Geochim Cosmochim Acta* **57:** 2725-2736.

WELCH SA and VANDEVIVERE P (1995) Effect of microbial and other naturally occurring polymers on mineral dissolution. *Geomicrobiol J* **12:** 227-238.

WELCH SA, BARKER WW and BANFIELD WW (1999) Microbial extracellular polysaccharides and plagioclase dissolution. *Geochim Cosmochim Acta* **63:** 1405-1419.

WIELAND E, WEHRLI B and STUMM W (1988) The Coordination chemistry of weathering, III. A generalization on the dissolution rates of minerals. *Geochim Cosmochim Acta* **52:** 1969-1981.

WIKIPEDIA (2006a) Wikipedia – The Free Encyclopedia. Apatite. http://en.wikipedia.org/wiki/Apatite.

WIKIPEDIA (2006b) Wikipedia – The Free Encyclopedia. Citric Acid. http://en.wikipedia.org/wiki/Citric_acid.

WIKIPEDIA (2006c) Wikipedia – The Free Encyclopedia. Citric Acid Cycle. http://en.wikipedia.org/wiki/Citric_acid_cycle.

WIKIPEDIA (2006d) Wikipedia – The Free Encyclopedia. Muscovite. http://en.wikipedia.org/wiki/Muscovite.

WIKIPEDIA (2007a) Wikipedia – The Free Encyclopedia. Dump Leaching. http://en.wikipedia.org/wiki/Dump_leaching.

WIKIPEDIA (2007b) Wikipedia – The Free Encyclopedia. Heap Leaching. http://en.wikipedia.org/wiki/Heap_leaching.

WRIGHT'S ROCK SHOP (2005) http://wrightsrockshop.com.

WROLSTAD RE and SHALLENBERGER RS (1981) Free sugars and sorbitol in fruits-a complication from the literature. *J Assoc Anal Chem* **64:** 91-103.

YARZÁBAL A, BRASSEUR G and BONNEFOY V (2002) Cytochromes *c* of *Acidithiobacillus ferrooxidans*. *FEMS Microbiol Lett* **209:** 189-195.

YIGITOGLU M and McNEIL B (1992) Ammonium and citric acid supplementation in batch cultures of *Aspergillus niger* B60. *Biotechnol Lett* **14:** 831-836.

YUSFIN YS, CHERNOUSOV PI, GARTEN V, KARPOV YA and PETELIN AL (1999) The role of alkali's and conserving resources in blast-furnace smelting. *Metallurgist* **43:** 54-58.