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# CHAPTER ONE

# LITERATURE REVIEW

## 1.1     Introduction – Biohydrometallurgy

Production, trading and utilisation of minerals have become a very important part of human lives. Existence of life without these minerals is almost unimaginable due to their vital contributions to industrial developments such as construction and manufacturing industries. Few minerals such as talc, asbestos and sulfur can be used just after mining without any processing. However, most of the minerals, especially the more important ones such as iron ore and gold, need to be refined and reprocessed to produce utilisable materials (Klein, 2002).

The current pace of development has led to increases in the demand for minerals and their by-products. This has subsequently resulted in rapid depletion and exhaustion of quality mineral ores, which means most mineral ores are no longer found in their pure forms (Dale, 1984). Over the last century, scientists have queried and investigated reasons for this disparity in existence of minerals. Long-term natural events such as mineralisation, deposition, solubilisation and weathering were suggested as possible causes of changes in the chemical constitution of minerals (Rawlings, 2002; Rawlings, *et al*., 2003; Astrup and Hammerbeck, 1998). Geologists, microbiologist or biotechnologists may have different definitions for these processes; but the inter-relationship between these processes cannot be disputed. For example, mineralisation and solubilisation have been described as possible stages in weathering. Meanwhile, weathering itself can be a natural way by which mineral ores can be purified or leached through solubilisation by some microbes or could lead to formation of more complex types of minerals such as the deposition or precipitation by anaerobic sulphate-reducing bacteria (Banfield *et al*., 1999; Rawlings, 2002). In any of these scenarios, microorganisms play active roles through their interaction with the mineral environment.

Different microbial functionalities have been exploited by scientists to develop processes that can mobilise and solubilise the elemental composition of mineral ore. These have been achieved through laboratory simulation and repetition of microbial activities to yield a technology known as biohydrometallurgy (Rawlings, 2002; Jain and Sharma, 2004; Rawlings, 2005). The word "biohydrometallurgy" has been used widely and interchangeably with "bioleaching" to describe

microbial processes utilised in the mineral industry such as metal extraction from low grade ores, metal detoxification, ore beneficiation, coal beneficiation and recovery of metals from waste materials (Olson *et al*., 2003; Jain and Sharma, 2004).

## 1.2    Relationship between biohydrometallurgy and weathering

Weathering is described as the breakdown of materials on the earth's crust, which leads to changes in the composition of the original materials yielding more stable products (Banfield *et al*., 1999; White *et al*., 1992; Burford *et al*., 2003). Weathering could be biological, physical or chemical (Banfield *et al*., 1999; White *et al*., 1992), but weathering described in this study is biological. This is in some situations referred to as bioweathering, defined by Sollas (1880) as a process that involves the erosion, decay and decomposition of rocks and minerals caused by living organisms. There are numerous advantages associated with weathering in nature. The most discussed topic pertaining to weathering is its natural effects, which ensure nutrient cycling and availability to both plants and microbes in the soil (Banfield *et al*., 1999; White *et al*., 1992; Burford *et al*., 2003; van Scholl *et al*., 2006b). Putting the pieces of this puzzle together, weathering could therefore be described as a natural bioleaching process that involves slow and gradual degradation or purification or solubilisation of some important minerals which can occur over a long period of time. In addition, it is also a process that could remove toxic metals from the environment (Willscher and Bosecker, 2003).

Apart from the fact that both biological weathering and bioleaching are natural processes, there are many other features that are also shared between them (Rawlings, 2002; Rawlings, 2005; Willscher and Bosecker, 2003). These include production of organic acid, mobilisation, binding and solubilisation of metals. Therefore, it may be true that some natural microbial weathering agents could also be bioleaching agents. In some situations, weathering roles of some microorganisms have been translated as bioleaching (Styriakova *et al*., 2003; Jain and Sharma, 2004). However, further studies on both processes could provide more information on how bioleaching occurs in nature. In addition, potential bioleaching agents may also be identified by investigating microorganisms that are known to participate in weathering.

## 1.3    Biohydrometallurgy as a technology

Biohydrometallurgy is a word that evolved from hydrometallurgy, which means biological hydrometallurgy. In hydrometallurgy, the system relies on dissolution of metallic artifacts by acids or alkalis to produce solubilisation effects that leach metallic ions from minerals into solutions (Bodsworth, 1994). Meanwhile, leaching conditions are normally adjusted to ensure the desired part of the mineral remains insoluble. If soluble, additional techniques are introduced to separate the desired portion from other compounds in the solution (Bodsworth, 1994). Processes involved in biohydrometallurgy are valued for being more environmentally friendly and cheaper compared to most physical and chemical methods of mineral extraction (Rawlings, 2002). Generally in biohydrometallurgy, minerals can be categorised into two types, i.e. the sulfidic and non-sulfidic minerals (Jain and Sharma, 2004). Chemolithoauthotrophic bacteria are in most cases used for bioleaching of sulfidic minerals, while heterotrophs, which could be bacteria or fungi, are used for the bioleaching of non-sulfidic minerals (Rawlings, 2005; Jain and Sharma, 2004). Currently, there is a growing distinction in terms of mechanisms involved between bioleaching and another related process known as biobeneficiation. Biobeneficiation involves the use of microbes to dissolve only unwanted parts of a mineral ore (Vasan *et al*., 2001; Jain and Sharma, 2004). However, both processes can be referred to as biohydrometallurgy technologies (Rawlings, 2002; Jain and Sharma, 2004).

## 1.4    Bioleaching methods

Processes involved in bioleaching are broadly divided into two types, namely irrigation- and stirred tank–type processes (Brierley and Brierley, 2001; Rawlings, 2002). Irrigation type processes involve infiltration of ores arranged in heaps, columns or dumps with the leaching solution (Schnell, 1997; Brierley and Brierley; 2001). The irrigation method has been successfully commercialised in different countries (e.g. Chile) for different minerals such as copper and cobalt (Brierley and Brierley, 2001). For the stirred tank process, tanks containing ores and leaching solution are exposed to aeration by continuous stirring. An example is the use

of this technology at the Youanmi plant, Australia, for bioleaching of gold-containing ores (Brierley and Brierley, 2001).

## 1.5    Bioleaching of sulfidic minerals

It is generally believed that activities of microbes for mining began a long time ago, around 1556 at the Rio Tinto mine, Spain, where copper and silver were said to have been mined in this ancient period (Rawlings, 2002). However, it was not until around half a century ago that proper documentation of biohydrometallurgical processes began (Zimmerley *et al*., 1958). After this period, most of the studies and technological developments in biohydrometallurgy focussed on sulfidic minerals. These are minerals that could provide sources of energy for bioleaching microorganisms through the sulfur and iron cycles (Jain and Sharma, 2004). Various groups of bacteria have been confirmed as having potential in the bioleaching industry. This diverse group of bacteria can be classified into two groups depending on their oxidation mechanisms. These comprise thiosulfate and polysulfide mechanisms. The thiosulphate mechanism is a type of oxidation mechanism proposed for solubilisation of acid insoluble metal sulphides such as pyrite and molybdenite, while the polysulfide mechanism is proposed for oxidation of acid- soluble metal sulphides (Rawlings, 2005). Examples of common sulfidic microbes are *Acidithiobacillus thiooxidans*, *Leptospirillum ferroxidans*, *Acidiphilium acidophilum*, *Acidianus infernus*, *Sulfobacillus thermosulfodooxidaans*, *Ferroplasma acidarmanus*, *Sulfolobus* sp. and *Metallosphaera sedula* (Rawlings *et al*., 1999; Rawlings, 2001; Rawlings, 2005).

## 1.6    Bioleaching of non-sulfidic minerals

Heterotrophs consist of a wide group of organisms, including fungi and some bacteria that use naturally occurring organic substrates for generation of energy (Jain and Sharma, 2004). Heterotrophs have been highlighted as having the best potential in bioleaching of non-sulfidic minerals (Jain and Sharma, 2004; Rawlings, 2005). This group of microorganisms can be used for the recovery of valuable metals from low-grade mineral ores, beneficiation of mineral raw materials, recovery of metals from wastes and detoxification of heavy metal-contaminated soils

and solid residues (Bosecker, 1997; Jain and Sharma, 2004; Rawlings, 2005). Heterotrophic microorganisms have been successfully used in mobilisation of elements such as Si, Al, Fe, K, Li, Ni, Zn and Mg from mineral ores (Table 1.1). Functional roles of heterotrophic microbes used in bioleaching strongly depends on their ability to produce low-molecular-weight organic acids, exopolysaccharides, amino acids and proteins that can be used to solubilise mineral ores through a variety of mechanisms (Burgstaller and Schinner, 1993; Jain and Sharma, 2004).

## 1.6.1  Mechanisms of biobeneficiation of non-sulfidic minerals

Jain and Sharma (2004) classified mechanisms involved in the solubilisation of non-sulfidic minerals into four different groups. These include: bioreduction, acidification, ligand production or complexolysis and alkalinisation. Bioreduction has been described as the process through which microbes could reduce some minerals through their activities such as the biological reduction of iron with *Pseudomonas* (Hoffman *et al*., 1989). Acidification is directly related to the production of organic acids such as citric, oxalic, acetic, propionic and tartaric acids by heterotrophic leaching microbes. This mechanism could also be due to selective utilisation of alkaline food substrates by these microbes (Jain and Sharma, 2004).

Another important mechanism of leaching non-sulfidic minerals by heterotrophs is complexolysis. This is the reaction that involves the organic ligands produced by microbes and some mineral constituents such as Fe, Al, Cu, Zn, Ni, Mn, Ca and Mg. The ability of these ligands to form stable ligand-metal complexes helps to increase solubilisation of non-sulfidic minerals (Amrhein and Surez, 1988; Bennett *et al*., 1988; Wieland *et al*., 1988; Jain and Sharma, 2004). Microbial extracellular polysaccharides that are produced by some microorganisms also help to increase the solubility of non-sulfidic minerals, because of their ability to complex with ions in solutions (Welch and Ullman, 1999; Welch and Vandevivere, 1994; Welch *et al*., 1999; Jain and Sharma, 2004).

## 1.6.2 Importance of organic acid to biobeneficiation of non-sulfidic minerals

Organic acids are low-molecular-weight carbon compounds such as oxalic, citric, acetic, lactic, tartaric, malic and malonic acids. The action of these acids could be explained in two different ways. This involves direct mineral attack by the metal complexing organic acid anion and protons (Gadd, 1999). Due to the high chelation constants of $[Al (C_2O_4)_3]^{3-}$; $2.0 \times 10^{16}$ and $[Fe (C_2O_4)_3]^{3-}$; $3.9 \times 10^{16}$, mineral sites containing $Al^{3+}$ and $Fe^{3+}$ are easy targets for attack by organic acid anions. For instance, oxalate chelation of these cations results in structural imbalance and therefore, may lead to the dissolution and release of different elements and nutrients contained in minerals such as Si, K and P (Yuan *et al*., 2004; Delvasto *et al*., 2009). Secondly, there is similarity (monovalent structures) of both protons from organic acids, and K from minerals such as muscovite, but the size of the protons ($0.32 \times 10^{-10}$ m) is much smaller than that of K ($2.03 \times 10^{-10}$ m) (Lapeyrie *et al*., 1987; Yuan *et al*., 2004). The size of the proton is therefore an advantage, as it enables them to replace interlayer K contained in layer minerals associated with iron ores investigated in this study.

Therefore, organic acids production has been highlighted as one of the most important factors that determines leaching ability of heterotrophs (Castro *et al*., 2000, Jain and Sharma, 2004). Sheng *et al*. (2008) reported an example of this phenomenon where high gluconic and acetic acid production were suggested to be responsible for the dissolution of feldspar by *Bacillus globisporus* Q12. In addition, Delvasto *et al*. (2008) also reported the possible effects of gluconic acid released by *Burkhoderia caribensis* FeGL03 on the mobilisation of P from iron ore. Furthermore, Williams (2008) utilised citric acid obtained from *Aspergillus niger* for the biobenefication of Sishen iron ore.

## 1.6.3 Factors affecting bioleaching of non-sulfidic minerals

There are various groups of heterotrophs that are involved in the biohydrometallurgy of non-sulfidic minerals. The diversity of these microbes has a great influence on their mode of action, because of the associated differences with regards to their requirements for growth and metabolism. Below are some of the previously investigated factors:

**Mineralogy:** Mineral composition has been found to significantly affect the type of microorganisms that can solubilise the minerals (Schippers and Sand, 1999). An important example here is the grouping of minerals into sulfidic and non-sulfidic minerals (Jain and Sharma, 2004). Presence of sulfur as an energy source in sufuldic minerals is a "privilege" to organisms such as *Thiobacillus ferrooxidans* that can utilise sulfur as their source of energy (Rawlings, 2002; Jain and Sharma, 2004). On the other hand, absence of sulfur in non-sufuldic minerals also provided a competitive advantage to heterotrophs that can utilise other forms of mineral constituents as their energy source (Rawlings, 2002; Jain and Sharma, 2004). It is however important to mention that ores can exist in different forms containing different minerals. For instance, iron ore can be categorised as sulfudic (Biswas, 1981) when it contains sulfur or non-sulfudic (Williams, 2008) when it contains no sulfur (e.g Sishen iron ore with no sulfur).

**Nutrient limitation:** Whether in a biohydrometallurgy process or in a normal growth condition, there is need for microorganisms to survive in order to grow and multiply. Whenever there is shortage or lack of essential nutrients, microbes reengineer their metabolic process in order to ensure availability of such nutrients (Willey *et al*., 2007). In the process of doing this, mechanisms for solubilisation of unavailable nutrients are triggered through processes such as increased production of organic acids and scavenging (Banfield *et al*., 1999; Sheng *et al*., 2008).

This method can therefore, be used to isolate potential bioleaching organisms. For example, K-limited media were used by Hutchens *et al*. (2003) for the isolation of *Serratia marcescens* that could solubilise feldspar. The isolate was then grown in this same medium for feldspar solubilising experiment. Furthermore, Sheng *et al*. (2008), also utilised K-limited medium to isolate the silicate-solubilising bacterium *Bacillus globisporus* Q12. They eventually discovered that using these silicate minerals as the sole K source of the bacterium increases the number of cells in the medium and subsequently an increase in the solubilsation rate was recorded. In another study by van Scholl *et al*. (2006a), K deficiencies significantly increased the oxalate production by tree seedlings colonised by the fungus *Paxillus involutus*, while Mg deficiencies increased the oxalate production in both mycorrhizal and non-mycorrhizal tree seedlings in the same experiment.

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**Microbial type:** Due to differences in their metabolic activities, there is a diverse range of mechanisms that microbes adopt in bioleaching processes and this invariably affects their rate, mode and manner of bioleaching. These differences can be intra- or inter-species, depending on other factors such as exposure to high levels of heavy metals. For instance, some species of *Aspergillus* and *Penicillium* have mutants that can withstand heavy metals and are essentially different from other strains in their group because of the genetic adaptation (mutation) they developed to heavy metals during exposure to high levels of heavy metal concentration. Rezza *et al*. (2001) also pointed out the differences in organic acid production among some hetrotrophs. In this situation, a decrease in concentration of organic acids was recorded for *Rhodotorula rubra* after 15 d of incubation during the dissolution of aluminosilicate mineral. However, an opposite result was obtained for *Penicillium purpurogenum* under the same conditions, where an increase in citric acid production was recorded after 15 d.

**Physicochemical factors:**

**pH (acidification or alkalinisation):** The acidity or alkalinity of the growth medium of bioleaching microbes plays an important role in the bioleaching potential of these microorganisms (Jain  and Sharma, 2004). This becomes a vital advantage when bioleaching organisms can only grow at very high or very low pH. This restricts the number of microbes that can grow under these conditions and eliminates the task and cost of carrying out the leaching experiment under sterile conditions (Vasan *et al*., 2001; Jain and Sharma, 2004). In a study conducted by Vasan *et al*. (2001), it was discovered that the increase in pH to near neutral levels of metabolite produced by *Paenibacillus polymyxa* reduces the Ca solubisation from bauxite. In another example, at pH 3 of the medium, Welch *et al*. (1999), reported increased feldspar dissolution compared to pH 4.

**Cultural composition and carbon source:** This factor is also related to nutrient limitation and mineralogy. Chemical constitution of the growth medium determines the substrate availability for utilisation by microbes for metabolic activities (Willey *et al*., 2007). For example, the source, type or quantity of carbon in the culture may determine the nature and quantity of organic acids

to be produced. This invariably affects the mineral dissolution, because mineral solubilisation is in most cases "organic acid specific". Various sources of carbon have been used in previous studies to grow the heterotrophs in bioleaching experiments. For instance, Hutchens *et al*. (2003) used 0.2 g of glucose in 100 ml of medium to leach potassium from feldspar. In their own study, Sheng *et al*. (2008) used 1% sucrose as carbon source in an experiment that resulted in dissolution of feldspar by *Bacillus globisporus* Q12. Meanwhile, the ambition of developing a good biohydrometallurgical method for non-sulfidic minerals may only be realised if a cheap form of carbon source utilizable by the microbes is integrated into the process (Jain and Sharma, 2004).

**Temperature:** High temperature exposure of minerals before and during bioleaching processes has been found to enhance bioleaching of these minerals. For example, in the study conducted by Groudev and Groudeva, (1986), it was discovered that initial heat treatment of clay mineral prior to the bioleaching process improved their vulnerability to leaching.  A decrease of 5 °C in temperature also led to improved feldspar weathering by a factor of 20 in a study conducted by Welch *et al*. (1999).

Other physical factors include shaking, aeration and time. Most of these remaining factors are interconnected. Franz *et al*. (1991) recorded increased solubilisation of zinc from an industrial filter with increasing shaker speed. The speed of the shaker was related to the aeration (oxygen supply) by these investigators as a cause of increased dissolution of zinc. Time (period of incubation) is another important factor. Rezza *et al*. (2001) reported a sharp decrease in the concentration of organic acid produced by some heterotrophs after 15 d of incubation. This decrease directly corresponded to a decrease in the dissolution of aluminosilicate by these microbes.

**Pulp density:** This factor has been found to be indirectly related to the rate of mineral solubilisation. For instance, Vasan *et al*. (2001) compared the bioleaching rates at two different pulp densities of 5% and 10% and concluded that the former produced a better leaching of Ca

from bauxite. Pradhan *et al*. (2008) also recorded good bioleaching of copper at a low pulp density.

**Particle size:** The size of the mineral particles has a great influence on the weathering (solubilisation) rate of minerals (White and Brantley, 1995; Rosling *et al*., 2004). This importance was indicated in the study conducted by Leake *et al*. (2008) where weathering was found to be dependent on particle size in *Pinus sylvestris* colonised by the root microbe *Paxillus involutus*. In another study by Modak *et al*. (2001), bioleaching of bauxite by *Paenibacillus polymyxa* was found to be better with finer particle size of bauxite compared to when the particle size is coarser. In contrast, Srihari *et al*. (1994) discovered that "grinding effect" on minerals can change this trend. Better leaching were obtained from coarser particle size pyrite minerals compared to finer particle size.

## 1.6.4  Bacterial leaching of non-sulfidic minerals

Bacteria were the first type of microorganisms to be commercially developed in biohydrometallurgy and they are still the most widely used (Rawlings, 2002; Rawlings, 2005). The high level of bacterial technology development in biohydrometallurgy has eluded those involved in biohydrometallurgy of non-sulfidic minerals. Only recently have investigators began in-depth studies on bioleaching of non-sulfidic minerals through adoption and adaptation of some natural processes such as weathering. For instance, 27 fast-growing aerobic heterotrophs were isolated by Hutchens *et al*. (2003) from feldspar-rich soil to investigate their potential in the dissolution of feldspar. *Serratia marcescens* emerged as the best isolate in the dissolution of this mineral and factors such as growth conditions, bacterial type, growth phase, metabolites production and subculturing were all highlighted to significantly affect the dissolution rate.

Furthermore, He and Sheng (2006) reported the ability of *Bacillus edaphicus* in the solubilisation of some K-bearing minerals (feldspar and Illite) and the subsequent release of K in the rhizosphere for utilization for plant growth. Such bacterial-solubilising abilities have been transformed into their bioleaching potentials in the laboratory by testing for their ability to dissolve artificial insoluble chemical compounds for final selection as a potential bioleaching

candidate. Delvasto *et al.* (2008) utilised this method to isolate and characterise different phosphate-solubilising bacteria that included *Leifsonia xyli* Fe G1 02, *Burkholdera cenocepacia* FeSu 01, *Burkholdera caribensis* FeG1 03 and *Burkholdera ferrariae* FeG1 01. Among the potential bioleaching microbes obtained from their study, *Burkholderia caribensis* FeGL03 was able to show high potential in mobilising P contained in iron ore (Delvasto *et al.*, 2009). A few other examples of bacteria that have been used in bioleaching are listed below in Table 1.1.

**Table 1.1:** Examples of bacteria that have been used for heterotrophic leaching of non-sulfidic minerals (adapted from Jain and Sharma, 2004).

| Bacteria | Mineral/Mineral ore | Bioleached metal | Reference |
|----------|--------------------|--------------------|-----------|
| *Bacillus* sp. | Manganiferous ore | Ag | Rusin, 1992 |
| *Paenibacillus polymyxa* | Calcite, hematite, corundum | Fe, Al, Ca | Deo and Natarajan (1998) |
| *Arthrobacter* sp., *Norcadia* sp., *Pseudomonas* sp | Spodumene | Li, Al, Si | Karavaiko *et al.*, 1980 |
| Mixed culture of *Agrobacter radiobacter*, *Spaphilococcus* sp., *Candida* sp. | Manganiferous ore | Mn | Veglio *et al.*, 1997 |
| *Bacillus circulans* | Iron ore | Al | Pradhan *et al.*, 2006 |
| *Bacillus globisporus* Q12 | Feldspar | K and Si | Sheng *et al.*, 2008 |
| *Burkholdera caribensis* FeGL03 | Iron ore | P | Delvasto *et al.*, 2008 |

## 1.6.5 Fungal leaching of non-sulfidic minerals

Not many studies have attempted to discuss or investigate fungal leaching (Burgstaller and Schinner, 1993; Jain and Sharma, 2004). Fungi generally have the capability of being used in separation of metals from low-grade ores, mine wastes or in the removal or reduction of contaminations from ores (Groudev, 1987; Burgstaller and Schinner, 1993; Valix *et al.*, 2001). Burgstaller and Schinner (1993), listed four factors that had probably discouraged intensive research into the use of fungi as bioleaching agents. These included the high carbon demand of

fungi needed for growth, lack of thorough knowledge about fungi, slower rate of leaching by the fungi and an inability to use refined genetic methods for improvement of the fungi. The most important of these factors is the provision of a carbon source for these microbes that can serve as their source of energy during the leaching process (Jain and Sharma, 2004).

Generally, in fungal bioleaching, only *Aspergillus* and *Penicillium* spp. have received much attention (Table 1.2). In a study conducted on *Aspergillus* and *Penicillium* by Valix *et al*. (2001), cobalt and nickel were effectively leached by direct microbial activities and the effects of organic acids produced by these microbes. In another study, Castro *et al*. (2000) confirmed the better potential of *A. niger* to bacteria (*Bacillus* sp. and *Pseudomonas* sp.) in the leaching of zinc and nickel.

Although great progress has been made in the use of fungi such as *Aspergillus* and *Penicillium* spp. for bioleaching processes; however the health implication of the aerosols formed by the spores of these fungi (Gorny, 2004) has raised a serious question about their safety. The focus on the use of fungi for bioleaching should therefore extend beyond their leaching potential, but should also include the cost implication, as well as their short and long time impacts on the environment.

One of the most studied groups of microorganisms involved in weathering is the ectomycorrhiza (ECM) fungi. The capacity of ectomycorhizae to participate in weathering through the production of organic acid has been proven in many studies (Jongman *et al*., 1997; Hoffland, 2003; Gadd, 1999). Both the ectomycorrhizal root tips and the ectomycorrhizal hyphae in the soil are capable of releasing organic acids into the soil environment (Landeweert *et al*., 2001). In addition, other important metabolites of ECM fungi produced during weathering are siderophores. These are low-molecular-weight iron-chelating compounds synthesised under conditions of low Fe availability. Siderophores are Fe-loving compounds that help in solubilisation of insoluble Fe compounds or minerals, thereby contributing significantly to weathering of minerals that contain such compounds. Several studies have confirmed the increased uptake of siderophores by mycorrhizal plants (Haselwandter *et al*., 1992; Haselwandter, 1995). A combination of siderophores and organic acids was said to be very effective in dissolution of iron-containing compounds such as goethite (Cheah *et al*., 2003; Haselwandter, 2008). Meanwhile, organic acids

could sometimes function like siderophores in the mobilisation of Fe (Guerinot *et al*., 1990; Carson *et al*., 1992 in Machuca *et al.,* 2007).

In one of the earliest studies on ECM weathering, Jongmans *et al. (*1997) suggested that tubular pores found in feldspars and hornblende in podzol E horizons and granitic bedrocks at some places in Europe were formed by ECM fungi. This is one of the strategies used by these fungi to search for $K^+$, $Mg^{2+}$ and $Ca^{2+}$ on behalf of their host plants that grow under severe nutrient conditions, thereby contributing to the weathering of these minerals. Wallander and Wickman (1999) attempted a study that focussed on the mobilisation of potassium from biotite and microcline using two ECM fungi, namely *Paxillus involutus* and *Suillus variegatus*. Absence of K in their control experiments in *P. involutus*-colonised soils resulted in a decreased biomass of the ECM fungi in the soil compared to the situation where biotite was added as the source of K. In addition, they suggested that the relationship that existed between the foliar K and the citric acid (in *S. variegatus*-colonised seedlings) was an indication that K was released from biotite by weathering and later transferred to the host plant of the fungus.

Hoffland *et al.* (2003) also established the direct relationship between feldspar tunneling and ECM root density as a proof of participatory role of these fungi in weathering of feldspar. In addition, the study also provided the correlation between high weathering and low nitrogen availability. The ability of ECM fungi to repeatedly gain assess to nutrient trapped inside different minerals such as Phlogopite, feldspar and biotite (Paris *et al*., 1995),  leaves no doubt that the weathering ability of these fungi could possibly indicate their high bioleaching and metal binding potentials.

Assuming time is not a factor (such as the case in mine wastes) and a cheap carbon source could be provided, then fungi could compete with bacteria as candidates in bioleaching. For example, fungi can grow in higher pH environments (created by materials contained in metals to be leached) (Willscher and Bosecker, 2003) compared to some bacteria that are used in bioleaching and thrives best in acidic environments. In addition, the production of organic acids, amino acids, proteins, protons and peptides by fungi increases the solubility of metals in solution, thereby aiding in the bioleaching process.

Nevertheless, prior to the full development of a technology where ECM fungi could be used as bioleaching agents, the immediate focus should be on screening of potential ECM fungi that could function in this area of biotechnology. Indeed, if there are ECM fungi that are bioleaching agents, then improvements could be made in their culturability whether in the presence or absence of a host plant, such as those developed in the mushroom industries where some mushrooms that are ectomycorrhizal could be cultivated without the host plant (Debaud and Gay, 1987; Hall *et al.*, 2003). Furthermore, there should also be investigations into different methods in which minerals could be leached with these fungi, bearing in mind the ease of separation of the fungi and mineral products after leaching, as well as the cost implication of the method should be low. For instance, the consequence of direct leaching with the fungi should be compared to the use of fungal filtrates for the leaching process. The use of fungal metabolite for this purpose was demonstrated by Williams (2008), that utilised citric acid produced by *A. niger* was utilised to reduce K of iron ore by 17.65%.

A difficult challenge regarding the use of ECM fungi in bioleaching will be the growth of the fungi under non-sterile conditions. The pH and temperature ranges at which fungi grow normally supports the growth of many contaminants (Jain and Sharma, 2004). In conclusion, use of fungi such as ECM in bioleaching could be feasible if there is thorough understanding of the metabolic pathways and genetics of these fungi (Burgstaller and Schinner, 1993; Jain and Sharma, 2004).

Since it has been established that performance of ECM fungi in pure culture does not necessarily translate to their role when in symbiosis (Smith and Read, 2008), separate investigations of the two scenarios should be encouraged. This would increase the knowledge about different potentials of the ECM fungi with or without the host plants. Therefore, there is a high prospect in the ability of ECM fungi to solubilise varieties of minerals, as proven in their weathering capabilities (Paris *et al.*, 1995; Landeweert *et al.*, 2001; van Scholl *et al.*, 2006b).

**Table 1.2:** Examples of fungi that have been used for heterotrophic leaching of non-sulfidic minerals (adapted from Jain and Sharma, 2004).

| Fungi | Mineral/Mineral ore | Bioleached metal | Reference |
|---|---|---|---|
| *Aspergillus* sp. | Manganese ore | Mn | Ghiorse, 1988 |
| | Laterite ore | Ni, Co, Mn | Tzeferis, 1994 |
| *A. clavatus* | Mercury compounds | Hg | Puerner and Siegel, 1976 |
| *A. niger* | Laterite ore | Ni, Co | Tzeferis *et al.* 1994 |
| *A. niger* | Manganese nodule | Cu, Ni | Ehrlich, 1980 |
| | Coal fly ash | Al | Singer *et al.*, 1982; Torma and Singh, 1993 |
| | Clay | Al | Groudev and Groudeva, 1986 |
| | Nepheline | Al | King and Dudeney, 1987 |
| | β-spodumene | Li | Ilger and Tormer, 1989 |
| | | Li, Al | Rezza *et al.*, 1997 |
| | Copper converter slag | Cu, Ni, Co | Sukla *et al.*, 1992 |
| | Silicate ore | Zn, Ni | Castro *et al.*, 2000 |
| | Iron ore | Al | Pradhan *et al.*, 2006 |
| *A. ochraceous* | Rocks | U | Munier-Lamy and Berthelin, 1987 |
| *Penicillium* sp. | Manganese ore | Mn | Ghiorse, 1988 |
| | Gold dust | Au | Groudev and Groudeva, 1988 |
| | Silver ore | Mn, Ag | Gupta and Erlich, 1989 |
| | Iron ore | Fe | Hoffman *et al.*, 1989 |
| | Laterite ore | Ni, Co | Agatzini and Tzeferis, 1997 |
| *P. funiculosum* | Rocks | U | Munier-Lamy and Berthelin, 1987 |
| *P. notatum* | Pagmetite rock | Li, Si, Al, Fe | Avakyan *et al.*, 1981 |
| *P. simplicissimum* | Rocks | Ti | Silverman and Munoz, 1971 |
| | Basalt rock | Al | Mehta *et al.*, 1978, 1979 |
| | Red Mud | Al | Vachon *et al.*, 1994 |
| *P. purporogenum* | Spodumene | Al, Li | Rezza *et al.*, 1997 |
| *P. variotti* | Lead zinc ore | Zn | Dave *et al.*, 1981 |
| *Trichoderma ligneruom* | Pagmetite ore | Li, Si, Al, Fe | Avakyan *et al.*, 1981 |
| *Yarrowia lipolytica* | Used catalyst | Cu, Pb, Sn | Hahn *et al.*, 1993 |
| *Candida* sp. | Gold dust | Au | Groudev and Groudeva, 1988 |

## 1.7    Iron ore

Iron ore is one of the oldest metals and an essential source of primary iron for the global iron and steel industries. Iron is one of the most common elements on earth, traded and consumed in different forms in many countries (Astrup and Hammerbeck, 1998). There are different types of iron-bearing minerals, but the highly exploited ones include: Magnetite – $FeO.Fe_2O_3$ (72% Fe), Haematite - $Fe_2O_3$ (70% Fe), Goethite - $FeO.OH$ (61% Fe), Lepidocrocite - $FeO.OH$ (61% Fe), Siderite – $FeO.CO_2$ (48% Fe) and Chamosite – $3FeO.Al_2O_3.2SiO_2.6H_2O$ (35% Fe). In addition, there are associated gangue minerals found in iron ores and these include feldspar, quartz, calcite, dolomite, clays and carbonaceous matter (Astrup and Hammerbeck, 1998). Iron ore could also contain some deleterious elements such as phosphorus, silica, potassium, zinc, sulfur and sodium (Astrup and Hammerbeck, 1998; Yusfin *et al*., 1999). Iron is desirable because of its physical properties such as hardness, strength, malleability, ductility, durability and the ease with which it can form alloys with other elements to form different types of steel (Klemic *et al*., 1973; Astrup and Hammerbeck, 1998).

Iron ore deposits in South Africa can be categorised into four types of deposits, namely: Banded iron formations (BIF), Magnetic deposits, Gossan and residual properties and Lode, vein and replacement deposits (Astrup and Hammerbeck, 1998). However, most of the world's iron ore currently being mined are from the high-grade haematite iron hosted by the Precambrian branded iron formations (BIF) (Gutzmer *et al*., 2001; Beukes *et al*., 2003). The BIF is generally divided into two groups, i.e. martite-goethite ores and martite hematite ores. The martite-goethite ores consist of a range of strongly indurated, brown goethite-rich material to friable yellow ochre, consisting of martite and early formed prismatic hematite. These deposits are characterised by variable preservation of BIF features such as banding and pseudomorphing of gangue minerals by goethite. This group is generally considered to result from supergene enrichment of BIF during Mesozoicto Tertiary lateritic weathering (Morris *et al*., 1980; Morris, 1985; Barley *et al*., 1999). The second group, martite-hematite (or microplaty hematite) ores, is characterised by well-preserved primary lamination, but with variable loss of internal texture and the growth of abundant secondary prismatic and microplaty hematite. Though the origin of these ore deposits is

not certain, it is generally believed that they represent metamorphosis of martite-goethite ore bodies that occurred during Palaeoproterozoic burial, where goethite is susbstituted for hematite at <100 °C (Morris, 1985). These types of deposits are found in South Africa and Brazil (Morris, 1985; Barley *et al*., 1999). In South Africa, these BIF consist of distinctive hills, stretching a distance of 400 km from the south in Prieska to Pomfret in the north (Carney and Mieney, 2003).

## 1.7.1 Iron ore in South Africa

Records have shown that mining must have started in South Africa during the middle stone age when black people obtained minerals either by picking them at the surface or digging holes to get these minerals (Mason, 1982). Iron and copper were the first two minerals mined by the early farmers in South Africa (Miller, 1995). Beumont (1973) estimated the exploitation of iron ore minerals in Southern Africa to be 40000 years old when specularite and haematite were extracted by ancient miners from Ngwenya, Swaziland. There are also documented traces of early mining of specularite in the Nothern Cape province of the country, whereas historical evidence of early smelting and fabrication were recorded from Tzaneen and Broederstroom, near Hartbeespoort dam (Astrup and Hammerbeck, 1998). However, proper documentation of mining in South Africa is only available for the last two centuries. South African iron ore mines include Sishen, Beeshoek, Thabazimbi, Palabora and Mapochs, having combined reserves of 2879 million tonnes of iron ore. Up to 80% of these reserves belong to the Sishen iron ore mine located in the Northern Cape (Fig. 1.1) (Astrup and Hammerbeck, 1998). This huge deposit means South Africa has the largest iron ore deposit in Africa.
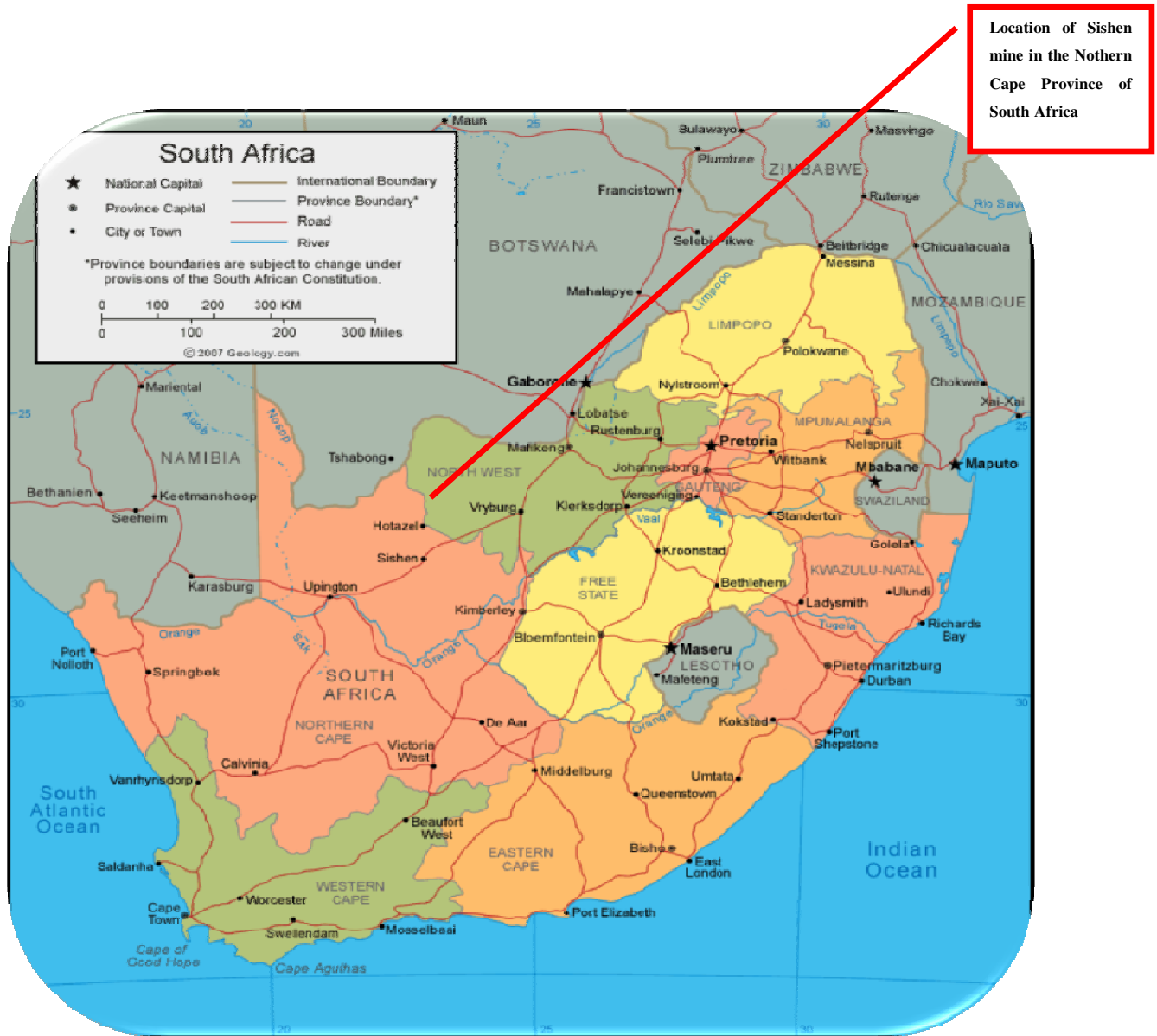
**Figure 1.1:** Map of South Africa showing the location of Sishen mine in the Northern Cape Province (adapted from http://geology.com/world/south-africa-satellite image.shtml).
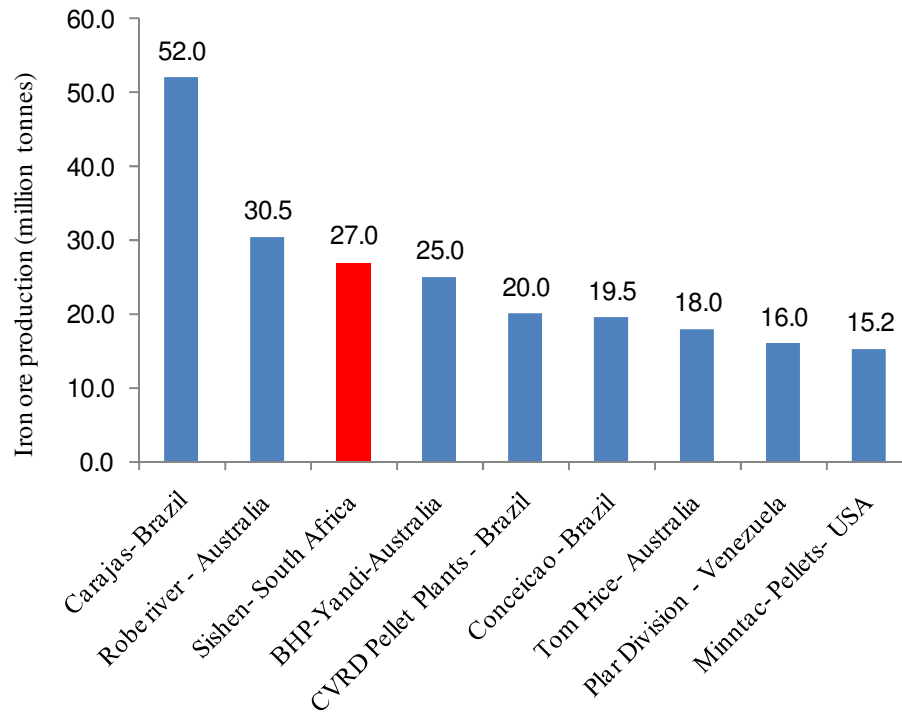
**Figure 1.2**: Top iron ore mining sites in the world, Sishen is 3$^{rd}$ on the list (adapted from (http://www.saldanhaportoperations.com/operations_io_sishen_largest.php?sid=)

South African iron ore mining is dominated by KUMBA resources that controls the two biggest iron ore mining sites in South Africa, i.e. Sishen in the Northern Cape and Thabazimbi in the Northern Province. Sishen was founded in 1953, and presently has the capacity to produce up to an average of 27 million tonnes of iron ore a year. This corresponds to 78% of the total iron ore in South Africa and it is the third largest iron ore company in the world (Fig. 1.2) (Internet 1). At Sishen, South Africa, the iron ore company faces the problem of increasing mining volume of impure iron ore minerals, which contain potassium and phosphorus that affect the commercial value of the ore.

## 1.7.2 Biobeneficiation of iron ore

Despite the popularity of iron ore, the biobeneficiation of this ore has not received much attention like the other mineral ores. This is mostly due to the cheap cost of this mineral, regardless of its importance (Williams, 2008; Delvasto *et al*., 2009). However, fast depletion and difficulty in finding the mineral in the pure form has increased awareness about the extraction and biobeneficiation processes of iron ore. Problematic elements such as P, K and Na have been identified to reduce the commercial value or render the mineral valueless. These elements have various ways of interfering with the processing of iron ore. Phosphorus, which is an element needed by both plants and animals, is, however, one of the most deleterious elements that can associate with iron ore mineral. High phosphorus levels in steel cause a reduction or loss of strength, hardness and ductility, as well as increasing the chances of corrosion (Marshall, 1984). High levels of alkaline - K and Na, can also affect iron ore processing, especially the smelting of iron ore in the blast furnace. The problem with alkalis occurs when they are volatilised as elements. Some parts of the vapour normally react with the refractory lining or burden material to generate compounds in relation to their relative stability. This mainly happens in the cooler regions where oxygen potential is high. The compounds formed progress to the hotter regions where they are reduced and rise again to form recirculating load of alkalis (Biswas, 1981; Elkasabgy, 1984; Yusfin *et al*., 1999). In summary, the adverse effects of alkalis on the blast furnace include an increase in coke rate, poor quality of hot metal and mechanical weakening of the furnace lining. They also cause a decrease in production; conditions that are more pronounced when there is low stability of coke and the iron ore (Davies *et al*., 1978; Elkasabgy, 1984; Yusfin *et al*., 1999). Due to these problems, iron ore with high contents of both P (>0.03%) and K (>0.24%) attracts penalties (low cost) in the international market. It has therefore become imperative for iron ore industries to find an economical and environmentally friendly method that could solve these problems.

To solve these problems, several methods have been proposed and these normally rely on the principle of dilution (with better quality ore) and adjustment of temperature, basicity and acidity inside the blast furnace. The method that was adopted by Sishen iron ore mine, South Africa, to deal with this problem prior to this time is the mixing of standard (low levels of K and P) and low

grade (high levels of K and P) iron ore to avoid penalty charges and meet international standards (Williams, 2008). However, with increasing volumes of iron ore that have high levels of K and P, this method has become unsustainable. Traditionally, different experiments have been conducted using chemical and physical methods to get rid of these contaminants found in iron ore (Delvasto *et al.*, 2005). The choice of method in this regard strongly depends on the basic characteristics of the iron ore and the type or degree of association that exist between the iron ore and the P content (Delvasto *et al.*, 2005). For example, Cheng *et al.* (1999) utilised sulfuric acid for the biobeneficiation of Australian iron ore with a phosphorus level of 0.126%. In this situation, more than 67% of P was leached within 5 h at 60 °C by a 0.1 M concentration of sulfuric acid. In addition, Changde iron ore with a high P content (1.125%) was successfully leached using an alkali and acid solution that contained sodium hydroxide, sulfuric, hydrochloric and nitric acids. Another hydrometallurgical method was proposed by Muhammed and Zhang (1989), where isoamyl alcohol, phosphoric acid and nitric acid were used in an integrated leaching process to remove P from the iron ore.

Unfortunately, further developments of these technologies have been hindered by cost implications and their potential negative impacts on the environment (Delvasto *et al.*, 2005). For this reason, this last decade has seen the gradual development of affordable and environmentally friendly methods of leaching iron ore. Focus is now more on the use of microorganisms for the leaching of this mineral, a process known as biobeneficiation (Jain and Sharma, 2004). Development in this area has been slow due to the non-sulfidic nature of some iron ore minerals (Williams, 2008), which has made it difficult to apply already detected sulfidic bioleaching microorganisms for leaching of the iron ore mineral. However, two decades ago, the use of microorganisms to leach iron ore was patented by Hoffman *et al.* (1989). In their work, domestic wastewater containing *Pseudomonas* 200 was used under anaerobic conditions to reduce the ferric iron in iron ore to ferrous iron and which was later precipitated from the solution with the help of a base to recover the iron mineral. Another study was carried out by Parks *et al.* (1995) where metabolites containing itaconic and oxalic acid produced by a *Penicillium* sp. significantly reduced the phosphorus contents of the iron ore. Further reduction of the P content was obtained by addition of a low concentration of hydrochloric acid. The only report regarding the use of

ECM fungi for the solubilisation of P from iron ore was reported by Buis (1995) in Delvasto *et al*. (2005), where *Paxillus involutus, Hebeloma crustiniforme, Thelopora terrestris* and *Laccaria bicolour* failed to solubilise P from iron ore, despite their ability to solubilise P from hidroxylapatite.

Recently, Delvasto *et al*. (2005) investigated the biobeneficiation of iron ore in greater detail. In this case, after bioactivation of the iron ore samples, one of the phosphate-solubilising fungi that was isolated from the iron ore, *Aspergillus niger*, was tested for its ability to solubilise the P content of the iron ore. Up to 30% desphosphorisation was attained, signifying high potential in the use of microorganisms for the bioleaching of iron ore. This was followed by another study by Delvasto *et al*. (2008), where four different phosphate-solubilising bacteria were isolated from the high phosphorus Brazilian ore using tricalcium phosphate [$Ca_3(PO_4)_2$] as the insoluble form of P. The bacteria isolated included *Leifsonia xyli* FeGl 02, *Burkholderia cenocepacia* FeSu 01, *Burkholderia caribensis* FeGl 03 and *Burkholderia ferrariae* FeGl 01. Further studies were then carried out on *Burkholderia caribensis* FeGl 03 to investigate its potential in desphophorisation of the iron ore. The results indicated that this bacterium was able to mobilise between 5% and 20% of the initial P value of the iron ore within a period of 21 d. Particle size, organic acid and exopolymeric substances production were all listed as possible factors that affected the leaching process (Delvasto *et al*., 2009).

The problem associated with this method is mostly related to the growth conditions of the bacteria. Heterotrophs are known to grow at temperature and pH ranges near neutral (around pH 7, ±2), a situation that encourages easy contamination of their growth medium (Jain and Sharma, 2004). Iron ore being an inexpensive mineral, it is very difficult to develop this processes into heap leaching that can be used in outdoor leaching. It has therefore been suggested that focus should shift to finding a better source of carbon such as organic wastes from food or agricultural industrial wastes. Another problem is the strong attachment of fungi used in biobeneficiation to the mineral to be leached. Separation in such scenario may be quite difficult and in this case, the use of metabolites produced by the fungi could be the solution. In addition, separating techniques could also be introduced into the fungal biobenefication process from the initial stage to prevent the fungal attachment to the surfaces of the mineral.

From the examples above, it can be said that technologies in the biobeneficiation of iron ore is gradually gaining momentum. However, what is not clear to investigators in this area of research is the exact type of microorganisms that can perform this task. Both fungi and bacteria have been used for the bioleaching process. A knowledge gap here is the absence of such studies on other contaminants of iron ore such as K. From the available literature, there has not been development or investigation of biobeneficiation for other impurities (such as K) found in iron ore. Information about P removal from previous studies provided direction on how to approach this study. This means technologies already developed for P-solubilising bacteria could provide a platform for studies concerning solubilisation of other P-containing mineral. The current study therefore, provides an elaborate investigation of both uninvestigated fungi and bacteria that have the potential to participate in the bioleaching of iron ore. The study started by investigating the bioleaching roles of some soil microbes. This was expected to provide insight into the relationship between bioleaching and weathering, an investigation that can lead to detection of potential bioleaching agents of iron ore. Furthermore, potential influences of iron ore-associated microorganisms (fungi and bacteria) were also investigated to establish the possibility of using indigenous microbes for the biobeneficiation of iron ore. Finally, a novel method was designed to initiate a future development of an economical and environmental friendly method of leaching iron ore. Based on the above-mentioned points, the aims of this study are as listed under the objectives.

## 1.8   Objectives of the study:

   a. To investigate the relationship between natural weathering and biohydrometallurgy potentials of ectomycorrhizal fungi

   b. To investigate the *in vitro* capabilities of pure cultures of different ectomycorrhizal fungi in mobilisation of potassium and phosphorus from iron ore

   c. To isolate and identify potential organic acid-producing fungi that can mobilise nutrients from iron ore minerals

d. To investigate indigenous bacterial flora of two types of Sishen iron ore and the potential of the bacterial isolates in mobilisation of nutrients from the ore

e. To initiate an economically viable method for biobeneficiation of iron ore using a cheap energy source and septic conditions.

## 1.9 References

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