CHAPTER ONE
INTRODUCTION

1.1. Motivation

The increase in the depletion of the world’s high grade ores has led to an increase in the attention being given to low-grade complex sulphide ores. Complex sulphide ores are however, complicated mineralogical associations of their constituent minerals, which are generally made up of intergrown minerals, often with interlocked and finely disseminated refractory and precious metals. Regardless of the form in which the different elements occur and are associated, they are usually very difficult to process (Deveci et al., 2004; Rubio and Frutos, 2002). This may be due to the close similarities in their mineralogical properties, which make them unsuitable for conventional methods of processing. Differential flotation does not release all the constituent phases (Majima, 1969) and so the different concentrates obtained are of poor quality with a low rate of metal recovery. This makes further pyrometallurgical processing of these ores difficult and costly (Rubio and Frutos, 2002) and renders them unattractive for commercialization (Sandstrisom and Petersson, 1997). As a result, metal value is preferably extracted from sulphide ores through hydrometallurgical processes.

Hydrometallurgical processing however, suffers a major drawback of inability to effectively dissolve sulphide ores due to their low solubility in many reagents and the complexities in their mineralogical associations. This drawback has lead to the use of large volumes of concentrated solutions for dissolving these ores, thereby resulting in a high consumption rate of reagents that often cause environmental hazards. The increase in the complexities during hydrometallurgical processing of complex sulphide ore and their cost implications have recently become a strong and formidable challenge to process engineers in designing effective and sustainable recovering routes for their constituent metals. Selecting and designing of an optimum technical and economic hydrometallurgical recovering process of their constituent metals therefore requires a complete knowledge of the ore, its chemical and mineralogical compositions, relative amounts, and grain size distribution.
The complexities in the mineralogical associations of sulphide ores necessitate a detailed mineralogical characterization of such ores to determine an optimal processing route for its constituent minerals and metals. This is due to the fact that the identification and characterization of minerals is of fundamental importance in the development and operation of mining and mineral processing systems (Hope et al., 2001), and it is very important in choosing a suitable flowsheet for recovering the constituent metals. It is also critical to optimize the actual plant for improving performance (Xiao and Laplante, 2004).

The growing need for detailed information about the mineralogical composition of a mineral deposit therefore determines that mineral characterization studies form an integral and often critical part of investigations of deposits (Cook, 2000). This is because knowledge of mineralogical/chemical composition, size, morphology and association with other minerals is very useful in providing insights and information on the characteristics, type, nature and amount of minerals and elements present within the ore at different locations. This knowledge would permit an assessment and determination of an optimal processing route for its constituent minerals/metals (Costigliola et al., 2003; Zhou and Cabri, 2004). Applied mineralogical studies would also play an important role in overcoming incorrect assumptions which may have disastrous consequences during process design (Reyneke and Van Der Westhuizen, 2001), and provide a sound background in understanding the behavior of the minerals during beneficiation and the designing of optimal beneficiation routes. It is considered critical to optimize the exploration of the mineral resource by providing a better knowledge of the ore characteristics and its behavior in the process (Sant’Agostino et al., 2001).

1.2. Problem Statement
The major problem with the hydrometallurgical processing of sulphide ores is that most sulphide minerals do not allow the optimum recovery of metal by direct chemical leaching (Hiskey and Wadsworth, 1975; Dutrizac, 1989), due to their low solubility in many leaching reagents. Therefore, for the metal content to be leached, the reagent must come into direct contact with metal atoms or metal containing compounds within the
mineral ore.

Amongst several methods, a suitable approach to ensure that the metal content of the ore comes in contact with the leachant, is to grind the ore fine enough to liberate all the mineral phases so as to enable them to be exposed to chemical attack. A limitation to grinding many sulphide ores is that the ore cannot practically be ground fine enough to expose the metals. For instance, chalcopyrite and sphalerite are frequently intergrown, with micro-size grains of 10-20 µm being dispersed within the pyrite (Gomez et al., 1999). Therefore, due to these specific mineralogical characteristics, it is necessary to finely grind and concentrate the ore prior to the solubilization of the valuable metals (Barbery et al., 1980). However, crushing and grinding of ore is a significant capital and operational cost in many mineral processing plants. According to Bilgili and Scarlett (2005), size reduction is an expensive and energy-inefficient process, however operated. Considering these factors, a small gain in comminution efficiency can have a large impact on the operating cost of a plant, while simultaneously conserving resources (Fuerstenau et al., 1999). Hence, it is important to fully determine the comminution parameters relevant to the crushing and milling of an ore to enable proper plant design to take place.

Owing to the complexities in the mineralogical associations of low-grade complex sulphide ores, the intergrown nature of the constituent mineral usually result in a poor liberation of the associated minerals. As a means for improving the efficiency of conventional grinding circuits, the liberation of minerals and to reduce costs associated with size reduction, several approaches could be adopted. A combination of electrical and ultrasonic energy has been successfully used to fracture the matrix of ores causing selective liberation (Parekh et al., 1984). Chemical additives which act as grinding aids during wet grinding have also been used to modify the mechanical properties of ores, facilitating breakage, as well as reducing cost of producing fine particles (Sacks and Tseng, 1983; Fuerstenau et al., 1985; Lu and Wei, 1992). An effective mineral breakage and liberation improvement approach which has been observed to be very promising is the application of microwave irradiation. The driving forces towards its application are the reduction in processing costs (Al-Harahsheh and Kingman, 2004) resulting from an improvement in the liberation of valuable minerals and a reduction in comminution
(Jones et al., 2005), and improvements of the yields of extracted metal and reduced processing time (Al-Harahsheh et al., 2005) due to its amazing capability of accelerating chemical reactions (Huang and Rowson, 2002; Hwang et al., 2002).

Apart from mineral liberation techniques, another useful approach for overcoming the problems of the low solubility of sulphide ores in the leaching reagent is a pre-treatment process whereby the desired metals are physically released and the ore made more amenable to leaching reagents (Chiacchiarini et al., 2003). The processes available for the pretreatment of complex sulphide ores involve the sulphide oxidation to form water-soluble sulphates or oxides. The sulphur oxidation processes including roasting, pressure oxidation and chemical oxidation, which are traditionally used, are complex, expensive (Gomez et al., 1997; Han and Meng, 2003) and mostly environmentally unsuitable (Ubaldini et al., 2000) because they produce poisonous sulphur dioxide gas. The bioprocess has proved to be the best alternative (Holmes, 1988; Smith and Misha, 1991; Acevedo, 2002). It is cheaper, environmentally benign and less complex (Anand et al., 1996).

Despite the encouraging incentives from bioleaching as an alternative to chemical leaching for metal recovery, there are still great challenges to be overcome. The slow kinetics of the reactions and high residence time hinder its industrial application and limit its economic viability (Rubio and Frutos, 2002). Several studies with mesophilic microorganisms such as Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum ferrooxidans have shown very slow metal leaching rates (Mehta and Murr, 1982; Sand et al., 1992). For this reason, current research into this field is focused on how to increase the bioleaching rate. Therefore, attention is drawn to the isolation and adaptation of new microorganisms (thermophiles) with a high capacity to leach these ores (Acar et al., 2005). When thermophilic microorganisms were used, leaching rates were considerably increased due to high temperatures, higher metal tolerance capacity and metabolic characteristics of this type of microorganisms (Brierley, 1993; Clark and Norris, 1996), which favours their use for bioleaching. However, the use of these thermophilic bacteria involves raising the temperature to 48 °C for moderate thermophiles and 68 °C or higher for extreme thermophiles, thus resulting in an initial high energy...
consumption and requiring high temperature corrosion resistant equipment. This leads to higher operating cost of the process, solution evaporation and possible environmental problems.

In order to prevent problems associated with thermophiles, there is therefore a need to investigate and optimise conditions favouring the dissolution rates of the mesophiles. Apart from the conditions of physical parameters (particle size, stirring speed, leaching time, pulp density, and volume of innoculum), a useful approach for optimising mesophilic leaching is the use of mixed cultures of mesophilic bacteria. The use of mixed cultures to optimize mesophilic leaching has been widely studied (Rodriguez et al., 2003a, 2003b, 2003c; Sampson, et al., 2005, Akcil et al., 2007). Mixed cultures were shown to have better bioleaching capacities than the pure cultures. Akcil et al. (2007) observed that the bioleaching capacity of mixed cultures of Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans and Leptospirillum ferrooxidans was higher that those of Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans.

Although a few research has been reported on the optimization of bioleaching parameters (Nemati et al., 1998; Gomez et al., 1999; Nemati et al., 2000; Third et al., 2000; Deveci, 2002; Deveci et al., 2004; de Kock et al., 2004; Hossain et al., 2004; Das et al., 2005), it is however observed that the effect of ore mineralogy, which is a major contributing factor during mesophilic leaching, has not been properly taken into account. Apart from the fact that ore mineralogy provides useful information for understanding the underlying mineralogical basis for microbial attack, it is also envisaged that its knowledge would be useful in revealing vital information about the process to allow for process optimization and guide decisions on processing parameters. As there is still no agreement on the mechanism of interaction of microwaves with hydrometallurgical systems (Al-Harahsheh and Kingman, 2004), knowledge of mineralogy would provide useful information in understanding microwave assisted leaching.

1.3 Purpose and Aims of the Research
This research is aimed at providing a mineralogical basis for understanding the
biohydrometallurgical route for processing sulphide ores and understanding the means by which microwave processing improves microbial recovery, using a Nigerian low grade complex sulphide ore as a case study. The research aims to achieve the following objectives:

- Investigate the geological and mineralogical characteristics of the bulk complex ore.
- Study the influence of applied mineralogy in determining an optimal route for hydrometallurgical processing of the ore.
- Investigate the influence of ore mineralogy on bioleaching of the ore using mixed cultures of mesophilic bacteria
- Study the role that ore mineralogy plays in understanding and optimizing the conditions favouring the bioleaching process of the ore
- Study the interplay of mineralogical variation and mineral phase distribution within varying particle sizes on the bioleaching mechanism
- Investigate the interrelationship of microwave irradiation and mineralogy on the heating characteristics, comminution behaviour and the bioleaching behaviour of the ore.

1.4 Hypothesis
Complex sulphide ores are composed of different types of both sulphidic and non-sulphidic minerals, which behave differently under the same and at varying processing conditions. These mineralogical differences affect:

i. ore response during comminution and microwave heating,
ii. their breakage and microwave heating characteristics,
iii. size distribution, and
iv. mineral and elemental deportment amongst varying particle size fractions.

Variations in the mineralogical properties at varying particle size fractions would thus results in differences in the bioleaching behaviour amongst the varying size fractions due to variation in galvanic interactions that could have resulted from the differences in their elemental distributions.
1.5 Justification for the Study
Several research studies have been reported on microbial leaching of sulphide ores, bioleaching capacities of pure and mixed cultures, and the application of microwave technology in bioleaching processes. However, applied mineralogy, which is an important area in hydrometallurgy and which is a major contributing factor during microwave processing and bioleaching, has not been adequately taken into consideration. Although some understanding of the galvanic interaction between minerals (resulting from mineralogical differences) in an aqueous system has been known in terms of its process and mechanism, it is observed that an understanding of low-grade complex sulphide ore systems, especially in microbial media, is yet to be fully understood and established. Studies reported on the influence of microwaves on bioleaching have not provided sufficient information from which industrial systems could be understood, and thus provide a basis for its industrial acceptability.

While mineralogy is critical to the behaviour of ores during breakage and dissolution, it is observed that not much work on its influence in biohydrometallurgical process development has been carried out. Few related research reports (Sampson et al., 2000b; Sampson and Philip, 2001; Valix et al., 2001) could not provide useful information that could aid a full understanding of the underlying principles governing the bioleaching process, and to clarify reasons for the recoveries and process effectiveness. It is also observed that much attention has not been given to studying the dual effects of ore mineralogy and microwave treatment and their probable influence on the bioleaching mechanism of the treated ores. Hence it is very necessary to conduct such studies.

1.6 Structure of the Thesis
A general introduction to the study, motivation, problems, aims and objectives of the research are presented in Chapter One. Chapter Two contains literature review of the
basic concepts and on previous and recent work relating to this study. A compilation of eight (8) articles from the investigations of this study published in or submitted to peer-review and reputable ISI journals is contained in Chapter Three. This Chapter consists of a synopsis of the articles showing how they relate to one another and the reprint versions of the articles as they appeared in the journals. The papers are sequentially arranged to coherently accomplish the objectives of the research.

Papers 1 to 3 comprise publications on applied/process mineralogy. Paper 1 brings together information on process mineralogy as applied to the processing of complex sulphide ores and with the view to form an integral part of the overall techniques for improving efficiency during sulphide processing and recovery. Paper 2 reports the detailed mineralogical study on the complex sulphide ore deposit located at Ishiagu in the South Eastern part of Nigeria that was used for this study, with a view to provide relevant mineralogical information on which the processing of the ore could be easily be achieved. In Paper 3, useful mineralogical data on size reduction characteristics, size distribution pattern, degree of mineral liberation, mineralogical and elemental distribution among different size fractions are interpreted and used for predicting parameters for which optimal hydrometallurgical recoveries of constituent metals could be obtained.

Investigations published on the role and interactions of mineralogy and the bioleaching process are presented in Papers 4 to 6. Mineralogical data on the variations in mineral and phase distribution within particle sizes of -53, 53, 75 and 106µm was utilized in Paper 4 to provide an understanding of the influence of particle size and ore mineralogy on the microbial dissolution behaviour of the ore. In Paper 5, a further understanding of the effects of particle size on bioleaching of the low-grade complex sulphide ore initially reported in Paper 4 is provided by studying the interplay of ore mineralogy, particle size, and mineral phase distribution within varying particle sizes on bioleaching behaviour through bioleaching experiments, electrochemical techniques and scanning electron microscopy methods. The role that ore mineralogy plays in understanding and optimizing the conditions favouring the bioleaching of the ore using mixed cultures is reported in Paper 6. The knowledge of mineralogical behaviour of the constituent minerals within
the ore was employed to aid the understanding of the microbial dissolution process, thereby revealing vital information for bioleaching process optimization and thus guiding decisions on processing parameters.

Publications on the interplay between ore mineralogy, microwave heating, mineralogical response to microwave heating, and overall effect on the dissolution process makes up Papers 7 and 8. The mineralogical basis for understanding the way in which microwave treatment affects the processing and recovery of a complex sulphide ore is reported in the Paper 7. The interplay of microwave irradiation and mineralogy on the heating characteristics, breakage response, and the mechanisms of dissolution of microwaved ore was investigated in sulphuric and hydrochloric acid as a case study using electrochemical techniques. In Paper 8, the effects of microwave irradiation on their biohydrometallurgical processing behaviour was studied through an investigation on the interrelationship of mineralogy, microwave irradiation, and bioleaching behaviour of the low grade complex sulphide ores. The influence of microwave irradiation on the bioleaching behaviour and mechanisms of the ore was investigated in a mixed mesophilic bacterial culture through bioleaching experiments and electrochemical study.


The conclusions drawn from the results of this study and recommendations for future work are contained in **Chapter Four**. This Chapter summarizes and synthesizes **Papers 1 to 8** and provide arguments for the originality and contribution of this study to the existing knowledge of process mineralogy as applied to biohydrometallurgy and microwave treatments of complex sulphide ores. Lastly, a comprehensive list of all referred materials to this thesis is given.
CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Biohydrometallurgical processing of sulphide ore

Biohydrometallurgy is essentially a microbial assisted dissolution of metals from an ore, especially sulphide ores, where metals are usually very difficult to dissolve in many aqueous solutions. It involves the use of microbes to transform minerals and make them amenable to dissolution in aqueous solutions. According to Acevedo (2000), biohydrometallurgy encompasses two related microbial processes that are useful in extractive metallurgy: bacterial leaching, also known as bioleaching, and biooxidation. Bioleaching involves either a direct or an indirect microbial assisted/mediated dissolution of metal value from the ore, as is the case in the bioleaching of base metals, while biooxidation involves an aerobic bacterial oxidation of reduced sulphur (insoluble metal sulphide), for example in the case of biooxidation of refractory gold. The recognition in the 1950’s that bacteria participated in the oxidative dissolution of metals from ores was the starting point for biohydrometallurgy (Technical Scope, 2001). Modern commercial application of biohydrometallurgy for processing ores became a reality in the 1950s with the advent of copper bioleaching (Brierly and Brierly, 2001), and nowadays, it is the most successful alternative in many mining projects (Frias et al., 2001).

Biohydrometallurgy has recently occupied an increasingly important place among the available mining technologies. It is no longer just a promising technology, but an actual economical alternative for treating specific mineral ores (Acevedo, 2002). It has been globally applied to the recovery of base and precious metals, piloted and commercialized in many countries, and intense research (Tipre and Dave, 2004; Qiu et al., 2005; Liu et al., 2007; Wang et al., 2007; Liu et al., 2008; Lavalle et al. 2008; Hita et al., 2008; Wang et al., 2008; Zhao et al., 2008) is presently focused on accelerating the kinetics of the process.

2.1.1 Principles of biohydrometallurgical processing

Biohydrometallurgy is based on the ability of autotrophic, acidophilic and
chemolithotrophic microorganisms to oxidize and dissolve insoluble metal sulphide ore, thus releasing the entrapped metal particles. The autotrophic bacteria for example, *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* which are frequently associated with sulphide minerals (Rawlings, 1997) obtain their energy from the oxidation of the sulphide ores and the oxidation of reduced sulphur compounds using oxygen as the final electron acceptor. The mechanisms and the underlying principles of the two aerobic processes involved in biohydrometallurgy are very similar and can broadly be described as:

\[
MeS + 2O_2 \xrightarrow{\text{Bacteria}} MeSO_4
\]  
(2.1)

The insoluble sulphide (MeS) on oxidation to sulphate (MeSO\(_4\)) becomes very soluble in aqueous solution and the metal ions are dissolved into the aqueous leaching solution. According to Gonzalez et al. (2004), the process is an autocatalytic system where cells and ferric ions are reactants and products in the global reaction. The simplified cooperative leaching process can be represented as (Gonzalez et al., 2004):

\[
Cells + MeS + Fe^{2+} + 2O_2 \xrightarrow{\text{Bacteria}} Cells + Fe^{3+} + SO_4^{2-} + Me^{2+}
\]  
(2.2)

According to Gonzalez et al., (2004), the process takes place through two general mechanisms: (i) the direct mechanism that accounts for the dissolution of reduced or partially reduced sulphur compounds (i.e. sulphide, elemental sulphur, and thiosulphate) by the action of the cells attached to the mineral particles, and (ii) the indirect mechanism (only possible for *Acidithiobacillus ferrooxidans*), that accounts for the oxidation of the mineral by ferric ions (Brierley and Brierley, 2001). In the indirect mechanism, the bacterial population oxidizes the ferrous ions to ferric ions that in turn oxidize the mineral particles. The iron (III) attacks the metal sulphide, forming iron (II) which is rapidly reoxidised to iron (III) by the bacterium (Giaveno and Donati, 2002; Rawlings, 2005). In both cases, metallic ions are released to the leaching medium (Donati et al., 1996). In the case of pyrite biooxidation for an example, direct and indirect bacterial leaching can be described according to the following reactions (Barret et al., 1993):
Direct mechanism:

\[ 2FeS_2 + 7O_2 + 2H_2O \xrightarrow{Bacteria} 2FeSO_4 + 2H_2SO_4 \]  

(2.3)

Indirect mechanism:

\[ FeS_2 + 2Fe^{3+} \xrightarrow{Bacteria} 3Fe^{2+} + 2S^0 \]  

(2.4)

The elemental sulphur produced during the oxidation of pyrite by ferric ions is oxidized by attached cells as represented by Equation 2.5 (Gonzalez et al., 2004).

\[ S^0 + 1.5O_2 + H_2O \xrightarrow{Bacteria} H_2SO_4 \]  

(2.5)

The role of the microorganisms in the solubilization of metal sulphides is, therefore, to provide sulphuric acid for a proton attack and to keep the iron in the oxidized ferric state for an oxidative attack on the mineral (Rawlings, 2005). The ferrous ions produced according to Equations (2.3) and (2.4) are oxidized to ferric ions by the bacterial population, as shown in the reaction.

\[ 4FeSO_4 + O_2 + 2H_2SO_4 \xrightarrow{Bacteria} 2Fe_2(SO_4)_3 + 2H_2O \]  

(2.6)

Acidothiobacillus ferrooxidans can also oxidise iron (II) to iron (III) under aerobic conditions, and even elemental sulphur using iron (III) as an electron acceptor under anaerobic conditions. The insoluble sulphide on oxidation to sulphate becomes very soluble in aqueous solution and the metal ions are dissolved into the aqueous leaching solution.

2.1.2 Bioleaching microbes

The microbes involved in the oxidation of sulphide ores are autotrophic and acidophilic in nature. They can grow in inorganic (minerals) media having low pH values and can tolerate high metal ion concentrations. They generate energy by fixing carbon dioxide as their carbon source and living through the oxidation of sulphide minerals. Through this,
they oxidize Fe(II) to Fe(III) and elemental sulphur to H₂SO₄, and these two reactions mainly control the reaction kinetics (Das et al., 1999). Depending on the tolerance of sulphide oxidizing microbes to temperature, bacteria commonly used can be classified into mesophiles and thermophiles.

2.1.2.1 Mesophilic microorganisms
Mesophiles are those bacteria that can grow, survive and are very active at a lower temperature of 28–37°C.

2.1.2.1.1 Acidithiobacillus ferrooxidans
The most widely used strain of mesophiles is Acidithiobacillus ferrooxidans (Sugio and Akhter 1996; Das and Mishra 1996; Semenza et al., 2002). Following the discovery that microorganisms played a role in the production of acid mine drainage (Colmer and Hinkle 1947), the first acidophilic iron-and-sulphur oxidizing bacterium, Acidithiobacillus ferrooxidans, was isolated and described (Temple and Colmer, 1951). According to Olson et al. (2003), it was not long before the microbial role in oxidation of sulphide minerals was investigated from an extractive metallurgy perspective. Acidithiobacillus ferrooxidans has been used in numerous early investigations into bioleaching of sulphide minerals because it has been the only known acidophilic iron-oxidizing bacterium until the discovery of others e.g. Leptospirillum ferrooxidans and Acidithiobacillus thiooxidans. Although many strains of Acidithiobacillus ferrooxidans have been isolated from different sources, most of the strains showed the following optimum growth conditions, i.e. pH 1.5–2.5 and a temperature range of 28–37 °C (Ahonen and Tuovinen, 1989). Acidithiobacillus ferrooxidans being a lithotroph, derives energy for its growth by oxidizing Fe(II) to Fe(III) and sulphur, sulphide and different oxyanions of sulphur to sulphate (Das et al., 1999). The natural tendency of Acidithiobacillus ferrooxidans to grow on surfaces makes it an adequate microorganism for cell immobilisation and useful to increase iron (II) oxidation rates (Myerson and Kline, 1983; Wakao et al., 1994).
2.1.2.1.2  *Acidothiobacillus thiooxidans*

*Acidothiobacillus thiooxidans* is another acidophilic microorganism like *Acidothiobacillus ferrooxidans*, which has the ability to derive energy for fixing carbon dioxide as their carbon source through the oxidation of sulphides. *Acidothiobacillus thiooxidans* has similar morphological characteristics as *Acidothiobacillus ferrooxidans*, but cannot oxidize Fe(II) to Fe(III). The inability of *Acidothiobacillus thiooxidans* to oxidize Fe(II) to Fe(III) was noted by Suzuki *et al.* (1992) to be as a result of sulphite, which inhibit the oxidation of sulphur by *Acidothiobacillus thiooxidans* cells under acidic conditions. Sulphite oxidation by *Acidothiobacillus thiooxidans* cells is inhibited by high total sulphite concentrations at acidic conditions probably because the lower pH increases the sulphurous acid concentration beyond the substrate inhibition level; hence the optimal pH for sulphite oxidation by *Acidothiobacillus thiooxidans* increases with increasing total sulphite concentration (Takeuchi and Suzuki, 1994). Despite the inability of *Acidothiobacillus thiooxidans* to oxidize iron from ionic sulphides in pure culture, it has been shown to be the most common sulphur (S\(^0\))-oxidizing bacterium isolated from continuous biooxidation reactors (Dopson and Lindström, 1999). Its presence in bioleaching media especially mixed cultures therefore forms a formidable synergy for sulphur oxidation.

2.12.1.3  *Leptospirillum ferrooxidans*

*Leptospirillum ferrooxidans* is an acidophilic chemolithotrophic ferrous oxidizing bacteria which behave similar to *Acidothiobacillus ferrooxidans* and *Acidothiobacillus thiooxidans* cells. This organism tolerates lower values of pH and higher concentrations of some metals like uranium, molybdenum and silver than *Acidothiobacillus ferrooxidans* cells, but is more sensitive to copper (Norris *et al.*, 1986; Sand *et al.*, 1992). The major drawback with *Leptospirillum ferrooxidans* is that it cannot oxidise sulphur to sulphate (Helle and Onken, 1988). On the other hand, it is observed that in continuous flow stirred tank processes where the steady state ferric iron concentration is usually high, *Acidothiobacillus ferrooxidans* is less important under such conditions than a combination of *Leptospirillum* and *Acidothiobacillus thiooxidans* (Rawlings *et al.*, 1999).
In order to effectively oxidize Fe(II) to Fe(III) and S to sulphate, it is advised to use a mixed culture of *Leptospirillum ferrooxidans*, *Acidothiobacillus ferrooxidans* and *Acidothiobacillus thiooxidans* during biodissolution.

2.2 Electrochemical basis for biohydrometallurgy of sulphide ores

The biohydrometallurgical process is believed to involve the interactions between microorganisms and ores for the recovery of metals through microbial modification of the surface chemistry of the minerals and microbial oxidation of the constituent minerals. Since it is an aerobic bacterial oxidation which involves either a direct or an indirect microbial assisted/mediated oxidation and dissolution of metal value from ore, Santhiya *et al.* (2001) observed that, when autotrophic bacteria interact with sulfide minerals, such as pyrite, chalcopyrite, galena and sphalerite, significant surface chemical changes occur both on the bacterial cells as well as on the mineral surfaces. According to Hansford and Vargas (2001), this dissolution process involves electrochemical and chemical reactions of the mineral with the leach liquor and the extra-cellular polysaccharide layers on the microorganisms, and can be interpreted using electrochemistry and chemiosmotic theory.

It is believed that the electrochemical phenomenon involved in metal dissolution from an ore depends on the electrical properties of the solid material and the redox characteristics of the solution (Paramguru, 2002). Since most sulphide minerals are semiconductors (Sand *et al.*, 2001) and the kinetics of dissolution is described by an electrochemical mechanism (Holmes and Crundwell, 2000), the semiconducting properties influence the kinetics of dissolution (Crundwell, 1988a, 1988b). Biegler and Swift (1979) noted that the principle of dissolution of semiconducting sulphide minerals in oxidizing solutions is an electrochemical corrosion process. The soluble oxidant is reduced at the mineral surface, the mineral oxidant itself behaves as an anode (Biegler and Swift, 1979). Since an electrochemical study would be best understood through the minerals’ electrochemistry, which would therefore contribute basic information on the sulphide ore dissolution mechanism, some theoretical background information on the interaction between bacteria and minerals, bacteria-minerals attachment, and mineralogical differences and influence
on bioleaching are discussed below.

2.2.1 Interaction between bacteria and minerals

Knowledge of the nature of the bacteria-mineral interface is essential for understanding the role of bacteria in influencing and accelerating dissolution of metals from minerals. It is believed that microbe-mineral interactions are governed by the cumulative effects of interfacial forces when microbes and minerals are separated by some finite distance, and by adhesion forces when in intimate contact (Tadanier and Lower, 2001). These interactions are thought to be influenced by several factors of ecological, biological, physical and chemical nature. Besides these factors, enhancement in dissolution rate by microbial action could possibly result from extracellular secretion of enzymes that promote oxidation. The production of very large amounts of exopolysaccharide glycocalyx material by organisms within the biofilm eventually buries the individual bacterial cells, and bacteria in fluid menstrua adhere avidly to the surfaces of the mineral to form a biofilm, which restricts flow and traps nutrients for use by the biofilm population (Shaw et al., 1985). Gehrke et al. (1998) observed that the attachment and adherence of bacteria to mineral surfaces and the subsequent formation of biofilms seemed to be the prerequisites for mineral dissolution.

According to Gehrke et al. (1998), the presence of extracellular polymeric substances (EPS) is believed to be a factor that improves the bridging between the microbial cells and the substratum, and permits the negatively charged bacteria to adhere to both negatively and positively charged surfaces. Moreover, glucose-1-phosphate which helps during the formation of extracellular polysaccharides serves as a hub of carbohydrate metabolism, channeling sugars to the formation of EPS and in turn promotes CO$_2$ fixation (Barreto et al., 2005) that is important for cell growth. Extracellular polymeric substances are actually produced during biofilm formation and they bridge the distance of a few microns between the cells and minerals.

Biofilms are primarily composed of heteropolysaccharides (i.e., sugar polymers) secreted by bacteria, forming a matrix or slime layer on solid surfaces in which bacteria become
embedded (Shaw et al., 1985). According to Horn and Meike (1995), biofilms aid in capturing and retaining moisture and organic and inorganic nutrients, thereby creating micro-environments where internal conditions are altered from the external environment. The localized environment within and beneath the biofilm is very complex because of multiple biochemical reactions and the generation of metabolic end products through bacterial activities (Horn and Meike, 1995). The conditions within a given biofilm are heterogeneous. Lee et al. (1995) noted that the region closest to the colonized surface is often anoxic, while the surface of the biofilm exposed to the surrounding media may be completely oxic. The relative immobility of the bacteria within the biofilm therefore allows them to form spatially defined relationships. Microbes always colonize a metal/solution interface through the formation of a biofilm that leads to the physical separation of anodic and cathodic sites. Depletion of the cathodic reactant (for example, oxygen) under the biofilm promotes anodic dissolution of the metals in this area, generating acidification through hydrolysis of metal cations, increasing the concentration of aggressive ions, and fostering oxidative dissolution of metal sulfides (Geesey, 1991).

2.2.2 Bacteria-minerals attachment
The interactions between acidophilic bacteria and mineral surfaces are very complex, and it has been an area of much research. Studies have shown that bacteria attach to the mineral surface by a variety of methods, e.g. protein binding receptors (Sakamoto et al., 1989; Ohmura et al., 1996; Ohmura and Blake, 1997), through chemical attachment (Schaeffer, 1963), through adsorption (Takakuwa et al., 1979), by hydrophobic interactions (van Loosdrecht et al., 1987a & b), with the use of glycolyax (Costerton et al., 1981 & 1978) or by attachment by means of pili (Weiss, 1973). Apart from physical parameters such as particle size, agitation and pulp density, which might affect bacteria-mineral attachment and interaction, it is believed that solution parameters also have a great influence on bacteria attachment onto minerals and the transport system by controlling the properties of interacting surfaces. Solution conditions controls both the hydrophilicity and hydrophobicity of bacteria-mineral interactions, and this therefore affects bacteria attachment and detachment probabilities. Vandevivere and Kirchman
(1993) reported that the attachment process is predominantly mediated by the extracellular polymeric substances (EPS) surrounding the cells and that attachment even stimulates EPS production.

The type of energy source in a growth media affects solution conditions and microbial attachment in some ways. Rohwerder et al. (2003) reported that cells grown on elemental sulphur do not attach to pyrite due to a considerably modified EPS composition. This was attributed to the total lack of complexed iron (III) ions or other positively charged groups due to the fact that EPS contain considerably less sugars and uronic acids but much more fatty acids than pyrite grown EPS. Consequently, exclusively hydrophobic interactions are relevant in attachment of Acidithiobacillus ferrooxidans to sulphur (Gehrke et al. 1998), hence the bacteria are able to adapt the composition and amount of their EPS according to the growth substrate (planktonic cells grown with soluble substrates, e.g., iron(II) sulphate, produce almost no EPS). Sampson et al. (2000a) also reported that Acidithiobacillus ferrooxidans (DSM 583) which had been grown on elemental sulphur was more hydrophobic than cells which had been cultured on ferrous iron. It was also noted that higher hydrophobicity of the sulphur-grown cells did not increase the amount of attachment to the pyrite and on a sample containing arsenopyrite and lodlingite, while less hydrophobic cells of Acidithiobacillus ferrooxidans which had been cultured on ferrous iron and a pyrite/chalcopyrite sample respectively, exhibited greater degrees of attachment. It was suggested that hydrophobic forces are not principally responsible for the attachment of Acidithiobacillus ferrooxidans to certain mineral sulfides. Notwithstanding the assumption by Sampson et al. (2000a), it should be understood that most hydrophobic minerals are easily oxidized.

Attachment can also be thought to occur as a result of electrostatic interaction between the positively charged cells with the negatively charged sulphide mineral. At the bacteria-mineral interface, several physicochemical interactions occur and are influenced by solution chemistry. The nature of attachment is also believed to be influenced by ore mineralogy. Mirajkar et al. (1997) investigated the growth of Acidithiobacillus ferrooxidans, their attachment to sulfide minerals and detachment during bacterial
leaching of sulphide ore containing galena and sphalerite. Bacterial growth and cell population were greater in sphalerite suspensions than in galena suspensions, but more cells were attached onto the galena mineral surface than to the sphalerite surface. Bacteria got attached and detached periodically from the sphalerite mineral surface, but most of the bacteria remain attached to the galena mineral surface. The detachment and attachment phenomenon was attributed to the type of product formed during microbial attack.

During oxidative conversion of the sphalerite, the solid sites responsible for bacterial attachment undergo dissolution by the formation of soluble zinc sulphate, which results in detachment of the cells into the liquid phase. Such cells from the solution could re-adhere to the fresh surface of the sulphide particles. In the case of galena, since the sulphate species is insoluble and continues to remain as part of the solid phase all through such oxidative conversion, the detachment does not easily occur. Santhiya et al. (2000) worked on the bio-modulation of galena and sphalerite surfaces using Acidothiobacillus thiooxidans, and reported that a greater amount of cells is adsorbed onto galena compared to sphalerite. This phenomenon was explained from the view point of elemental sulphur and the nature of the precipitate formed during dissolution, as it affects the hydrophobicity/hydrophilicity of the surfaces. During biodissolution of sulphide ore, elemental sulphur is always formed, although varied with ore type. It has been proposed by Takakuwa et al. (1979) that the process of oxidation of elemental sulphur by Acidothiobacillus thiooxidans involves two main mechanisms, namely reaction of sulphur with chemical or enzymatic reagents excreted by the bacteria, resulting in the formation of soluble compounds, and the reaction between sulphur and a cellular component at the surface of bacterial cells. From the view point that zinc sulphate formed from sphalerite due to bacterial interaction is soluble unlike the case of lead sulphate, these affected the surface morphologies of galena and sphalerite during bacterial interaction and adhesion.

2.2.3 Factors affecting microbial-mineral interaction

The effectiveness of mineral dissolution resulting from the interaction between microbes and minerals depend on several factors. Optimum recovery of metal from ore can only be
achieved through bacterial interaction when the dissolution environment is most suitable for optimal growth of organisms. The need to increase the dissolution kinetics of microbial aided leaching is therefore a challenge to the process engineer and has drawn the attention of many researchers in understanding and optimising parameters favouring microbial-mineral interaction. Apart from the mineralogical and chemical composition of the ore, various factors affecting the dissolution of minerals in the presence of mesophilic acidophilic microorganisms that are relevant to this study are discussed below.

2.2.3.1 Culture and bioleaching media

Microorganisms involved in interaction and modification of minerals surfaces for promoting metal dissolution from sulphide ores are chemolithoautotrophic in nature, hence, it is very important that conditions suitable for optimum growth and survival be maintained. Rawlings (2005) noted that mineral degradation processes differ from the vast majority of other commercial processes that employ microorganisms where an organic substrate is necessary to provide the carbon source and energy required for microbial growth. It was also envisaged that if it were necessary to feed the microorganisms required for mineral degradation with a carbon source (e.g. molasses), commercial mineral biooxidation processes would most likely not be viable.

Apart from carbon and oxygen that are needed for derivation of energy and as sources of food, a very important element is nitrogen, although a high concentration may inhibit iron oxidation. Nitrogen is always provided by ammonium. Tuovinen et al. (1971) reported that an ammonium level of 0.2 mM is sufficient to satisfy the nitrogen requirement of mesophiles. Mesophile like *Acidithiobacillus ferrooxidans* can also reduce atmospheric nitrogen to ammonia, but according to Rawlings (1988), its activity can be inhibited by oxygen. Mackintosh (1978) reported that *Acidithiobacillus ferrooxidans* growing on iron do not fix nitrogen when aerated, but will fix nitrogen once the oxygen concentration had decrease. The sensitivity of nitrogenase to oxygen poses a special problem for *Leptospirillum* because, as far as is known, it uses only iron as its electron donor and is probably obligately aerobic (Rawlings, 2005).
The presence of certain anions is also believed to inhibit iron and sulphur oxidation activities of mesophiles. Harahuc et al. (2000) studied the selective inhibition of the oxidation of ferrous iron or sulphur in *Acidithiobacillus ferrooxidans* and found that the oxidation of either ferrous iron or sulphur by *Acidithiobacillus ferrooxidans* was selectively inhibited or controlled by various anions, inhibitors, and osmotic pressure. Iron oxidation was more sensitive than sulphur oxidation to inhibition by chloride, phosphate, and nitrate at low concentrations (below 0.1 M) and also to inhibition by azide and cyanide. Similar observations were made on the inhibitive effects of chloride and nitrate on cell growth and ferrous oxidation (Razzell and Trussell, 1963; Lazaroff, 1977), and the limiting effects of phosphate on cell growth (Seeger and Jerez, 1993a &b). From the work of Harahuc et al. (2000), it was reported that sulphur oxidation was more sensitive than iron oxidation to the inhibitory effect of high osmotic pressure. These differences were evident not only between iron oxidation by iron-grown cells and sulphur oxidation by sulphur-grown cells, but also between the iron and sulphur oxidation activities of the same iron-grown cells.

As a result of the inhibitive effects of varying anions and elements on the growth of bioleaching microbes, a standard growing culture, the medium 9K is commonly used for their culturing and growth, especially the mesophiles. Some studies, however, have indicated that the 9K medium contains excessive concentrations of phosphate, magnesium and ammonium ions (Jones and Kelly, 1983; Tuovinen and Kelly, 1973), and as a consequence modified media have been developed and used by different researchers (Nemati et al., 1998). Askari Zamani et al. (2005) evaluated the possibility of using the Norris medium by comparing microbial activities in Norris medium with the 9K medium. It was found that the lag period was about 30 days in Norris medium, and this was longer than the lag period of the experiments with 9K medium. However, the amounts of extracted molybdenum and rhenium were almost the same in both media.

### 2.2.3.2 Oxygen and carbon dioxide supply

An important bioleaching condition is the requirement for carbon dioxide and a suitable
dissolved oxygen concentration. According to Bosecker (1997), an adequate supply of oxygen is a prerequisite for good growth and high activity of the leaching bacteria. Since acidophilic micro-organisms are obligate aerobes and hence, a low concentration of oxygen would impose constraints on the rate of oxidation by them (Das et al., 1999). Insufficient oxygen concentration will hinder ferrous oxidation, which would affect bacterial growth. The requirement of oxygen during the bioleaching process has drawn the attention of many researchers. Liu et al. (1988) noted that less than 0.2 g/dm³ dissolved oxygen concentration in the solution could be insufficient for the bacterium to retain metabolic activities. It was also reported that oxygen becomes a limiting substrate when the concentration of dissolved oxygen is less than 0.29 mg/l and Acidithiobacillus ferrooxidans does not grow in cultures with dissolved oxygen concentrations less than 0.2 mg/l.

Shrihari and Gandhi (1990) modeled Fe²⁺ oxidation by Acidithiobacillus ferrooxidans and found that O₂ was not a limiting substrate for bacterial growth in experiments carried out at 30 °C in 500 ml flasks containing 200 ml broth shaken at 240 rpm. Bosecker (1997) noted that the oxygen requirement in laboratory tests can be achieved by aeration, stirring, or shaking, while on a technical scale, particularly in the case of dump or heap leaching, insufficient supply of oxygen may cause some difficulties. de Kock et al. (2004) noted that under high-sulphide loading conditions, as is the case with high-grade concentrates, the microbial and chemical demand for oxygen is significantly increased during the bioleaching process in agitated tank reactors, and the increased demand for oxygen under these conditions can be facilitated by increased aeration rates, higher impeller agitation rates and improved agitator designs. It was observed that agitation speeds cannot be indefinitely increased to improve oxygen and carbon dioxide mass transfer limitations, as cell damage to bioleaching microbes becomes a limiting factor at high agitation speeds and power inputs in the presence of high pulp densities. Moreover, it was pointed out that mass-transfer limitations are not routinely encountered in commercial bacterial tank leaching operations under mesophilic (35– 40 °C) conditions when sparging with air. With thermophilic (65–80 °C) bioleaching conditions, especially for bioleaching of copper ore like chalcopyrite (CuFeS₂), de Kock et al. (2004) also noted
that mass-transfer limitations due to reduced oxygen solubility are a significant process challenge that cannot be overcome by increasing agitation speeds and aeration rates. Rawlings (1997) therefore suggested that sparging with enriched oxygen gas would offer a potential solution to overcome the mass-transfer difficulties at such elevated temperatures.

Since microbes involved in bioleaching are chemoautotroph and obligate, they therefore require a certain amount of carbon dioxide since it is the only source of carbon for growth. Several studies have been carried out to determine optimum carbon dioxide requirements for the bioleaching process. de Kock et al. (2004) noted that microbial cells are only sensitive to dissolved carbon dioxide concentrations, rather than gaseous carbon dioxide concentrations. In view of the limited solubility of carbon dioxide, Nemati et al. (1998) observed that at the pH required for the optimum growth of Acidithiobacillus ferrooxidans, cultures could be predicted to become limited by carbon dioxide availability. Kelly and Jones (1978) showed that limitation of carbon dioxide in growing cultures of Acidithiobacillus ferrooxidans shifts the exponential kinetics of ferrous iron oxidation to a linear relationship, which is due to the growth-uncoupled oxidation by a standing population of bacteria.

Although carbon dioxide is very necessary for bacterial growth, it is believed that a higher amount of carbon dioxide may have negative effects. Barron and Luecking (1990) studied the influence of carbon dioxide availability on the growth of Acidithiobacillus ferrooxidans and found that the level of carbon dioxide that supported the maximal rate of growth was in the range 7-8%. An increase beyond 8% resulted in the inhibition of culture growth, though higher cell yields were obtained as the percentage of carbon dioxide was elevated to inhibitory levels. Holuigue et al. (1987) investigated the importance of carbon dioxide availability for achieving optimal growth rates and maximum cell yields. It was found that culture sparging with a level of carbon dioxide around 5% in air showed maximal stimulation of growth. Significantly higher cell yields were obtained as the level of carbon dioxide was increased, although growth rate was unaffected. de Kock et al. (2004) also investigated oxygen and carbon dioxide kinetic
challenges for thermophilic mineral bioleaching processes and found that carbon dioxide concentrations of more than 17% carbon dioxide vol/vol supply have a significant effect on bacterial activity, as microbial growth rate becomes limiting and inhibited. This inhibiting effect of carbon dioxide on microbial growth rate was reported by Nagpal (1997) to be due to the formation of intracellular bicarbonates.

2.2.3.3 pH and Fe (III) concentration

The pH of the growth and leaching medium is an important condition for effective growth on bacteria and is decisive for its subsequent interactions with minerals. This is because pH significantly affects the growth and activity of acidophilic microorganisms (Das et al., 1999). Generally, these organisms operate at low pH and therefore tolerate metals at low pH. Amaro et al. (1991) reported that *Acidothiobacillus* usually grows in a pH range of 1.5 to 3.5 and they respond to external pH changes by regulating the synthesis of several of its cellular components. According to Cox et al. (1979), lower pH values would present a problem for the bacterium to keep its cytosolic pH close to 7, and for this reason, the bacteria generate a pH gradient, which is almost fully dependent on the proton extrusion through a proton-impermeable membrane (Amaro et al., 1991). Apel and Dugan (1978) noted that the growth of bacteria is usually initiated at a very low pH range and as the growth continues, the pH of the medium also increases without affecting the bacterial activities. Since biological oxidation of ferrous ion involves the movement of hydrogen ions and electrons, Apel and Dugan (1978) noted that *Acidothiobacillus ferrooxidans* takes up H\(^{+}\) from its external environment during the oxidation of Fe(II) to Fe(III). It can therefore be concluded that H\(^{+}\) is an important nutrient for its growth, and higher pH can also be presumed not to be suitable for the activities of acidophilic bacteria. The tolerance of acidophiles to most metals in low pH media probably results from effective competition by H\(^{+}\) ions for negatively-charged sites at the cell surface (Hossain et al., 2004).

The concentrations of ferrous ion in the bioleaching medium significantly affect the growth and activities of acidophilic bacteria and its ability to oxidize ferrous iron. Ferrous ions are needed for bacterial growth, because it is readily oxidized to ferric iron which...
serves as an electron donor for microbial activity. Rawlings (2005) extensively reviewed the importance of ferrous ions as reported below. The Fe$^{2+}$/Fe$^{3+}$ redox couple has a very positive standard electrode potential (+770 mV at pH 2), and as a result, only oxygen is able to act as a natural electron acceptor. In the presence of protons, the product of the reaction is water (O$_2$/H$_2$O +820 mV at pH 7). The use of iron as an electron donor will therefore occur only during aerobic respiration. However, under aerobic conditions, Rawlings (2005) noted that ferrous ions spontaneously oxidize to ferric ions unless the pH is low, and therefore, extremely acidophilic bacteria are able to use the ferrous ion as an electron donor in a manner that is not possible for bacteria that grow at neutral pH. Because the difference in redox potential between the Fe$^{2+}$/Fe$^{3+}$ and O$_2$/H$_2$O redox couples is small and because only one mole of electrons is released per mole of iron oxidized, vast amounts of ferrous iron needed to be oxidized to produce relatively little cell mass. These large quantities of iron are not transported through the cell membrane but remain outside of the cell and each ferrous ion simply delivers its electron to a carrier situated in the cell envelope. Ferric ion could inhibit bacterial growth. According to Das et al. (1999), a low Fe(III) concentration enhances the oxygen uptake by the acidophilic microorganisms. Nyavor et al. (1996) observed that a higher concentration of Fe(III) could competitively inhibit ferrous iron oxidation by Acidithiobacillus ferrooxidans. Similarly Curutchet et al. (1992) reported that in the absence of other sources of energy, the presence of soluble Fe(III) inhibits the growth of Acidithiobacillus ferrooxidans.

### 2.2.3.4 Physical and process parameters

Microbial aided dissolution is influenced by various factors. These include a number of various physical and process parameters such as particle size, pulp density, agitation, volume of inoculum, and microbe-mineral contact. These factors control the growth and activities of the microbes, and could therefore, affect their interaction with minerals and the resultant oxidation of sulphide minerals.

#### 2.2.3.4.1 Particle size

Ore size is an important factor that determines the effectiveness of bacteria on mineral
dissolution. Its effects have been studied by various researchers. Gonzalez et al. (1999) investigated the influence of particle size, ferric ion and suspended cells concentration on the attachment of *Acidothiobacillus ferrooxidans* to a high pyrite and enargite concentrate and reported a marked effect of particle size on the equilibrium concentrations. Smaller particles bring about higher amounts of attached cells, which were reported to be due to the increased surface area of the finer particles that gave an increase in the number of active attachment sites. Addition of ferric ions increased the concentration of attached cells, while the amount of attached cells and suspended cells at equilibrium was reported to be dependent upon the total amount of cells in the system. An inhibitory phenomenon was observed at elevated values of suspended cells concentration by which a further increase in concentration caused a decrease in the attached cells concentration.

Several results have been reported contrary to those by Gonzalez et al. (1999). Nemati et al. (2000) studied the effect of mineral particle size on the bioleaching of pyrite by acidophilic thermophiles in a batch bioreactor, and it was observed that decreasing the particle size from a mean diameter of 202 micron (size fraction: 150±180 micron) to a mean diameter of 42.5 micron (size fraction: 25±45 micron) enhanced the bioleaching rate from 0.05 kg m\(^{-3}\) h\(^{-1}\) to 0.098 kg m\(^{-3}\) h\(^{-1}\). Decreasing the particle size of the mineral to a mean diameter of 6.40 micron (size fraction <25 micron) adversely influenced the activity of the cells. The decrease in biodissolution at lower particle size was attributed to the fact that the presence of fine particles apparently damaged the structure of the cells, resulting in their inability to oxidize pyrite. Mazuelos et al. (2001) also studied the effects of particle size on ferrous iron biooxidation in a packed bed bioreactor, and reported that ferric iron productivity increased with an increase in the particle size of the bed. The decrease in ferric iron production at smaller particle sizes was attributed to hindrance in biofilm development and consolidation, as biofilm is preferentially formed in the space between particles (Nikolov et al., 1988). The work of Deveci (2004) on the effect of particle size and shape of solids on the viability of acidophilic bacteria during mixing in stirred tank reactors shows that the rate and extent of loss in the viability appeared to increase with decreasing particle size from -180 + 125 to - 63 + 45 µm over a mixing period of 4 hours. Since both higher and lower particle sizes have inhibitory effects on
both bacterial activity and ferrous oxidation, there is therefore a need to develop an understanding of the mean ore size for optimal relationship between microbial attack on minerals and oxidation behaviour.

2.2.3.4.2. **Pulp density**

The solids concentration for the operation constitutes one of the most critical parameters of the bioleaching process in terms of impact on the size of the equipment (d’Hugues et al., 1997). Gomez et al. (1999) studied the influence of pulp density, ranging from 5 to 20%, on the bioleaching of a Spanish complex sulphide ore bulk concentrate, and reported a significant decrease in both bioleaching rate and extractions of copper, zinc and iron when the pulp density was increased, being maximal at 5% pulp density. The decrease in the percentage of metal dissolution when the pulp density is increased was attributed to a lower availability of oxygen and carbon dioxide, which are essential nutrients for microbial growth and activity. Nemati and Harrison (2000) reported that the ability of the bacteria to oxidize pyrite in a conventional stirred tank bioreactor is dependent on the mineral pulp density. Pulp densities were varied in the order of 3%, 6%, 9%, 12%, 15%, and 18%. The highest recovery was obtained at 9%, while 18% gave the least recovery. In the laboratory system used, three regions of operation with respect to minerals pulp density were identified. It was reported that in the 3 to 9% solids region, the pulp density did not influence the rate of iron solubilization, but the rate of iron solubilization was significantly reduced in the presence of partial pyrite suspension at a pulp density of 18%. Makita et al. (2004) found that an increase in pulp density generates a decrease in the dissolved metal concentration.

Rubio and Frutos (2002) studied the bioleaching capacity of copper concentrates by a moderate thermophilic culture at different pulp densities such as 1%, 5%, 10%, 15% and 20% (w/v). Pulp with a density of 1% gave the highest recovery of 98% at the shortest time of 2 days, while 98 and 96% were respectively recovered at the 7th and the 10th day for 5 and 10% pulp densities. A copper recovery of 80% was obtained at the 14th day. Since higher pulp density is needed on the industrial scale for economic justification of bioleaching, the batch tests showed that it is possible to work both with high pulp density
(20%) obtaining good copper extraction (80%), and with lower pulp density (10%) obtaining better copper extraction (94%) in 14 and 10 days respectively. The general reduction in the bioleaching rates at higher pulp densities can be attributed to the fact that higher concentrations of solids caused an increase in the friction between particles, and probably decrease the adhesion between the particles and bacteria (Chong et al., 2002). Deveci (2002) noted that the friction may consequently cause some mechanical damage to the cells.

2.2.3.4.3  **Bacterial concentration and counts**

Bacterial concentration and counts in the solution is one of the controlling factors in determining the oxidation kinetics for sulfide minerals both in the direct as well as the indirect mechanism. The volume of inoculum should be increased in order to increase the kinetics of microbial attack. This can be done effectively by increasing the dissolved oxygen concentration within the system and effective washing of the cells before inoculation. Third et al. (2000) found that parameters other than bacterial numbers are important in the bioleaching of sulphide ores. Washed bacterial cells before inoculation was found to improve the leaching rate, and it was suggested that a separation process e.g. centrifuging and washing bacterial cells is needed to exclude any effect by chemical species of the bacterial culture.

It should be noted that at higher volumes of inoculum, bacteria interaction capacity with minerals and thus the oxidation power will reduce. This was supported by the work of Hiroyoshi et al. (1997 and 1999) where it was noted that in some cases, volume of inoculum inhibit rather than stimulate leaching of sulphide ore. Hiroyoshi et al. (1999) concluded that the bacterial suppression of chalcopyrite leaching in ferrous sulphate solution was mainly due to the bacterial consumption of ferrous ions. This act as a promoter for chalcopyrite oxidation with dissolved oxygen, and that co-precipitation of copper ions with jarosite causes the bacterial suppression of copper extraction. Third et al. (2000) corroborated this assumption where the copper release rate from chalcopyrite in bacterial cultures inoculated with 1%, 10% and 50% v/v was studied, and highest
copper recoveries were found in the flask inoculated with 10% v/v. Similarly, Nestor et al. (2001) noted that, with the continuous exposition to the sulphide concentrate, bacterial cells progressively loose their capacity to oxidize iron, but when the amount of mineral is higher, cells linked to the mineral require a larger time period of exposition.

2.2.3.4.4 Agitation

The effectiveness of the bioleaching process requires adequate agitation to maximize the recovery of metals. According to Acevedo (2000) agitation has a double purpose: to increase the rate of transfer operations, such as oxygen and carbon dioxide transfer and heat transfer, and to mix the reactor contents. Insufficient agitation may lead to limitations in the transfer operations (Gonzales et al., 2003), and under conditions of insufficient agitation, the transfer operations may become limiting and the overall reaction performance will decline because of the appearance of zones of the fluid with insufficient nutrients or inadequate temperature or pH (Namdev et al., 1994). On the other hand, higher agitation is detrimental to bacterial growth and activities. Several research studies have therefore been reported on optimal agitation suitable for microbial-mineral interactions and growth. Gomez et al. (1999) studied the effect of stirring by using two types of reactors: mechanically stirred reactors and shaked flasks. The rates of dissolution and yields of copper, zinc and iron were higher with mechanically stirred reactors. Hossain et al. (2004) studied the effect of shaking speeds on bioleaching of sphalerite in a shaked flask at varying shaking speeds of 30 rpm, 45 rpm, 60 rpm, 75 rpm and 90 rpm. Maximum bioleaching recoveries of 74.85% (w/w) and 71.50% (w/w) for 25 kg/m$^3$ and 30 kg/m$^3$ initial loading were obtained at a shaking speed of 60 rpm. The secretion of enzymes responsible for the bioleaching was highest at the same shaking speed with maximum bioleaching of the zinc sulphide ore.

Das et al. (2005) determined the effect of biological iron oxidation vis-à-vis agitation speed that varied from 50 to 200 rpm. It was found that the iron oxidation rate increased with increasing agitation until 150 rpm was reached, and thereafter it remained constant. It was reported that beyond 150 rpm, the oxidation reaction was completely chemically
controlled. d’Hugues et al. (1997) observed that high agitation rates could affect the efficiency of oxidation by limiting bacterial productivity and by reducing bacterial contact with the solid substrate. This corroborated the reports by Hackl et al. (1989), Griffin and Luinstra (1989), and Oolman (1993) that the increase in the agitation rate, i.e. shear, led to a limitation in the oxidation rate or bacterial activity using mesophilic cultures of acidophilic bacteria. Deveci (2002) attributed the lower oxidation at higher agitation rates to a deactivation of cells that occur during mixing. This can be attributed predominantly to the collisions between the particles promoted by the intensity of agitation. It was supposed by Gormely and Branion (1989) and Hackl et al. (1989) that the mechanical and hydrodynamic forces limit bacterial productivity by seriously damaging the bacterial cell wall and/or by inhibiting bacterial contact with the solid substrate. Bailey and Hansford (1993) also identified the excessive hydrodynamic turbulences caused by aeration as a physiological stress factor for the bacteria.

This induced shear by agitation has been identified by Rossi (2001) and Rawlings et al. (2003) as one of the most important factors limiting the use of high pulp density on industrial scale. To overcome the problems associated with high agitation, de Kock et al. (2004) suggested that sparging with enriched oxygen gas would offer an alternative process option to increase agitation and to overcome the mass transfer difficulties. This is because agitation speeds cannot be indefinitely increased to improve oxygen and CO$_2$ mass transfer limitations, as cell damage to bioleaching microbes becomes a limiting factor at high agitation speeds and power inputs in the presence of high pulp densities.

### 2.3 Mineralogical differences and influence on bioleaching

The differences in the atomic structure of minerals resulting from the type of bonding and the distance between the atoms within different minerals, affect their mineralogical properties. Mineralogical differences in turn affect minerals’ response and behaviour in different aqueous media. It was found (Berry et al., 1978; Mehta and Murr, 1982; Attia and El-Zeky, 1990a; Attia and El-Zeky, 1990b; Holmes and Crundwell, 1995) that when various sulphide minerals such as chalcopyrite, galena, sphalerite, pyrite, etc. are present together in an acidic solution, selective dissolution occurs due to electrochemical galvanic
interactions resulting from the differences in the electrical conductivities of the minerals. Cruz et al. (2005) noted that these galvanic interactions depend on the mineralogical association between the phases present in the ore. It was also found that these galvanic effects, occurring between conducting and semi-conducting minerals in aqueous systems, play an important role in the aqueous processing of ores and minerals, such as in flotation and leaching.

Galvanic interactions have been studied for several chemical leaching and bioleaching systems (Mehta and Murr, 1983; Madhuchhanda et al., 2000; da Silva et al., 2003). In these systems, Nowak et al. (1984), Paramguru and Nayak (1996) and Abraitis et al. (2004) observed that galvanic interactions could substantially increase the leaching of one or both of the minerals that constitute the galvanic cell, depending on the electrochemical characteristics of the minerals and on the occurrence of the distinct sulfides contained in the concentrates.

For semiconductive minerals, such as sulphides, direct contact of different minerals with dissimilar rest potentials initiates the galvanic effect (Cruz et al., 2005). According to da Silva et al. (2003), when two minerals of different conductivity come into contact in aqueous solution, current flows through the solution from the mineral of highest to lowest rest potential, forming a galvanic cell. This galvanic interaction causes the mineral of lower rest potential to be sacrificed (Equation 2.7), while the mineral of higher rest potential acts as the cathode and is galvanically protected (Majima, 1969), as shown in Equation 2.8. These interactions occur between sulphides, involving the flow of electrons from grains with a higher potential to grains with lower potentials, modifying the Fermi level of both minerals (Shuey, 1975):

**Anodic dissolution**

\[ \text{MS} \longrightarrow M^{2+} + S^0 + 2e^- \]  

**Cathodic reduction**

\[ \frac{1}{2}O_2 + 2H^+ + 2e^- \longrightarrow H_2O \]
Studies have shown that, during bioleaching, chalcopyrite (CuFeS$_2$) is selectively leached from pyrite (FeS$_2$), while sphalerite is selectively leached from both chalcopyrite and pyrite (Berry et al., 1978; Mehta and Murr, 1982; Natarajan, 1992; Torres et al., 1995). Attia and El-Zeky (1990b) have shown that chalcopyrite, sphalerite and galena are all selectively oxidised from pyrite during bioleaching, though any selectivity between galena and sphalerite was not determined.

Munoz et al. (1998) investigated the electrochemical behaviour of chalcopyrite in the presence of silver and Sulfolobus bacteria and found that the presence of silver improves the chalcopyrite oxidation in the presence of thermophilic bacteria. Initially, particulate electrodes were studied to determine the electrochemical response of reactants and products involved in the silver-catalyzed bioleaching process. It was demonstrated that the electrodeposition of metallic silver on an elemental sulphur surface enhanced the conductive properties of sulphur. Besides, this electrodeposition took place at lower potentials on S$^0$ than on Ag$_2$S. It was also reported that the dissolution of Ag$_2$S produced a decrease in the current due to the formation of a poorly conductive Ag$_2$SO$_4$ layer on the electrode surface, which was more marked with an increase in silver concentration. The contact of a silver solution with a chalcopyrite electrode produces a film of Ag$_2$S on the ore surface as well as metallic silver. When the time of contact between the solution and the ore increased, both the thickness of the Ag$_2$S film and the quantity of Ag$^0$ increased. The addition of Fe$^{3+}$ to the solution in the system Ag$^+$-chalcopyrite favoured ore attack as a consequence of the Ag$_2$S layer breakdown. Romano et al. (2001) carried out a comparative study on the selective chalcopyrite bioleaching of molybdenite concentrate with mesophilic and thermophilic bacteria and observed that though chalcopyrite is a refractory ore, the presence of molybdenite speeded up the chalcopyrite dissolution due to the formation of a galvanic couple.

The work of da Silva et al. (2003) on electrochemical passivation of sphalerite during bacterial oxidation in the presence of galena revealed that galena was passivated by sphalerite during bioleaching, due to the formation of a galvanic cell. The rest potential of
both galena and sphalerite in the bioleaching medium were measured and found to be 325 and 372 mV, respectively. It was observed that during bioleaching, galvanic current flowed from galena to sphalerite when in solution together, resulting in the selective oxidation. It was also observed that the effect of the galvanic interaction increased with decreasing ferric concentrations, resulting in the selectivity for zinc observed during bacterial oxidation to be greater than that witnessed during ferric sulphate leaching. It was found during whole-ore bioleaching that increased lead grades resulted in decreased rates of zinc extraction. Also, sphalerite was seen to be able to leach in the presence of anglesite, though it is believed that diffusion limitation may limit oxidation rates. Shi et al. (2006a) studied the bioleaching processes of three zinc sulphides (marmatite, sphalerite and ZnS synthetically prepared) with Acidithiobacillus ferrooxidans and a moderately thermo-acidophilic iron-oxidizing bacterium. It was observed that the bioleaching process was significantly affected by the different mineralogical properties and chemical compositions. Experimental results showed that marmatite, having the highest amount of Fe within the crystal lattice, had a higher leaching rate than the sphalerite and synthetic ZnS. The electrochemical measurements confirmed that marmatite had higher reaction activity than the sphalerite and synthetic ZnS. It meant that in addition to mineral properties, the electrochemical behaviors of zinc sulphides could be also influenced by the concentrations of Fe$^{2+}$ and Fe$^{3+}$ ions in the leaching solution during the bioleaching process.

### 2.4 Microwave Treatment of Ores

#### 2.4.1 Interaction of microwave irradiation and minerals

Microwave energy is a form of electromagnetic energy that travels at high frequencies of about 300 - 300 000 MHz and wavelengths of 1 - 1000 mm. Its properties are similar to that of sunshine; the wavelength ranges between infrared radiation and radio wave radiation of the electromagnetic spectrum, while the velocity of wave is the same as that of light ($3 \times 10^8$ m/s) (Li et al., 2008). Microwaves can be transmitted, absorbed or reflected (Uslu et al., 2003). The most commonly used frequencies for heating purposes are 915 MHz and 2.45 GHz, which correspond to wavelengths of 33.5 and 12.2 cm,
respectively (Al-Harahsheh et al., 2005). These frequencies were chosen by international agreement in order to minimize the interference with microwave communication signals (Meredith, 1998).

When material is heated in a microwave field, the electromagnetic energy causes friction within the material lattice, which gives rise to heat. According to Jones et al. (2007), some minerals heat extremely rapidly within a microwave field, whereas other mineral species remain transparent and do not heat. Depending on the response to microwave heating, materials can be classified into three groups with respect to the interaction with the microwave field: transparent or low loss materials where microwaves pass through without any losses; conductors which reflect microwaves without any penetration; and absorbing or high loss materials, which absorb microwaves and dissipate them into heat depending on the value of the dielectric loss factor (Al-Harahsheh et al., 2005). The fourth category is materials that contain two or more phases with different dielectric properties. Therefore, microwaves will selectively heat the high loss phase of these materials, and pass through the low loss phase without considerable loss of heat.

The materials that absorb microwave radiation are commonly called the dielectrics, and have two important properties (Osepchuk, 1984). They have very few free charge carriers, and thus, when the external electric field is applied there is very little charge carried through the material matrix. The molecules or atoms comprising the dielectric also exhibit a dipole movement. A dipole is essentially two equal and opposite charges separated by a finite distance (Kingman et al., 2000; Jones et al., 2002), and may be a natural feature of the dielectric or they may be induced (Kelly and Rowson, 1995; Kelly, 1996). Microwave heating of dielectric materials therefore arises from the ability of the electric field to polarize the charge of the material in cases where polarization cannot follow the rapid change of the electric field (Metaxas and Meredith, 1983). According to Kingman et al. (2000), the distortion of the electron cloud around non-polar molecules or atoms through the presence of an external electric field can induce a temporary dipole moment. This movement generates friction inside the dielectric and the energy is subsequently dissipated as heat (Jones et al., 2002).
The interaction of dielectric materials with electromagnetic radiation in the microwave range results in energy absorbance, and the ability of a material to absorb energy while in a microwave cavity is related to the loss tangent of the material (Jones et al., 2002). This depends on the relaxation times of the molecules in the material, which, in turn, depends on the nature of the functional groups and the volume of the molecule (Gabriel et al., 1998). Generally, the dielectric properties of a material are related to temperature, moisture content, density and material geometry (Metaxas and Meredith, 1983).

The behaviour of material when placed in microwave field largely depends on its permittivity \((\varepsilon, \text{F/m})\), permeability \((\mu, \text{H/m})\) and conductivity \((\sigma, \text{S/m})\) (Al-Harahsheh et al., 2006). Materials with low values of conductivity are classified as dielectrics, whereas, those with high values of conductivity, are called conductors (Meredith, 1998). The permittivity of a dielectric material is composed of a real part \((\varepsilon', \text{dielectric constant})\) and an imaginary part \((\varepsilon'', \text{dielectric loss factor})\) (Al-Harahsheh et al., 2006). The dielectric constant \((\varepsilon')\) and dielectric loss factor \((\varepsilon'')\) are used to express the dielectric response of materials in a microwave field (Al-Harahsheh et al., 2005). The dielectric constant measures the ability of the material to store microwave energy or in other words it measures the ability of material to be polarized (Al-Harahsheh et al., 2005). The dielectric loss factor measures the ability of material to dissipate the stored energy into heat (Metaxas and Meredith, 1983). They are expressed in terms of the complex dielectric constant \((\varepsilon^*)\):

\[
\varepsilon^* = \varepsilon' - j\varepsilon''
\]

Permittivity \((\varepsilon^*)\) can also be expressed as:

\[
\varepsilon^* = \varepsilon_0 \left( \varepsilon_r - j\varepsilon_{\text{eff}}'' \right)
\]

where \(\varepsilon_0\) is the permittivity of free space \((8.86 \times 10^{-12} \text{ F/m})\), \(\varepsilon_r\) is the relative dielectric constant, \(\varepsilon''_{\text{eff}}\) is the effective relative dielectric loss factor,

\[
j = \sqrt{-1}
\]
The propagation of an electromagnetic wave through the dielectric material depends on its dielectric properties, as well as microwave frequency, and can be characterized by the penetration depth \((D_p)\). The Penetration Depth \((D_p)\) is the distance from the material surface at which the absorbed power falls to \(1/e\) of the absorbed power at the surface. Penetration depth is inversely proportional to frequency, whereas the greatest heating is achieved at high frequencies. For example, the greatest heating for water occurs at 18 GHz where dielectric loss factor is at its maximum value. However, the penetration depth \((D_p)\) at such frequencies is so low that only a thin layer of the material will heat (Metaxas and Meredith, 1983 and Mingos and Baghurst, 1991). \(D_p\) is given by

\[
D_p = \frac{c}{2\pi f \sqrt{2\varepsilon'\left[1 + \tan^2 \delta - 1\right]}}
\]

where \(c\) is the speed of light \((3 \times 10^8 \text{ m/s})\), \(f\) the microwave frequency \((\text{Hz})\), \(\varepsilon'\) the dielectric constant, and \(\tan \delta\) is the loss tangent.

Loss tangent provides an indication of how well the material can be penetrated by an electric field and how it dissipates energy into heat:

\[
\tan \delta = \frac{\varepsilon''}{\varepsilon'}
\]

**2.4.2 Application of microwave technology in sulphide ore processing**

The use of microwave heating for processing minerals is not a new concept (Kingman, 2006). Its applications in mineral processing and extractive metallurgy have been of great and particular research interest for over the past three decades. Microwave energy is extremely efficient in the selective heating of materials as no energy is wasted in ‘bulk heating’ the sample, which thus gives it a clear advantage over conventional methods (bulk heating in furnaces) (Jones et al., 2002). Microwave treatment of minerals offers several advantages over conventional method of heating as they only heat the "responsive" phases. According to Kingman et al. (2000), microwaves heat considerably faster than conventional heating methods as the heating is in situ rather than relying on radiation and conduction heat transfer mechanisms. The speed at which materials heat has
been shown to be particularly important in both conventional and microwave thermally assisted liberation (Holman et al., 1926; Kingman, 1999). Rapid heating has been shown to be particularly effective in applied microwave fields as differential expansion due to the different heating rates of the constituent minerals is encouraged, creating stress in the mineral lattice (Kingman et al., 1998).

Several investigations on the application of microwave radiation for improving minerals processing and recovery have been reported. Kingman et al. (2000) studied the influence of mineralogy on the response of ores to microwave radiation, the reduction in their grinding energy, and the suitability of ore types for possible economic exploitation of microwave assisted grinding. It was found that grindability of ores after microwave treatment are related to the specific mineral species present, grain size of the specific mineral and the degree of dissemination. Ores that have consistent mineralogy and contain a good absorber of microwave radiation in a transparent gangue matrix are the most responsive to microwave treatment. Wang and Forssberg (2000) performed tests on a low grade copper ore (0.22–0.4% Cu) from Aitik, and showed that thermal stress cracks occurred readily along the sulfide-gangue mineral grain boundaries when the ore was first treated by microwaving, and that a better liberation of sulfide minerals in the ore matrix was obtained.

Huang and Rowson (2002) investigated the dielectric heating characteristics of pyrite and marcasite and found that microwave power, crystal structure and particle size had an effect on dielectric heating rate. Marcasite could be heated at a higher rate than pyrite and could be more readily decomposed than pyrite. After microwave irradiation marcasite and pyrite decomposed into elemental sulphur and porous pyrrhotite. Amankwah et al. (2005) used microwave pretreatment to augment the grinding of a free-milling gold ore containing quartz, silicates and iron oxides. Under microwave irradiation, selective and differential heating of different minerals phases of the ore resulted in thermal stresses cracking and made the ore more amenable to size reduction, resulting in a decrease in the work index. In addition to the enhanced grindability, gold was released from the matrix of the host minerals at a coarser size, resulting in a significant increase in free gold recovery.
The simulation work by Jones et al. (2005) using a 2-phase mineral model of a microwave absorbing species within a microwave transparent matrix, analyzed the stress regime around the absorbing phases. It was concluded that fractures are likely to occur around the grain boundaries between absorbent and transparent species, and that microwave treatment would likely lead to increased intergranular fracture. It was suggested that the liberation of valuable minerals can be improved and that a reduction in comminution energy is possible after microwave treatment. Further, it was determined that particle shape of the microwave absorbent phase was important and that regularly shaped particles were much less likely to incur transgranular fracture as a result of microwave treatment than irregular particles. However, microwave treatment might be less efficient at smaller particle sizes for a fixed applied power density. Jones et al. (2007) also simulated the weakening mechanism of a simplified pyrite/calcite system using a quasi-static thermo-mechanical approach in commercial finite difference numerical modelling software. The effects of microwave power density and exposure time on simulated uniaxial compressive strength were quantified. Microwave induces differential thermal expansion of certain mineral phases that is sufficient to cause stresses that exceed the strength of the material, and pulsed treatment is effective for weakening rocks. A decrease in exposure time will lead to greater reductions in strength for a given total energy input, which could suggest that a diminishing rate of reduction of strength exists as power density is increased. It was observed that there may be a power density above which no further reduction in strength is possible.

Li et al. (2008) studied the temperature rise characteristics of carbon-containing chromite ore fines in the microwave field. It was observed that carbon-containing chromite ore fines have better temperature rise characteristics in the microwave. This behaviour was attributed to the difference in the dielectric property of the constituents’ minerals. Scott et al. (2008) used quantitative mineralogical analysis to study the effects of microwave treatment on the liberation of a low-grade copper carbonatite ore. The treated ore was processed for 0.5 s at 10.5 kW in a single mode microwave cavity in batches of 1 kg. It was reported that microwave treated ore showed a significant increase in the amount of
liberated copper minerals in the relatively coarse particle size range (106 to 300 μm). The fracture pattern and mineral liberation of the carbonatite ore was changed as a result of the microwave pretreatment, and more importantly did so in a manner which improved the recovery of valuable copper minerals from the ore. Almost full liberation of copper sulphides was achieved in particles smaller than 53μm. It was suggested that intergranular fracture occurred between microwave susceptible and non-susceptible minerals, and also that a fracture pattern was induced which alters the size distribution of the gangue material, favouring the production of larger particles.

Although, significant numbers of studies reviewed above have been reported on the application of microwaves for improving bioleaching, these studies are observed to be centered on microwave leaching. While studies have been reported on means by which microwave improves size reduction and mineral liberation (Kingman et al., 2000; Wang and Forssberg, 2000; Amankwah et al., 2005), attention has not been paid to studying the biohydrometallurgical processing behaviour of the microwave treated ores.

**Summary**

A review of relevant reported literature on the mineralogy of complex sulphide ores, their response to microwave processing and their biohydrometallurgical processing behaviour is made. Reported studies showed that several process parameters including particle size, pulp density, temperature, types of microorganisms, pure and mixed cultures favour the biohydrometallurgical recovery of these ores. Studies also revealed that the application of microwave technology could enhance their bioleaching potentials. A major gap in knowledge that is common to all the reported studies is the lack of information on how ore mineralogy affects size reduction and distribution, microwave heating process, and the overall bioleaching process. Since ore mineralogy is a fundamental influencing factor during size reduction, microwave heating and very crucial to the understanding of the behaviour of ore during dissolution, this work will be focussing on process mineralogy as its influences biohydrometallurgical processing of complex sulphide ores.