

MICROBIAL PRE-TREATMENT OF DOUBLE REFRACTORY GOLD ORES

by

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Dedication

I dedicate this thesis to my ever loving and caring wife, Vivian Gidiglo, for being a strong pillar of support and our three children, Elinam, Edem and Delali for enduring my absence during the first 18 months of the research.

Abstract

The use of microorganisms notably bacteria in mineral processing industry is presently one of the leading emerging pre-treatment techniques being employed for the processing of double refractory gold ores and concentrates. Currently numerous studies are in progress to further improve upon the efficiency of the bacterial process and to investigate the potential of other microorganisms.

In this study, microbial pre-treatment of double refractory gold ore (sample A) and concentrate (sample B) was investigated using a white-rot fungus, *Trametes versicolor* (ATTC 20869). Pulp density, temperature, pH and retention times were the process variables considered.

Preliminary studies investigated the amenability of selected pure sulfide sulfur minerals, various types of coal and ore sample A to fungal degradation. Various pre-treatment scenarios were also studied to optimize gold extraction. These were single stage, a two-step and two stage processes involving the well known chemolithotrophic bacteria *Acidithiobacillus thiooxidans* (ATTC 15494), *Acidithiobacillus ferrooxidans* (ATTC 19859) and *Leptospirillum ferrooxidans* (ATTC 53992), the bacterium, *Streptomyces setonii* (ATTC 39116) and the white-rot fungus, *Trametes versicolor*.

Preliminary results for sample A indicated that *T. versicolor* did not degrade sulfides significantly at its optimum growth conditions (pH range of 4.5 – 5.0) and carbonaceous matter was not degraded but rather passivated as preg-robbing decreased significantly. Lignite was inert to passivation by *T. versicolor* unlike bituminous coal and to lower

extent anthracite. Stimulated alkaline conditions (pH range of 9.5 – 10.5) recorded the overall best sulfur oxidation.

Results of both the single stage and two-step processes confirmed that carbonaceous matter was passivated by *T. versicolor*; as preg-robbing decreased significantly from 18.1% to $\leq 1.0\%$. Gold extraction by cyanidation of the pre-treated sample A was 82.5% for the two-step as against 80.5 % for the single stage and 15.0% for the untreated sample. Application of the two-step and single stage process conditions to sample B resulted in 93.3% and 89.9% gold extraction respectively as against untreated concentrate of 30.5%. For the various two stage pre-treatment processes investigated, the abiotic - *S. setonii* process recorded the best gold extraction of 81.5% for sample A.

S. Setonii degraded carbonaceous matter unlike *T. versicolor* which passivated it. However, it takes only 3 - 7 days for *T. versicolor* to effect passivation and eliminate preg-robbing while 14 - 56 days is required for *S. setonii* to degrade carbonaceous matter significantly.

The result of the novel microbial pre-treatment process indicated that sulfide sulfur was degraded under alkaline conditions and carbonaceous matter passivated by *T. versicolor* at its optimum growth conditions. This led to a significant improvement in gold extraction from the double refractory gold ore and concentrate investigated.

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I also express my gratitude to Maritza Bailey, Laboratory Technician of the Mining Engineering Department, for coming in readily to help with some the sample analysis throughout the research. I owe a lot of gratitude to Hull Lyndsay and Charlie Conney of the Chemistry and Geological Engineering Department respectively for running Infra red and X-ray Diffraction test on my samples. I thank my fellow graduate students for their motivation, warm friendship and advices.

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Chapter 1

Introduction

1.1 Background Information

Refractory gold ores usually require very complex and sophisticated pre-treatment processes. Thus most gold processing plants treated non-refractory ores before 1970. However, there has been growing interest in refractory gold deposits around the world since 1971 due to depleting reserves and decreasing grades of non-refractory gold ores, inefficiency of selective mining, increasing quantity of stockpiled refractory material and advances in refractory gold extraction technology (Scheiner et al., 1971; Guay and Peterson, 1973; Guay, 1980; Jackson, 1982).

Gold ores may be broadly classified as non-refractory and refractory. Non-refractory ores are relatively easy to treat and the available gold may be recovered by gravity concentration and/or direct cyanidation. Refractory ores, however, require pre-treatment before cyanidation (Boyle, 1979; Guay, 1981).

Gold ores and concentrates are classified as refractory when a significant portion of the gold content cannot be extracted effectively by conventional cyanidation, even after fine grinding. The refractory behavior of ores is generally attributed to two main factors: the presence of carbonaceous matter and the occurrence of sulfides, tellurides and cyanicides (Boyle, 1979; Guay, 1981). When the refractoriness is due to the presence of sulfides and carbonaceous matter, the ore or concentrate can be referred as “double refractory” (Nyavor and Egiebor, 1992).

The most important classes of carbonaceous matter are organic carbon (hydrocarbons, humic acids and other organic substances) and graphitic or amorphous elemental carbon (Radtke and Scheiner, 1970; Osseo-Asare et al., 1984; Hausen and Bucknam, 1985; Stenebraten et al. 1999, 2000; Rees and Van Deventer, 2000). However, graphitic carbon is the main carbonaceous matter in double refractory gold ores and poses serious recovery concerns during leaching. The carbonaceous matter tend to adsorb gold from leach solutions, a phenomenon known as “preg-robbing” and the sulfide minerals which may occlude gold also limit the access of leaching reagents and thereby reducing recoveries.

Thus pre-treatment methods which seek to eliminate or passivate the preg-robbier during gold dissolution (Henley, 1975; Osseo-Asare et al., 1984; Demopoulos and Pangangelakis, 1987; Afenya, 1991; Linge, 1991) and to decompose the mineral matrix to liberate gold will have to be employed. The most common pre-treatment processes used for double refractory sulfide ores include the conventional roasting, pressure oxidation, chlorination and the more recent bacterial oxidation (Arriagada and Osseo-Asare, 1984; Berezowsky and Weir, 1989; Marsden and House, 1992).

Presently, focus is being shifted from the conventional pre-treatment processes to biooxidation due to problems of high capital and operating costs, inefficient gold recovery and unfriendly environmental management practices. Bacterial oxidation is gaining popularity as it offers a cost effective and environmentally friendly alternative for the processing of gold-bearing sulfide ores. Commercial biooxidation of refractory

gold ores was introduced in 1986 and by the end of 2007 there were 10 operating plants around the globe with very bright prospects of more future applications.

1.2 Bacterial Oxidation of Mixed Sulfides

During microbial pre-treatment of refractory gold, chemolithotrophic or rock-eating bacteria are employed to break down the sulfide matrix to liberate gold particles. Several types of bacteria are used in bio-mining but the prominent ones that are known to be involved in the oxidation of sulfide ores include *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* (Brierley and Brans, 1994; Brierley, 1995; Hackl, 1997; Rawlings, 1998). These bacteria are acidophilic and work very well in the pH range of 1.2 to 1.8 and a temperature range between 35°C and 45°C. The biooxidation process may be carried out in continuously stirred tank reactors (CSTR) or by bio-heap leaching.

The microorganisms catalyze the oxidation of sulfide minerals which otherwise do not proceed at a practical rate under the mild ambient conditions used during biooxidation. Due to the bacteria-minerals interaction, ferrous iron in the ore is converted to the ferric state and the sulfide sulfur is ultimately converted to sulfate and sulfuric acid. Any arsenic present in the ore may be converted to arsenite or arsenate depending on the oxidation potential of the system. Gold is released from the matrix of the sulfides as relatively free particles or exposed for the subsequent leaching process (Murr, 1980; Livesey-Goldblatt et al., 1983; Sugio et al., 1985; Kelly and Harrison, 1989; Nicholson et al., 1994; Hackl, 1997; Rawlings, 1997).

Due to varying galvanic interactions, sulfide minerals such as pyrite, arsenopyrite and chalcopyrite respond differently to microbial oxidation. Hence, the concentration of each mineral in the feed material to a biooxidation plant may influence the oxidation process. Since ores are not homogenous, the contribution of each sulfide mineral to the total sulfur content of the feed may vary and their different responses to biooxidation could influence the physicochemical factors such as redox potential, pH, dissolved oxygen and the ratio of Fe^{3+} to Fe^{2+} concentration. It may also have an impact on the process-related factors such as residence time and degree of oxidation

1.3 Biodegradation of Carbonaceous Matter

Some types of carbonaceous materials present in refractory gold ores are not oxidized significantly during biooxidation and continue to act as preg-robbers in the subsequent cyanidation process. Some carbon containing compounds that are known to undergo biodegradation include hydrocarbon oils, humic acid (Lakshmanan et al., 1989; Riser-Roberts, 1992; Solano-Serena et al., 2000), low rank coals, lignin, (Antai and Crawford, 1981; Strandberg and Lewis, 1988a; Quigley and Dugan, 1989; Srivastava et al., 1997) and tire rubber (Linos and Steinbuchel, 1998; Tsuchii and Tokiwa, 1999). Oxidation or passivation of the preg-robbler is the focus of most research in Biomining in recent times. Some investigations into the microbial degradation of carbonaceous matter have been reported. A recent novel two stage approach to the degradation of sulfide sulfur and carbonaceous matter in double refractory ore was investigated (Amankwah and Yen, 2003). In the study, *Streptomyces setonii*, a coal degrading bacteria (solubilizing

actinomycete) was used to degrade the carbonaceous matter after sulfide oxidation in the first stage by employing the three well known chemolithotrophic bacteria. Gold recovery during cyanidation increased from 81.1 to 94.7% after carbonaceous matter degradation.

A mixed regime of heterotrophic bacteria, many of which are from the *Pseudomonas* family and naturally associated with gold ores, could deactivate the active sites on carbonaceous components leading to increase in gold extraction during cyanidation (Brierley and Kulpa, 1992; Kulpa and Brierley, 1993). Portier (1991) used other heterotrophic bacteria and some fungi to deactivate carbonaceous matter and reported increases in gold recovery due to microbial action.

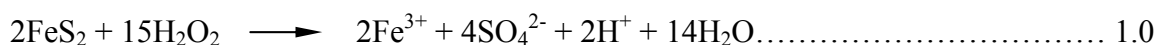
The use of fungi in biomining has not been studied much until recent times. Some recent research into the use of fungi include leaching and precipitation of nickel and iron from laterites (Alibhai et al., 1993), solubilization of manganese from ores (Baglin et al., 1992), leaching of lateritic nickel ores (Bosecker, 1985; Sukla et al, 1993) and mineral leaching of non-sulfide nickelferrous ores (Agatzini and Tzeferis, 1994). The fungal strains used in all the above investigations were *Aspergillus sp* and *Penicillium sp*. Results on nickel oxide ores (Bosecker, 1985; Tzeferis et al 1991) have shown the amenability of this mineral to leaching by fungal strains. The efficiency of leaching depended on the type of ore and the kind and concentration of organic metabolite produced by the fungi (Bosecker, 1985; Alibhai, et al 1993). Most of the investigations have been in processing of non-auriferous ores. Thus this present investigation into the biodegradation of sulfides and carbonaceous matter in double refractory gold ores by

Trametes versicolor presents a new challenge in the application of fungi in mineral processing.

1.4 Motivation for Research Project

The motivation for this research stems from the abilities of the versatile fungus *Trametes versicolor* to degrade coal by the generation of a regime of mixed oxidative enzymes including manganese peroxidase and lignin peroxidase (Kirk and Farrel, 1987).

Manganese peroxidase and lignin peroxidase have been known to produce strong oxidants for the oxidation of coal and other carbon compounds in wood. Peroxidases require H_2O_2 to complete their catalytic degrading activities. Extracellular H_2O_2 -generating enzymes are secreted simultaneously with lignin modifying enzymes. H_2O_2 has also been reported to have the ability to deactivate active sites on carbonaceous matter (Nyavor and Egiebor, 1992) and has been studied for the dissolution of many sulfide minerals including pyrite, chalcopyrite and sphalerite (Antonijevic, et al., 1997). H_2O_2 reaction with pyrite is shown in equation 1.0:



Since the structure of carbonaceous matter in gold ores is similar to some of these carbon-containing substances, it is therefore possible for the oxidative enzymes generated

by *T. versicolor* to degrade both carbonaceous matter and sulfides in double refractory gold ores.

1.5 Objectives of this Investigation

First, the ability of *T. versicolor* to degrade both sulfide sulfur and carbonaceous matter in coal, sulfide minerals and double refractory gold ores was studied. The effect of alkaline medium and the fungal culture on the overall sulfur oxidation were also investigated. The objectives of this research were to:

- Determine the optimum pH range of *T. versicolor*.
- Study the effect of carbonaceous characteristics on fungal degradation and gold adsorption using carbonaceous rocks such as lignite, bituminous coal and anthracite
- Study the sulfide sulfur degrading ability of *T. versicolor* with samples of sulfide minerals associated with refractory gold ores.
- Investigate the fungus' ability to degrade carbonaceous material present in gold ores and to establish the conditions for fungal degradation. The variables to be considered are temperature, retention time, pH, pulp density and ore type,
- Biooxidize the sulfides and carbonaceous matter in an ore sample in a two stage pre-treatment using the well known bacterial oxidation process and *T. versicolor*. The oxidation product will serve as feed material for biodegradation of carbonaceous matter by *T. versicolor*,
- Determine the conditions for leaching gold from the ores after microbial pre-treatment.

Results of this research work will offer the following benefits to both the academic circle and the mineral processing industry:

- (1) Better understanding of the reactions of *T. versicolor* and carbonaceous matter associated with refractory gold ores.
- (2) Better understanding of the reactions of *T. versicolor* and sulfide minerals associated with refractory gold ores.
- (3) Better insight into the effects of fungal pre-treatment on the gold adsorption behavior of various carbonaceous matter and
- (4) Present a better alternative for eliminating the effect of preg-robbars in double refractory gold ores and concentrates.

1.6 Format of the Thesis

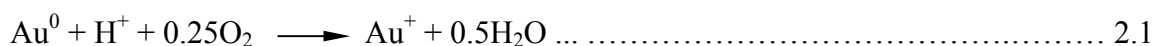
The following chapter reviews the pertinent literature on refractory gold ores, pre-treatment processes in use with much emphasis on the biological oxidation. Chapter 3 outlines the chemistry and mechanism of preg-robbing, gold leaching and the interactions between biomining microorganisms and sulfide minerals present in refractory gold ores. Chapter 4 summarizes the materials, equipment and methods used during the experimental phase of the project. The results are presented and discussed in detail in Chapter 5. Conclusions, processing proposals and recommendations for future research are outlined in Chapter 6.

Chapter 2

Literature Review

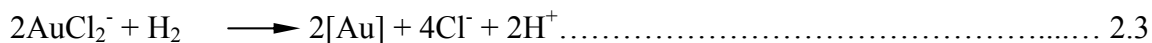
2.1 Geochemistry of Gold Ores

Generally gold is dissolved and transported by oxidizing hydrothermal fluids and is precipitated when the fluid meets reducing conditions. The reactions that lead to gold deposition from hydrothermal solutions are controlled by the nature of the rocks through which the hydrothermal fluid passes. Redox reactions mainly influence the solubility of gold as shown in equation 2.1 (Boyle, 1979).

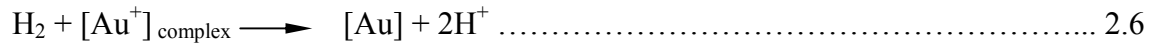
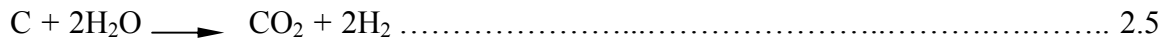


Sulfides and carbon containing minerals are able to provide the reducing environment necessary for gold deposition from the solutions. Gold precipitation may occur according to the following reactions (Boyle, 1979; Lewis, 1982):

(i) Gold precipitation by pyrite;



(ii) Gold and quartz precipitation by reaction with carbon:



(iii) Precipitation of quartz by a reduction in water activity



Sulfides, carbonaceous matter and other dissolved components in the environment are also embedded in the host rock when hydrothermal fluids solidify.

2.2 Classification of Gold Ores

Classification of gold ores could be done in several ways; according to either the extraction technique or the associated geological environment (McQuiston and Shoemaker, 1975; Boyle, 1979; Boyle, 1987, Guay, 1981; Guay and Gross, 1981; Yannopoulos, 1991). However, one generally accepted method of classification based on the extraction method classifies gold ores as either refractory or non-refractory. Non-refractory ores include placer, free milling and oxidized ores from which about 95% of the gold is recoverable by simple gravity concentration and/or direct cyanidation.

2.2.1 Refractory Gold Ores

Refractory ores are of hydrothermal origin and are deposited by hot aqueous solutions obtained from an external source such as a volcanic intrusion. The hydrothermal solutions may also carry several dissolved mineral components such as sulfides, tellurides, and arsenides. These associated minerals usually make the gold ore not amenable to extraction by conventional methods. The term refractory is thus applied when a significant proportion of the gold cannot be recovered by conventional gravity concentration or direct cyanidation even after fine milling.

The nature of the gold bearing minerals and the gangue minerals present are the two main mineralogical factors that determine the refractoriness of gold ores.

Compounds in which gold is in chemical combination with other elements such as tellurides (AuTe_2 – calaverite), antimonides (AuSb_2 – aurostibnite) and selenides (Ag_3AuSe_2 – fischesserite) cause chemical refractoriness. These gold compounds are found in nature and their rate of dissolution in cyanide solution is relatively low (Fersman, 1939; McQuiston and Shoemaker, 1975; Boyle, 1979; Guay and Gross, 1981).

Other gold bearing minerals that cause refractoriness include sulfides, silicates, carbonaceous matter and compounds of bismuth. The occlusion and inclusion of fine or colloidal gold particles in the matrix of these minerals cause physical refractoriness since this association makes it impossible to fully liberate gold by mechanical means (Boyle, 1979; Guay and Gross, 1981; Afenya, 1991; Linge, 1991; Marsden and House, 1992; Henly 1993).

Refractoriness of gangue minerals is caused by reacting with and depleting the free cyanide and oxygen required for gold dissolution or by adsorbing dissolved gold from the solution (preg-robbing). These minerals may also passivate the gold surface thereby preventing contact with the cyanide solution. Based on the degree of refractoriness, Linge (1991) suggested the classification in Table 2.1.

Table 2.1: Classification of Refractory Gold Ores (Linge, 1991)

Classification	Gold recovery
Free milling	More than 95%
Mildly refractory	80 - 95%
Moderately refractory	50 - 80%
Highly refractory	Less than 50%

2.3 Global Refractory Gold Ores Deposits

Double refractory gold ores are found in several mining areas around the world including Prestea and Obuasi in Ghana, Witwatersrand in South Africa, Nevada and the mother lode districts of California in the United States of America, McIntyre Porcupine and Kerr Anderson in Canada and Bakyrchik and Natalinsk in the Soviet bloc. Others are Morro Velho and Queiroz Mine in Brazil, Cosmo Howley and Fortnum in Australia, Waihi/Paeroa in New Zealand and Laizhou and Neilanggou in China (Lever and Wolfe, 1930; Radtke and Scheiner, 1970; Zhuchkov et al., 1968; Nice, 1971; Adamson, 1972; Afenya, 1976; Kesse, 1985).

2.4 Potential Preg-robbing Components in Gold Ores

The term preg-robbing was first used by Smith (1968) to describe poor gold recovery during cyanidation, due to adsorption by some components of the ore. Though several minerals may act as preg-robbers, the term has been attributed mainly to the presence of carbonaceous matter (Radtke and Scheiner, 1970; Osseo-Asare et al., 1984; Hausen and Bucknam, 1985; Stenebraten et al., 1999, 2000; Rees and Van Deventer, 2000).

Preg-robbing is the phenomenon whereby gold cyanide complex, $\text{Au}(\text{CN})_2^-$, is removed from solution by the constituents of the ore. The preg-robbing components may be carbonaceous matter of geologic or non-geologic source. Geologic carbonaceous matter includes humic acids and elemental carbon. Non-geological sources may include substances such as decaying mine timbers, woodchips; fine activated carbon generated in carbon adsorption circuits and particularly in tailings re-treatment, burnt plant growth and boiler ash discarded on dumps (Hausen and Bucknam, 1984; Rees and Van Deventer, 2000).

Preg-robbing components are classified as either carbonaceous or non-carbonaceous material. The non carbonaceous component consists of clays, sulfides and silicates while the carbonaceous are made up of graphitic carbon, long chain hydrocarbons and humic acids. Examples of non carbonaceous preg-robbers are some heavy minerals such as pyrite (FeS_2), chalcopyrite (CuFeS_2) and Pyrrhotite (Fe_{1-x}S) where the value of x ranges from $0 < x \leq 0.125$, khaki shale, quartz and pyrophyllite. Others include thucolite and clay

minerals such as kaolin, ball clay, fuller's earth, and bentonite (Olphen, 1963; Urban et al., 1973; Hausen and Bucknam, 1984).

It is well documented that the graphitic carbon component is similar to activated carbon in that it is capable of adsorbing gold cyanide from solution (Adams et al. 1996; Hausen and Bucknam 1984; Pyke et al., 1999; Radtke and Scheiner 1970; Stenebråten et al., 2000). Hausen and Bucknam (1984) found that the carbonaceous material had a lower capacity for gold adsorption than activated carbon, but the rate of adsorption was up to four times faster.

The second component deemed to play a role in the preg-robbing behavior of carbonaceous material is long-chained hydrocarbons. This mixture of high molecular weight hydrocarbons, predominantly long-chain alkanes, is thought to passivate the graphitic carbon in the carbonaceous material (Radtke and Scheiner 1970).

The third component implicated in the preg-robbing behavior of carbonaceous material is humic acids. Humic acids are complex, macromolecular, organic substances of poorly known composition (Hatcher et al., 1985). Pyke et al. (1999) indicated that humic acids have a skeleton of aromatic rings and contain carboxylic acid groups, phenolic and alcohol hydroxyls, ketone and quinone groups.

Carboxyl groups are one of the few definitively known components of humic acids, and are the most important from the standpoint of gold ore deposits (Hatcher et al., 1985). It

has been reported these functional groups have the ability to complex metal and metal containing cations (Hatcher et al., 1985).

The examination of the literature suggests that graphitic carbon is the most consequential component in carbonaceous material, due to its ability to adsorb gold cyanide. The presence of humic acids and hydrocarbons were initially reported to contribute to the preg-robbing behavior of carbonaceous material (Radtke and Scheiner 1970), but numerous studies have proved unsuccessful in attempts to clarify this finding (Abotsi et al., 1986; Nelson et al. 1982; Osseo-Asare et al., 1984; Pyke et al., 1999; Sibrell et al., 1990).

2.4.1 Carbonaceous Matter in Gold Ores

The term ‘carbonaceous’ has been applied freely to ores of varying carbon characteristics. Other terms that have been used to describe carbonaceous matter are ‘graphitic schist’, ‘carbonaceous shale’ and ‘black or carbonaceous slate’ (Feldtmann, 1914; Avery, 1916; Fischer, 1916). The most important classes of carbonaceous matter are organic carbon, and graphitic or amorphous elemental carbon. Organic carbon is made up of hydrocarbons, humic acids and other organic substances while graphitic and amorphous carbon is comprised mainly of elemental carbon. Not all the components of carbonaceous matter may be present in a given carbonaceous ore and thus extreme variations may exist in carbonaceous material concentration from one deposit to another, composition and preg-robbing behavior from one location to the other within deposits. Variations in carbon activity are also great as may be expected from a surface phenomenon (Nelson et al., 1982; Leventhal and Hofstra, 1990; Sibrell et al., 1990).The

pioneering work in isolating and identifying the ‘preg-robbing’ components in carbonaceous material were performed by Radtke and Scheiner (1970). The carbonaceous material was classified into three main components, a carbon similar in nature to activated carbon, long-chained aliphatic hydrocarbons and humic acids. Numerous studies (Abotsi et al., 1986; Leventhal and Hofstra 1990; Nelson et al. 1982; Osseo-Asare et al., 1984; Stenebråten et al., 1999) have followed since, all aimed at characterizing the carbonaceous material with respect to its possible graphitic carbon, humic acid and/or hydrocarbon content.

Many researchers are of the view that the carbonaceous matter in gold ores is similar to that of coals. For example, many of the organic compounds in carbonaceous matter associated with gold ores such as long chain hydrocarbons and humic acids are present in lignite while the more matured aromatic and graphitic carbon is also present in bituminous coal and anthracite (Radtke and Scheiner, 1970; Wells and Mullens, 1973; Zaitseva et al., 1973; Afenya, 1976; Zumberge et al., 1978; Nelson et al., 1982; Osseo Asare et al., 1984; Hausen and Park, 1985; Abotsi and Osseo-Asare, 1986; Hallbauer, 1986; Asiam, 1988; Sibrell et al., 1990; Leventhal and Hofstra, 1990; Afenya, 1991; Stenebraten et al., 1999, 2000; Wilkomirski, 2003).

Studies by investigators using ores from all over the world have identified that all forms of preg-robbing carbonaceous material contain a component that has been reported as being between amorphous carbon to graphite. However the nomenclature used to describe this ‘carbon’ varies from naturally occurring activated carbon (Radtke and Scheiner 1973; Sibrell et al., 1990) to elemental (Afenya 1991; Nelson et al., 1982; Osseo-Asare et al., 1984) or native carbon (Pyke et

al., 1999). Strictly speaking elemental carbon refers to the element carbon, activated carbon refers to the partial oxidation of a carbon material to increase internal surface area, and graphite by itself is a material with a perfect crystalline structure with no defects. The carbonaceous material consists of 'graphitic carbon', which is carbon with a graphitic structure, but with a number of defects.

2.5 Refractory Gold Ores Processing Options

Each type of refractory carbonaceous gold ores has its peculiar problem which needs to be addressed specifically. A number of processing options have been developed over the years for treating refractory carbonaceous ores and these include gravity concentration, carbon flotation or depression prior to leaching, the use of a highly competitive material which can adsorb the dissolved gold faster than the carbonaceous matter, passivation of preg-robbes using blanking agents and dissolved gold capture during leaching (Adamson, 1972; Henly, 1975). Any one method could be used or in combination with others depending on the ore mineralogy.

For deposits in which the particle size of gold is coarse enough to be separated by gravity, gravity concentration is recommended. Carbon flotation or depression has also been used in situations where the carbonaceous matter is not mineralized. In situations where the carbonaceous matter contains gold, the floated carbon may be processed in another stream. Carbon flotation or depression has also been used successfully. Blanking agents such as kerosene and fuel oil may also be used to coat carbonaceous matter and render the preg-robbes passive during the subsequent gold leaching step.

Dissolved gold adsorption, which makes use of activated carbon, has been used with great success. Technically, depending on the preg-robbing characteristics of the ore, activated carbon may be added in the course of leaching, a process known as carbon-in-leach (CIL) or after leaching in the carbon-in-pulp (CIP) process. Activated carbon may also be loaded into columns or up-flow contactors before the solution is passed through and it is referred to as carbon-in-column (CIC). CIL has advantages when carbonaceous materials are present in the ore since it brings about competitive adsorption between the added carbon and the naturally occurring carbonaceous matter and it has been embraced by gold processing plants (Davidson, 1979; Muir, 1982; Ruane, 1982).

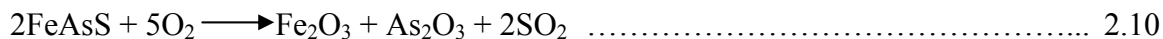
2.6 Pre-treatment Options

The purpose of pre-treatment is to target and decompose selected associated minerals and opens up the molecular structure so that leaching reagents can make contact with and extract gold. It also eliminates, passivates or drastically reduces the presence of the gangue minerals, which cause refractoriness. These pretreatment methods are broadly classified into pyrometallurgical and hydrometallurgical oxidation processes (Guay, 1981; Hayden et al., 1987; Taylor et al., 1989; Yannopoulos, 1991; Afenya, 1991; Linge, 1991; Marsden and House, 1992)

2.6.1 Pyrometallurgical Oxidation

Roasting is the main pyrometallurgical oxidation method and for many years has been a very reliable method of treating refractory gold ores to achieve significant recovery.

Roasting involves the heating of refractory ores and concentrates in a controlled oxidizing atmosphere resulting in the decomposition of volatile components and oxidation of sulfide minerals simultaneously. Roasting thus converts sulfur and arsenic into volatile components and iron into hematite. The liberated gold particles are concentrated in the calcine, which may consist mainly of hematite and siliceous gangue minerals. The temperatures for roasting most refractory sulfidic ores vary from 450°C to 750°C with ample supply of oxygen (Arriagada and Osseo-Asare, 1984; Stanley, 1987; Robinson, 1988). The major reactions of pyrite, arsenopyrite, pyrrhotite and carbonaceous materials can be expressed as follows:



The roasting process is quite complex and the desired end product may not necessarily be achieved due to the presence of several minerals in refractory ores. Temperature control is quite difficult as the various mineral components oxidize at different temperatures. High temperatures are experienced if the ore contains carbonaceous material which oxidizes at a much slower rate and at high temperatures. The carbonaceous material

when not fully oxidized becomes activated making it a more efficient preg-robbing agent. High temperatures lead to the recrystallization of hematite and subsequent physical encapsulation of gold, leading to a reduction in the porosity of the calcine and inefficient gold leaching (Arriagada and Osseo-Asare, 1984; Swash and Ellis, 1986; Grimsey and Aylmore, 1990). More often the roasting temperature employed is between 600°C and 700°C. However, the roasting temperature depends very much on the type of sulfide minerals present.

Other options such as very fine grinding, the two-stage roasting process and quenching of calcine have been proposed to help produce calcine that is more amenable to leaching. Lime is sometimes added to the roaster feed to act as a heat sink for the exothermic reactions that take place and allow better temperature control. In addition, it reacts with sulfur dioxide and helps to reduce emissions by about 75%. The use of roasting has suffered a setback in recent times due to heightened environmental concerns over its byproducts and high power cost. However, it may still be considered as a suitable pre-treatment option due to recent improvements in furnace and process design such as scrubbing of roaster gases and development of fluidized-bed roasters.

2.6.2 Hydrometallurgical Oxidation

Currently much of research and development activities in the mineral processing industry is focused on hydrometallurgical methods of treatments and this has the potential to eventually replace the traditional roasting. The three main hydrometallurgical oxidation

techniques are: hydrochemical oxidation, pressure oxidation (autoclave leaching) and bioleaching (biochemical leaching)

2.6.2.1.1 Hydrochemical Oxidation

(1) Chlorine Oxidation

Aqueous solutions of chlorine has the potential of oxidizing carbonaceous material and associated sulfides especially pyrites and dissolving the free gold in the process. Chlorine gas easily dissolves in water to form hydrochloric acid and hypochlorous acid (Snoeyink and Jenkins, 1979).

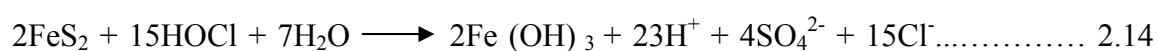


Both chlorine gas and hypochlorous acid have high oxidizing abilities but hypochlorous acid is the preferred agent for the oxidation and deactivation of carbon and sulfides. Hypochlorous acid is added in the form of sodium hypochlorite which is inexpensive, readily available, easy to handle and can be added in specific quantities. Chlorine oxidation is effectively achieved at temperatures between 50°C and 60°C and a pH range of 3 and 5 since the activity of hypochlorous is highest in this range. Sodium hypochlorite could be regenerated directly in the pulp by electrolysis to maintain a high level of the reactant at all times (Marsden and House, 1992).

The exact mechanism involved in the deactivation of carbonaceous matter is not fully comprehended but it is believed that the surfaces of the carbonaceous matter are modified by chlorine either by the formation of a chlorohydrocarbon layer or by the formation of

carbonyl structures composed mainly of carboxyl groups. These surface groups passivate the carbon by blocking active adsorption sites (Brunk et al., 1988; Sibrell et al., 1990).

For sulfidic and carbonaceous ores, chlorine consumption during the process could be over 50 kg/t. Gold also reacts with and consumes the oxidant (Nagy et al, 1968; Brunk and Atwood, 1987; Birak and Deter, 1987). Pyrite reacts according to the equation:



The very high operating cost of the process due to excessive consumption of chlorine make the process less attractive and thus the double oxidation process was introduced to help reduce the chlorine consumption.

(2) Double Oxidation

The initial oxidation process involves vigorous agitation of the pulp with air at temperature of 70°C to 80°C and air pressure of 6-8 psig until a considerable amount of pyrite and other sulfides are oxidized. Lime or sodium hydroxide may be introduced during the initial aeration step for any sulfuric acid produced by pyrite oxidation to be neutralized. Subsequent chlorine consumption is significantly reduced after pre-aeration. The process of aeration followed by chlorination is thus referred to as double oxidation (Marsden and House, 1992).

Despite this modification and the high gold recoveries of 90-93%, excessive consumption of chlorine as ores becomes more refractory, the toxic and corrosive nature

of chlorine coupled with its expensive tailings disposal have limited the use of this process technique. Currently there is virtually no processing plant that uses this process.

2.6.2.2 Arseno Process

The arseno process is a pressure oxidation process that utilizes a nitrate catalyzed oxygen leach system. It is best suited for ores or concentrates in which the gold is locked in minerals such as marcasite or arsenopyrite. The catalytic nature of the process results in very high reaction rates and sulfide decomposition is completed in less than 15 minutes (Fair et al., 1986; Beattie and Raudsepp, 1988; Van Weert, et al., 1988; Beattie and Ismay, 1990).

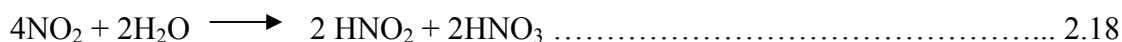
Temperatures of up to 120°C and an oxygen pressure of 700 kPa are required to maintain the process reactions. During the process, nitric acid dissociates completely in water to produce the nitrate ion, which is a strong oxidizing agent (Beattie and Raudsepp, 1988; Foo and Bath, 1989).



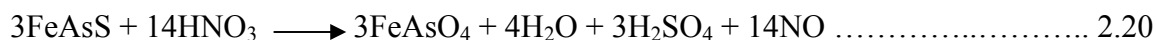
The nitrate ion also undergoes reactions to form another oxidizing agent according to the equation:



Both the nitrate and nitrite species are strong oxidizing agents capable of oxidizing sulfides yielding nitrous oxide gas (NO) as the reduced species. Nitrous oxide reacts rapidly with oxygen to form nitrogen dioxide, which dissolves in water to regenerate the reactants.



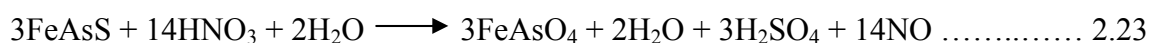
The reactions with pyrite and arsenopyrite proceed at a very fast rate at temperatures above 60°C, pH below 1.7 and an acid strength between 70 and 180 g/l. The reactions are given by (Fair et al, 1986):



The drawbacks for this process are the generation of arsenide and elemental sulfur which are detrimental to the cyanidation process. High pressure and temperature (180°C) are employed to arrest these problems.

2.6.2.3 Nitrox Process

The nitrox process decomposes pyrite and arsenopyrite concentrates using nitric acid as an oxidant under atmospheric conditions. Neither air nor oxygen is involved in these reactions and temperatures are relatively lower. The reactions of pyrite and arsenopyrite in the process are given by:



Unlike the arseno process, iron nitrate is one of the leach products. The nitric acid used is regenerated and recycled according to the reactions:



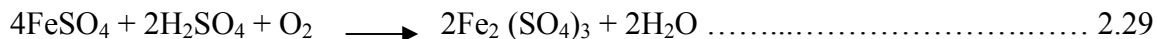
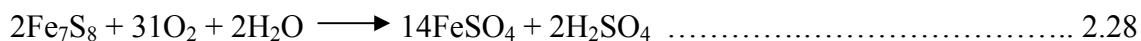
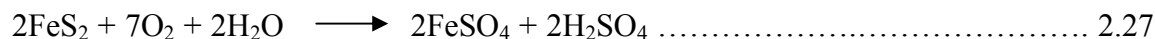
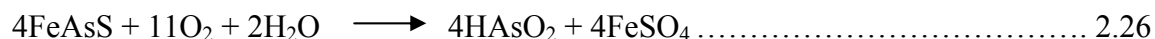
Due to the very corrosive nature of nitric acid, processes that use this oxidant are not very popular in the gold industry and no plant uses either the nitrox or the arseno process.

2.6.2.4 Pressure Oxidation

Pressure oxidation techniques employs high pressure oxygen and temperatures to treat refractory ores or concentrates and thereby oxidizing the gold bearing sulfide minerals

and precipitation of leach products. The process can take place in highly acidic or alkaline medium depending on the mineralogy of the ore. However, high-pressure acidic oxidation is the most widely used pressure oxidation process (Weir and Berezowsky, 1984, 1987; Peters, 1986; Berezowsky and Weir, 1989a, 1989b). Non-acidic or alkaline pressure oxidation is applied to acid-consuming carbonate rich refractory ores. The process is best suited for ores with low sulfide content.

The principal reactions involve the total oxidation of sulfidic sulfur and arsenic to sulfate and arsenate respectively (Berezowsky and Weir (1989a, 1989b.) These reactions take place at higher temperatures (between 180 and 225°C) and in strongly acidic environments (pH less than 2). The major reactions are represented as:



Oxidation is carried out at temperatures above 160°C to prevent the formation of hydronium jarosite ($\text{H}_3\text{OFe}_3(\text{SO}_4)_2(\text{OH})_6$) and elemental sulfur and promote complete

oxidation of the sulfides to sulfates. Ferrous salts, which form, are also converted to the ferric state.

Refractory ore treatment by this method normally gives gold extraction in excess of 95%.

2.6.2.5 Bacterial Oxidation

The bacterial oxidation process is the latest processing technique developed for pre-treating double refractory gold ores. The technology which was commercialized in 1986 under the name, Biox[®], employs a mixed population of chemolithotrophic bacteria to break down the sulfide mineral matrix, thereby liberating the occluded gold for subsequent cyanidation. The carbonaceous matter on the other hand, is only partially oxidized and therefore not rendered totally inactive. Bacterial oxidation of gold ores is differentiated from bacterial leaching in that the bacteria used do not dissolve the gold.

Several types of bacteria are known to oxidize sulfides but the most common ones in biomining are *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* (Livesey-Goldblatt et al., 1983; Hutchins et al., 1987; Rawlings, 1997). These bacteria are acidophilic mesophiles and perform optimally within the pH range of 1.2-1.8 and temperature range of 35 and 45°C. The oxidation reactions of sulfide minerals are exothermic. The process is cooled with water to maintain the temperature within the optimum range.

The oxygen demand for sulfide oxidation is high and thus large volumes of air has to be injected and dispersed into the slurry and bioreactors are designed to provide efficient and rapid oxygen uptake. Dissolved oxygen levels are kept 2 ppm and above.

Biooxidation of gold ores may take place in continuously stirred tank reactors (CSTR) or in heaps and dumps but the use of stirred tank bioreactors is more prominent. Feed to bioreactors is mainly flotation concentrate and the processing time is between 3 and 5 days if high sulfide oxidation levels are expected.

During biooxidation, the bacteria require nutrients to sustain growth. Essential elements such as nitrogen; phosphorus and potassium are added into the primary reactors as solution of fertilizer grade ammonium sulfate and potassium phosphate or phosphoric acid (Rawlings, 1997; Hackl, 1997).

Thermophiles having growth activity from 45 to 55°C and from 50 to 80°C have also been studied. The extremely thermophilic *Sulfobulus* species which grow at temperatures of 50 to 80°C have been used to pre-treat both sulfidic and carbonaceous gold ores and recoveries in excess of 80% were achieved. Using *Sulfobulus* for treating various carbonaceous ores and blanking the carbon before cyanidation, recoveries of 84 to 94% were achieved (Hutchison et al., 1988)

Biooxidation process is efficient and cost effective making it an attractive alternative to the conventional roasting and pressure oxidation. The bacterial culture is robust; the plants are simple to operate and have proven high scale up potential. More so the process is environmentally friendly as neutralization of plant effluents produces precipitates that meet the most stringent environmental regulations.

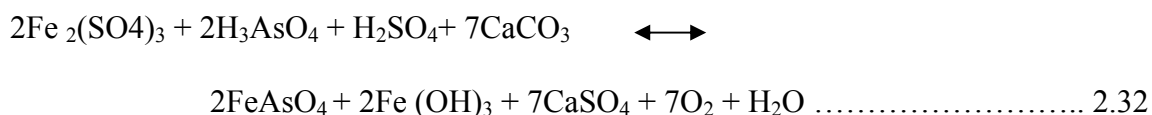
2.6.2.5.1 Post Bacterial Oxidation Processes

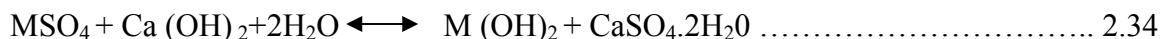
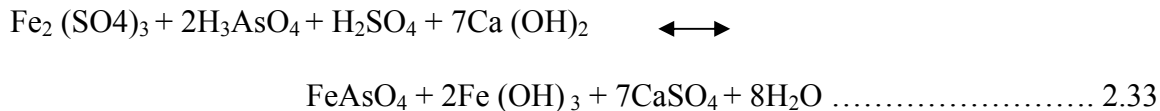
The product of bacterial oxidation which contains high concentrations of sulfuric and arsenic acid, ferrous and ferric compounds and other dissolved ions is washed in a three or four stage counter current decantation (CCD) circuit to separate the oxidized gold bearing solids from the acidic liquor before conditioning to pH 10-11 and cyanide leaching. The washed biooxidized product would normally contain less than 1 g/l total iron in solution with an acid pH of 1-3. Iron removal is necessary before cyanide leaching to promote gold recovery and reduce cyanide consumption.

The arsenic rich CCD effluent is acidic and very toxic and it is a known carcinogen. It is neutralized with limestone and lime in a two stage process to a pH between 6 and 7 to precipitate arsenic, iron and sulfates as a stable precipitate of ferric arsenates. The environmentally acceptable waste sludge is then impounded safely at the tailings dam.

Extensive research and development has gone into the optimization of the design of the neutralization section of the Biox[®] process in recent years. This was to ensure that the effluents comply with the most stringent international environmental regulations with respect to product stability and residual arsenic concentrations.

The chemistry of the arsenic precipitation process is as follows:





M= Mg, Cu, Ni, Zn, etc



Equation 2.31 represents free acid neutralization with limestone or seashells (pH 1.4 to 2.0) to form gypsum and Equation 2.32 is the precipitation of ferrate arsenate with limestone (pH 2.0 to 4.0) during the first stage. Ferric iron and other dissolved tramp metals are precipitated in the second stage as the pH is further raised with lime to 7 as represented by equations 2.33 and 2.34. Equation 2.35 represents neutralization of the excess acid present by further lime addition to pH 8.

The thickened biooxidized product is conditioned to pH 10-11 and gold recovered by traditional cyanidation at the leaching circuit. The cyanide tailing which is the second waste stream generated by the process joins the neutralized sludge at the tailings pond. Some of the plants now use part of the leach circuit tailings by virtue of its alkalinity as a neutralizing media to cut down on cost of lime consumption.

The gold is adsorbed onto activated carbon by either the carbon in leach (CIL) or carbon in leach (CIP) process. The carbon is eluted with strongly basic cyanide solution, and the

gold is electro won from solution onto stainless steel wool cathodes, then calcined, smelted and cast into bullion.

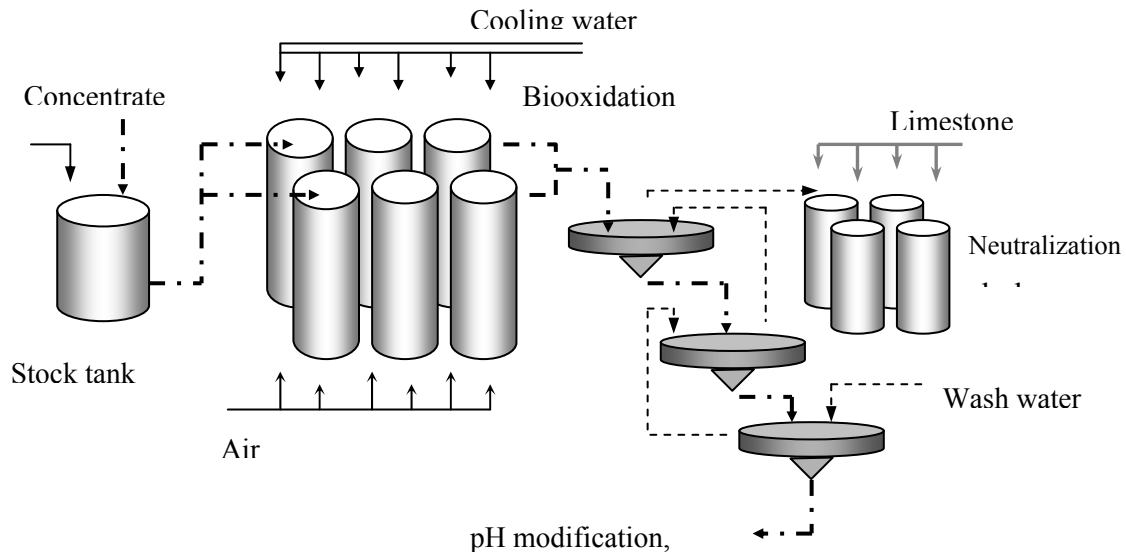


Figure 2.1: Typical Flow sheet for Genmin's Biooxidation Plant for Treating Refractory Gold Concentrates (after Hackl, 1997)

2.7 Recent Microbial Pre-treatment Approach

Due to the partial oxidation of the carbonaceous matter in double refractory gold ores and its detrimental effect during cyanidation, various attempts have been made to further oxidize the carbon matter or deactivate the active sites on the carbonaceous component to improve gold recovery. Bierley and Kulpa (1992) and Kulpa and Bierley (1993) demonstrated that a consortium of heterotrophic bacteria, many of which are from the *Pseudomonas* family and naturally associated with gold ores could deactivate the active sites on carbonaceous matter leading to increase in gold recovery during cyanidation. Portier (1991) used other heterotrophic bacteria and some fungi to degrade carbonaceous

matter and reported increases in gold recovery due to the bacteria's action. In recent research by R. Amankwah et al (2005), gold recovery improved by 13.6% when a double refractory gold concentrate was subjected to a two-stage microbial process to degrade both sulfides and carbonaceous matter. In the first stage, chemolithotrophic bacteria were used to oxidize sulfides and carbonaceous matter destroyed in the second stage with the bacterium *Streptomyces setonii*.

This current investigation is a novel attempt to oxidize both sulfides and carbonaceous matter in double refractory gold ores and concentrates using a versatile white-rot fungus, *Trametes versicolor*.

2.8 Background Information on Fungi

2.8.1 Historical Background, Importance and Applications

The use of fungi for the production of commercially important products has increased rapidly over the past half century. The exploitation of fungi by man is not a recent phenomenon. The fermentation of alcoholic beverages is one of the earliest known examples of the exploitation of the biochemical activities of a fungus by humans. The use of yeast to leaven bread also dates back to biblical times (Papagianni, 2004). Fruit bodies of basidiomycetes and ascomycetes have been collected and eaten by civilizations throughout the world (Hayes and Nair, 1978). The production of alcoholic beverages through the action of fungi known as yeasts, biomass and the manufacture of therapeutic compounds such as antibiotics, together with the production of simple organic compounds, still remains the major fields in which fungi are used. Ecologically fungi act

as decomposers and nutrient recyclers in the soil. Fungi also are important crop parasites, causing loss of food plants, spoilage of food and some infectious diseases.

The deliberate growth of fungi for commercial purposes did not commence until well into the 20th century. The development of the sulfite process for the production of glycerol by a yeast fermentation which was widely used during the First World War probably marked the beginning of industrial mycology (Papagianni, 2004). However, it is since the advent of the submerged culture techniques used in the penicillin fermentation that the greatest expansion in the use of fungi in the industry has taken place. At present, increasing numbers of commercially important products are being produced from fungi.

2.8.2 Biotechnology and Fungal Versatility

Biotechnology according to UN Convention on Biological Diversity is any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use. One section of biotechnology is the directed use of organisms for the manufacture of organic products (examples include beer, milk-products, and skin). Naturally present bacteria are utilized by the mining industry in bioleaching. Biotechnology is also used to recycle, treat waste, cleanup sites contaminated by industrial activities (bioremediation), and produce biological weapons.

Biotechnology offers an efficient, environmentally benign and relatively cost-effective remedy for many chemical processes and it is applied in a wide variety of fields. The

principles are applied in many areas including food processing, municipal composting, industrial leaching processes aimed at metal recovery and in the environmental clean-up of mining sites. Other uses are upgrading of petroleum and other fuels, remediation of soils and sediments contaminated with heavy metals, cleaning up massive oil spills, human therapeutics, agriculture, hazardous waste management and feedstock chemicals (Zajic and Knetting, 1971; Summers and Wilver, 1978; Kohler et al., 1984; Misra et al., 1991; Brierley, 1993; Glazer and Nikaido, 1995; Bosecker, 2001; Volesky, 2001)

Microorganisms are generally described as organisms that are unicellular or multicellular though are not composed of differentiated tissues. Microorganisms can be classified into five major groups: bacteria, viruses, fungi, algae and protozoa. Bacteria and fungi had been used extensively in microbial biotechnology than the others due to their metabolic versatility (Stanier et al., 1986; Glazer and Nikaido, 1995).

Fungi consist of unicellular, multinucleate, and multicellular forms. Fungal cells have cell walls containing the carbohydrate chitin. They are classified on the basis of their reproductive spores and the nature of their multinucleate or multicellular filaments known as hyphae. Fungal hyphae have a small volume but large surface area, enhancing the fungal absorptive capacity. Fungal hyphae are collectively termed a mycelium. Fungi are either saprophytic or parasitic in nature. Saprophytes obtain their food from the decaying bodies of plants and animals. Generally fungi are absorptive heterotrophs: they breakdown food by secreting digestive enzymes onto substrates and then absorbing the resulting small food molecules.

2.9 Microorganisms Used in this Investigation

2.9.1 Fungus used for Sulfide and Carbonaceous Matter Degradation

Trametes versicolor is a white-rot fungus which belongs to the phylum, Basidiomycetes. White-rot fungi are a physiological grouping of fungi that are capable of degrading a lignocellulose substrate. They are called white rot because the degradation process results in a bleaching of the wood substrate. White-rot fungi are robust organisms that have a high tolerance to toxic environments, making them ideal to use for bioremedial purposes. These basidiomycetes are aerobic and withstand high temperatures and a wide range of pH, further enhancing their versatility. The branching, filamentous mode of fungal growth allows for more efficient colonization and exploration of substrate.

White rot fungi is reported to cause the most rapid and extensive degradation of lignin, a resistant bio-polymer (Kirk and Farrell 1987). They have been reported to degrade persistent organic pollutants via their lignin degrading system which is extracellular and non-specific (Cameron et al., 2000; Lamar 1992; Pointing 2001). These fungi cannot use lignin as a source of energy, however, and instead require substrates such as cellulose or other carbon sources used to enhance degradation rates.

Among the white-rot fungi, *Trametes versicolor* and *Phanerochaete chrysosporium* have been particularly studied in detail due to their high ligninolytic activities. The production of high amounts of laccase by *Trametes versicolor* has been known for decades (Fahraeus et al., 1958)

2.9.2 Bacteria used for Sulfide and Carbonaceous Matter Degradation

The well known chemolithotrophic bacteria and *Streptomyces setonii* were also employed during the investigations to study various two stage pre-treatment processes.

The chemolithotrophic bacteria used in oxidizing sulfides are *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. *A. ferrooxidans* and *L. ferrooxidans* primarily oxidize ferrous iron to the ferric state and *A. thiooxidans* oxidizes elemental sulfur and sulfide sulfur ultimately to sulfate. This mixture of three bacteria is the most useful in biomining and is found in most commercial biooxidation plants.

The bacterium *A. ferrooxidans* is rod-shaped and about 0.5 μm in diameter by 1-2 μm long. The typical habitat pH of these bacteria is 1.5 to 3.5 and the metabolic activity is temperature dependent, peaking at about 30-45°C.

A. thiooxidans is a strictly aerobic bacterium and it is similar to *A. ferrooxidans* in physiological and morphological properties. . It is more acid-tolerant and more efficient in oxidizing elemental sulfur. The bacterium is able to oxidize sulfides, elemental sulfur, thiosulfate, and polythionite. It is adapted to highly acidic environments and thrives optimally in temperature ranges 25-35°C. The morphological features of *A. ferrooxidans* are different from the *thiobacilli* and its form ranges from a helix to a curved rod to a vibrio. It is typically about 0.2-0.4 μm in diameter and 1-2 μm in length. *L. ferrooxidans* is highly efficient at oxidizing ferrous to ferric iron but inert to elemental sulfur.

A. ferrooxidans and *L. ferrooxidans* oxidize Fe^{2+} to Fe^{3+} , which is a very strong oxidizing agent. Even at a $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio of 1:1,000,000, a Redox potential of greater than +0.4 V is generated that is sufficient for the attack of most base metal sulfides. Consequently, by generating Fe^{3+} , these bacteria are indirectly responsible for the dissolution of base metal sulfide minerals and the mobilization of metallic cations (Chapelle, 1993; Paul and Clark, 1996).

The chemolithotrophic bacteria utilize carbon from an inorganic source, which may be atmospheric carbon dioxide or carbon dioxide from the simultaneous acid dissolution of carbonates that may be present in the ore. The bacteria are commonly found in environments where sulfur compounds are abundant, such as mining areas, sewer treatment and marine habitats. They convert residual sulfides in the natural environment to sulfuric acid leading to acid mine drainage.

The bacterium used to investigate biodegradation of carbonaceous matter is from the genera *Streptomyces*. *Streptomyces* is a genera belonging to a large group of filamentous bacteria, usually gram positive, known as actinomycetes. *Streptomyces* filaments are usually 0.5 to 1.0 μm in diameter and may be indefinite in length.

These actinomycetes are aerobic and generally mesophilic. However, some are known to survive above the mesophilic range to between 50 and 60°C. *Streptomyces* is nutritionally highly versatile and they can use a wide variety of carbon sources. These include sugars, alcohols, organic acids and amino acids. *Streptomyces setonii* is known to solubilize lignin and low rank coals (Hirsh and McCann-McCormick, 1985; Strandberg and Lewis,

1987; Quigley and Dugan, 1989). It has also been reported to degrade carbonaceous matter in double refractory gold ores (Amankwah, 2003).

Chapter 3

Chemistry of the Process

3.1 Fungal Growth

Filamentous fungi are morphologically complex micro-organisms, exhibiting different structural forms throughout their life cycles. The basic vegetative structure of growth consists of a tubular filament known as hypha that originates from the germination of single reproductive spore. As the hypha continues to grow, it branches repeatedly to form a mass of hyphal filaments referred to as mycelium. When grown in submerged culture, these fungi exhibit different morphological forms, ranging from dispersed mycelial filaments to densely interwoven mycelial masses referred to as pellets (Papagianni, 2004).

The particular form exhibited is determined not only by the genetic material of the fungal species but also the nature of the inocula as well as the chemical (medium constituents) and physical (temperature, pH, mechanical forces) culturing conditions (Atkinson and Daoud, 1976; Kossen, 2000).

3.1.1 Growth Mechanisms in Filamentous Fungi

The vegetative mycelium under appropriate conditions, gives rise to a reproductive mycelium that supports the production of reproductive spores. The type of sporulation and the morphology of the spores and spore-bearing structures are key characteristics in fungus identification. The fungal spore, therefore, can be considered as the beginning and the end of the differentiation process. In mycelial fungi, hyphae extend by a highly polarized process of cell extension known as tip extension. As the tip extends, periodic

branches are formed at or near the apex of the tip. These branches also extend in a polarized manner as new tips. The two processes of tip extension and branching permit the organism to colonize and efficiently utilize the substrate, and they are rarely found in organisms other than fungi, leading to their being termed hallmarks of the fungal kingdom (Heath, 1995).

Microbial growth is normally associated with exponential increases in biomass when conditions are favorable for growth and when nutrients are in excess. Exponential growth requires that all, or a constant percentage of the mass of the microorganisms present, contribute to new growth. If all growth takes place in the apical segment of the hyphae and the individual hypha extends at a constant linear rate, then exponential growth will require that new branches are produced at a rate proportional to the rate of increase in cell mass. Exponential growth therefore occurs through an exponential increase in the number of branches, each of which extends at the same constant rate.

3.1.2 Growth in Submerged Culture

It is often stated that growth kinetics of filamentous fungi in submerged culture are quite similar to those of unicellular organisms that reproduce by binary fission. In fact, due to practical difficulties that hinder studies of filamentous organisms in submerged culture, growth kinetics are based mainly on studies with unicellular organisms. Attachment and growth on bioreactors' walls, agitators, probes and baffles lead to a degree of heterogeneity within the biomass which is more pronounced in the case of pelleted growth. Areas of growing and non-growing biomass inside the bioreactor influence the

overall growth kinetics. In addition to this, the mechanism of hyphal growth itself contributes to heterogeneity with extension, but little de novo biosynthesis at the tip, active biosynthesis behind the tip and reduced activity in more distant regions as hyphae age and vacuolated (Prosser, 1995).

3.2 Growth Cycles in Batch Culture

Growth in a batch culture growth is typically divided into four distinct phases namely lag exponential, stationary and death phases (Figure 3.1).

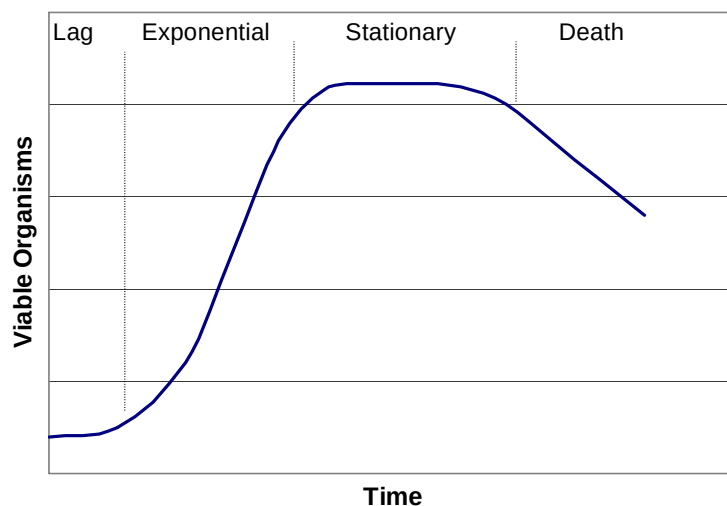


Figure 3.1 Typical growth curve for a fungal population

The lag phase represents a period during which the fungal cells or spores adapt to a new environment. It is therefore the period between inoculation and the beginning of growth. Adaptation includes formation of enzymes and intermediates to support resumption of growth. The length of this phase is dependent not only on the physiological state of the fungus, but also on the morphology and level of inoculum. Spore inocula require a germination period (Smith and Calam, 1980), while pelleted inocula may require a

certain degree of mechanical disruption prior to inoculation (Greasham, 1991). Physiological adaptation of the organism includes synthesis of enzyme systems required for substrate utilization, or removal of inhibitory compounds carried over with the inoculum (Prosser, 1995).

The exponential phase is characterized by a significant increase in cell mass. The rate of hyphal growth depends not only on the strain of the fungus, but also on the physicochemical environmental conditions. As on solid media, exponential growth results from autocatalysis through exponential production of branches, each of which extends at a linear rate. A reduction in the specific growth rate occurs when the fungus begins to experience an unfavorable growth environment such as the limitation of a required nutrient, the development of an adverse pH value or the accumulation of end products of metabolism that are inhibitory.

During the deceleration phase, growth kinetics during this phase is largely uncharacterized, despite the importance of this phase for biotechnological processes, as the period when secondary metabolite production begins. Deceleration in growth rate due to oxygen limitation is of particular importance for cultures of filamentous fungi because of the influence of their morphology on rheological properties. Dispersed growth leads to non-Newtonian rheological behavior. The apparent viscosity increases with growth, reducing the transport of nutrients, oxygen and heat. Nutrient limitations can accelerate entry into the deceleration phase, in comparison with unicellular cultures of equivalent biomass and activity.

The stationary phase may be defined simplistically as the balance between hyphal mass increase and decrease. However, if the hyphal mass accumulates intracellular storage material during the reduced growth phase, a slight increase in hyphal mass may be observed during endogenous metabolism of these storage materials. Also, if the hyphae begin to autolyse, new growth could be expected from the products of autolysis, e.g., release of a limited nutrient.

Death phase is the period preceding the stationary phase where the fungus cell dies. In some cases, death leads to loss of cellular integrity with the release of cytoplasmic constituents or lysis. However incubation may continue after the stationary phase and cells may remain alive and continue to metabolize.

3.3 Biochemistry and Mechanism of Lignin Degrading System

3.3.1 Historical Background

The first attempts to delignify wood by means of white-rot fungi were made in the early 1930s. According to Campbell (1930) and, independently, to Wiertelak (1932), *Trametes versicolor* and *Trametes pini* respectively transformed wood efficiently. Among the white-rot fungi, the polyporous fungus *Trametes versicolor* have been particularly studied in detail due to their high ligninolytic activities.

3.3.2 Classification of White-rot Fungi

Wood-rotting basidiomycetes penetrate wood tissues in order to come into contact with

the easily assimilable carbohydrate constituents of the lignocellulosic complex. The white rot group of these basidiomycetes, which has a versatile machinery of enzymes co-operating with certain secondary metabolites of fungi, is capable of attacking the lignin barrier efficiently. These fungi use a multi-enzyme system including the so-called “feedback” type enzymes to transform and degrade all structural elements of the lignocellulosic complex (polysaccharides and lignin).

The currently known enzymes of white-rot fungi involved in wood degradation can be classified into three groups. The first is capable of attacking the wood constituents or their primary degradation products directly; this group includes among others laccase, different peroxidases, protocatechuate-3,4 dioxygenase, *etc.* The second group co-operates with the first group by providing H₂O₂ for the peroxidases, but they do not attack wood components directly with enzymes; aryl alcohol oxidase and glyoxal oxidase among others constitute this group. The third enzyme group represented by glucose oxidase and quinone oxidoreductase (cellobiose dehydrogenase) includes feedback type enzymes which play a key role in linking the metabolic chains during the biotransformation of high-molecular mass wood constituents. All these enzymes, including laccase, can act separately or in co-operation (Leonowicz et al, 2001).

3.3.3 The Lignin-degrading System

Lignin degrading system of white rot fungi is extra-cellular and non-specific. Scientists have therefore looked at exploiting the non-specificity of the lignin degrading system of white rot fungi in bioremediation. Several investigations reported that white rot fungi

were able to degrade a wide range of organic pollutants and synthetic polymers (Cameron et al., 2000; Lamar, 1992). Lignin degradation occurs during the secondary metabolic phase (idiophase) when carbon, nitrogen, phosphorous or sulfur sources are limiting. The fungi produce and secrete several extracellular hydrolytic and non-hydrolytic digestive enzymes under these "ligninolytic" conditions. Three lignin-modifying enzymes (LMEs) are thought to catalyse lignin degradation: lignin peroxidase (LiP), manganese (II)-dependent peroxidase (MnP) and laccase (phenol oxidase) (de Jong, 1993; de Jong et al. 1994). These LMEs have the ability to catalyze both the cleavage of carbon-carbon or carbon-oxygen bonds in complex lignin polymer or lignin model compounds (Kirk and Hammel, 1992; Hammel et al., 1993; Marzullo et al., 1996) and also one-electron oxidations resulting in the formation of radicals which undergo numerous spontaneous reactions. These, in turn, lead to various bond cleavages including aromatic ring fission (Shoemaker and Leisola, 1990; Tuor et al, 1992; Akthar et al., 1997; Zapanta and Tien, 1997). Several isoforms of each enzyme are secreted simultaneously by the fungus at different stages during lignin degradation. However, not all white rot fungi produce all LMEs.

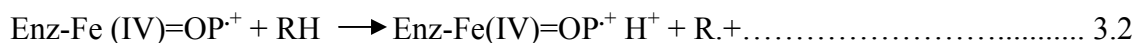
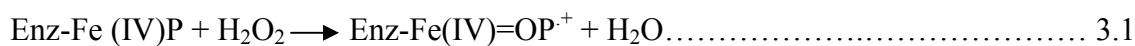
3.3.4 The Lignin-Modifying Enzymes (LMEs)

3.3.4.1 Lignin Peroxidase

LiP, originally designated as ligninase, was discovered for the first time in cultures of *Phanerochaete chrysosporium* (Tien and Kirks, 1983). Lignin peroxidase (LiP) is a monomeric N- and probably O-glycosylated protein expressed in several iso-forms. LiP contains one iron protoporphyrin IX as a prosthetic group and its molecular weight varies

from 38 and 47 kDa. The enzyme is acidic having an iso-electric point between 3 and 5 and its optimum pH which is unusually low is between 2.5 and 3(Cho et al., 2000). LiP requires H_2O_2 to be active.

Reactions catalyzed by LiP occur in cycles and often involve the oxidation of small molecular weight substrates such as veratryl alcohol to veratraldehyde where an aryl cation radical intermediate is generated as shown in equations 3.1 – 3.3 (Piontek et al., 2002; Boominathan and Reddy, 1992). The aryl cation radical is highly reactive and subsequently oxidizes lignin subunits.



LiP catalyzes several reactions such as oxidations in the alkyl side chains of lignin related compounds, C-C cleavages in the side chains of lignin subunits and oxidation of veratryl alcohol and related substances to aldehydes or ketones (Tien and Kirks, 1983). LiP can depolymerize dilute solutions of lignin in vitro, oxidize and depolymerize a variety of dimers and oligomers structurally related to lignin (de Jong et al., 1994; Spadaro and Renganathan 1994; Tien et al., 1986). Finally, LiP can catalyze the generation of oxygen activated species in the presence of veratryl alcohol (de Jong et al., 1994).

3.3.4.2 Manganese (II)-Dependent Peroxidase

Manganese (II)-dependent peroxidase (MnP) is a glycoprotein containing an iron protoporphyrin group IX as a prosthetic group and is also expressed in several iso-forms. Its molecular weight varies from 40 to 46 kDa. Its iso-electric point varies from 2.9 to 7.0 (Cho et al., 2000). MnP optimum pH varies from 3.5 to 5 for *T. versicolor*, (Nyman and Johansson, 1997). The catalytic cycle of MnP shown in Figure 3.2 is similar to that of LiP including native ferric enzyme as well as peroxidase compound I and II red-ox state. However, MnP requires Mn^{2+} as an electron donor which reduces compound I and II to complete the cycle. Mn^{3+} -chelator complexes mediate the oxidation of aromatic rings of lignin subunits once produced and chelated to organic acids such as oxalate.

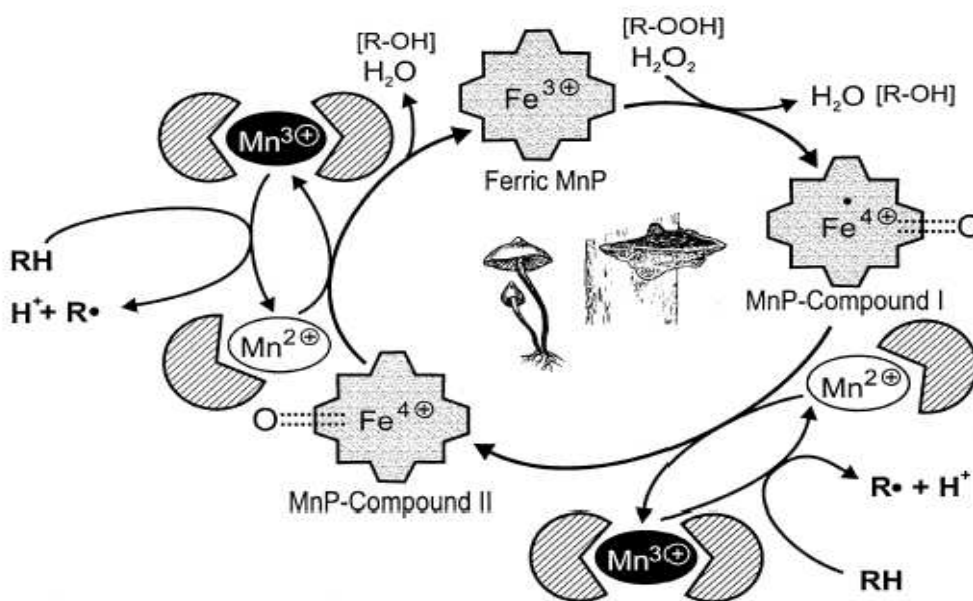


Figure 3.2: Catalytic Cycle of Mn (II)-Peroxidase (Hofrichter 2002)

Mn^{2+} preferentially reduces MnP compound II to the native enzyme and a variety of

phenols are capable of directly reducing the oxidized enzyme (Cho et al., 2000; de Jong et al., 1994; Tien and Kirks 1983). However, this reduction of MnP compound II by phenolic compounds is less efficient than with Mn^{2+} probably because the Fe-IV=O center of compound II is partially buried and not available to large organic substrates (Boominathan and Reddy 1992).

MnP is reported to generate H_2O_2 during oxidation of certain thiols such as glutathione. Recently investigations have revealed that the enzyme is capable of completing its catalytic cycle efficiently in the absence of external H_2O_2 by oxidizing organic acids such as oxalate and malonate in an "oxidase-like" auto-catalytic reactions involving the transient formation of several radical species (Hofrichter et al., 1998; Urzúa et al. 1998).

3.3.4.3 H_2O_2 -Generating Enzymes

H_2O_2 is required by peroxidases to complete their catalytic cycle. The following extracellular H_2O_2 -generating enzymes are secreted simultaneously with LMEs:

(1) Glucose-1 oxidase: This is a flavin adenine dinucleotide (FAD)-dependent oxidase located in the cytosol and secreted by fungal cells. During the enzymatic catalysis, glucose is initially oxidized to gluconolactone with the simultaneous reduction of FAD to $FADH_2$. Then, $FADH_2$ is oxidized back to FAD by oxygen and the latter is converted to H_2O_2 (Cho et al., 2000).

(2) Aryl alcohol oxidase: Aryl alcohol oxidase is an extracellular FAD-dependent enzyme with a molecular weight between 71 to 78 kDa. It is capable of oxidizing various

aromatic alcohol such as aryl α - and α - β -unsaturated, γ -aromatic alcohols. Aryl alcohol oxidase concomitantly reduces oxygen to H_2O_2 while oxidizing the various substrates (Cho et al., 2000).

(3) Cellobiose dehydrogenase (CDH): This enzyme is made up of an avin and heme domain with its molecular weight ranging from 81 to 97 kDa. The avin-containing domain was originally named cellobiose-quinone oxidoreductase produced by the proteolytic cleavage of cellobiose dehydrogenase. The enzyme catalyzes the 2-electron oxidation of cellobiose to 1,5-cellobionolactone as indicated in reaction 2.1 (Hilden et al., 2000; Roy et al., 1996).



Cellobiose dehydrogenase is also capable of catalyzing the reduction of a wide range of quinones such as 2,6-dichlorophenolindolphenol. Furthermore, CDH also oxidizes the reducing end groups of cello-dextrins and cellulose (Hilden et al., 2000; Roy et al., 1996). MnP and laccase cannot directly oxidize certain non-phenolic lignin structures efficiently and it is suspected that the bio-polymer is hydroxylated before breakdown (Hilden et al., 2000). Cellobiose dehydrogenase also oxidizes cello-dextrins and manno-dextrins to their corresponding lactones. Its exact biological role is not yet fully understood and it is suggested that it:

- prevents product inhibition during cellulose degradation by oxidizing cellobiose,

- contributes to hydroxyl radicals production through a Fenton-type reaction that can take part in lignin and polysaccharide degradation
- inhibits polymerization of cation radicals produced by LiP
- provides MnP with Mn^{2+} by reducing MnO_2 precipitates (Hilden et al., 2000).

The Fenton reaction as shown by reaction 3.5 plays a very important role in lignin degradation and it is dependent on the presence of sufficient amount of catalytic ferrous iron in the solution.



Cellobiose dehydrogenase sustains the Fenton reaction by producing continuously the unfavored Fe^{2+} which readily oxidizes to Fe^{3+} in aerobic environments (Bollag et al., 1999). H_2O_2 is produced by cellobiose dehydrogenase itself but can also be produced by other oxidases from the fungus. Hydroxyl radicals are very reactive and can hydroxylate both phenolic and non-phenolic lignin model compounds (Abadulla et al., 2001).

3.3.4.4 Laccase

Laccase is a multi-copper poly-phenol oxidase which can be a monomeric, dimeric or tetrameric glycoprotein. Its molecular weight varies from 55 to 390 kDa and usually contains four copper ions (Cu^{2+}) per monomer (Solomon et al., 1996). Laccase activity is highly dependent on Cu^{+2} which are distributed to 3 red-ox sites and defined according to their spectroscopic properties. The type 1 site contains the type blue copper (Cu1) whose

tight coordination to a cysteine is responsible for an intense absorption band around 600 nm, giving its blue color to the enzyme. The type 2 site contains a type 2 copper (Cu₂) with a characteristic electron paramagnetic resonance (EPR). In the T3 site, the pair of strongly coupled type 3 coppers (Cu_{3a} and Cu_{3b}) are EPR-silent in the presence of oxygen, indicative of a strongly antiferromagnetically coupled Cu pair bridged by a hydroxide.

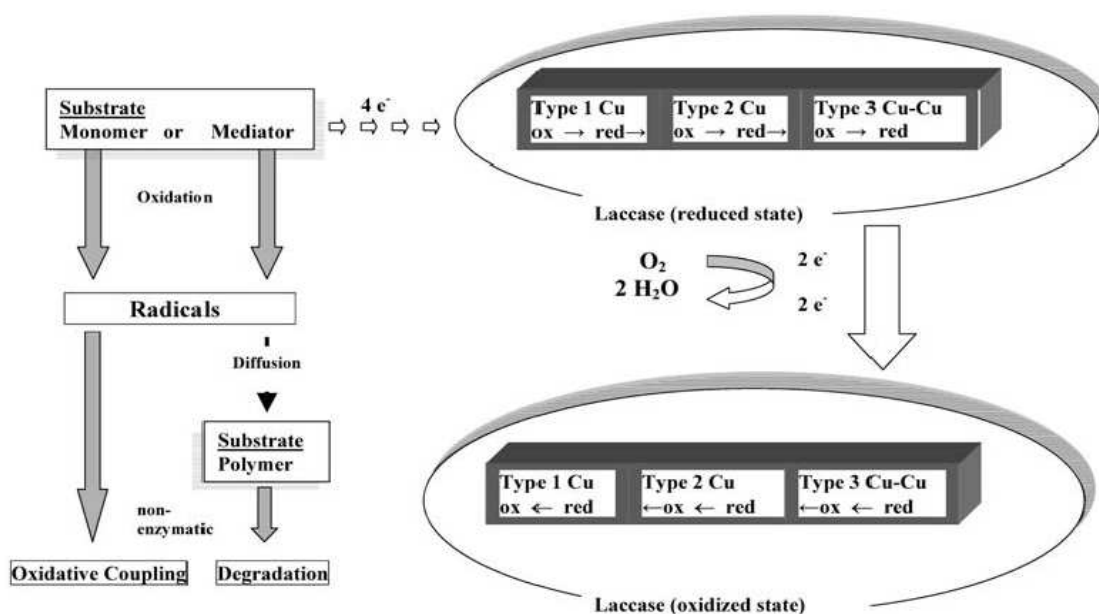


Figure 3.3: Catalytic Cycle of Laccase (Claus, 2003)

The mononuclear type 1 extracts electrons from the reducing substrate and mediates their transfer to the tri-nuclear type1/type3 cluster center where molecular oxygen is reduced as shown in Figure 3.3 (Solomon et al. 1996; Bertrant et al., 2002; Claus, 2003).

Laccase oxidizes preferentially phenolic lignin structures to phenoxy radicals which subsequently form quinones. This spontaneous rearrangement can also lead to the fission of carbon-carbon or

carbon-oxygen bonds inside the lignin phenyl-propane subunits resulting either in the degradation of both side chains (Fukuzumi, 1960) and aromatic rings (Kawai et al., 1988b), or in demethylation processes (Harkin and Obst 1974; Ander et al., 1983, Leonowicz et al., 1984 and 1991a). In this respect, laccase can co-operate with various FAD containing oxidases like glucose oxidase (Szklarz and Leonowicz 1986), veratryl alcohol oxidase (Marzullo et al., 1995), cellobiose:quinone oxidoreductase (Westermarck and Eriksson, 1975) and cellobiose dehydrogenase (Ayers et al., 1978). Laccase catalyzes the oxidation of various aromatic compounds, more specifically phenols and anilines, while reducing molecular oxygen to water at the same time. It removes a hydrogen atom by a one-electron abstraction from hydroxyl or amino groups of ortho- and para-substituted phenolic substrates to form free radicals capable of undergoing further de-polymerization, re-polymerization, de-methylation, or quinone formation (Abadulla et al., 2001). Although the actual role of laccase in lignin biodegradation is still under discussion and not completely understood, several authors have reported that laccase acts on the lignin polymer in ways different from ligninolytic peroxidases. In order to present the role of laccase more clearly several conceptions of biochemical routes in lignin biodegradation have been reviewed, especially with respect to the co-operation of laccase with other biocatalysts.

A summary of the properties of the key enzymes from *T. versicolor* (PRL572) (Nyman and Johansson 1997) and from *T. versicolor* (ATCC 20869) (Roy et al., 1996) is presented in Table 3.1. Properties such as the optimal pH and the iso-electric point (pI) may vary from one strain to another.

Table 3.1 : Properties of some Lignin-Modifying Enzymes of *T. versicolor* (¹Nyman and Johansson 1997; ²Roy et al., 1996)

	Enzymes MW (kDa)	Optimal pH	Isoelectric(pI)
Lignin peroxidase ¹	40.9 - 43.7	3.0	3.1 - 3.7
Mn(II)-dependent peroxidase ¹	44 - 45	4.5	3.2 - 3.9
Laccase ^{1,2}	67 - 70	4.6	n.a.
Mn(II)-independent peroxidase ²	40.1	3.2	n.a.
Cellobiose dehydrogenase ²	(1)81 (2)97	(1) 4.5-5.0 (2) n.a.	(1) 4.2 (2) 6.4

3.3.4.5 Low Molecular Weight Mediators

It has been established that LMEs are too big to enter the lignin matrix and it is suggested that the bio-polymer is broken down by small molecular weight metabolites produced by the enzymes. These activated metabolites can modify or oxidize lignin subunits rendering the bio-polymer more susceptible to the LMEs catalyzed reactions. Veratryl alcohol is an aryl alcohol produced by most white rot fungi. LiP binds veratryl alcohol and the latter is oxidized to a radical which can in turn oxidize other organic substrates of lower red-ox potential. During this coupled red-ox reaction, veratryl alcohol is converted to veratraldehyde. It is believe that the radical Veratryl alcohol can act as potential mediator in degradation of lignin but its short half life hampers this hypothesis. As mentioned previously, veratryl alcohol can protect LiP from inactivation by H₂O₂ (Cho et al, 2000; Kuan et al., 1993).

MnP produces manganic chelates by first oxidizing Mn²⁺ to Mn³⁺. Then it binds a chelator such as oxalate, tartrate and malonate to catalyze the generation of Mn³⁺-chelates. Once produced, these complexes can diffuse through the lignin matrix and oxidize phenolic subunits (Kuan et al., 1993; Nyman and Johansson 1997; Wariishi et al., 1992). MnP can also oxidize other metabolites to produce radicals. For example, Aken et al., (2000) reported the oxidation of glutathione by MnP

to its radical and the latter degraded 2-amino-4,6-dinitrotoluene. It was also found that MnP could bind larger molecules such as textile dyes however with a restricted affinity.

Tanaka et al. (1999) demonstrated that *T. versicolor* can produce a small molecular weight compound of approximately 5 - 8 kDa under specific growth conditions. It contains a heme-iron center which mediates the production of hydroxyl radicals. These radicals can thereafter modify compounds such as lignin subunits or other substrates. Thereafter, the hydroxylated compounds can be available to LMEs for breakdown.

3.4 Background Studies into Hydrogen Peroxide

Hydrogen peroxide is a strong oxidizing agent which is being used in recent times in the mining industry under specific conditions to enhance cyanidation in leach circuits and to detoxify cyanide in mine effluent prior to disposal. The peroxide assisted leach process has been developed to introduce oxygen necessary for cyanidation in a liquid form (Loresch et al., 1990). Hydrogen peroxide and calcium hydroxide are used to improve the extraction of gold/silver and reduce cyanide consumption in heap leaching and milling operations (Castrantas and Gurunathan, 1992). The efficacy of hydrogen peroxide as oxidant for gold dissolution during the cyanidation of pyritic ore has been evaluated (Stoychevski and Williams, 1993). Oxidation by Hydrogen peroxide was selected as the most appropriate route during an investigation into a means of detoxifying cyanide in tailings slurry from a gold mine (Castrantas et al., 1988; Kitis et al., 2005). Hydrogen peroxide assisted leaching led to significant increases in gold recoveries and reduced leach residence times with no cyanide attack by the hydrogen peroxide (Loresch et al., 1990). The peroxides maximized gold dissolution when added at the lower end of the pH range 9.5 – 11.5 such that an Eh range of -50 to 0 mV was maintained (Stoychevski and Williams, 1993). As a cyanide detoxifier, target reductions in cyanide levels to 50 mg/l or less were achieved with only 15% stoichiometric excess of hydrogen peroxide (Loresch et al., 1990) and gold and silver

values in the reclaim waters were not affected by the hydrogen peroxide treatment (Castrantas and Gurunathan, 1992)

The use of hydrogen peroxide as a first line reagent for pre-treating refractory ores has not been investigated much and thus little information on its abilities in this area of application. However, it has been used as a leaching agent for uranium ores and its use has been investigated with zinc-lead bulk sulfide concentrate as well as concentrates of chalcopyrite, sphalerite and pyrite.

Hydrogen peroxide had been used during an investigation into the effectiveness of selected acids assisted pressure oxidation on the cyanide extractability of gold from a double refractory concentrate. Results of the investigation indicated that hydrogen peroxide after 12 hours contact with the concentrate at ambient temperature gave no detectable sulfur and carbon oxidation and cyanidation of residue indicated no change in the ore characteristic with respect to its leach ability and preg-robbing activity compared with the untreated concentrate.

Hydrogen peroxide assisted pressure oxidation at pH of 6.5 resulted in much lower sulfur and carbon oxidation compared to the acids. It however, gave pre-treated residues with decreased levels of preg-robbing activities and higher gold recoveries during subsequent cyanidation, thus indicating some deactivation of the preg-robbing carbon during pre-treatment with hydrogen peroxide (Nyavor and Egiebor, 1992).

The most recent study of hydrogen peroxide has been for the dissolution of pyrite in sulfuric acid (Antonijević, et al., 1997). Stirring speed, temperature, particle size, concentration of hydrogen peroxide and sulfuric acid were the parameters studied. It was found that the leaching rate of pyrite decreases with increasing stirring speed and sulfuric acid concentration while addition of hydrogen ions enhanced the pyrite dissolution.

3.5 Biochemistry and Mechanism of Sulfide Biooxidation

A. thiooxidans, *A. ferrooxidans* and *L. ferrooxidans* are the main bacteria used to catalyze oxidation of gold bearing sulfide ores. *T. ferrooxidans* and *L. ferrooxidans* oxidize ferrous iron to ferric while *A. thiooxidans* specializes in the oxidation of sulfides to sulfate. *A. ferrooxidans* is also known to oxidize sulfur to some extent (Hackl, 1997; Rawlings, 1997).

The mechanisms and sequence of reactions occurring during bacterial sulfide oxidation are extremely complex and are not fully understood. There are various schools of thought on the mechanisms and sequence of reactions during bacterial oxidation due to its complexity. One prominent mechanism suggests that oxidation of iron and sulfur occurs in the cell envelope of the bacteria as shown in Figure 3.4 which illustrates the schematic representation of biochemical reactions that occur during Fe (II) oxidation by *T. ferrooxidans* (Hackl, 1997; Marsden and House, 1992).

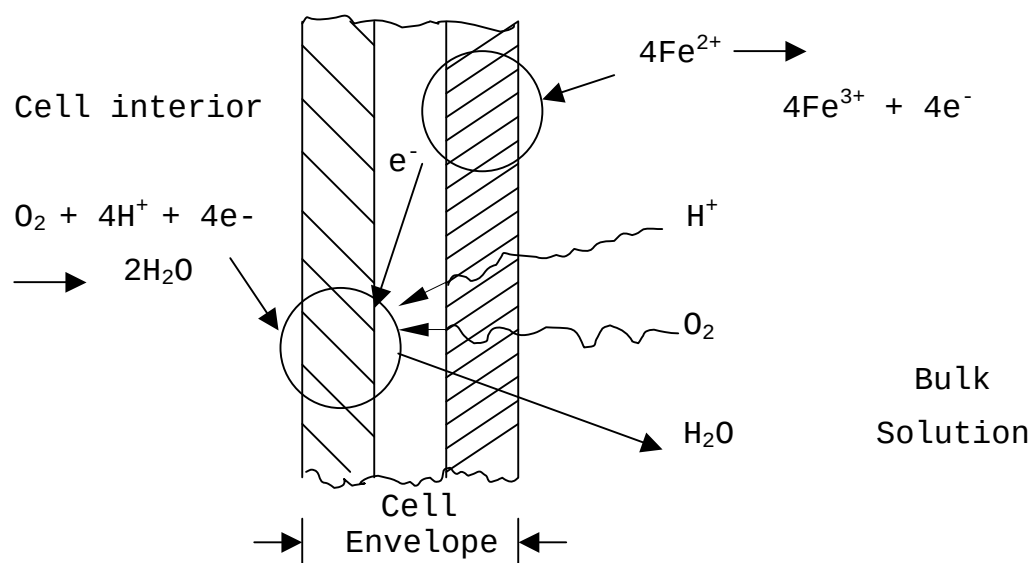
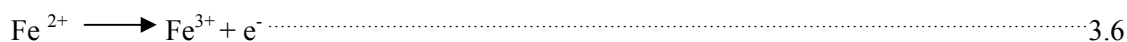


Figure 3.4 : Illustration of Biochemical Reactions occurring during Fe (II) Oxidation by *A. ferrooxidans* (after Murr, 1980).

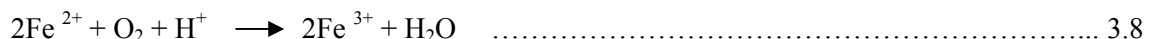
The bacterial cell envelope contains three zones. The inner layer cell envelope consists of a cytoplasmic membrane bordering the interior cytoplasm. The central zone consists of a rigid peptidoglycan layer and the periplasmic space. The outer layer consists of lipopolysaccharide and lipoprotein. Ferrous ions give up their electrons in the outer layer and are oxidized to Fe (III) according to the anodic half-cell reaction:



The electrons migrate through the central zone to react with oxygen and hydrogen ions in the inner layer. This reaction produces water, which migrates out as represented by the half-reaction:



The overall reaction for the oxidation of ferrous iron is:

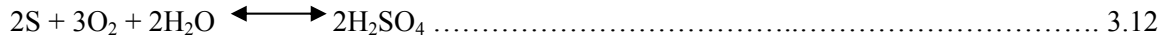
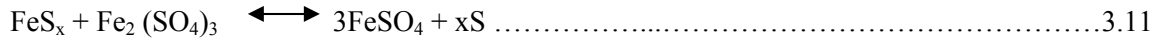
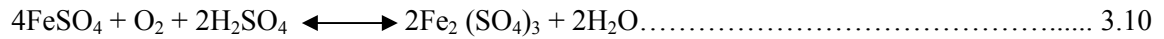
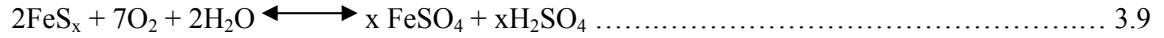


The standard free energy of formation for reaction 3.15, is $\Delta G^\circ_f = -88 \text{ kJ/mol}$, hence theoretically the reaction should proceed spontaneously to the right. However, due to slow kinetics, the reaction does not proceed at a significant rate in the absence of bacteria. According to Lacey and Lawson (1970), oxidation is increased by a factor of 500,000 times in the presence of bacteria.

Oxidation of sulfide sulfur proceeds in a manner similar to that of iron (II). The sulfide is converted to sulfur by the loss of two electrons. The sulfur is then polymerized, and the

polymeric sulfur is oxidized to thiosulfate, sulfite and ultimately to sulfate (Lundgren and Tano, 1978).

For a typical generic iron sulfide mineral, FeS_x in acidic media, the oxidation reactions are given as follows (Marsden and House, 1992).



Investigations have shown that reactions 3.9 and 3.11 are mainly chemical reactions and can occur with little or no bacterial involvement. Reactions 3.10 and 3.12 do not proceed without bacteria under mild conditions. The conversion of sulfur to sulfate as evidenced in reaction 3.12 is a very important reaction since sulfur, if not oxidized will build up on the surface of gold and other minerals, passivating them and preventing further oxidation reactions.

Ferric ion is an oxidizing agent and the bacterial oxidation of ferrous to ferric introduces an agent for indirect oxidation of other constituents. In addition, the ferric-ferrous couple dictates the redox potential and its value can be deduced from the Nernst equation:

$$E_h = E_h^o - \frac{RT}{nF} \ln \left(\frac{a(\text{Fe}^{3+})}{a(\text{Fe}^{2+})} \right) \dots\dots\dots 3.13$$

where,

E_h is the measured potential for the reaction on the hydrogen scale

E_h^0 is the standard Fe^{3+}/Fe^{2+} reduction potential on the hydrogen scale (0.77)

R is the gas constant, 8.314 joules K^{-1} mole $^{-1}$

T is absolute temperature

n is the moles of electrons transferred per mole of reactant

F is Faraday's constant, which is approximately 965000 C mole $^{-1}$

$a(Fe^{3+})$ and $a(Fe^{2+})$ are the activities of Fe^{3+} and Fe^{2+} respectively

At 25°C equation 3.11 may be simplified as:

$$E_h = 0.77 + 0.059 \log \left(\frac{a(Fe^{3+})}{a(Fe^{2+})} \right) \dots\dots\dots 3.14$$

From equation 3.11, it can be deduced that when the $[Fe^{3+}]$ to $[Fe^{2+}]$ ratio for a given system is high, an equally high potential will be generated leading to very high oxidizing conditions.

Reactions that do not take place without bacterial intervention are known as direct reactions while those that can take place as chemical reactions are referred to as indirect. In direct leaching, there is bacterial attachment to the surface of the sulfur or sulfide particle, followed by direct oxidation of the sulfur or sulfide with oxygen serving as the oxidant. Bacteria adhere to and form bonds with the surface of mineral particles through the formation of an extracellular, sticky polymer called glycocalyx. This bond, commonly called a 'slime layer' is very stable and can only be broken by vigorous mechanical treatment. Hackl (1997) presented some of the simplified direct leaching reactions of sulfur, pyrite and arsenopyrite as:



In indirect reactions, the role of bacteria is to oxidize the products Fe (II), As (III) and sulfur generated as shown in equations 3.16 to 3.21.



In addition to direct and indirect leaching, bacterial leaching is also believed to proceed through galvanic conversions (Lawrence, 1990; Morin et al., 1995). Since sulfide minerals are electrically conductive, when two sulfide minerals with different rest potentials come into physical contact, a galvanic cell is created with the more noble mineral acting as the cathode. For example, in a system containing pyrite and arsenopyrite, the less noble, arsenopyrite acts as the anode and is preferentially oxidized.

3.6 The Chemistry of Preg-robbing by Carbonaceous Matter

The components of carbonaceous matter come in varying proportions and thus it may not always behave like any of the individual constituents. Various studies has reported that carbonaceous

matter is 'similar' to activated carbon in structure but the former does not always behave like activated carbon. For example, the equilibrium adsorption isotherms for gold cyanide on activated carbon for temperatures up to 80°C show that increasing temperature has a negative effect on gold adsorption, a fact that has been utilized in designing elution circuit systems (McDougal, 1988).

Work done by Zaitseva et al. (1973), within a similar temperature range gave a contrary result regarding carbonaceous matter. These investigators are of the view that the humic acid component has some functional groups that are responsible for the different behavior from that of graphitic carbon. Humic acid was confirmed to be the dictating factor in the interaction of carbonaceous matter with aurocyanide.

3.6.1 Preg-robbing Chemistry of Individual Constituents of Carbonaceous Matter

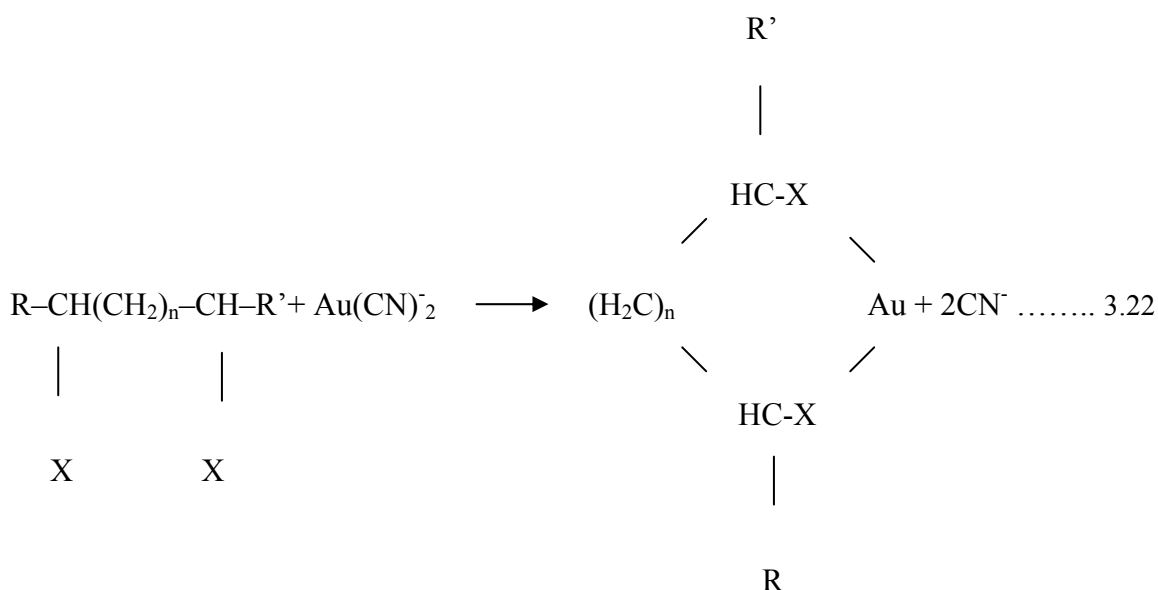
Humic acid, elemental carbon and hydrocarbons are the main constituents of carbonaceous matter in refractory gold ores. Each of these constituents plays different roles in preg-robbing and as such demonstrates different chemistry and mechanisms. The preg-robbing behavior of carbonaceous matter is thus discussed in relation to studies conducted on the individual components.

(1)Humic Acid

Humic acid is capable of adsorbing the aurocyanide complex. Zaitseva et al. (1973), in an infrared spectroscopic investigation of the interaction between humic acids extracted from carbonaceous gold ores with aurocyanide solutions, revealed that two new gold adsorption bands were formed on the product while one disappeared. The 1720 cm^{-1} peak for the original carboxyl

group disappeared while the new bands at 1580 and 1400 cm^{-1} showed the presence of carboxylate-ion. These new adsorption bands were considered to give evidence of the coordination of Na (AuCN)_2 with the carboxyl and phenolic groups in humic acid. The formula $\text{NaAu (CN)}_2\text{RX}$ was thus proposed where X represents COO^- or CO^- . This suggests that the gold cyanide ion does not dissociate when adsorbed onto humic acid (Zaitseva et al., 1973).

Studies indicated that when 5 g of humic acid, derived from lignite, was introduced into 100 g of non carbonaceous ore, gold extraction decreased from 95% to 21% (Radtke and Scheiner, 1970). Osseo-Asare et al. (1984) developed the following reaction for the interaction of humic acid and aurocyanide solutions based on the work of Radtke and Scheiner (1970):



where X represents any ligand capable of reacting with Au (I), R is the general skeletal structure of humic acid and may include the carboxyl group (COOH), R' equals R or H and n equals 0 or 1 (Radtke and Scheiner, 1970; Osseo Asare et al., 1984).

(2)Elemental Carbon Fraction

Many researchers are of the view that the mechanism and chemistry of adsorption is similar for both elemental and activated carbons. Prominent among them are Stenebraten et al. (1999, 2000) and Schmitz et al. (2001) who investigated naturally occurring elemental carbon and suggested that this material is chemically equivalent to commercial activated carbon activated at temperatures above 750°C. The conventionally accepted mechanisms for the adsorption of gold onto activated carbon are: (1) reduction of aurocyanide complex to metallic gold, (2) adsorption of the complex as an ion-pair, (3) precipitation of insoluble Au(CN) as a result of chemical degradation of the aurocyanide complex (McDougall and Hancock, 1981).

Solvation is the specific interaction that is responsible for gold cyanide adsorption (Cho and Pitt, 1979). Their deduction was based on the relatively large size of the aurocyanide complex, which renders it hydrophobic. Data supporting their assertion indicated that adsorption of anions by activated carbon increases with ionic size. However, the basis of their assertion was later refuted by McDougal and Hancock (1981) who found that the addition of a relatively large ion ClO_4^- , had little effect on gold adsorption when compared with Cl^- and CN^- ions.

A chemisorption mechanism involving a surface reduction of Au (I) was consequently proposed (McDougall and Hancock, 1981). These researchers found that the adsorbed gold had an oxidation state of 0.3, using X-ray photoelectron spectroscopic analysis. The nature of these species were unknown as their oxidation state value lies between zero (the

oxidation state for metallic gold) and unity (the oxidation state of gold in AuCN and AuCN₂⁻). The authors have suggested a similarity between these species and other gold cluster compounds.

Recent studies indicate that there is no chemical change in the aurocyanide complex after adsorption onto activated carbon (McDougal et al., 1987; Adams et al., 1987; Klauber, 1991; Sibrell and Miller, 1992; Ibrado and Fuerstenau, 1995). These authors agree that the major mechanism of gold adsorption is aurocyanide adsorption as an ion-pair. Adsorption is also thought to take place along the edges of microcrystallites (Miller and Sibrell, 1991; Sibrell and Miller, 1992) or along the basal planes of the graphitic sheets (Klauber, 1991; Ibrado and Fuerstenau, 1995).

Various studies into the surface charges of elemental carbon have been done. One test by Osseo-Asare et al., (1984) revealed that the surfaces are negatively charged in the range $2 \leq \text{pH} \leq 12$ during investigations on the electrophoretic mobility of the graphitic/amorphous carbon found in carbonaceous matter. This finding is contrary to the normal behavior of negatively charged surfaces and calls for explanation on how a negatively charged carbon surface could attract and adsorb another negatively charged ion, (AuCN)₂⁻. In spite these contradictions, Osseo-Asare et al., (1984) were of the view that since the two surfaces are negatively charged any factor that reduces the electrostatic repulsion should increase the gold uptake. The surface chemistry of the material is controlled by oxygen functional groups such as carboxylic (R-COOH), phenolic

hydroxyl (R–OH) and quinine-type carbonyl (R–C=O) (Donnet, 1968; Puri, 1970; Mattson and Mark, 1971; Snoeyink and Weber, 1972; Shergold and Hartley, 1982).

(3) Hydrocarbons

Hydrocarbon fractions in carbonaceous matter have been extracted and analyzed by many researchers. Based on the functional groups detected by infrared analysis, the fraction is known to contain long chain alkanes. In addition, C=C bonds, and cyclic CH₃ and CH₂ scissoring vibration frequencies were also detected (Radtke and Scheiner, 1970; Zaitseva, 1973; Afenya, 1976; Osseo-Asare et al., 1984).

The hydrocarbon fraction is always extracted into a liquid phase; therefore preg-robbing determination has not been possible. However, it is known that the hydrocarbon fraction, when present, acts as a film around the activated carbon fraction and its presence reduces the preg-robbing behavior of carbonaceous matter (Radtke and Scheiner, 1970).

3.6.2 Biochemistry of Coal Degradation by *Streptomyces setonii*

A major physical limitation on microbial activity on coal is its impermeability as extensive microbial degradation requires access to internal surfaces (Faison, 1992). Coal is relatively porous, but pore diameter of many, between 0.04 to 30 µm, is too small to allow microbial access (Bale et al., 1984). Low rank coals have more of the larger diameter pores than the other coals, though there is no clear correlation between rank and porosity, according to Valkovi (1983).

Some authors have asserted that due to the structure of coal, a nonenzymatic and hence extracellular mechanism is necessary for its degradation (Fakoussa, 1981, 1988; Scott et al., 1986; Cohen et al., 1990; Fakoussa and Hofrichter, 1999). Lignite, a low rank coal is seen as dehydrated lignin and can be degraded only by a non-specific extracellular attack (Durie et al., 1960; Hayatsu et al., 1979; Hatcher, 1990).

Fakoussa (1981) and Cohen and Gabriele (1982) reported the ability of microorganisms to alter the structure of coal metabolically in the early 1980s. Various theories have been put forward to explain the bacterial degradation of coal. However, enzymatic and nonenzymatic mechanisms are the two main schools of thought. Laccase and peroxidase are the predominant enzymes involved in the enzymatic mechanism. Nonenzymatic mechanisms include among others alkali solubilization and chelation (Bollag and Leonowicz, 1984; Pyne et al., 1987; Cohen et al., 1987; Kawai et al., 1988; Gupta et al., 1988; Strandberg and Lewis, 1987; Wariishi et al., 1989; Kersten et al., 1990)

Strandberg and Lewis (1987, 1988a, 1988b), Quigley and Dugan (1989) and Quigley et al. (1987, 1988a, 1988b, 1989a, 1989b) proposed the prominent theories in support of coal degradation by *S. setonii*. In an investigation by Strandberg and Lewis (1987, 1988b), it was found that the *S. setonii* secretes an extracellular component that was capable of solubilizing coals. The extracellular component was heat stable and retained over 60% of its activity after the broth was heated for 1 hour at 121°C. The cell-free active components were found not to be proteins and this suggests that coal solubilization by *S. setonii* is nonenzymatic. A substantial loss in the physical integrity of coal particles

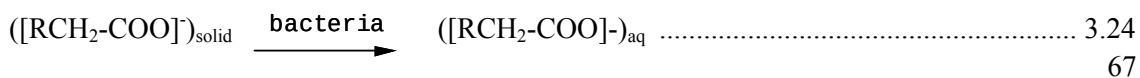
was also reported. In the study, the pH of the active culture broth was between 8 and 10 and the molecular weight of the extracellular component was found to be between 1,000 and 10,000. These findings suggest that the active components could be basic polypeptides or polyamines (Strandberg and Lewis, 1987, 1988b). The liquid fractions obtained were referred to as “soluble coal products”.

Quigley et al. (1987, 1988a, 1989a) asserted that, alkaline substances like; biogenic amines and similar compounds are involved in microbial solubilization of coal. These alkaline components can solubilize acidic groups in coal by deprotonation, which enhances the hydrophilicity of coal. Further studies by the same authors (Quigley et al., 1988b, 1989b) showed that the presence of multivalent cations such as Ca^{2+} , Fe^{3+} and Al^{3+} influence coal solubilization by serving as bridging elements between carboxylate groups.

Amankwah (2005) proposed the following reaction mechanism for the biodegradation of lignite by *S. setonii*. The initial neutralization reaction, at the coal surface, which led to a drop in pH immediately after samples are introduced into the culture, may be represented as:



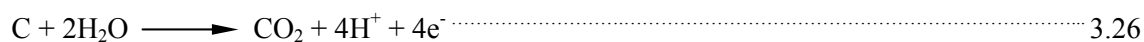
where R represent variable groups in the coal structure. This deprotonation reaction makes lignite more hydrophilic and after the neutralization reaction, solid coal particles may be solubilized according to equation 8.6.



Part of the dissolved components may be utilized directly by the bacterium *S. setonii* and further degradation of the solubilized material may proceed as:



Bacterial metabolic activities resulted in solubilized products with lower carbon contents and higher oxygen containing groups than the parent samples. At the elemental level, the proposed redox pair for the aerobic metabolism of carbon is given by:



Overall redox reaction:



3.7 Aqueous Chemistry of Gold

Gold is a noble metal and is not easily affected by water, acids or oxygen. Principally, gold forms two series of compounds with oxidation states +1, aurous, and +3, auric. These simple aqueous ions do not occur free at practically useful concentrations and any gold ions found in solution are always in the form of complexes. Though it is possible to dissolve metallic gold in aqueous solution using powerful oxidants such as ozone, the process is quite hazardous. In addition the dissolved gold is stable only if the potential of the system remains above the reduction potential

of gold ions, a situation that requires significant excess of the reagent at all times (Xue and Osseo-Asare, 1985; Nicol et al., 1987).

The measured reduction potential is a function of concentration of gold ions, thus any decrease in their activity would lead to lowering of the potential as predicted by Nernst's equation. For the equation:



Nernst's equation is given by:

$$E = E_o - \frac{RT}{nF} \ln \frac{C^m D^w}{A^x B^y} \dots\dots\dots 3.30$$

where,

E_o is the standard reduction potential for the reaction on the hydrogen scale

R is the gas constant, $8.314 \text{ joules K}^{-1} \text{ mole}^{-1}$

T is absolute temperature

n is the number of electrons

F is Faraday's constant, which is approximately $965000 \text{ C mole}^{-1}$

The driving force for the precipitation of a metal from a solution of its ions can be expressed quantitatively in terms of the reduction potential. Therefore the reaction of a metal, M , in contact with a solution of its ions, M^{n+} , is given by:



and from Nernst's equation, the reduction potential, E, at equilibrium is given by:

$$E = E_o - \frac{RT}{nF} \ln \frac{[M]}{[M^{n+}]} \dots\dots\dots 3.32$$

Since the activity of a pure solid is unity, Nernst's equation becomes:

$$E = E_o + \frac{RT}{nF} \ln [M^{n+}] \dots\dots\dots 3.33$$

With the exception of the metal ion concentration, $[M^{n+}]$, all the other parameters are constants and therefore the value of E depends to a large extent on the metal ion concentration. If the metal M is gold, then the reduction potential of aurous and auric ions is given by:

$$E_1 = 1.73 + 0.0591 \log [Au^{+}] \dots\dots\dots 3.28$$

$$E_2 = 1.50 + 0.0197 \log [Au^{3+}] \dots\dots\dots 3.34$$

Figure 3.5 shows the potential-pH diagram for the gold water system at 25°C. It indicates the regions of predominant stability of various gold compounds and the conditions under which the gold compounds indicated could be transformed.

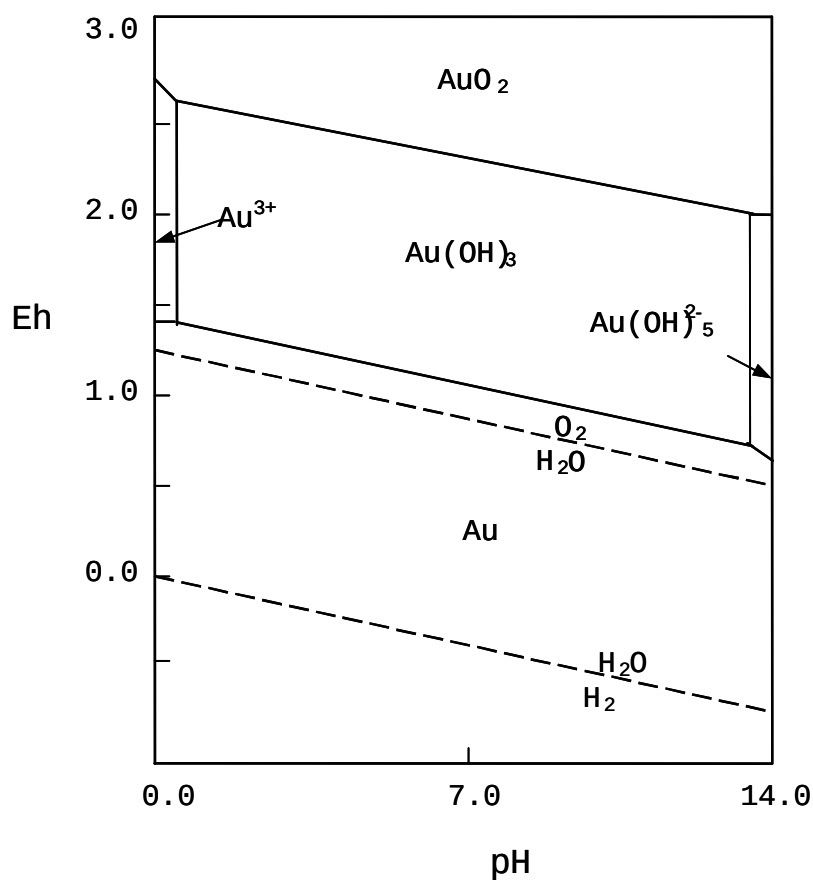


Figure 3.5 : Potential-pH Diagram for the Au-H₂O System at 25°C with 10⁻³ Kmol/m³ Gold Concentration (after Xue and Osseo-Asare, 1985).

Gold occurs in the stability region of water as an insoluble metallic substance. The potentials E_1 and E_2 in equations 3.28 and 3.29 should be decreased by reducing the concentration of free gold ions to achieve dissolution of gold. In an oxidizing environment containing a suitable complexing agent, gold will dissolve and the free metal ion concentration will be reduced by complex ion formation hence lowering the values of E_1 and E_2 (Finkelstein, 1972; Xue and Osseo-Asare, 1985; Nicol et al., 1987).

Presently, cyanide is the most extensively used complexing reagent for extracting gold from ores. The wide acceptance of cyanidation for gold processing is due to its effectiveness in treating a wide range of ores at a relatively low cost compared with other leaching reagents. Other factors include the high stability of the gold cyanide complex, higher capacity and lower corrosive nature (McArthur et al., 1889; Davis and Tran, 1991).

Environmental concerns and the desire for faster leaching kinetics in recent times have led to the consideration of other leaching reagents. The emerging leaching reagents that have the potential to replace cyanide include among others thiourea, $(\text{CS}(\text{NH}_2)_2)$; thiosulfate, $(\text{S}_2\text{O}_3^{2-})$; chlorine, (Cl_2) ; bromine, (Br_2) and malononitrile, $(\text{CH}_2(\text{CN})_2)$. The stability of the various species is shown in Table 3.2 (Groenwald, T., 1976; McGrew and Murphy, 1985; Hiskey and Atluri, 1988; Xia and Yen, 2003; Arima et al., 2003; Yen et al., 2003)

Table 3.2 : Stability Constants of some Gold Compounds

Complex	Au (I)	Complex	Au (III)
$\text{Au}(\text{CN})_2^-$	2.0×10^{38}	$\text{Au}(\text{CN})_4^-$	1.0×10^{56} to 1.0×10^{80}
$\text{Au}(\text{S}_2\text{O}_3)_2^{3-}$	5.0×10^{28}		
AuI_2^-	4.0×10^{19}	AuI_4^-	5.0×10^{47}
$\text{Au}(\text{SCN})_2^-$	1.3×10^{17}	$\text{Au}(\text{SCN})_4^-$	1.0×10^{32}
AuCl_2^-	1.0×10^9	AuCl_4^-	1.0×10^{26}

3.7.1 Cyanide Leaching

Cyanide leaching of gold has been demonstrated to be driven by electrochemical reactions (Habashi, 1966; Nicol, 1980; Xue and Osseo-Asare, 1985). The process involves anodic dissolution of gold with reduction of oxygen at the cathode. Nicol (1980) gave the reactions as:

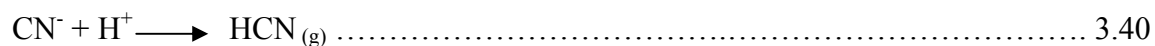


It has also been deduced that the anodic reaction proceeds via an intermediate product which further reacts with cyanide to give the aurocyanide ion as given in equations 3.32 and 3.33



The rather slow kinetics of gold dissolution has been attributed to the solid intermediate product, AuCN, which coats gold particles and passivates their surfaces. Cyanidation is

carried out in alkaline media because the presence of hydrogen ions or even water causes cyanide to hydrolyze according to equations:



Hydrogen cyanide (HCN) gas generation is highly undesirable since it is very toxic and also leads to a reduction in the quantity of cyanide available for leaching. The acceptable pH for effective cyanidation is between 10.5 and 11 and it can be adjusted using lime or caustic soda. Lime is usually used because it is cheaper and also serves as a viscosity modifier (Finkelstein, 1972; Brunk and Atwood, 1987; Brooy et al., 1994). The Au-H₂O-CN system in Figure 3.6 shows that the gold cyanide complex lies in the stability region of water.

The kinetics of gold dissolution is influenced by the size of gold particles, dissolved oxygen levels, cyanide concentration, temperature and the quality of water used. Others are agitation, time, pulp density and the formation of gold coatings and compounds (Finkelstein, 1972; Yannopoulos, 1991)

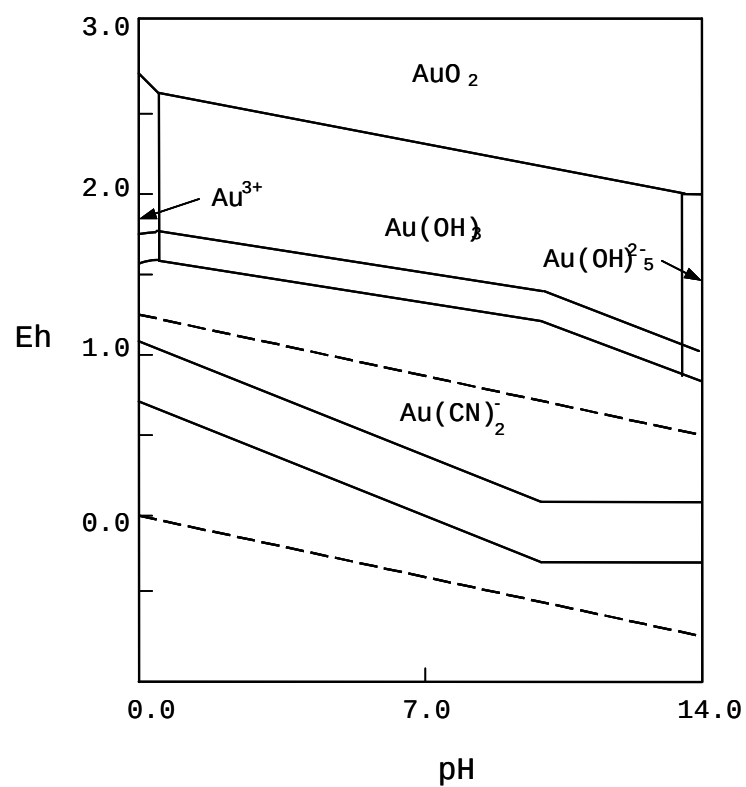


Figure 3.6: Potential-pH Equilibrium Diagram for the System Au-H₂O-CN⁻ at 25°C. Concentration of all Soluble Gold Species is 10⁻⁴M (After Xue and Osseo-Asare, 1985).

Chapter 4

Materials and Methodology

4.1 Samples

Double refractory gold ore samples, named A and B were sourced from two gold processing plants. Sample A is an ore sample while B is a flotation concentrate and had been milled to 90% passing 75 μm . During the studies, Sample A was crushed and milled to 90% passing 75 μm . Sample A was used for all the investigations while Samples B was used to study effect of ore type on fungal action.

For the studies into the fungus' ability to degrade sulfide sulfur, relatively pure pyrite and arsenopyrite were obtained from Ward's Natural Science Company. The purity of pyrite and arsenopyrite were about 99% and 95% respectively. The major impurities in arsenopyrite were quartz and pyrite while that of pyrite was quartz. Samples were pulverized to 90% passing 75 μm .

Lignite, bituminous coal and anthracite were used in investigating the carbon degrading ability of the fungus. These samples were also obtained from Ward's Natural Minerals Company. The carbon, sulfur and ash content of the coal samples are presented in Table 4.1.

Table 4.1: Carbon and Sulfur Content of Sulfide Minerals and Coal Samples Used

Sample	Carbon , %	Sulfur , %
Pyrite	0.41	54.10
Arsenopyrite	5.67	12.20
Lignite	62.8	0.75
Bituminous coal	85.5	0.67
Anthracite	99.44	0.56

4.2 Characterization of Ore Samples

4.2.1 Mineralogical Study

A Philips X'Pert Pro Multi Purpose Diffractometer was employed to determine the main mineral constituents of the ore samples. The X-ray diffraction pattern of sample A shows the presence of pyrite, quartz and muscovite and dolomite (Appendix A). Traces of kaolinite were also detected. The relative concentrations of the minerals in increasing order was dolomite < kaolinite < pyrite < muscovite < quartz. The X-Ray diffraction pattern of the biooxidation product still showed pyrite peaks just as chemical analysis indicated due to incomplete sulfur oxidation. The same major phases present in the untreated ore showed up in the biooxidized product. However, no peaks were detected for carbon (dolomite) though chemical analysis indicated their presence; this may be due to loss of crystallinity after microbial contact.

The major phases present in sample B were quartz, pyrite, arsenopyrite, dolomite, gypsum and augite. Traces of Pyrrhotite, albite, rutile and muscovite were also detected. But for arsenopyrite, all the peaks in the untreated sample showed up in the treated sample. The diffraction patterns are presented in Appendix A.

4.2.2 Chemical Analyses

The major elemental constituents in samples A and B and their bioleach products were determined using several techniques. Conventional fire assaying followed by atomic absorption spectroscopy was used to determine the grade of gold. Sulfur and carbon contents were determined by the combustion volumetric method using a LECO titrator SC-444DR.

For all others, 1- 2 grams of each ore sample was digested with aqua-regia and the resulting solutions filtered into a 25 - 50 ml volumetric flask and topped up with distilled water. Metal concentrations were determined by atomic absorption spectroscopy (AAS) using a Perkin Elmer Spectrophotometer model 3300. Shown in Table 4.2 are the results of the chemical analyses of the samples.

Table 4.2: Results of Chemical Analyses of Samples

Component	Sample A	Sample B
Gold, g/t	2.33	65.75
Silver, g/t	Trace	8.00
Iron, %	3.19	14.98
Copper, %	0.006	0.065
Total sulfur, %	3.80	8.95
Elemental carbon, %	0.592	5.28
Total Carbon, %	0.63	6.84

4.2.3 Demineralization and Identification of Functional Groups

Ore samples were demineralized to concentrate carbonaceous matter, reduce interference from other minerals and also enhance the resolution of spectroscopic analysis. Demineralization involves the digestion of samples with hydrochloric acid in the first stage followed by

hydrofluoric acid and boric acid in the second stage as described by Tafuri (1987) and Stenebraten et al. (1999).

Infrared analysis was used to determine the functional groups present on the carbonaceous matter in gold ores and their microbial degradation products. Samples were prepared by mixing with Potassium bromide in a ratio of 1:150 and analyzed using the Bomem MB-120 spectrometer. The infra red patterns of the biotreated product were then compared with the as received samples.

4.3 Microorganisms Used for the Studies

The amenability of the various sulfide, coal and ore samples to microbial sulfide and carbon degradation was initially investigated using the white-rot fungus *Trametes versicolor* (ATCC 20869). Further investigations into sample A's amenability to microbial sulfide degradation was done utilizing the known chemolithotrophic bacteria *Acidithiobacillus thiooxidans* (ATCC 15494), *Acidithiobacillus ferrooxidans* (ATCC 19859), *Leptospirillum ferrooxidans* (ATCC 53992). These bacteria are well known to oxidize sulfides and their enumeration and morphological features have been observed and documented (Hackl, 1997; Rawlings, 1997). The bacterium *Streptomyces setonii* (ATCC 39116) was also used to study sample A's carbon degradation amenability in combination with the abiotic pre-treatment step.

4.3.1 Maintenance of White-rot Fungus

The fungus, *T. versicolor* (ATCC 20869) was maintained in an incubator at 30°C on plates containing Kirk's medium, 1.5 % agar and 3 % malt extract. Several agar plugs of the actively growing fungus were used to inoculate 500 ml of Kirk's medium in 1000 ml Erlenmeyer flasks. The medium contained 10.1 g/l glucose, 1.2 g/l ammonium tartrate, 0.05 g/l MgSO₄ .7H₂O, 0.01

g/l $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1 mg/l thiamine, 1 ml/l of trace minerals (Kirk et al., 1978). The medium was also supplemented 2.92 g/l 2, 2-dimethylsuccinate to serve as pH buffer. The trace mineral solution contained 30 g/l $\text{MnSO}_4 \cdot 7\text{H}_2\text{O}$, 10 g/l NaCl, 5g/l $\text{MgSO}_4 \cdot \text{H}_2\text{O}$, 1 g/l CoSO_4 , 1 g/l $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1 g/l $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.82 g/l CaCl_2 , 0.1g/l $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.1 g/l $\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$, 0.1 g/l H_3BO_3 and 1 g/l EDTA. The shake-flask cultures were grown at 30 °C with continuous agitation (180 rpm) for 10 – 14 days.



Figure 4. 1: Maintenance of *T. versicolor* on Agar Plates in an Incubator, Fisher Isotemp® Dual CO₂ incubator Model 413D.

4.3.2 Maintenance of Chemolithotrophic Bacteria

Frozen bacteria cultures were initially recovered at room temperature, vortexed and transferred aseptically into fresh medium containing 0.5g/l of $(\text{NH}_4)_2\text{SO}_4$, K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/l KCl, 0.01 g/l CaNO_3 , 15.0 g/l $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.0 g/l sulfur and 0.25 ml/l of Wolfe's solution. Culture transfer into fresh medium was done every 4 - 6 weeks. Cultures for biooxidation experiments were prepared by transferring 10% (v/v) of the maintenance culture into a fresh

growth medium containing 0.5g/l of $(\text{NH}_4)_2\text{SO}_4$, K_2HPO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/l KCl and 0.01 g/l CaNO_3 .

4.3.3 Growth and Maintenance of *S. setonii*

Spores of the bacterium *S. setonii* in freeze-dried state were received in vial from the American Type Culture Collection (ATTC). The spores were suspended in 5 mls of sterilized growth medium aseptically in a laminar-flow hood and used to inoculate transferred into culture medium in a 250 ml Erlenmeyer flask and incubated on an orbital shaker at 180 rpm at ambient conditions. Fresh cultures are prepared by periodic transfer of 10% (v/v) of a liquid culture into fresh growth medium containing 5 g/l tryptone and 3 g/l yeast extract, supplemented with 0.5 g/l KCl, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g/l $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and K_2HPO_4 as inorganic nutrients. Cultures for degradation of coal samples and carbonaceous matter in gold ores had a similar composition.



Figure 4.2: Microbial Cultures in Contact with Samples in Erlenmeyer Flasks mounted on an Orbital Shaker, Barnstead Max^Q.

4.4 Fungal Sulfide and Carbonaceous Matter Degradation Studies

The pure sulfide mineral (pyrite, arsenopyrite, pyrrhotite) and coal samples (lignite, bituminous coal and anthracite) of varying maturity were contacted with 90 mls of freshly grown fungal culture in Erlenmeyer flasks for 2 weeks and flask is agitated at 180 rpm. Temperature was maintained at 28 - 30°C and the pulp density was 10% solids. The test was conducted at the unadjusted sample-fungal culture pH, 3.0, 4.5, 7.0 and 10.5.

Sodium hydroxide (10N) and hydrochloric acid (6M) were the pH modifying agents used.

The microbial pre-treatment products were thoroughly washed with water and dried at 60 - 70°C.

A similar test was conducted using the ore samples to investigate simultaneous carbon and sulfide sulfur degradation by *T. versicolor*. Both sterilized and non-sterilized samples were used in the microbial-mineral interactions and the differences in results were not significant. Duplicate experiments were run and the differences in the values were within 1-3%. Other evaluation techniques employed included mass changes of samples, infrared analysis, gold sorption before and after microbial contact and visual observations. For the gold sorption tests, gold adsorption by commercial grade activated carbon was used as a standard.

4.4.1 Establishing Process Conditions for Optimal Fungal Action

To establish process conditions for optimal fungal action on sulfides and carbonaceous matter, various process variables such pH, retention time, pulp density and temperature were investigated using sample A since sulfide and carbon degradation trends for the

pure sulfide and coal samples were similar to sample A. Process optimization studies were conducted at pulp density of 10% to 50% solids, 3 to 28 days retention time, temperature of 23 to 45°C at pH range of 3.5 to 10.5. Tests were conducted in 250mls Erlenmeyer flasks and agitated at 180rpm. The fungal culture composition remains the same as the preliminary investigation.

4.4.2 Process Conditions Optimization

Sample A was solely used during the process optimizing studies. Pulp density, temperature, pH and contact time were the variables considered. Two types of microbial pre-treatment process were investigated to optimize gold extraction during cyanidation. These are the single stage and the two-step processes.

During the single stage process (known as biotic), 75 to 120 g of sample was contacted with the fungal culture in 2000 liters Erlenmeyer flasks at alkaline pH conditions at the predetermined process variables. The flask was agitated continuously on an orbital shaker at 180 rpm. The initial pH of 4.5 to 5.0 was adjusted to between 9.5 and 10.5 and maintained daily with 10 M sodium hydroxide solution. For the two step process, ore was first contacted with the culture medium (known as abiotic) in 2000 liters Erlenmeyer flasks for 2 weeks at a pulp density of 20% and temperature of 45°C at alkaline conditions. The product of the abiotic leaching was filtered and repulped to 20% solids with the fungal culture in Erlenmeyer flasks and agitated continuously on an orbital shaker at 180 rpm for further 7 days. Daily monitored pH for the second step was

between 4.5 and 6. The sequence of the two-step process was reversed to study the effects of the sequence order.

At the end of the predetermined contact time, the pre-treated ore sample was filtered; solids washed several times with water to get rid of the alkali and dried. Duplicate experiments were run and the difference in values was within 1-3%.

4.4.3 Fungal – Bacteria Pre-treatment Process Sequences

Fungal-chemolithotrophic bacterial, abiotic – bacterial (*S. setonii*) and chemolithotrophic bacterial- *S. setonii* sequence of pre-treatment were also investigated using sample A. For the chemolithotrophic bacterial-fungal sequence, the ore was first contacted with the chemolithotrophic bacteria at 30 to 45°C to destroy the sulfides and the biooxidized product served as feed for the passivating step by the fungus. These sequences were also reversed to investigate the effect of the sequence order.

During the abiotic-bacterial process, the 100 to 120 g of ore sample is first contacted with the fungus culture medium at 45°C, 20% pulp density and 2 weeks retention time. The oxidized product for the abiotic step is used as feed for the carbon degrading step by *Streptomyces setonii* at 45°C for 2 weeks.

For the bacterial step, 75 to 80 g portion of the samples were weighed into 1000 mls Erlenmeyer flasks containing culture of *S. setonii* to form a pulp density of 20% solids, which were tested for degradation of carbonaceous matter. The culture medium was prepared with tryptone and yeast extract, supplemented with 0.05% KCl, MgSO₄.7H₂O,

0.01% $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ and K_2HPO_4 as inorganic nutrients. At the end of the predetermined processing time, the ore samples were filtered, washed with water and dried. Details of pre-treatment results can be found in Appendices B, C and D.

4.5 Studies into Culture Alkaline Medium pH

The effect of the alkaline medium on the oxidation of sulfide and gold sorption was investigated for Sample A using the culture medium alone at the unadjusted pH, water adjusted to alkaline pH (9.5 – 10.5) and the cultural medium adjusted to alkaline pH (9.5 – 10.5) The studies were conducted at 30°C, 20% pulp densities and retention time of 2 weeks. At the end of the predetermined processing time, the ore samples were filtered, washed with water and dried. The pre-treated samples were then subjected to gold sorption test and cyanidation.

4.6 Fungal Viability Studies at Various pH

The viability of the fungus at pH 3.0, 4.5, 7.0, 9.5 and 11.0 was studied. Variable pH test were conducted on sample A by contacting with the fungal culture at various pH mentioned above for 2 weeks, 20% solids and temperatures of 30°C.

After the 2 weeks contact time, 0.1 - 0.3 mls of the product pulp were placed on agar plates and incubated at 30°C for 10 to 14 days. Agar plates were then physically inspected for growths of white patches of fungus.

4.7 Fungal Biomass Quantification

The fungal biomass in a known volume of culture is settled by gravity and the solid-free liquid medium decanted. The biomass is pooled and vigorously shaken to ensure homogeneity. 10 mls of the homogeneous biomass is sampled and solids are recovered by centrifugation at 10,000 rpm for 10 to 15 minutes. The recovered solids were put on a pre-weighed aluminum dish and dried in an oven at 70 - 100°C for 24 hours. The fungal biomass is determined by difference in weight.

4.8 Studies into Fungal Medium Constituents

Carbon source and nitrogen are the major medium component responsible for cell growth and activity. The impact of varying these components on cell biomass was investigated by varying the amount of glucose and ammonium tartrate added to the culture medium. Glucose-ammonium tartrate ratios (w/w) of 1:1, 1:2 and 2:1 were studied and the corresponding biomass determined. The effect of these cultures' composition on pre-robbing and gold extraction was also investigated.

4.9 Post Microbial Pre-treatment Investigations

The product of microbial treated ore samples was filtered washed with water and dried in an oven at 70 to 100°C for 24 hours after the pre-treatment process. Dry weight of the ore sample before and after microbial contact were noted to monitor changes in weight as a result of microbial action. Percentage reduction in carbonaceous matter and sulfur was determined quantitatively using the Leco volumetric combustion method. Filtrate of

microbial treated samples was also analyzed for gold and residual thiosulfate. Details of thiosulfate analytical method can be found in Appendix G.

4.9.1 Digestion of Microorganisms' Biomass

The influence of microbial biomass (organic carbon) on carbon analysis was eliminated by dissolution of the biomass from the pre-treated ore sample prior to analysis. Biomass digestion was done for 30 minutes using 5 - 6 % hypochlorite as described by Berger et al. (1989) and Ramsay et al. (1990); the samples were then thoroughly rinsed with distilled water.

4.9.2 Evaluation of Gold Extraction after Microbial Contact

Preg-robbing and cyanidation tests were used to evaluate the gold extraction properties of products obtained from the microbial pre-treatment processes. All pre-treated samples used in these experiments were washed with water and dried before evaluation.

Preg-robbing tests were conducted on both pre-treated and untreated ore samples. 3 g samples were placed in 27 mls solution of potassium gold cyanide containing 5.0 mg Au/l in 50-mls flasks. The pH was kept at 10.5 and free cyanide concentration of 0.5 g/l. Gold dissolution was not observed in the course of the preg-robbing tests. The samples were agitated at 75 rpm for 24 hours. The ore was then separated from the solution by filtration and the final solution gold determined using an atomic absorption

spectrophotometer (AAS). The difference in gold concentration before and after the solution-ore contact is the gold preg-robbing percentage.

Cyanide leaching was used to determine the effect of fungal pre-treatment on gold extraction. Cyanidation was conducted on 50 to 100 g sample after both carbonaceous matter degradation/passivation and sulfide oxidation. Samples were leached at a pulp density of 33% by weight for 24 hours at pH of 10.5 - 11.5. The pH was adjusted using industrial grade calcium hydroxide (lime) and cyanide strength was 1.0 g/l. The dissolved gold concentration was determined using atomic absorption and the solid residual gold was determined by conventional fire assay method followed by atomic absorption analysis. Residual cyanide and lime after cyanidation were determined by titration method. Details of cyanidation results can be found in Appendix E and sample calculation of gold extraction in Appendix F.

Chapter 5

Results and Discussion

5.1 Preliminary Studies

5.1.1 Fungal Action on Sulfides

The ability of *T. versicolor* to degrade sulfide sulfur was investigated using the pure sulfide minerals, pyrite and arsenopyrite. The pulverized sulfide mineral was contacted with the fungal medium at various pH of 3.0, 4.5, 7, 9.5 and the unadjusted fungal medium-ore pH(4.25). Other process conditions were 10 % solids, 2 weeks retention time and temperatures of 28 - 30°C.

Preliminary results indicated that sulfur oxidation was insignificant for both arsenopyrite and pyrite for the fungus at its natural pH conditions. However at alkaline conditions there was an improvement in sulfide oxidation to 11.3% and 1.64% respectively for pyrite and arsenopyrite. Acidic conditions recorded 1.43% for pyrite and insignificant for arsenopyrite. Sulfur oxidation recorded for the unadjusted pH conditions for pyrite and arsenopyrite averaged 5.7 and 6.6% respectively. In decreasing order of sulfur oxidation, we have the alkaline medium > acidic > neutral > unadjusted pH (control). This means that enzymatic secretions of *T. versicolor* at its optimum pH state cannot oxidize sulfur significantly while the alkaline medium pH of 9.5 -10.5 enhanced sulfur oxidation. This preliminary finding led to the adoption of alkaline medium for the research.

5.1.2 Fungal Action on Carbonaceous Matter

Lignin, bituminous coal and anthracite were the coal rock used to investigate the carbon degrading abilities *T. versicolor* since these classes of coal represent the different ranks or maturity of carbon in coal minerals which are similar to those found in carbonaceous

matter in gold ores. The pulverized coal samples were contacted with the fungal medium at the same conditions as the sulfide minerals discussed.

Results showed that carbonaceous matter was not degraded for all the three types of coal minerals studied but rather there was an increase in the carbon content which was due to the fungal biomass content. However, upon digestion of the fungal biomass with sodium hypochlorite, the carbon assayed for the pre-treated samples were almost the same as the as received sample. This means the *T. versicolor* did not degrade carbonaceous matter irrespective of the level of maturity of the coal sample and the pH conditions.

5.1.3 Simultaneous Sulfur and Carbon Degradation Studies

The ability of *T. versicolor* to simultaneously degrades sulfides and carbonaceous matter was studied using ore sample A under the same process conditions as the pure sulfides and coal samples. Sulfur oxidations trends for the various pH conditions investigated followed the same trend as the pure sulfide minerals. 35.6% sulfur oxidation was achieved for the alkaline medium followed by acidic, neutral and natural system pH with 18.3, 6.1% and 1.2% respectively; Carbon degradation also followed a similar trend as the coal samples. Generally increases in carbon contents were observed. There was a remarkable 96.3 % increase in the bioresidue carbon of the unadjusted pH due to the favorable pH of 5.5 which favored fungal growth. However, carbon values of the pre-treated samples dropped to values closer to the untreated ores (0.63%) upon fungal biomass dissolution.

5.1.4 Preg-robbing Studies on Pre-treated Samples

Pre-treated samples of the coal samples and ore sample A were subjected to gold sorption studies. Results of the studies indicated a decrease in gold sorption for bituminous coal and anthracite while lignite showed as an upward trend. Bituminous coal gave the best results with gold sorption dropping from 40.3% for the untreated sample to a low of 8.3% for the neutral pH conditions. Anthracite under alkaline condition dropped from 99.7 to 27.7%. Results for Sample A indicated a decrease for all the various pH conditions studied with values ranging from 0.4% for neutral pH to 2.3% for alkaline pH.

Generally, pre-treated samples recorded dips in gold sorption but for lignite as indicated in Table 5.1. In spite of the stable values of carbon content after pre-treatment, gold sorption values dropped significantly indicating passivation of the carbonaceous matter after microbial pre-treatment.

Table 5.1: Gold Sorption Data for Coal Samples and Sample A.

Sample	Gold Sorption, % at various pH				
	Untreated	Acidic (3.0)	Neutral (7.0)	Alkaline (10.5)	Unadjusted (4.2)
Lignite	33.5	59.8	35.8	40.1	56.9
Bituminous Coal	40.3	9.4	8.3	13.1	11.1
Anthracite	99.5	30.7	79.1	27.7	85.9
Ore Sample A	18.1	1.00	0.4	2.3	1.4

5.1.5 Analysis of Infra red Patterns after Fungal Contact

Untreated and pre-treated samples A were subjected to infra-red analysis and their diffraction patterns compared. The peaks assignments, on the untreated samples and the pre-treated samples were deduced by comparing with earlier works by Rao (1963), Silverstein et al (1981) and Amankwah (2005).

Amankwah et al. (2004) reported that lignite have more oxygen containing groups such as the -C=O and -COOH groups than bituminous coal and anthracite according to infra red analysis. Significant difference in their surface characteristics in terms of functional groups and exposed ion was also observed. Summarized in Table 5.2 are the characteristic peaks of the various components present in untreated lignite, bituminous coal and anthracite. Peaks of various carbon components present in Sample A are also shown in Table 5.3. The OH peak is generally attributed to the presence of water while CH_3 are the aliphatic hydrogen groups. Amankwah (2005) reported that oxidation or biodegradation of carbonaceous matter resulted in the formation of more oxygen containing groups (C=O) with the disappearance of aliphatic hydrogen groups (CH_2 and CH_3).

Table 5.2: Peaks of Carbon Components in Untreated Coal Samples (Amankwah, 2004)

Coal Sample	Peaks Wave number, cm^{-1}				
	C=O	CH_2	CH_3	OH	C=C
Lignite	1650	-	2850 – 2980	3000 – 3800	-
Bituminous Coal	1620	1460	2980	-	-
Anthracite	-	1460	-	-	1580

Table 5.3: Peaks of Carbon Components Present in Sample A

Pre-treatment	Peaks Wave number, cm ⁻¹				
	C=O	CH ₂	CH ₃	OH	COOH
Untreated	1618-1658(5)	-	-	3426(1)	1092(2)
Abiotic(pH 10.5)	1638- 1655(2)	-	-	3439(1)	1165-1091(2)
Biotic(Natural)	-	-	2895(1)	3253-3439(2)	1096(1)
Biotic(pH 10.5)	1636(1)	-	2544(1)	-	1089-1170(2)

Numbers of peaks are indicated in bracket

Comparing the untreated sample's peak components with the coal samples it could be deduced that the level of carbonaceous matter maturity in sample A is between lignite and bituminous coal as none of the peaks in anthracite was detected. Comparing the microbial treated samples with the untreated, the product of the abiotic process showed similar peaks as the untreated except that there was reduction in the C=O peaks from 5 to 2 and the formation of another COOH group. This indicated that not much change has taken place in the carbonaceous matter components. For the biotic pre-treatment at unadjusted pH conditions, no C=O peak was detected but rather the formation of an aliphatic group and a reduction in the COOH group. For the biotic process under alkaline conditions, only one C=O group was detected while an aliphatic group formed. The graphs of sample A infra red diffraction patterns can be found in Appendix A.

Analysing the data, biodegradation of the carbonaceous matter was not observed as no new oxygen groups were formed after the *T. versicolor* contact. However, the pre-robbering activity of the biotreated samples decreased significantly; thus confirming some

surface passivating phenomenon rather than degradation. Carbon values after biotreatment indicated no significant changes as well. Both the fungal culture and the medium alone have carbon passivating property.

5.2 Studies into Effects of Various Process Variables on Fungal Action

Pulp density, pH, retention time and temperature effects on fungal degradation of sulfur and carbonaceous matter were investigated after the preliminary investigation with the aim of establishing feasible microbial pre-treatment conditions for refractory gold ores and concentrates.

Sample A was used for the studies and was contacted with the fungal culture in a single stage (biotic) pre-treatment process. A control test with only the culture medium (abiotic) was also run for the various process variables. *T. versicolor* thrives optimally at temperature ranges of 28 – 35°C at pH range of conditions of 3.5 to 5.5.

5.2.1 Effect of Pulp Density

Sulfide sulfur oxidation was studied at various pulp densities between 5% and 50% solids for the biotic process at fixed alkaline pH of 9.5 -10.5 and temperature of 30 °C for 2 weeks. The changes in sulfide oxidation with increasing pulp density were not very significant; all were in the range 48% to 44% as density increased from 5% to 50%. This indicates that pulp density is not a very sensitive variable in the biotreatment of sulfides using *T. versicolor*. This finding is of major significance to plant operation as throughput could be maximized without compromising on sulfur oxidation and gold recovery.

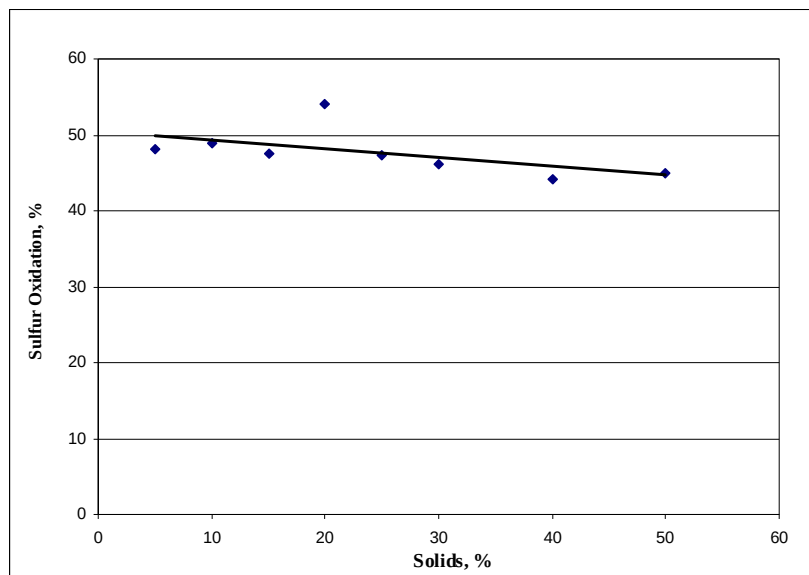


Figure 5.1: The Effect of % Solids on Sulfur Oxidation after *T. versicolor* contact. (30°C; pH of 10.5; 2 weeks retention time; shaker Speed of 180 rpm)

Fluctuations in carbon values were observed over the pulp density range studied. Gold sorption was remarkably low at 0.7% at 5% solids. It was equally low for all pulp densities used and even after pre-treatment at 50% solids gold sorption was 6.5%. Increasing preg-robbing with percent solids might be due to high attrition rate and thereby hindering effective passivation of the particles' surface by the fungal metabolites which include among others hydrogen peroxide. It was observed that preg-robbing values do not correlate with unoxidized carbonaceous matter in the ore sample which confirms surface passivation rather than degradation.

5.2.2 Effect of pH

The effect of pH was investigated for pHs 3.5, 4.5 and 10.5 for both abiotic and biotic process for 2 weeks at a temperature of 30°C. Sulfide oxidation for both processes followed the same trend but values for the abiotic were statistically higher than the biotic.

The alkaline pH gave the overall best sulfide degradation followed by 4.5 and 3.5 in that order. The lower sulfur oxidation by biotic process may be due to coating of sulfide mineral surfaces by

the metabolites produced by *T. versicolor*. The higher oxidation at the alkaline pH conditions may be attributed to the presence of hydroxyl ions in solution.

No significant carbon degradation was observed after pre-treatment but generally carbon values of the pre-treated samples were higher than the untreated ore due to the introduction of organic carbon by the fungal biomass and culture medium. However, preg-robbing trends were clear. Generally decrease in gold sorption for the biotic process was better than the abiotic. This indicates that there is microbial involvement in the carbon passivation process. The drop in gold sorption for the abiotic process indicated that the culture medium has some passivating abilities which need to be investigated during future studies.

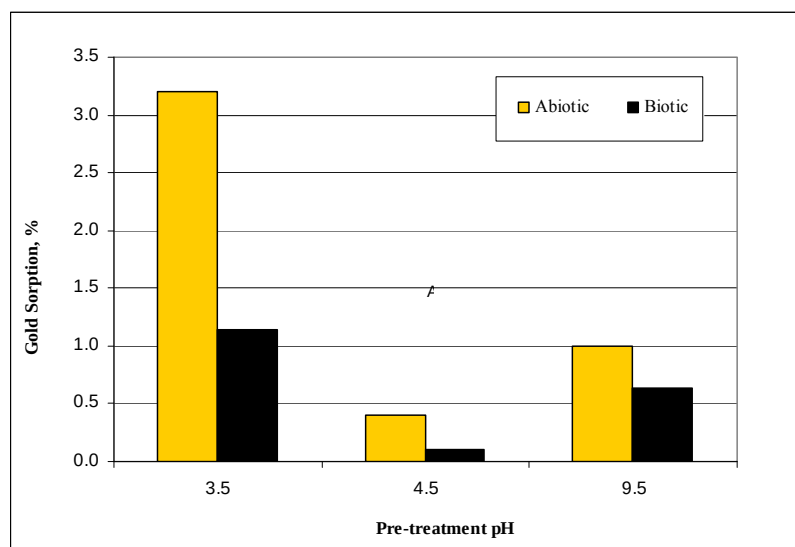


Figure 5.2: The Effect of pH on Gold Sorption after *T. versicolor* contact. (30°C; 10% Solids; 2 weeks retention time; shaker speed of 180 rpm)

5.2.3 Effect of Contact Time

Sulfur oxidation increased with retention time (Figure 5.3). About 45% sulfur oxidation was achieved after 2 weeks and 73% after 9 weeks during the preliminary studies. The rate of increase

however, slowed down as the retention time increased. Since a batch culture was used, the decrease in sulfur oxidation rate can be attributed to reduction in microbial activity due to exhaustion of growth medium. Though investigations did not continue after 9 weeks, it is expected that sulfur oxidation will continue for some time before stabilizing.

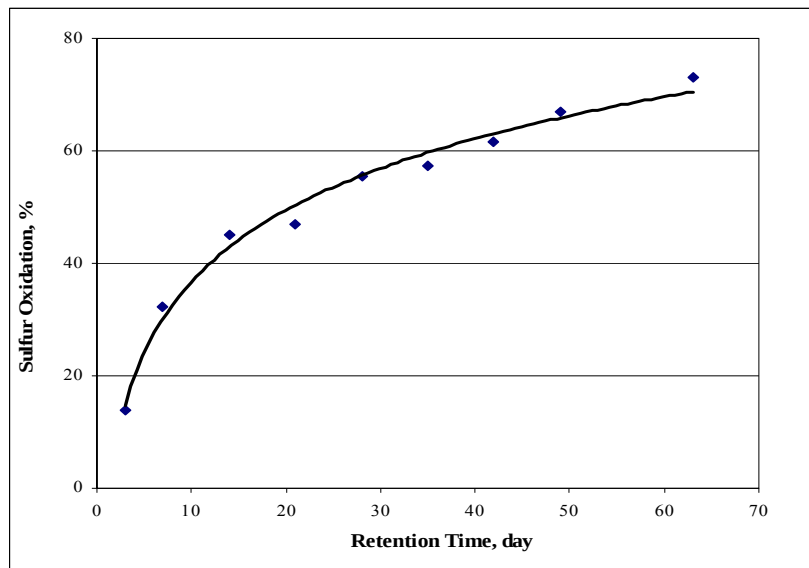


Figure 5.3: The Effect of Retention time on Sulfur Oxidation after *T. versicolor* contact (Sample A; 30°C; pH of 10.5; 10% solids; shaker speed of 180 rpm)

Passivation of carbonaceous matter was higher at shorter contact times. As the contact time increased, passivation decreased and the ability of the biotreated material to preg-rob increased as shown in Figure 5.4. Since passivation is a surface phenomenon, it is possible that the continuous agitation and subsequent increase in attrition lead to a reduction in the passivating effect of the fungal metabolites over the period of time studied.

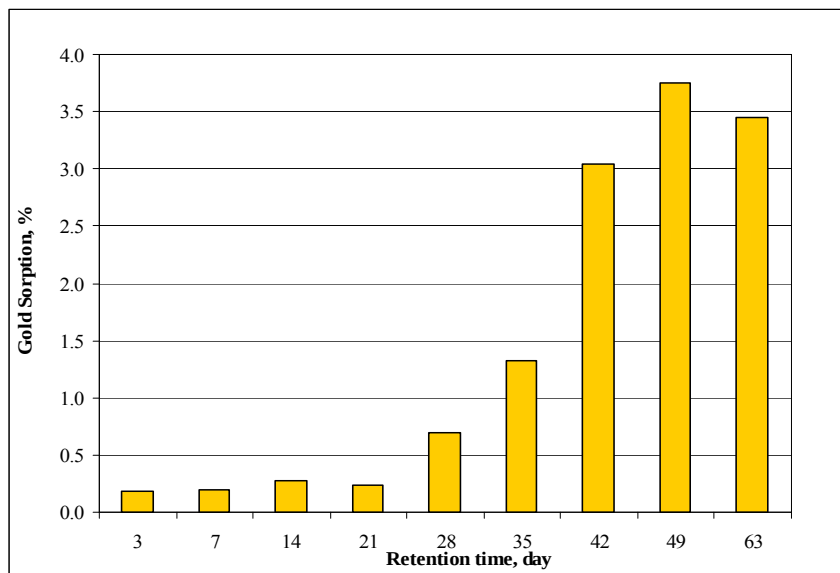


Figure 5.4: The Effect of retention Time on Gold Sorption after *T. versicolor* Contact
(Sample A; 30°C; pH of 10.5; 10% solids; shaker speed of 180 rpm)

5.2.4 Effect of Temperature

Temperature effect on sulfur oxidation was studied at alkaline pH for the biotic and abiotic processes at ambient temperature (25°C), 30 and 45°C. Sulfur oxidation tends to increase with temperature as shown in Figure 5.5. 76.7% sulfide oxidation was achieved for abiotic process at 45°C as against 44.7% and 50.0% at 26°C and 30°C respectively. Biotic results achieved 42.7%, 45.8% and 73.4% sulfide oxidation at room temperature, 30°C and 45°C respectively. The increasing sulfur oxidation trend can be attributed to improved kinetics at higher temperatures. These results were pivotal in investigating the two step process of abiotic at 45°C followed by biotic at 30°C. Some degree of carbon degradation was observed at 45°C which is likely due to breaking of carbon bonds as temperatures increase above 30°C.

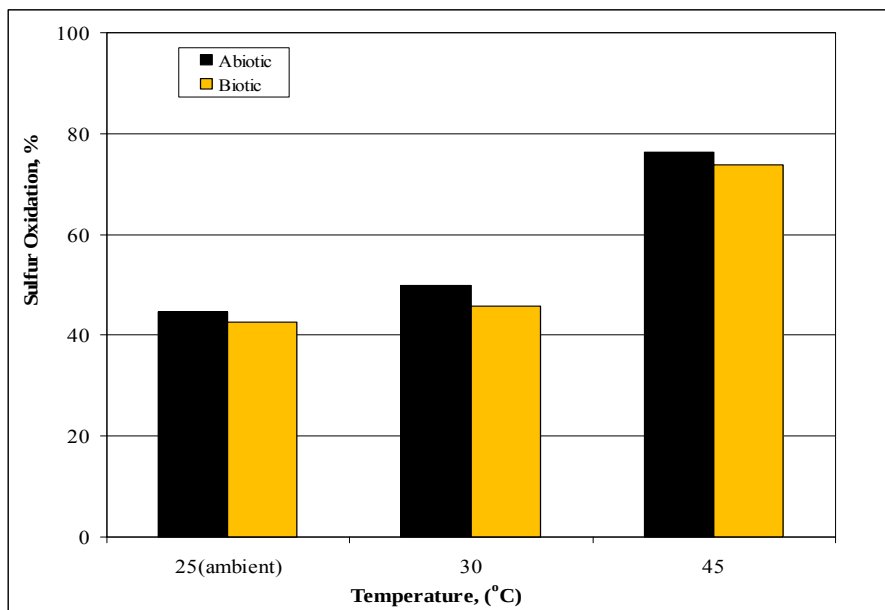


Figure 5.5: The Effect of Temperature on Sulfur Oxidation after *T. versicolor* Contact (Sample A; pH of 10.5; 2 week retention time; 10% solids; shaker speed of 180 rpm)

5.2.5 Deductions from Process Conditions Optimization

It could be deduced from the trends of the process variables investigated that alkaline conditions at temperature range of 30 to 45°C and 3 – 4 weeks retention time were feasible conditions for optimal microbial pre-treatment. Pulp density did not impact much on sulfur oxidation; however, 20% solids which gave a slightly higher sulfur oxidation was adopted for most of the latter test conditions.

5.3 Pre-treatment Process Optimization

Samples A was solely used during the pre-treatment process optimizing studies. Conditions established during the process optimization studies were applied. Two types of microbial pre-treatment were investigated during this stage of the research to optimize sulfur oxidation and

enhance gold extraction during cyanidation. These are the single stage process and the two-step process.

5.3.1 The Single Stage Process

The single stage samples are microbially treated at alkaline conditions to simultaneously oxidize sulfur and passivate carbonaceous carbon. The single stage process was studied at alkaline pH, 20% pulp density, retention time of 3 to 28 days and temperatures of 25°C (ambient) to 43°C. Pre-treated samples were then subjected to gold leaching by cyanidation.

Observed sulfur oxidation and gold sorption trends were similar to those of the process optimization studies. However, sulfur oxidation figures were slightly higher and gold sorption figures were generally below 1.0%. Sulfur oxidation achieved after 28 days of single stage pre-treatment was 79.2% as against 76.2% for 3 weeks at 30°C (Table 5.4). As a result of improved kinetics at 45°C, % sulfur oxidation of 82.7 and 83.1% were recorded after 21 and 28 days retention time respectively. It could be deduced that 3 - 4 weeks of single stage pre-treatment is enough to achieve significant sulfur oxidation for sample A. 3 to 7 days retention time is enough to decrease preg-robbing from 18.1% to 0.18% for sample A.

Table 5.4: Single Stage Sulfur Oxidation and Gold Sorption at 30°C for Sample A

Retention time (days)	Sulfur Oxidation, %	Gold Sorption, %
3	21.8	0.18
7	30.5	0.22
14	55.8	0.27
21	76.2	0.23
28	79.2	0.69

When the single stage process was applied to sample B, a flotation concentrate, 34.19% and 52.85% sulfur oxidation were achieved at 30 and 45°C respectively after 3 weeks retention.

5.3.2 The Two-step Pre-treatment Process

The two-step pre-treatment process involves an abiotic process and biotic process. Generally, sulfur oxidation for the abiotic process was better than the biotic and the reduction in preg-robbing for the biotic process was better than the abiotic at the various temperatures studied. Thus the two stage process was studied to take advantage of the best conditions for sulfur oxidation and gold sorption reduction. Thus, the aim of this process is to oxidize the sulfur during the abiotic step and passivate the carbonaceous matter during the biotic step. Generally sulfur oxidation for sample A was slightly higher for the two step process (Table 5.5) and gold sorption remained below 1.0%. This pre-treatment option was studied at 30°C - 45 °C at 20% pulp density under alkaline conditions for 3 – 4 weeks retention time.

Table 5.5: Two-step Pre-treatment Process Sulfur Oxidation for Sample A

Pre-treatment Process	Sulfur Oxidation, %
1. Abiotic (2 weeks) at 30°C followed by Biotic (1week) at 30°C	62.3
2. Abiotic (2weeks) at 45°C followed by Biotic (1week) at 30°C	72.1
3. Abiotic at 45°C (2weeks) followed by Biotic (1week) at 45°C	75.1
4. Abiotic at 45°C (3wks) followed by Biotic(1week) at 30°C	82.3

The two-step pre-treatment scenario involving abiotic process at 45°C followed by biotic at 30°C gave the overall best sulfur oxidation of 82.3% for sample A after 4 weeks. Gold sorption remained below 1% for all the scenarios studied. When the best case scenario for the two-step pre-treatment process was applied to sample B, only 35.7% sulfur oxidation was recorded.

Reversal of the two-step process in the order, biotic followed by abiotic resulted in slightly lower sulfur oxidation compared with the abiotic followed by biotic at the

various temperatures (Table 5.6). This may be due to the coating of the sulfide mineral surfaces by metabolites generated by the fungus during the biotic step and thereby reducing access to sulfide minerals by thiosulfate formed during the abiotic process.

Table 5.6: Sulfur Oxidation for Reversed Two-step Pre-treatment Process for Sample A

Pre-treatment Process	Sulfur Oxidation, %
Biotic at 30°C (1 week) followed by Abiotic(2 weeks) at 30°C	57.40
Biotic at 30°C (1 week) followed by Abiotic(2 weeks) at 45°C	68.42
Biotic at 45°C (1week) followed by Abiotic (2 weeks) at 45°C	71.35
Biotic at 30°C (1week) followed by Abiotic (3 weeks) at 45°C	75.60

5.3.3 Fungal – Bacterial Pre-treatment process sequences

Various two step pre-treatment scenarios were studied using sample A. The sequence considered were abiotic followed by carbon degradation by *S. setonii*, sulfur degradation using chemolithotrophic bacteria followed by biotic process, chemolithotrophic bacteria followed by *S. setonii*. The aim is to investigate how gold extraction could be improved by combining the sulfur degrading abilities of the abiotic process or the well known chemolithotrophic bacterial with carbonaceous matter passivating ability of *T. versicolor* or carbon degradation by *S. setonii*.

The pre-treatment process involving sulfur degradation by abiotic process followed by carbon degradation by *S. setonii* at 45°C recorded the best sulfur degradation in this category of studies. Table 5.7 summarizes the sulfur degradation data for the various two stage pre-treatment scenarios studied.

Table 5.7: Sulfur Oxidation for Two-step/ Stage Pre-treatment Processes for Sample A

Pre-treatment Scenario	Sulfur Oxidation, %
Chemolithotrophic Bacterial Only(2 weeks) at 30°C	21.8
Chemolithotrophic Bacterial Only(2 weeks) at 43°C	39.7
Abiotic Only at 2 weeks at 30°C	50.0
Abiotic Only at 2 weeks at 45°C	76.4
Biotic (1 week) followed by Chemolithotrophic Bacterial(2 weeks) at 30°C	7.4
Chemolithotrophic Bacterial (2 weeks) followed by Biotic(1week) at 30°C	27.8
Chemolithotrophic Bacterial (2eweeks) followed by Biotic(2 week) 43°C	45.5
Chemolithotrophic Bacterial (2weeks) followed by <i>S. setonii</i> (2 weeks) at 43oC	58.2
Abiotic(2 weeks) at 45°C followed by <i>S. setonii</i> (2 weeks)at 45°C	81.7

For the bacterial pre-treatment stage, sulfur oxidation was not very good compared to the abiotic step at the 30 - 45°C temperature range considered. Optimal bacterial activity could not be achieved for the bacterial step as indicated by the redox potential of the tests which averaged 420 mV. This might be due to the relatively low sulfur grade (3.8%) of Sample A. High redox potential is necessary to obtain highly oxidizing conditions (Rawlings, 1997; Hackl, 1997). Generally chemolithotrophic bacteria oxidize sulfides optimally at redox potentials of 500 mV and above. Due to the low sulfur grade of Sample A, the pH of the system could not be sustained at the optimal 1.2 to 1.8 due to low sulfuric acid generation. Sulfuric acid was therefore added to maintain the pH.

Unlike the abiotic stage, the reactions responsible for sulfur oxidation were mainly chemical and were induced by the alkaline conditions and with the high temperature of 45°C, the reaction kinetics were enhanced leading to the high sulfur oxidation of 81.7%.

5.4 Evaluation of Gold Extraction after Microbial Contact

The degree of amenability of pre-treated ore samples to gold leaching is a measure of how effective the pre-treatment was. In this study the conventional cyanidation method of leaching was used. The product of the single stage, the two-step and the other two stage pre-treatment scenarios investigated were therefore subjected to 24 hours cyanidation. The effects of retention time, pH, and temperature and pulp density on gold extraction were also investigated after single stage pre-treatment for 2 weeks.

Gold Extraction for the untreated ore samples were 15 and 30% for samples A and B respectively. Sample B has some free gold and this might accounted for the high extraction for the untreated sample.

5.3.4 Effect of Process Variables on Gold Extraction

5.3.4.1 Effect of pH

Gold extraction for the pH conditions studied, i.e. unadjusted, acidic and alkaline indicated similar trends as the sulfur oxidation. There is therefore a strong correlation between pH and gold extraction. The alkaline pH gave gold extraction of 54% after 2 weeks of single stage pre-treatment as indicated in Figure 5.6. Acidic and unadjusted pH followed that decreasing order with 23.6% and 30.6% gold extraction respectively.

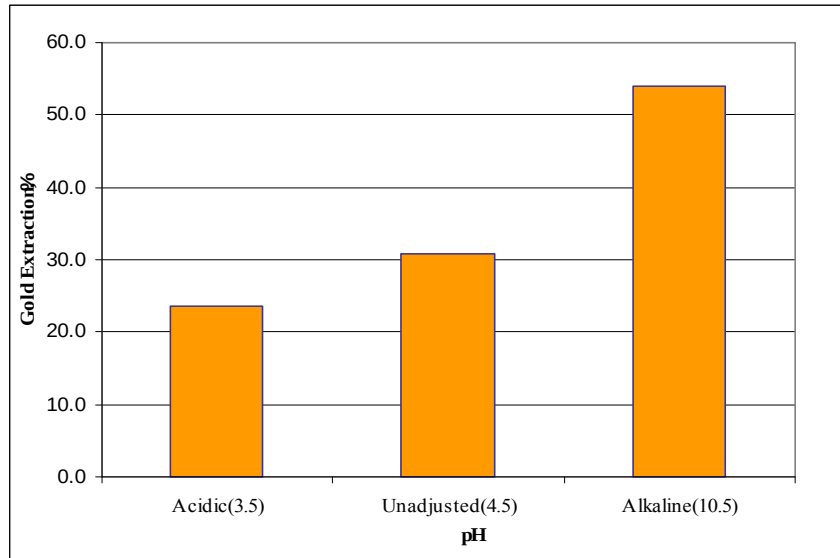


Figure 5.6: The Effect of Pre-treatment pH on Gold Extraction (Sample A; 30°C; 20% solids; 2 weeks retention time; shaker speed of 180 rpm; 24 hours cyanidation at 33% solids; 1 g/l NaCN)

5.3.4.2 Effect of Retention Time

Gold extraction tends to increase with retention time for the 3 to 28 days processing time studied for the single stage process. This extraction trend agrees with that of sulfur oxidation. Gold extraction improved from 36% for 3 days to 80.5% for 28 days as indicated in Figure 5.7. 78.95% was recorded for 21 days which confirms that 3 – 4 weeks of pre-treatment is sufficient to achieve significant gold extraction as only 2.05% increase was recorded after 21days.

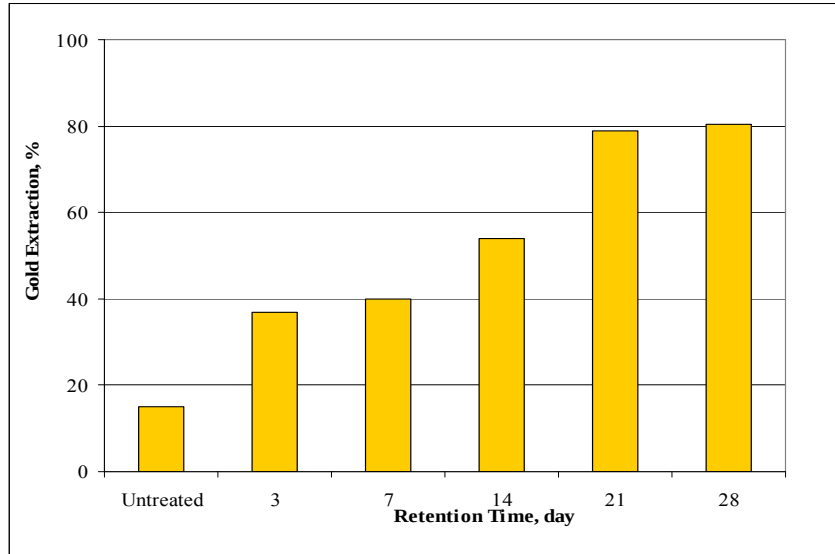


Figure 5.7: The Effect of Pre-treatment Time on Gold Extraction(Sample A; 30°C; 20% Solids; pH of 10.5; shaker speed of 180 rpm; 24 hours cyanidation at 33% solids; 1 g/l NaCN)

5.3.4.3 Effect of Temperature

Increase in temperature during pre-treatment enhanced sulfur oxidation which consequently improved gold leaching kinetics. 40.5%, 54.1% and 66.4% gold extractions were recorded for ambient, 30°C and 45°C after 2 weeks of single stage pre-treatment as indicated in the Figure 5.8

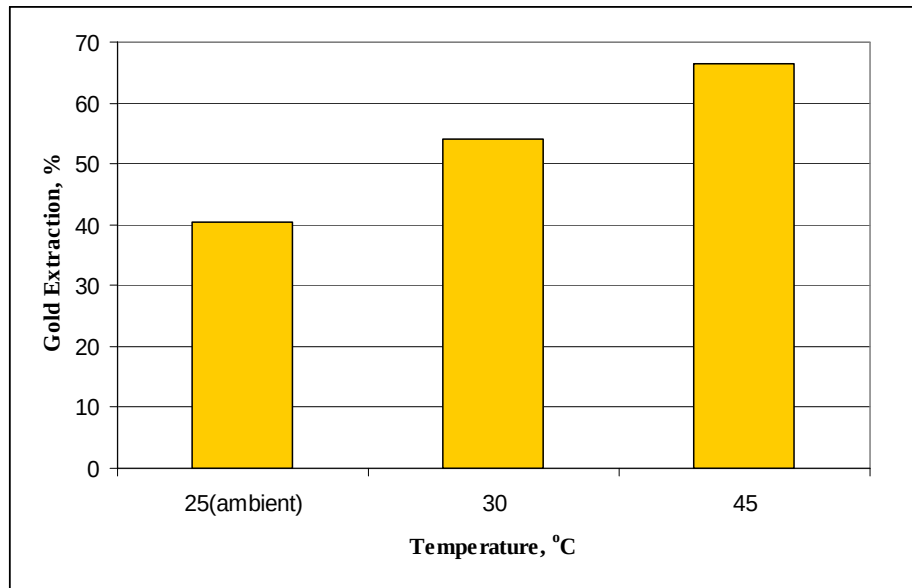


Figure 5.8: The Effect of Pre-treatment Temperature on Gold Extraction (Sample A; pH of 10.5; 20% solids; 2 weeks retention Time; shaker speed of 180 rpm; 24 hours cyanidation at 33% Solids; 1 g/l NaCN)

5.3.4.4 Effect of Pulp Density

Leachability of gold from pre-treated ore sample seems not to be influenced by increasing pulp density (Figure 5.9). This observation is quite interesting and quite against normal trends. Sulfur oxidation for this investigation at the pulp densities of 10, 20, 40 and 50% also followed the same trend. This trend will give room for high throughput without much compromise on recovery during plant operations.

Analysis of the filtrate of the pre-treatment process revealed increasing gold content with increasing pulp density. Since thiosulfate is responsible for sulfur oxidation and gold leaching during the pre-treatment, formation of thiosulfate is possibly enhanced at higher pulp densities. However, lower extraction was recorded with increasing pulp density

during cyanidation which is logical since the grade of pre-treated samples decreased with pulp density.

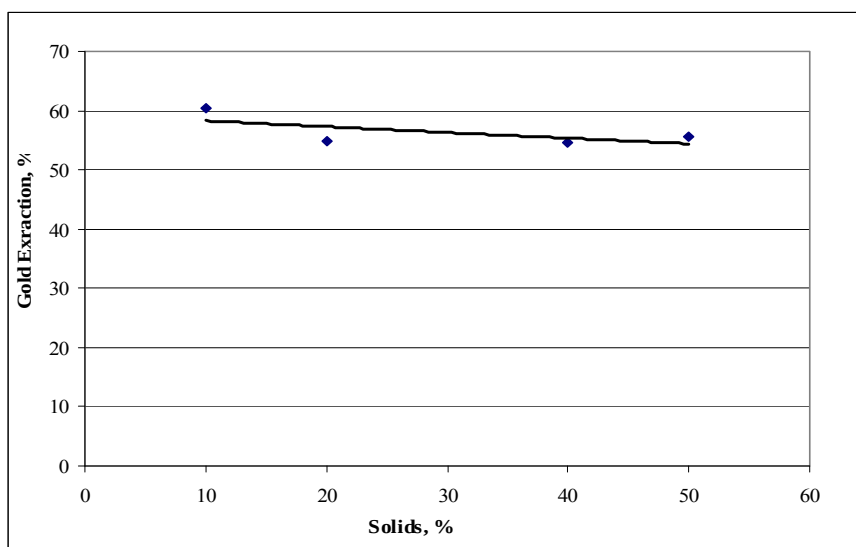


Figure 5.9: The Effect of Pre-treatment %Solids on Gold Extraction (Sample A; 30°C; pH of 10.5; 2 weeks retention time; shaker Speed of 180 rpm; 24 hours cyanidation at 33% solids; 1 g/l NaCN)

5.3.5 Effect of Two-step Pre-treatment on Gold Extraction

Generally there was an improvement in gold extraction for the two-step pre-treatment as shown in Figure 5.10. The overall best gold extraction of 82.5% was achieved after three weeks abiotic process at 45°C followed by biotic at 30°C for 1 week. Just like the single stage process, increase in temperature enhanced sulfur oxidation and consequently improved gold extraction. 3 – 4 weeks of pre-treatment is enough to achieve significant gold extraction. Application of the two-step process to sample B resulted in 93.3% gold extraction. Shown in Figure 5.12 is the Gold extraction trend of sample B for various pre-treatment scenarios studied.

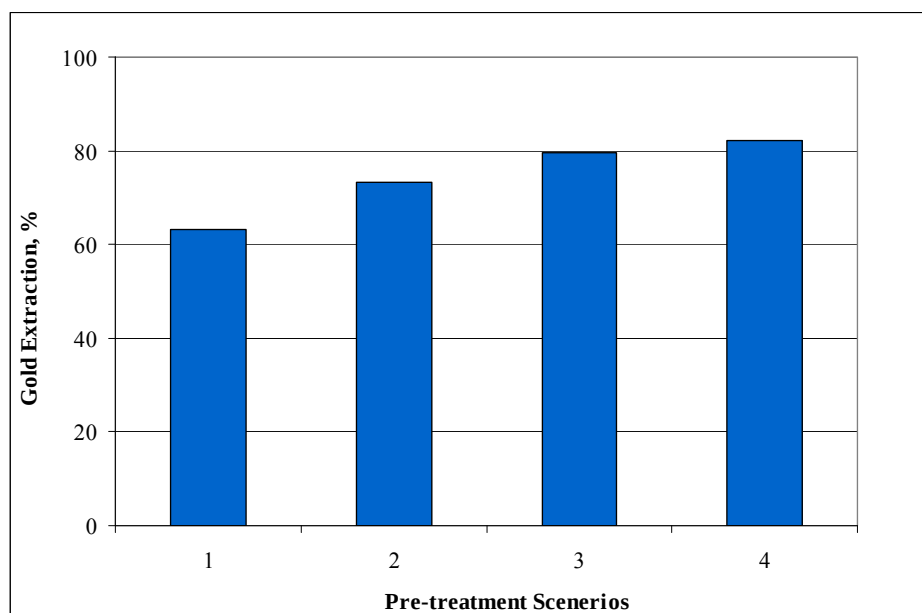


Figure 5.10: The Effect of Various Two-step Pre-treatment Processes on Gold Extraction (Sample A; 30°C; 20% Solids; 2 weeks retention time; shaker speed of 180 rpm; 24 hours cyanidation at 33% solids; 1 g/l NaCN)

1. Abiotic at 30°C (2 weeks) followed by Biotic (1week) at 30°C
2. Abiotic at 45°C (2weeks) followed by Biotic (1week) at 30°C
3. Abiotic at 45°C (2weeks) followed by Biotic (1week) at 45°C
4. Abiotic at 45°C (3weeks) followed by Biotic(1week) at 30°C

Reversal of the above pre-treatment scenarios resulted in a decrease in gold extraction during cyanidation just as there was a decrease in sulfur oxidation. Reversal of the three weeks abiotic process at 45°C (3weeks) followed by biotic at 30°C for 1 week resulted in 79.5 % gold extraction. Thus abiotic process followed by biotic is therefore the preferred order of the two-step pre-treatment process.

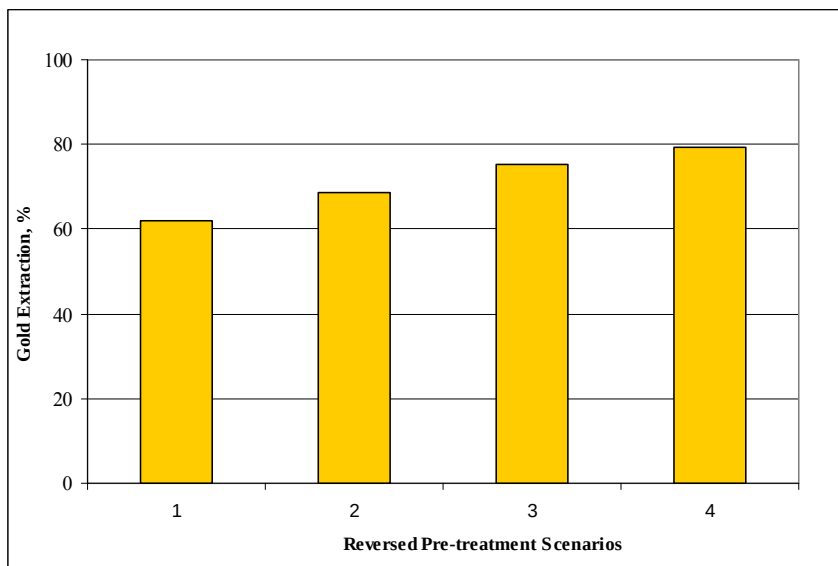


Figure 5.11: The Effect of Various Reversed Two-step Pre-treatment Processes on Gold Extraction (Sample A; 30°C; 20% solids; 2 week retention time; shaker speed of 180 rpm; 24 hours Cyanidation at 33% solids; 1 g/l NaCN).

1. Biotic(1 week) at 30°C followed by Abiotic(2 weeks)at 30°C
2. Biotic(1 week) at 30°C followed by Abiotic(2 week) at 45°C
3. Biotic at 45°C(1week) followed by Abiotic(2 week) at 45°C
4. Biotic at 45°C(3 weeks) followed by Abiotic (1 week)at 45°C

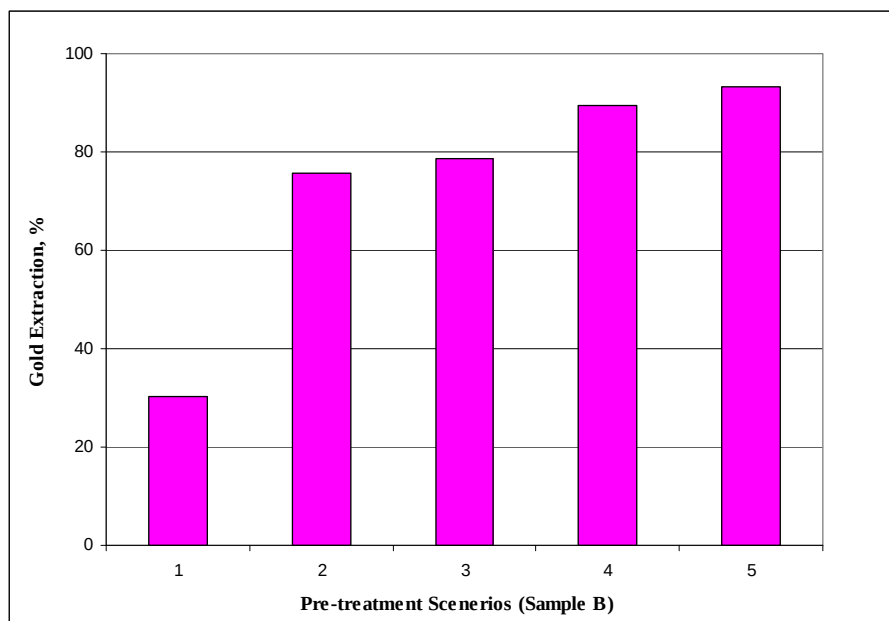


Figure 5.12: The Effect of Various Two-step Pre-treatment Processes on Gold Extraction

(Sample B; 30°C; 20% Solids; 2 weeks retention time; shaker speed of 180 rpm; 24 hours cyanidation at 33% solids; 1 g/l NaCN).

1. Untreated
2. Biotic(1 week) followed by Abiotic (2 weeks) at 30°C
3. Biotic(3 weeks) at 30°C
4. Biotic(3 weeks) at 45°C
5. Abiotic(3 weeks) followed by Biotic(1 week) at 30°C

5.3.6 Effect of Other Two stage Pre-treatment Process on Gold Extraction

When the microbial treated product of the other two stage scenarios were subjected to cyanidation, the highest gold extraction of 81.5% was recorded for the pre-treatment process involving 2 weeks of abiotic sulfur degradation followed by two weeks of carbon degradation by *S. setonii* at 45°C (Figure 5.13). A drastic decrease in gold extraction was observed for the biotic followed by chemolithotrophic bacterial pre-treatment; only

26.6% gold extraction was achieved and this further confirms that biotic process after any of the abiotic or chemolithotrophic bacterial pre-treatment stage is a preferred sequence order.

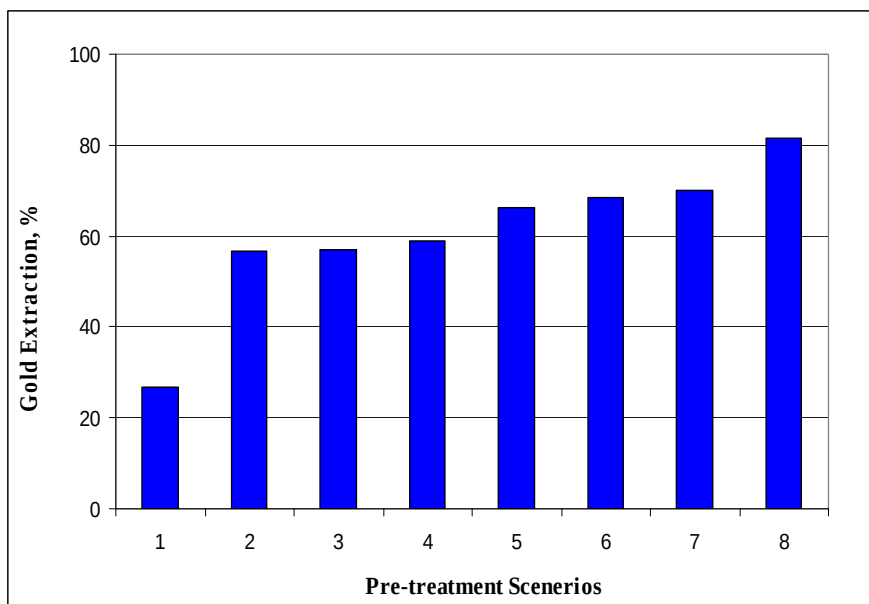


Figure 5.13: The Effect of Various Two Stage Pre-treatment Processes on Gold Extraction(Sample A; 30 – 45°C; 20% solids; 2 weeks retention time; shaker speed of 180 rpm; 24 hours cyanidation at 33% solids; 1g/l NaCN).

1. Biotic (1 week) followed by Chemolithotrophic Bacterial (2 weeks) at 30°C
2. Bacterial (2 weeks) at 43°C
3. Abiotic only at 2 weeks at 30°C
4. Bacterial (2weeks) followed by Biotic(1 week) at 30°C
5. Abiotic (2 weeks) at 45°C
6. Chemolithotrophic Bacterial (2 weeks) followed by Biotic (2 weeks) at 43°C
7. Chemolithotrophic Bacteria (2 weeks) followed by *S. setonii*(2 weeks) at 43°C
8. Abiotic (2 weeks) at 45°C followed by *S. setonii* (2 weeks) at 43°C

5.4 Studies into Fungal Culture Media

5.4.1 Medium Composition

The medium used in submerged industrial fermentations favor both growth and product formation at high yields: The basic requirements of fungi are water, molecular oxygen, an organic source of carbon and energy, a source of nitrogen other than molecular nitrogen and several other elements and buffers. At least 13 elements are essential for growth, namely oxygen, carbon, hydrogen, nitrogen, phosphorus, potassium, sulfur, magnesium, manganese, iron, zinc, copper and molybdenum. The first eight are needed in relatively large quantities (macronutrients). The latter five are required in small amounts (micronutrients). The use of complex media in submerged fermentations of filamentous fungi has been shown to influence the morphology and growth kinetics. Solids in the liquid substrate have been shown in many cases to induce pellet formation in filamentous fungi (Braun and Vecht-Lifshitz, 1991; Schügerl et al., 1998).

The use of complex media may affect not only the formation but also the degradation rate of the product.

5.4.2 Type and Concentration of Carbon and Nitrogen Source

Like all fungi, *T. versicolor* is heterotrophic. This means that they require organic compounds as a source of carbon and energy. Organic compounds supporting most growth are usually sugars (e.g., D-glucose, D-fructose, sucrose) which are rapidly taken up. Polysaccharides, amino acids, lipids, organic acids, proteins, alcohols and hydrocarbons are also used. A small amount of exogenous carbon may be required to maintain the fungus even when it is not growing. Carbon limitation contributes greatly to the process of cell aging and autolysis in fungal cultures.

Nitrogen may be supplied as ammonia, as nitrate or in organic compounds, such as amino acids or proteins. Beet or cane molasses, corn-steep liquor, whey/powder, soy flour, yeast extract and others are used as industrial raw materials, rich in nitrogen. Nitrogen plays an important role in metabolite overproduction and affects fungal morphology (Papagianni, 2004).

5.4.3 Studies into Fungal Culture Carbon and Nitrogen Concentration

Carbon and nitrogen are very important components of the culture medium and play crucial roles in cell growth and metabolite production. For the *T. versicolor* medium, carbon is added as glucose (10.1 g/l) and nitrogen as ammonium tartrate (0.44 g/l). The impact of varying the amount of these two components on fungal cell biomass and activity were thus investigated at various glucose-ammonium tartrate weight ratios. The impact of these cultures on sulfur oxidation, gold sorption and gold extraction were investigated for ore sample A for 2 weeks contact time. The result is summarized in Table 5.8.

Table 5.8: Fungal Culture Glucose -Nitrogen Studies Data for Sample A

Glucose: Nitrogen (w/w)	Biomass g/l	S Oxidation %	Au Sorption %	Au Extraction %
2G:1N	1.0	47.89	1.12	41.52
2G:2N	19.0	40.26	1.39	36.06
1G:2N	3.0	40.26	0.89	30.04
1G:1N	3.0	48.42	0.98	54.02

The fungal biomass determined for glucose: ammonium tartrate ratio (w/w) of 1:1 and 1:2 were both 3.0 g/l while 2:1 was 1.0 g/l. Though ratio of 2: 2 recorded the highest biomass of 19 g/l, it did not translate into superior sulfur oxidation and gold extraction as these figures were lower compared to 1:1 and 1:2 ratios. It is worth noting that though cell biomass is important, conditions of carbon and nitrogen limitations must be created to trigger off metabolite production needed for passivating carbonaceous matter. Possibly the 10 – 14 days of culturing prior to pre-treatment was not sufficient for the fungal cells in the medium with 2G: 2N ratio to utilize all the nitrogen and carbon present in the medium and this might have affected metabolite production.

The culture of the highest biomass ratio was very viscous and this might have affected the bioleaching kinetics as shown by the low sulfur oxidation and consequently low gold extraction. It was also observed that culturing at temperatures below the optimum range led to pelleted growth of the fungal cells rather than filamentous. Based on the results of this investigation, the medium composition of 1:1 glucose-ammonium tartrate ratio was adopted for *T. versicolor* culturing.

5.4.4 Studies into Culture Medium Alkaline pH

The effect of the alkaline pH i.e. presence of hydroxyl ions on sulfur oxidation, gold dissolution and preg-robbing was studied using sample A. During the investigation, the fungal medium was studied at the both the unadjusted and alkaline pH conditions while water was observed at only alkaline pH. 26% sulfur oxidation was recorded for water at alkaline medium as against 42% and 54% for fungal culture at the unadjusted and alkaline pH respectively after 2 weeks retention time. Summarized is the result in Figure 5.14.

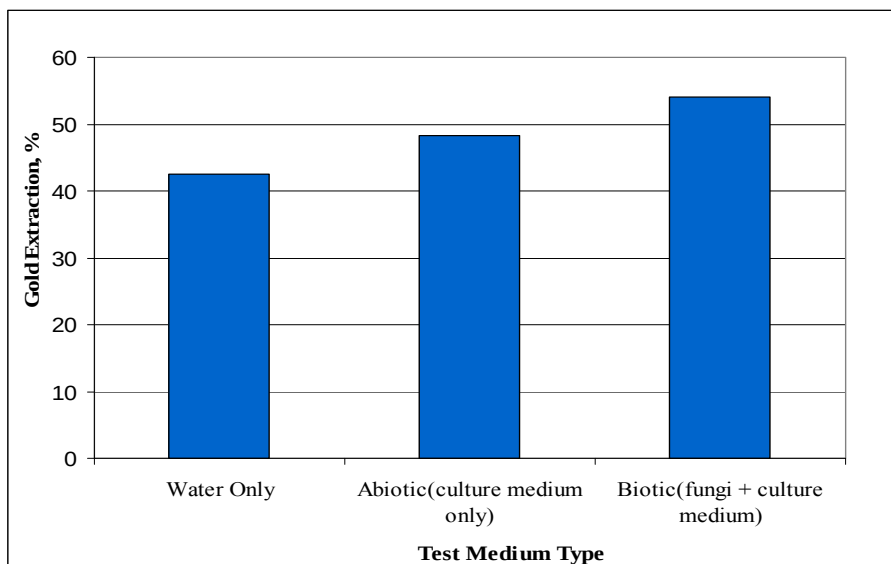


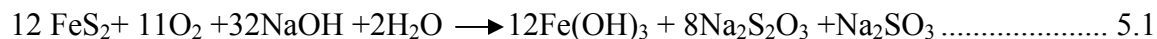
Figure 5.14 : The Effect of Media Type on Gold Extraction (Sample A; 30°C; 20% solids; pH of 10.5; 2 weeks retention time; shaker speed of 180 rpm; 24 hours cyanidation at 33% solids; 1 g/l NaCN).

Analyses of the bioleach filtrate after pre-treatment indicated the presence of gold and 2.5 - 5.0 mol/dm³ residual thiosulfate was detected in filtrate as well. The mechanism of gold solubilization appears to be associated with the in situ formation of thiosulfate as a leachant. Formation of thiosulfate is of great importance because gold in association with pyrite and arsenopyrite minerals was complexed with thiosulfate and dissolved in the alkaline solution.

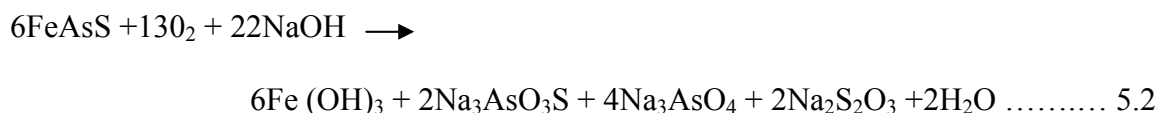
On the average, 10 to 50% of the sample gold was leached after 2 - 3 weeks pre-treatment. Gold sorption for the pre-treatment process involving water under alkaline condition was 14% which is closer to the untreated sample of 18.1%. Both fungal medium and culture recorded low gold sorption during preg-robbing test. It could

therefore be deduced that the fungal medium also has some carbon passivating abilities and alkaline medium alone did not decrease gold sorption significantly.

Thiosulfate has been reported to be the dominant product of pyrite oxidation (Zhang, 2004). The formation of thiosulfate ions in alkaline solutions has also been found during atmospheric oxidation of gold bearing arsenical ores (Rossvosky, 1993). Ferric hydroxide in the form of porous layers on the mineral particle is the product of both pyrite and arsenopyrite oxidation. An appropriate stoichiometry for the overall process of pyrite oxidation in alkaline solutions with air is given by the following reaction:



In the case of arsenopyrite, the overall oxidation reaction suggested is given by:



Alkaline oxidation of sulfides is well studied but has not been used commercially for sulfidic ores because of high reagent cost involved. Though the cost aspect of this research was not investigated, high caustic consumption was observed. However, the initial high consumption was compensated for by low lime consumption during leaching of the pre-treated ore due to residual hydroxyl ions. Low cyanide consumption was also

recorded during leaching as a result of gold dissolution during the alkaline pre-treatment. Caustic and cyanide consumption figures can be found in Appendix E.

5.5 Fungal viability at various pH

Observations of the agar plates at the various pHs for growths showed no *T. versicolor* growth for pH 9.0 and 11.0. Sparse growth was noted for pH 3.0 and scattered for pH 7.0. Optimum growth was observed for 4.5. However, *T. versicolor* can be viable at pH range 3.0.to 7.0 as results indicated.

T. versicolor did not thrive under alkaline conditions but since the test is a batch process, the only option is to adjust the pH from the fungal optimum of 4.5 - 5.0 to 9.5 - 10. 5 few minutes after ore contact with the culture. For normal plant operations, the ore will be contacted with the fungal culture in the leading reactor while the overflow pH is adjusted to 9.5 – 10.5 in the next reactor and this way continuous growth of fungal cells will be achieved.

5.6 Passivation/Degradation of Carbonaceous Matter

5.6.1 Carbon Analysis after fungal contact

Analyses of the carbon content in the feed materials and microbial treated residues were used to monitor the extent of passivation /degradation. The three carbon determinations made were: the original carbon content of the ore, that remaining immediately after pre-treatment and after digestion of pre-treated sample biomass. Increases in carbon content

biomass were observed after fungal-mineral interactions. However, carbon values dropped closer to that of the untreated ores after biomass digestion with hypochlorite as shown in Table 5.9.

Table 5.9: Carbon Content Analysis of Selected Samples after *T. versicolor* Contact

Sample number	Carbon content, %		
	Before Pre-treatment	After Pretreatment	After Biomass digestion
A	0.63	0.72	0.63
A	0.63	1.10	0.67
A	0.63	0.84	0.64
B	6.84	6.58	6.38

Slight fluctuations in carbon content were observed after the *S. setonii* - mineral interactions but after biomass digestion with hypochlorite, carbon values dropped below the untreated value. 17.6% carbon degradation was observed for sample A after 2 weeks contact with *S. setonii* culture (Table 5.10). The low degradation may be due to the low carbon content of sample A.

Table 5.10: Carbon Content Analysis of Selected Samples A after *S. setonii* Contact

Sample number	Carbon content, %			
	Before Pre-treatment	After Pretreatment	After Biomass digestion	%Degradation
1	0.63	0.67	0.59	5.70
2	0.63	0.62	0.52	17.62
3	0.63	0.67	0.58	7.00

Amankwah (2005) reported 59% degradation of carbonaceous matter by *S. setonii* after 14 days contact and 80% after 56 days. The higher degradation may be due to the higher carbon content (7.03%) of the double refractory gold concentrate used for the studies.

Comparing carbon passivation by *T. versicolor* and degradation by *S. setonii*, it could be deduced that it takes only 3 – 7 days for effective passivation by *T. versicolor* while relatively longer periods are required for *S. setonii* to degrade carbonaceous matter significantly. However, the impact of microbial passivation or degradation of carbonaceous matter is similar as both led to improved gold extraction.

When a sample that had not been contacted with *T. versicolor* or *S. setonii* was digested with hypochlorite, there was only a 0.23% reduction in carbon content. This indicates that the decrease in carbon content was not due to hypochlorite digestion but rather to microbial activity. When pure silica flour was contacted with bacteria and digested the same way, samples retained 0.068% of biomass carbon. The residual carbon content from biomass was therefore assumed negligible.

Chapter 6

Conclusions, Proposals and Recommendations for Future Works

6.1 Conclusions

The main conclusions that could be drawn from this novel investigation into microbial pre-treatment of double refractory gold ores are presented as follows:

Nitrogen and carbon sources which in this research were derived from glucose and ammonium tartrate respectively are the major components of the culture medium which affect cell growth and activity. It was demonstrated that increasing the amount of glucose and ammonium tartrate lead to increase in fungal biomass population. However, high fungal biomass did not translate into superior bioleach kinetics. There was no difference in gold sorption behavior of the various glucose to ammonium tartrate weight ratios studied.

The studies indicated that carbonaceous matter in lignite, bituminous coal and anthracite was not decomposed after contact with *T. versicolor*. However, gold sorption reduced significantly for bituminous coal and to a lower degree for anthracite. Lignite was inert to passivation and moreover, its gold sorption tendency increased for all the pH ranges studied.

The bacterium *S. setonii* degraded carbonaceous matter in Sample A after abiotic pre-treatment step and this confirms earlier work by Amankwah (2005). Gold extraction

improved from 57% to 81.5% after *S. setonii* contact. *S. setonii* could therefore be used to effectively degrade carbonaceous matter in refractory gold ore or concentrate.

T. versicolor did not degrade sulfides significantly at its optimum growth conditions. Only 12.9% sulfur oxidation was achieved after single stage pre-treatment for 2 weeks. Similarly investigation of arsenopyrite and pyrite at various pH conditions, revealed similar sulfide degradation trend. The culture medium at alkaline pH range of 9.5 to 10.5 demonstrated high sulfur oxidizing potential. Effectively the fungus, *T. versicolor* passivated carbonaceous matter while the presence hydroxyl ions set in motion the necessary reactions for sulfur oxidation. The pre-treatment process is therefore a combination of fungal and chemical actions.

Samples A contains only pyrite while sample B, a flotation concentrate has both arsenopyrite and pyrite present. Both samples exhibited similar characteristics as the pure sulfide minerals with respect to sulfide degradation by *T. versicolor*. The best sulfur oxidation was achieved for both sample A and B after 3 to 4 weeks of two stage pre-treatment under alkaline conditions. Though sulfur oxidation of sample B was relatively low, gold extraction was 93.3%; dissolution of free gold content might contributed to the high extraction.

Alkaline medium enhanced sulfur oxidation and promoted gold dissolution during pre-treatment. The presence of hydroxyl ions led to the formation of thiosulfate which is key to sulfide sulfur oxidation and gold dissolution process. Thiosulfate formation was

confirmed during the bioleach process in the alkaline pH range. 2.5 - 5.0 mol/dm³ residual thiosulfate was detected in the bioleach filtrate after pre-treatment for both samples A and B. 10 - 50% gold was leached into the bioleach medium at 30 - 45°C. An interesting feature of the bioleach filtrate is its color which varies from colorless to deep reddish brown. The color characteristics could not be investigated further but has the potential to reveal interesting trends.

Optimum pre-treatment conditions thus involves a two step or stage microbial pre-treatment process at pH range 9.5 - 10.5 (abiotic) and 4.5 - 5.0 (biotic), 20% solids, 3 - 4 weeks retention time at 30 - 45°C since significant sulfide degradation and carbonaceous matter passivation was achieved for the ore samples studied under these conditions. Samples A and B achieved an overall best of 82.5 and 93.3% gold extraction respectively after 24 hours cyanidation. Increasing the cyanide concentration and leaching time did not impact on gold extraction.

6.2 Processing Proposal

The results of the novel approach to microbial pre-treatment of double refractory gold ores indicated that sulfur was degraded and carbonaceous matter passivated. Double refractory ores may undergo a single stage or a two-step pre-treatment process. The single stage process could also be used to passivate carbonaceous matter in carbonaceous non sulfide ores. The carbon passivation step of the two-step pre-treatment process can be substituted with carbonaceous matter degradation by the bacterium *S. setonii*.

The single stage process may be a preferred choice over the two-step since it would significantly reduce the processing time and make the process flow simpler

This novel approach to extracting gold from ore or concentrate can be implemented via one of the following process flow sequences:

(1) Single stage:

- Biotic carbonaceous matter passivation/ sulfide sulfur oxidation
- Filtering of oxidized/passivated microbial pretreatment product
- Recovery of gold from filtrate by adsorption.
- Repulping and thickening of solid residue.
- Cyanidation and gold recovery from solid residue

(2) Two-step or stage:

- Abiotic sulfide sulfur oxidation at 45°C
- Filtering of oxidized product and recovery of gold from filtrate by adsorption.
- Washing and thickening of solid residues
- Passivation of carbonaceous matter by using fungus *T. versicolor* or degradation by *S. Setonii*
- Washing of product and safe disposal of effluent
- Cyanidation and gold recovery from solid product

The novelty of this microbial pre-treatment process is the drastic reduction of pre-robbing in double refractory gold ores by the passivating action of *T. versicolor*. The fungal passivation of carbonaceous matter can be used to complement the traditional processes like pressure oxidation and bacterial leaching where the carbonaceous matter is not usually oxidized significantly.

The application of this process to passivate carbonaceous may not be universal and limits will need to be set. As an initial step, the carbonaceous gold ores may be investigated to find out whether the carbonaceous matter present is amenable to passivation by *T. versicolor*. Lignite did not respond well to passivation by *T. versicolor* metabolites and as such carbonaceous matter of this rank may not respond to passivation. Double refractory gold ore with carbonaceous matter maturity of bituminous coal may not be passivated extensively but gold sorption could be reduced considerably.

Anthracite did not respond well to passivation by *T. versicolor* but better than lignite. The process is therefore recommended for ores in which the carbonaceous matter maturity is similar to bituminous coal and to a lesser extent anthracite. Gold ore samples containing lignite grade of carbonaceous matter may not be suitable for this process.

S. setonii could also be used for degrading carbonaceous matter in gold ore or concentrate in place of *T. versicolor*. *S. setonii* could not degrade all levels of maturity of carbonaceous matter in gold ore and concentrate. Only gold ores containing carbonaceous matter of maturity similar to bituminous coal and lignite or both would be

amenable to degradation by *S. setonii*. Anthracite grade of carbonaceous matter is not suitable for *S. setonii* degradation (Amankwah, 2005).

6.3 Recommendations for Future Works

T. versicolor did not passivate lignite and moreover, its peg-robbing ability increased after microbial interaction. It is recommended that the surface of lignite coal be studied after fungal contact to establish reasons for its behavior.

The surface passivation mechanism of bituminous coal and anthracite by *T. versicolor* need to be studied.

Other species of the white rot fungi such as *T. versicolor* (ATTC 12679) and *Phanerochaete chrysosporium* with similar characteristics as the *T. versicolor* (ATTC 20869) need to be studied.

All the tests conducted during the research were batch processes in shake flask cultures. It is therefore recommended that continuous process conditions be investigated to serve as a spring board for pilot tests.

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Appendices

Appendix A

X-ray and Infrared Diffraction Patterns

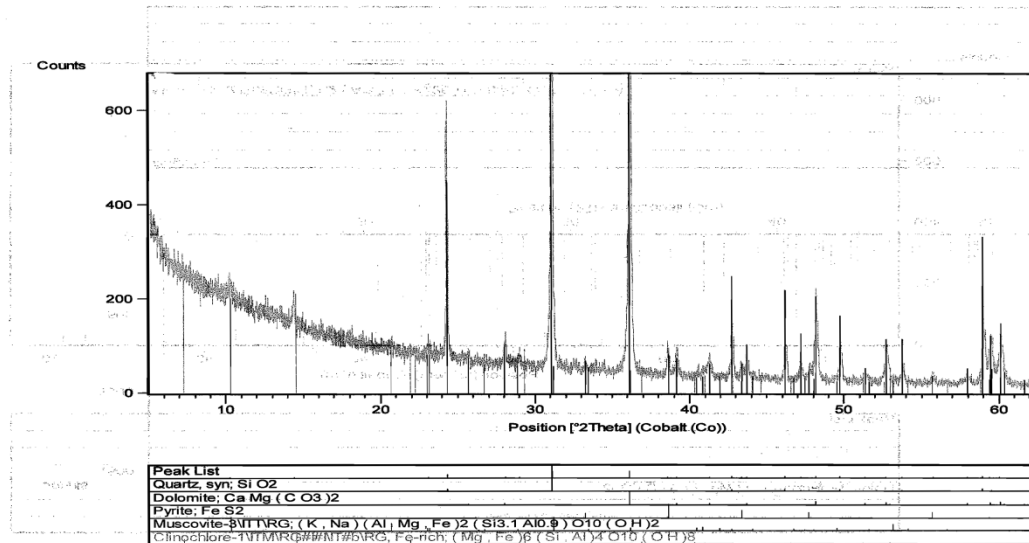


Figure A.1: Sample A (untreated) X-ray Diffraction Pattern

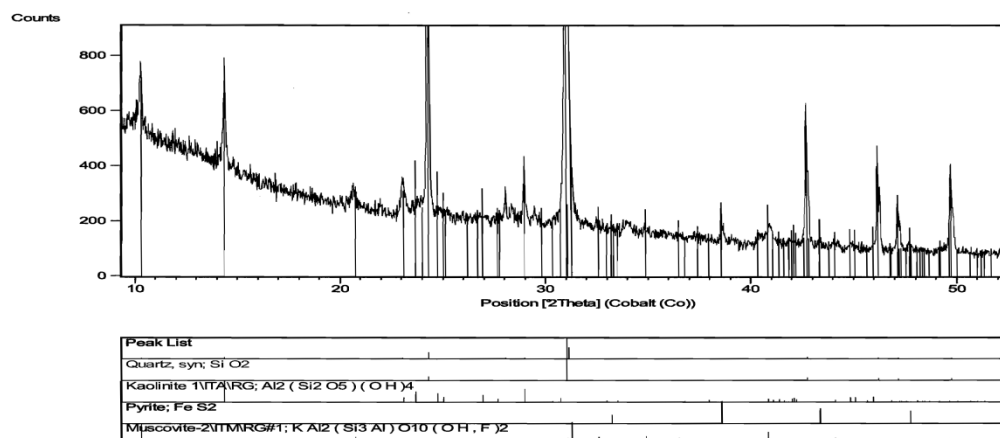


Figure A.2: Sample A treated X-ray Diffraction after *T. versicolor* Contact at Biotic Alkaline Pre-treatment Conditions (30°C; 20% solids; 3 weeks retention time; pH 10.5; shaker speed of 180 rpm).

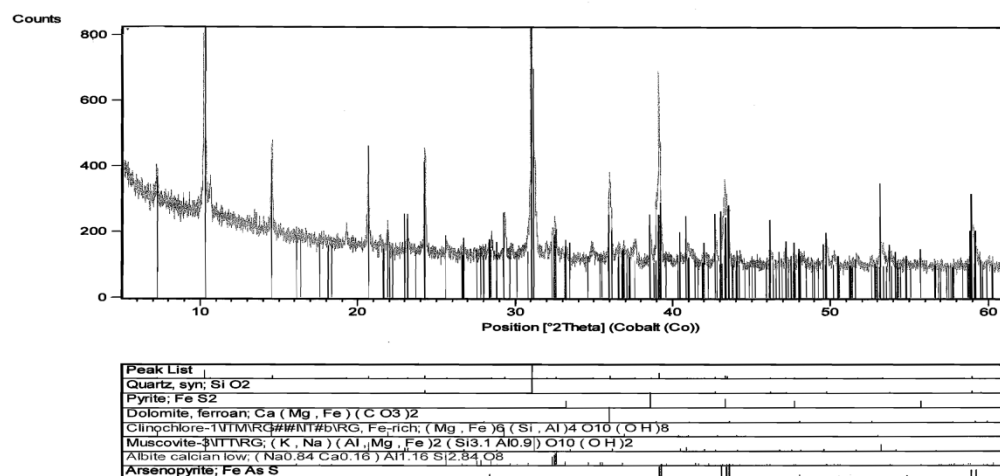


Figure A.3: Sample B (untreated) X-ray Diffraction Pattern

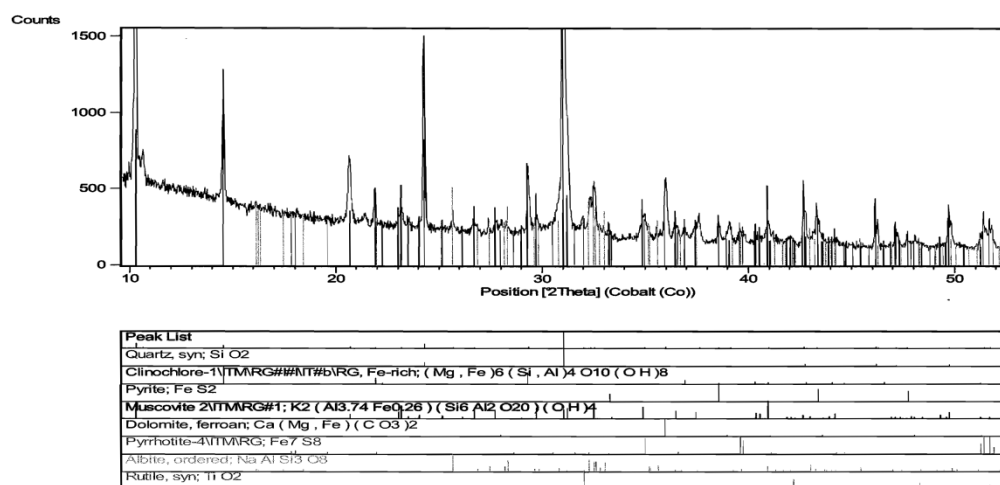


Figure A.4: Sample B X-ray Diffraction Pattern *T. versicolor* contact at Biotic Alkaline Pre-treatment Conditions (30°C; 20% Solids; 3 weeks retention time; pH 10.5; shaker speed of 180 rpm)

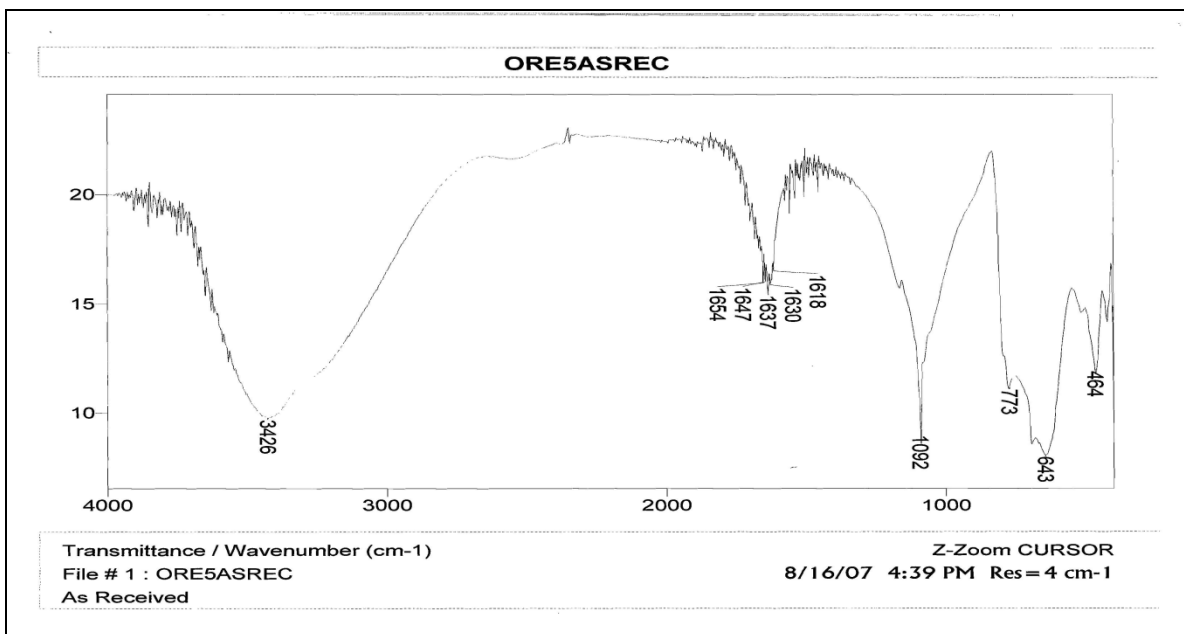


Figure A.5: Sample A Untreated Infrared Diffraction Pattern

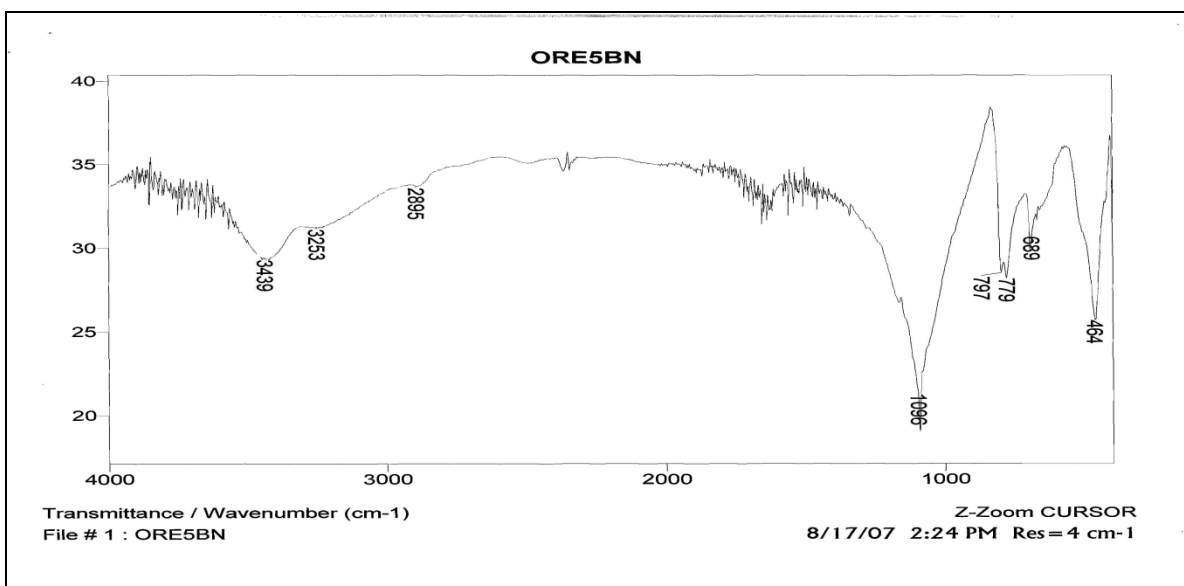


Figure A.6: Sample A Infrared Diffraction Pattern after *T. versicolor* Contact at Optimum Growth Conditions (30°C; 20% solids; 2 weeks retention time; pH 4.25; shaker speed of 180 rpm).

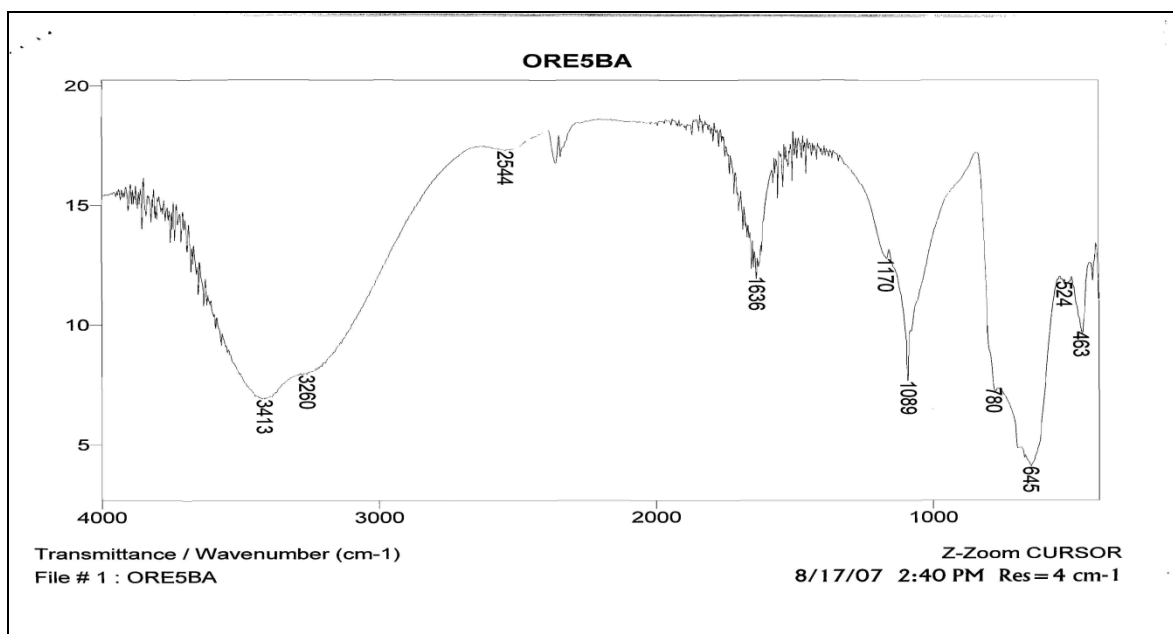


Figure A.7: Sample A Infrared Diffraction Pattern after *T. versicolor* contact under biotic alkaline pre-treatment conditions (30°C; 20% solids; 2 weeks retention time; pH 10.5; shaker speed of 180 rpm).

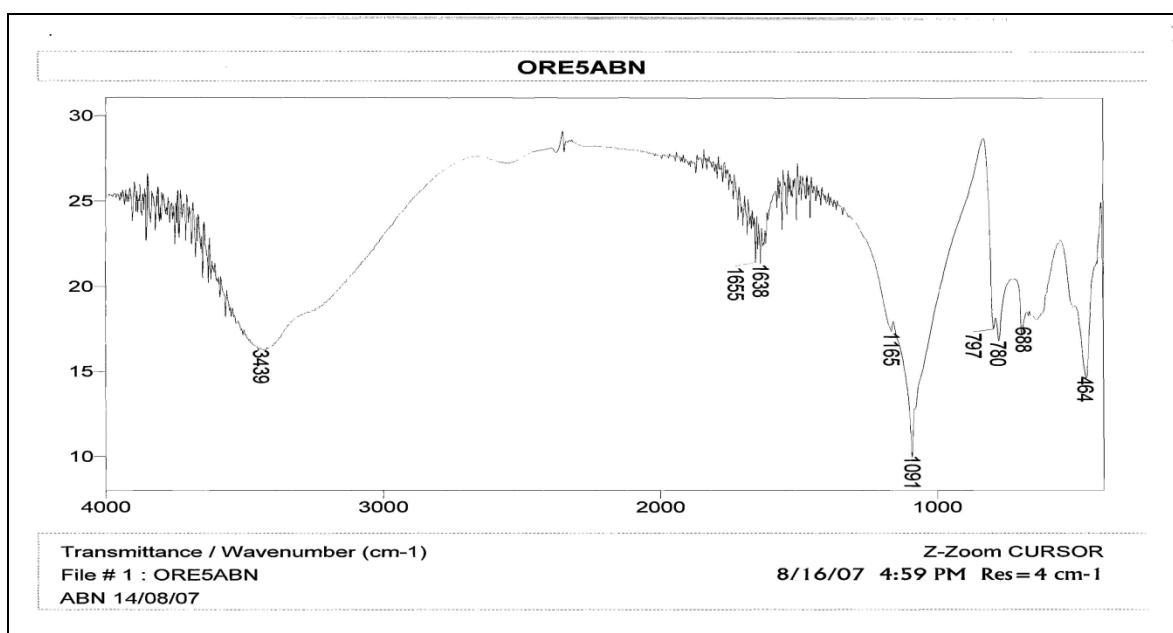


Figure A.7: Sample A Infrared Diffraction Pattern after *T. versicolor* Contact under Abiotic Alkaline Pre-treatment Conditions(30°C; 20% solids; 2 weeks retention time; pH 10.5; shaker speed of 180 rpm).

Appendix B

Test Data for Establishing Process Conditions

B.1: The Effect of pH on Sulfide and Carbonaceous Matter Degradation

Test	pH	Sample Wt(g)	%Mass Loss	%S Oxidation	%Preg-robbing
1	3.5	10.12	1.60	11.32	1.14
2	Natural(4.25)	10.13	2.30	12.89	1.33
3	4.5	10.15	2.50	9.06	0.10
4	10	10.14	3.00	45.20	0.64

B.2: The Effect of Temperature on Sulfide and Carbonaceous Matter Degradation

Test	Temperature	Sample Wt(g)	%Mass Loss	%S Oxidation	%Preg-robbing
5	Room	10.12	2.49	42.63	2.50
6	30	10.10	3.10	45.79	0.98
7	45	10.13	9.63	73.40	0.63

B.3: The Effect of Retention Time on sulfide and Carbonaceous Matter Degradation

Test	Day	Sample Wt(g)	%Mass Loss	%S Oxidation	%Preg-robbing
8	3	10.10	3.2	13.89	0.18
9	7	10.10	3.56	32.19	0.20
10	14	10.10	3.96	45.16	0.27
11	21	10.10	4.75	47.01	0.23
12	28	10.10	4.26	55.58	0.69
13	35	10.10	4.46	57.43	1.33
14	42	10.10	4.66	61.72	3.04
15	49	10.10	5.45	66.95	3.75
16	63	10.11	4.29	72.95	3.45

B.4: The Effect of Pulp Density on Sulfide and Carbonaceous Matter

Test	%	Sample Wt(g)	%Mass Loss	%S Oxidation	%Preg-robbing
18	5%	5.10	1.67	48.17	0.73
19	10%	8.50	4.38	48.86	0.90
20	15%	15.10	3.98	47.48	0.10
21	20%	20.00	3.96	53.96	1.00
22	25%	25.10	3.08	47.24	4.50
23	30%	30.10	2.70	46.09	6.00
24	40%	40.00	2.50	44.23	5.27
25	50%	50.10	4.02	44.92	6.50

Appendix C

Sample A's Test Data for Fungal Culture Medium Investigation (Abiotic Test)

C.1: The effect of pH on Sulfide and Carbonaceous Matter Degradation

Test	pH	Sample Wt(g)	%Mass Loss	%S Oxidation	%Preg-robbing
1	3.5	100.0	3.06	11.32	3.20
2	Natural(4.25)	100.0	1.56	37.63	1.76
3	4.5	100.0	3.95	26.45	0.40
4	10	100.0	5.12	55.26	1.00

C.2: The Effect of Temperature on Sulfide and Carbonaceous Matter Degradation

Test	Temperature	Sample Wt(g)	%Mass Loss	%S Oxidation	%Preg-robbing
5	Room	150	1.85	44.74	1.16
6	30	100	1.88	50.00	1.03
7	45	100	10.9	76.40	0.98

C.3: The Effect of Retention time on Sulfide and Carbonaceous Matter Degradation

Test	Day	Sample Wt(g)	%Mass Loss	%S Oxidation	%Preg-robbing
8	3	10.12	5.84	13.16	3.30
9	7	10.12	4.46	35.53	4.55
10	14	10.12	4.46	55.26	1.00
11	21	10.13	4.49	64.47	0.95
12	28	10.12	3.78	75.34	4.52

Appendix D

Test Data for Various Pre-treatment Scenarios

D1: Test Data for Single Stage Process (Biotic)

Test	Sample	Sample Wt(g)	Temperature, °C	%Mass Loss	%S Oxidation
1	A	100.10	30	4.26	76.52
2	A	100.12	30	4.92	79.18
3	A	100.08	45	9.63	82.68
4	B	100.01	45	6.72	52.85

D. 2: Test Data for Two Step Process (Abiotic followed by Biotic)

Test	Sample	Sample Wt(g)	Temperature	%Mass Loss	%S Oxidation
5	A	100.10	30(30)	4.52	62.25
6	A	100.05	30(45)	4.62	57.40
7	A	100.08	45(45)	6.79	75.13
8	A	100.50	45(45)	6.47	79.45
9	B	100.20	45(30)	6.80	52.34

D.3: Test Data for Reversed Two Step Process (Biotic follow by Abiotic)

Test	Sample	Sample Wt(g)	Temperature	%Mass Loss	%S Oxidation
10	A	100.10	30/30	3.67	57.40
11	A	100.05	30/45	4.58	68.42
12	A	100.08	45/45	5.10	71.35
13	A	100.50	45/45	6.54	75.60
14	B	100.20	30/45	5.75	41.78

D.4: Sample A Test Data for Various Two Stage Pre-treatment

Test	Sample Wt(g)	Temperature	%Mass Loss	%S Oxidation
15	100.05	43	8.87	52.92
16	100.20	30	4.65	50.00
17	100.15	45	5.12	76.40
18	100.23	30(30)	4.58	21.84
19	100.45	30(30)	6.50	7.37
20	100.36	45/(43)	7.89	81.66
21	100.30	43(45)	8.21	58.16
22	100.25	43(45)	8.57	45.52

Appendix D Test Description

1. Biotic (3 weeks) at 30°C
2. Biotic (4 weeks) at 30°C
3. Biotic (3 weeks) at 45°C
4. Biotic (3 weeks) at 45°C (Sample B)
5. Abiotic(2 week) followed by biotic at 30°C
6. Abiotic(2 weeks) at 45°C followed by biotic(1 week) at 30°C
7. Abiotic at 45°C (2 weeks) followed by biotic at 45°C (1week)
8. Abiotic at 45°C (3 weeks) followed by biotic at 30°C (1week)
9. Abiotic at 45°C (3 weeks) followed by abiotic at 30°C (1 week)-Sample B
10. Biotic (1 week) at 30°C followed by Abiotic(2 weeks) at 30°C
11. Biotic (1 week) at 30°C followed by Abiotic(2 week) at 45°C
- 12 Biotic at 45°C (1 week) followed by Abiotic at 45°C (2 week)
13. Biotic at 30°C (1 week) followed by Abiotic at 45°C (3 weeks)
14. Biotic at 30°C (1 week) followed by Abiotic at 45° C (3 weeks)-Sample B
15. Bacterial(2 weeks) at 43°C
16. Abiotic (2 weeks) at 30°C
17. Abiotic (2 weeks) at 45°C
18. Bacterial (2 weeks) followed by Biotic(1 week) at 30°C
19. Biotic (1 week) followed by Chemolithotrophic Bacterial (2 weeks) at 30°C
20. Abiotic(2 weeks) at 45°C followed by *S. setonii* (2 weeks) at 43°C
21. Chemolithotrophic Bacteria (2weeks) followed by *S. setonii* (2 weeks) at 43°C
22. Chemolithotrophic Bacteria (2weeks) followed by Biotic (2 week) 43°C

Appendix E

Gold Extraction Conditions and Test Data

In this appendix, data on the various cyanidation tests conducted during the research is presented. The following procedure was used for all the cyanidation tests:

The sample was pulp to 33% solids with water in a 2.5 L bottle

The pH was adjusted to 10.5 - 11.0 with lime $\text{Ca}(\text{OH})_2$

NaCN was added to attain a concentration of 1.0g/L

The gold leaching test was then run for 24hrs.

No lime or NaCN was added throughout the 24hrs period

The cyanidation product was filtered at the end of the test period

The filtrate (Pregnant solution) was collected and volume noted

The filter cake was washed with 2-3 times bed volume with dilute NaCN solution

The pregnant solution and Washed solution were mixed and gold value assayed

E.1: Data on Effect of Pre-treatment pH on Gold Extraction for sample A

Cyanidation Feed: 50 - 90g of pre-treated Sample A

Solution Volume: 305 - 488 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 10.7 – 11.1 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.125 – 1.343

CaO (kg/t): 1.08 – 6.33

Test #	Processing pH	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			NaCN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
1	3.5	80.06	0.163	0.78	0.156	0.51	0.049	0.003	0.108	0.507	3.97	11.10	10.29
2	4.3(Unadj)	90.04	0.183	0.37	0.176	0.24	0.128	0.007	0.048	0.233	5.76	10.70	9.40
3	10.5	70.05	0.142	0.13	0.136	0.136	0.128	0.009	0.009	0.076	9.26	11.08	10.70

Results

Test	Assay Head, Au g/t	Calculated Head, Au g/t	Solids Residue, Au g/t	Actual Recovery, %	Calculated Recovery, %	Au Distribution, %			Total Au, %
						Bioleach filtrate	Pregnant soln	Solids residue	
1	2.33	1.73	1.33	42.92	23.28	9.23	14.05	76.72	100.0
2	2.33	1.89	1.31	43.78	30.82	17.95	12.87	69.18	100.0
3	2.33	2.34	1.07	54.29	54.08	23.07	31.22	45.71	100.0

E.2: Data on Effect of Pre-treatment Temperature on Gold Extraction for Sample A

Cyanidation Feed: 50 - 90g of pre-treated Sample A

Solution Volume: 427 - 488 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 10.84 – 11.08 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 1.16 - 1.29

CaO: 0.13 - 0.63

Test #	Processing Temp(°C)	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			NaCN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
4	Room Temp	80.05	0.163	0.17	0.156	0.11	0.106	0.007	0.051	0.104	8.61	10.84	10.28
5	30	70.05	0.142	0.14	0.136	0.009	0.128	0.009	0.009	0.081	9.26	11.08	10.75
6	45	80.08	0.163	0.14	0.156	0.009	0.138	0.009	0.018	0.081	9.59	11.08	10.75

Results

Test	Assay Head, Au g/t	Calculated Head, Au g/t	Solids Residue Au g/t	Actual Recovery %	Calculated Recovery %	Au Distribution, %			Total Au, %
						Bioleach filtrate	Pregnant soln	Solids residue	
4	2.33	2.28	1.35	42.02	40.69	11.25	29.44	59.31	100.00
5	2.33	2.34	1.07	54.29	54.08	23.07	31.22	45.71	100.00
6	2.33	2.41	0.80	65.67	66.85	29.00	37.85	33.15	100.00

E.3: Data on Effect of Pre-treatment Time on Gold Extraction for Sample A

Cyanidation Feed: 50 - 90g of pre-treated Sample A

Solution Volume: 305 - 488 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range : 10.7 – 11.5 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.125 – 0.937

CaO: 1.03 – 2.69

Test #	Processing time. days	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			NaCN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
7	3	50.05	0.102	.009	0.098	0.06	0.086	0.06	0.011	0.009	8.69	10.89	9.91
8	7	50.05	0.102	0.14	0.098	0.09	0.071	0.09	0.027	0.007	9.02	11.14	10.25
9	14	50.05	0.102	0.14	0.098	0.09	0.092	0.09	0.006	0.009	9.70	11.45	10.54
10	21	80.06	0.163	0.34	0.156	0.22	0.081	0.22	0.075	0.005	9.53	10.70	10.40
11	28	80.07	0.163	0.26	.156	0.17	0.146	0.17	0.010	0.009	9.61	10.81	10.23

Results

Test	Assay Head, Au g/t	Calculated Head, Au g/t	Solids Residue, Au g/t	Actual Recovery %	Calculated Recovery %	Au Distribution, %			Total Au %
						Biobleach filtrate	Pregnant soln	Solids residue	
7	2.33	2.32	1.47	39.91	36.71	23.59	13.11	63.29	100.00
8	2.33	2.33	1.40	39.85	39.85	29.39	10.47	60.15	100.00
9	2.33	2.35	1.07	54.29	54.10	17.00	37.53	45.47	100.00
10	2.33	1.94	0.41	82.40	78.92	28.80	50.12	21.08	100.00
11	2.33	1.91	0.38	83.69	80.11	41.86	38.25	19.89	100.00

E.4: Data on Effect of Pre-treatment Pulp Density on Gold Extraction for Sample A

Cyanidation Feed: 40 - 80g of pre-treated Sample A

Solution Volume: 244 - 488 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 10.85 – 11.12 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.125 – 0.531

CaO: 1.08 – 2.52

Test #	Processing Pulp Density	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			NaCN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
12	10%	40.04	0.081	0.14	0.078	0.09	0.057	0.007	0.021	0.083	9.28	11.12	9.93
13	20%	70.05	0.142	0.13	0.137	0.085	0.128	0.009	0.009	0.076	9.26	11.08	10.75
14	40%	80.04	0.163	0.31	0.156	0.20	0.122	0.008	0.034	0.193	8.95	10.85	9.99
15	50%	80.05	0.163	0.32	0.156	0.21	0.130	0.008	0.026	0.202	9.13	10.94	10.32

Results

Test	Assay Head, Au g/t	Calculated Head, Au g/t	Solids Residue, Au g/t	Actual Recovery, %	Calculated Recovery, %	Au Distribution, %			Total Au, %
						Bioleach filtrate	Pregnant soln	Solids residue	
12	2.33	2.33	0.92	60.52	60.45	19.02	41.12	39.55	100.00
13	2.33	2.36	1.07	54.29	54.79	23.77	31.02	45.21	100.00
14	2.33	1.92	0.87	62.66	54.59	26.44	28.15	45.41	100.00
15	2.33	2.18	0.93	60.09	55.57	29.64	25.93	34.43	100.00

E.5: Data on Gold Extraction after 2 weeks Abiotic and Chemolithotrophic bacterial Pre-treatment of Sample A

Cyanidation Feed: 75 - 95g of pre-treated Sample A

Solution Volume: 457 - 579 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 10.7 – 11.1 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.17-1.34 CaO: 1.00 – 4.84

Test #	Type	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			NaCN	Ca (OH) ₂	NaC N	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
16	Abiotic(30)	75.05	0.152	0.12	0.15	0.08	0.091	0.006	0.06	0.074	9.51	11.10	10.26
17	Abiotic(45)	95.05	0.163	0.28	0.16	0.18	0.142	0.009	0.01	0.17	9.29	10.66	10.22
18	Abiotic(H ₂ O)	80.07	0.163	0.20	0.16	0.13	0.138	0.009	0.02	0.12	9.43	10.74	10.31
19	Chem Bact(45)	90.06	0.183	0.42	0.18	0.27	0.146	0.008	0.03	0.262	2.91	10.95	9.00
20	Untreated	150.10	0.305	1.12	0.29	0.73	0.091	0.003	0.20	0.727	4.54	10.88	9.68

Results

Test	Assay Head, Au g/t	Calculated Head, Au g/t	Solids Residue, Au g/t	Actual Recovery, %	Calculated Recovery, %	%Au Distribution			Total Au, %
						Bioleach filtrate	Pregnant soln	Solids residue	
16	2.33	2.28	0.98	57.94	57.08	21.03	36.03	42.94	100.00
17	2.33	2.56	0.58	75.11	66.31	45.08	21.23	33.69	100.00
18	2.33	1.80	1.03	55.79	42.63	22.28	20.35	57.37	100.00
19	2.33	2.35	1.06	52.35	52.67	8.51	44.26	47.23	100.00
20	2.33	2.33	1.98	15.02	15.12	0.00	15.12	84.98	100.00

E.6: Data on Effect of Two Step Process sequence Abiotic-Biotic (AB/B) and Biotic-Abiotic (B/AB) on Gold Extraction

Cyanidation Feed: 60 - 90g of pre-treated Sample A

Solution Volume: 366 - 549 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 10.7– 11.2 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.23 – 0.43

CaO: 0.95 – 2.63

Test #	*Test Description	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			NaCN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
21	AB/B(30)	70.02	0.142	0.21	0.137	0.14	0.122	0.001	0.02	0.13	6.50	10.95	9.75
22	AB/B(45/30)	90.06	0.183	0.32	0.1176	0.21	0.155	0.0085	0.02	0.20	6.34	10.88	9.64
23	AB/B(45/45)	60.05	0.122	0.23	0.1176	0.15	0.100	0.009	0.02	0.14	6.56	10.70	10.2
24	Ab/b(45/45)	60.05	0.122	0.22	0.1176	0.20	0.089	0.02	0.03	0.18	6.58	11.14	10.65
25	B/AB(30)	80.06	0.163	0.24	0.156	0.16	0.102	0.002	0.054	0.14	9.54	11.12	10.75
26	B/AB(45/30)	60.08	0.122	0.23	0.1176	0.15	0.098	0.00	0.02	0.15	9.65	10.98	1045
27	B/AB(45/45)a	60.08	0.122	0.23	0.117	0.15	0.104	0.0085	0.01	0.142	9.23	10.95	10.71
28	B/AB(45/45)b	60.02	0.122	0.23	0.117	0.15	0.091	0.0075	0.03	0.143	9.41	11.07	10.67

*Pre-treatment Temperature (°C) are indicated in brackets

Results

Test	Assay Head, Au g/t	Calculated Head, Au g/t	Solids Residue Au g/t	Actual Recovery, %	Calculated Recovery, %	Au Distribution, %			Total Au, %
						Bioleach filtrate	Pregnant soln	Solids residue	
21	2.33	2.11	0.78	66.52	63.05	28.42	34.63	36.95	100.00
22	2.33	2.26	0.60	74.25	73.42	22.15	51.27	26.58	100.00
23	2.33	2.29	0.47	79.83	79.46	26.22	53.24	20.54	100.00
24	2.33	2.16	0.38	83.69	82.43	12.02	70.41	17.57	100.00
25	2.33	2.24	0.85	63.52	62.10	24.08	38.02	37.90	100.00
26	2.33	2.44	0.76	67.38	68.79	26.28	42.52	31.21	100.00
27	2.33	2.23	0.55	76.39	75.29	28.76	32.69	20.49	100.00
28	2.33	2.05	0.42	81.97	79.51	46.82	32.69	20.49	100.00

E.7: Data on Effect of Two Stage Pre-treatment Process on Gold Extraction

Cyanidation Feed: 50 - 90g of pre-treated Sample A

Solution Volume: 426 - 549 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 11.0 – 11.5 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.33 – 1.34

CaO: 1.17 – 3.16

Test #	Test Description	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			NaCN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaC N	CaO	Before lime	start	End
30	B/BACT(30)	90.02	0.183	0.450	0.176	0.29	0.091	0.005	0.08	0.285	3.5	11.04	9.17
31	BACT/SET(45)	70.08	0.142	0.138	0.137	0.09	0.114	0.008	0.02	0.262	8.11	11.01	9.91
32	BACT/B(30)	90.06	0.183	0.354	0.176	0.23	0.146	0.008	0.03	0.222	4.9	11.15	9.45
33	BACT/B(45)	60.10	0.122	0.180	0.117	0.117	0.037	0.003	0.08	0.114	4.9	11.45	10.17
34	AB/SET(45)	70.06	0.142	0.160	0.137	0.104	0.114	0.0085	0.002	0.095	9.07	11.04	10.20

Results

Test	Assay Head, Au g/t	Calculated Head, Au g/t	Solids Residue, Au g/t	Actual Recovery, %	Calculated Recovery, %	Au Distribution, %			Total Au, %
						Bioleach filtrate	Pregnant soln	Solids residue	
30	2.33	2.17	1.60	31.33	26.11	9.24	16.88	73.89	100.00
31	2.33	2.56	1.10	52.70	56.96	9.37	47.58	43.04	100.00
32	2.33	2.71	1.11	52.36	59.05	7.38	51.68	40.95	100.00
33	2.33	2.66	1.06	54.51	65.40	11.75	53.65	34.60	100.00
34	2.33	2.33	0.43	81.55	81.51	34.39	47.12	18.49	100.00

E.8: Gold Extraction Data for Sample B

Cyanidation Feed: 50 - 90g of pre-treated Sample A

Solution Volume: 365 - 548 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 10.65 – 11.1 with Ca (OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.23 - 1.95

CaO: 0.94 - 3.28

Test #	Description	Cyan Feed (g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			Na CN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
35	Untreated	90.0	0.183	0.461	0.176	0.3	0.082	0.005	0.09	0.295	6.35	10.65	9.85
36	B(30)	75.0	0.152	0.123	0.147	0.08	0.125	0.010	0.02	0.07	10.00	10.83	10.08
37	B(45)	80.1	0.163	0.22	0.156	0.142	0.130	0.008	0.03	0.135	10.19	11.09	10.26
37	B(45)	80.0	0.163	0.22	0.156	0.142	0.130	0.008	0.03	0.135	10.19	11.09	10.26
38	B/AB(45/30)	60.0	0.122	0.30	0.117	0.20	0.104	0.009	0.03	0.19	9.44	10.73	9.94
39	AB/B(30/45)	80.0	0.162	0.32	0.156	0.21	0.106	0.007	0.05	0.20	7.04	10.88	9.64

Results

Test	Assay Head Au g/t	Calculated Head Au g/t	Solids Residue Au g/t	Actual Recovery %	Calculated Recovery %	Au Distribution %			Total Au %
						Bioleach filtrate	Pregnant soln	Solids residue	
35	65.70	63.07	46.64	44.23	30.23	0.00	30.98	69.02	100.00
36	65.70	55.00	11.76	82.10	78.62	25.46	53.16	31.38	100.00
37	65.70	67.27	7.07	89.24	89.49	27.95	61.54	10.51	100.00
38	65.70	56.53	13.73	79.10	75.71	1.06	74.65	24.29	100.00
39	65.70	61.41	3.12	95.25	93.30	21.49	73.42	6.70	100.00

E.9: Data on Effect of Culture Glucose: Nitrogen (G: N) ratio on Gold Extraction

Cyanidation Feed: 70 - 95g of pre-treated Sample A

Solution Volume: 426 - 457 mls

Pulp Density: 33%solids

Solution Composition: 1g/L NaCN

pH Range: 10.5 – 11.5 with Ca(OH)₂

Grind: 20min/kg in a rod mill at 65%solids

Reagent Consumption (kg/t of cyanidation feed) NaCN: 0.12 - 1.04

CaO: 0.55 - 1.08

Test #	Description	Cyan Feed(g)	Reagent added(g)				Residual(g)		Consumed(g)		pH		
			Actual		Equivalent								
			Na CN	Ca(OH) ₂	NaCN	CaO	NaCN	CaO	NaCN	CaO	Before lime	start	End
40	2G:2N	95.1	0.193	0.092	0.186	0.06	0.145	0.008	0.04	0.05	9.38	10.94	10.3
41	2G:1N	95.1	0.193	0.108	0.186	0.07	0.097	0.005	0.09	0.06	9.04	10.98	10.28
42	1G:2N	80.1	0.163	0.123	0.156	0.08	0.073	0.005	0.08	0.04	8.74	10.86	9.97
43	1G:1N	70.1	0.142	0.131	0.137	0.09	0.128	0.009	0.01	0.08	9.26	11.08	10.75

Results

Test	Assay Head Au g/t	Calculated Head Au g/t	Solids Residue Au g/t	Actual Recovery %	Calculated Recovery %	Au Distribution %			Total Au %
						Bioleach filtrate	Pregnant soln	Solids residue	
40	2.33	2.50	1.28	48.8	36.04	16.42	19.66	63.92	100.00
41	2.33	2.25	1.16	50.21	48.56	16.15	32.41	51.44	100.00
42	2.33	2.33	1.63	30.04	30.15	17.10	13.05	69.85	100.00
42	2.33	2.31	1.06	54.51	54.17	17.30	36.87	45.83	100.00

Appendix F

Sample Calculations

F1: Sample Calculation of Au (g/t) in Bioleach Filtrate

Sample	Bioleach feed (g)	Assay Head Au g/t	Filtrate Vol mls	Filtrate Au mg/L	Au in sample			*Bioleach Residue Au g/t
					mg	mg/t	g/t	
A	100.0	2.33	400.0	0.10	0.004	400.0	0.4	1.93

*Bioleach residue Au (g/t) serves as Cyanidation feed grade

Au in bioleach filtrate (mg) = [Filtrate vol (mls) X Filtrate Au (mg)/1000mls]

Au leached from bioleach feed (g/t) = [10^3 g X Au in bioleach filtrate (10^{-3} mg)]/bioleach feed(g)

Bioleach residue grade (g/t) = Sample Head grade (g/t) – Au Leached from bioleach feed (g/t)

F.2 Sample Calculation of Gold Extraction (%) for Single Stage Process

Sample	Cyan Feed (g)	Bioleach Residue, Au g/t	Filtrate Vol (Preg + Wash) mls	Filtrate Au, mg/l	*Au in sample			CN Residue ,Au g/t	Calculated Head Au g/t	Actual Recovery, %	Calculated Recovery, %
					mg	mg/t	g/t				
A	70.0 5	1.93	462.7	0.14	0.06	870	0.87	1.06	2.31	54.5	54.1

*The Au (g/t) of feed leached during Cyanidation is calculated the same way as the bioleach.

Calculated Head = Au leached from bioleach feed (g/t) + Au in Cyanidation filtrate (g/t) + Cyanidation Residue (g/t)

Actual Recovery = [Actual Head grade (g/t) – Cyanidation residue (g/t)]/Actual Head grade (g/t)

Calculated Recovery = [Calculated Head grade (g/t) – Cyanidation residue (g/t)]/Calculated Head grade (g/t)

Appendix G

Determination of Thiosulfate ion in Bioleach filtrate

Procedure

Residual thiosulfate ion in bioleach filtrate was assayed by the idiometric titration method after microbial pre-treatment. (Skoog et al., 1996) Following is the procedure for the titration method:

- 10 mls of thiosulfate solution (bioleach filtrate) was taken into a 50ls Erlenmeyer flask and the pH measured. Should the pH be greater than 8, the solution pH is neutralized to the range of 7.0 to 7.5 with 10 %(v/v) H_2SO_4 acid .The volume of the H_2SO_4 acid added is noted.
- The neutralized filtrate is then transferred into a 25mls burette for the idiometric titration.
- 5mls I_2 solution was prepared into a 50mls beaker and 2 to 3 drops of 0.05g/dm^3 starch is added. The previously neutralized filtrate was then subjected to idiometric titration with the I_2 -starch. A color change from deep brown to colorless indicated the end point of the titration.
- The total volume of filtrate added to reach the end point is noted and used to evaluate the thiosulfate concentration.

From the data obtained from the titration, the thiosulfate concentration is calculated by the following equation;

$$M_T = (2 \times M_1 \times L_1) / \{L_2 \times (V_1/V_2)\} \dots\dots\dots A.1$$

Where, M_T : Thiosulfate concentration [mol/dm³]

M_1 : Iodine concentration [mol/dm³]

L_1 : Initial Iodine Volume

L_2 : Thiosulfate volume used for titration

V_1 : Initial thiosulfate volume (mls) before pH neutralization

V_2 : Final thiosulfate solution volume (mls) after pH neutralization

The 0.05 mol/dm³ I₂ solution was prepared by dissolving 20g of KI in 1 liter flask.

The starch solution was prepared by dissolving 1.0 g of soluble starch in 500 mls flask by heating.