SIMULTANEOUS BIOTRANSFORMATION OF CARBONACEOUS MATTER AND
SULFIDES IN DOUBLE REFRACTORY GOLD ORES USING THE FUNGUS,

*PHANEROCHAETE CHrysosporium*

A Dissertation in

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by

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Abstract

Double refractory gold ores (DRGO) contain metal sulfides that encapsulate gold and prevent its dissolution by cyanide, and carbonaceous matter (CM) that adsorbs gold cyanide complex. Pretreatment is therefore required to decompose the sulfides and liberate gold before cyanidation, and to deactivate CM and prevent it from adsorbing gold during cyanidation. In this research, the fungus *Phanerochaete chrysosporium*, has been used to effectively reduce the gold adsorption ability of CM on one hand, and decompose metal sulfides on the other, to ultimately enhance gold extraction.

Different ranks of coal (lignite, subbituminous, bituminous and anthracite) were used as surrogates to investigate the capability of the fungus to reduce the gold adsorption ability of CM. The extent of biotransformation was assessed primarily by the difference in the ability of CM to adsorb gold before and after the treatment. It was realized that relative to the other coals, anthracite possessed about 10 fold gold adsorption power in the as-received state, and after biotransformation, reduction in gold adsorption was more pronounced in anthracite. *P. chrysosporium* reduced the ability of anthracite to absorb gold cyanide complex by over 90% in different growth media including glucose, millet and wheat bran, and within a wide range of processing periods (up to 21 days), pulp densities (15-75 %), temperatures (25 °C and 37 °C), pH (4 and 6.5), and level of agitation (0 and 150 rpm). A processing time of 5-7 days in a mixture of millet and wheat bran (MWB) medium at 37 °C and pH 4 constituted the best conditions for optimum biotransformation. The best pulp densities for stationary and shake culturing respectively were 60 % and 25 %. Using the non-cyanide gold complexes; thiourea and thiosulfate, biotransformation by *P. chrysosporium* in MWB medium led to reduction of 50-92 % in gold adsorption by the carbonaceous surrogates and carbon-containing gold concentrates.

Analysis of as-received and processed anthracite using Raman, FTIR and XANES spectroscopies indicated that fungal-treatment led to a reduction in carbon and a boost of oxygen-containing groups such as carboxyls and carbonyls. In addition, surface area and pore volume reduced by about 75 % and 80 % respectively, whereas average pore diameter increased by 65 %. The results suggest that *P. chrysosporium* biotransforms anthracite by surface oxidation, which leads to a decrease in C/O ratio and disruption of the continuous nature of graphitic planes, thus decreasing the active sites necessary for adsorption. Another factor is reduction in surface area via plugging of pores possibly by fungal hyphae and spores, and biofilms formed through fungal interaction with the substrate, and hence
decreasing accessibility of gold to the adsorption sites. Fungal action also led to pore enlargement, thus rendering the pores relatively unsuitable for adsorption of aurocyanide ions, which are generally known to adsorb preferentially into micropores.

*P. chrysosporium*, was also assessed for its ability to decompose sulfides in DRGO. Using surrogate pyrite and arsenopyrite, and sulfide-containing gold concentrate, biotransformation led to high concentrations of iron, sulfur and arsenic in incubation solutions, and reduction in sulfide sulfur in the residual solids. Overall, decomposition of sulfide sulfur in the samples was 15 wt%, 35 wt% and 57 wt% respectively for pyrite, arsenopyrite and the gold concentrate after 21 days of treatment. Changes in sulfide sulfur concentration and formation of oxide phases during fungal treatment were confirmed using Raman spectroscopy and X-ray diffraction analysis. Oxide phases formed included hematite and ferric arsenate.

Fungal treatment of flotation concentrate (FC) of DRGO and bacteria-oxidized FC (BFC) resulted in enhancement of cyanidation gold recovery. An increase of 38 % and 13 % for FC and BFC respectively over the as-received samples was realized. The single stage fungal treatment of FC recorded 78 % in gold recovery which compares very well with the 81 % obtained from the commercial biooxidation treatment of FC. Preliminary investigations on the use of cell-free extracts from *P. chrysosporium* to effect biotransformation of sulfides and CM in DRGO led to 26 % increase in gold recovery during cyanidation over the as-received, after a 3-day bioprocessing period.

The results suggest that *P. chrysosporium* is a promising microorganism for oxidative decomposition of metal sulfides associated with refractory gold ores and for deactivation of CM. In addition, it demonstrates an innovative and a potentially alternative pretreatment process for DRGO using either whole cells (*in vivo*) or cell-free extracts (*in vitro*) of *P. chrysosporium* to simultaneously biotransform sulfides and CM, and consequently enhance gold extraction.
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Chapter 1

General Introduction

1.1 Problem definition

1.1.1 Gold ores

A mineral is basically a naturally occurring solid that has a distinct chemical composition and structure, and an aggregate of minerals constitutes a rock. An ore is a type of rock mass from which a mineral of interest, such as gold can be recovered economically, given the available level of technology (Kesse, 1985; Boyle, 1987). Gold ores can be classified broadly as non-refractory (alluvial and free-milling) and refractory from a metallurgical standpoint depending on the mineralogy and the ease of gold extraction. In alluvial ore, gold exists as discrete particles naturally liberated by weathering. Gold in alluvial ores may be recovered by scrubbing and concentration processes, where the high specific gravity of gold is exploited for separation. Free-milling ores are those from which about 95% of the gold can be recovered by gravity concentration and/or simple cyanidation (dissolution via aurocyanide, \( \text{Au(CN)}_2^- \), formation) after milling (grinding) to 80% passing 75 µm (Marsden and House, 2006).

Refractory gold ores are more difficult to treat and depending on the degree of refractoriness, recovery could be below 50% (Hausen, 2000; Vaughan and Kyin, 2004). Since gold ores are fixed resources, continuous mining and subsequent depletion of non-refractory gold ores have generated profound interest in research on gold recovery from refractory ores. These hard-to-treat ores occur in several gold mining regions throughout the world (Baako, 1972; Boyle, 1979; Kesse, 1985; Brierley, 1995; Vaughan, 2004). The annual world gold production increased by 47% from 1677 t in 1989 to over 2464 t in 2004. Within this period, gold production from refractory gold ores increased by 165% from 94 t to 249 t, which is
approximately 10% of global production of the precious metal (Marsden 2006; Yen et al, 2008).

In refractory ores, gold usually exists as extremely fine particles (< 1 µm) (Marsden and House, 2006) in intimate association with gangue (waste) materials, and the major problem of refractoriness centres on the presence of metal sulfides and/or carbonaceous matter. In sulfidic refractory gold ores, tiny gold particles may be highly disseminated and locked up within the grain boundaries or fractures of sulfides such as pyrite and arsenopyrite. For this reason, decomposition of the sulfides is required to liberate the gold (Boyle, 1979; Guay, 1981; Arriagada and Osseo-Asare, 1984; Cabri et al., 1989; Marsden and House, 2006; Benzaazoua et al, 2007). The presence of carbonaceous matter (CM) in gold ores leads to two main deleterious effects: (a) confinement of gold with attendant difficult release from the CM matrix and (b) loss of dissolved metal via the ability of CM to adsorb gold from gold-impregnated solution. Adsorption of gold by CM leads to gold losses due to the fineness of the carbon (0.002–2 µm) which does not permit it to be screen-separated for gold recovery, and this phenomenon is termed as preg-robbing (Osseo-Asare et al., 1984; Hausen and Bucknam, 1985; Hutchins et al., 1988; Afenya, 1991; Kohr, 1994; Adams and Burger, 1998; Pyke et al, 1999). Due to the similarities between CM and the various coal ranks, coal is normally used as a surrogate in the characterization of this carbonaceous material (Ibrado and Fuerstenau, 1992; Van Krevelen, 1993; Van Vuuren et al., 2000).

If the same refractory ore contains sulfides and carbonaceous matter, the ore is referred to as double refractory (Nyavor and Eg挹or, 1992; Amankwah et al., 2005). Recovery of gold from double refractory ores can be less than 50 % when subjected to the conventional cyanidation process due to (a) the inability of cyanide to permeate sulfides and dissolve the encapsulated gold, and (b) the uptake of dissolved gold by CM (Osseo-Asare et al., 1984; Abotsi and Osseo-Asare, 1986).
1.1.2 Relevance of microbial pretreatment of double refractory gold ores (DRGO)

For gold locked up in the refractory components, especially sulfides, to be amenable to cyanidation, pretreatment is required to decompose these shielding minerals, thus liberating the gold. The processes that have been used commercially to pretreat refractory gold ores include roasting, pressure oxidation and bacteria oxidation (Arriagada and Osseo-Asare, 1984; Berezowsky et al., 1988; Komnitsas and Pooley, 1989; Afenya, 1991; Rawlings et al., 2003). Biooxidation employs the use of iron and sulfur chemolithoautotrophic bacteria such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, to catalyze the oxidation of metal sulfides, thus liberating gold for subsequent cyanidation (Livesey-Goldblatt et al, 1983; Brierley, 1995; Sand et al., 1995; Hackl, 1997). Since the bacteria are not organotrophs they do not utilize the organic carbon (Silver, 1970; Madigan and Martinko, 2006) which is consequently routed into the cyanidation circuit where it preg-robs dissolved gold.

Current research efforts aimed at improving gold extraction from double refractory gold ores have been focused on two-stage processes to first oxidize sulfides and then deactivate the CM (Portier, 1991; Brierley and Kulpa, 1992; Amankwah et al., 2005). Microorganisms employed in deactivating CM include the bacteria *Pseudomonas sp.*, *Achromobacter sp.* and *Streptomyces setonii*, and fungi *Aspergillus bruneio*, *Penicillium citrinum* and *Trametes versicolor* (Portier, 1991; Brierley and Kulpa, 1993; Amankwah and Yen, 2006; Afidenyo, 2008; Yen et al., 2008). It is expected, however, that a single-stage microbial process which simultaneously deactivates the CM and solubilizes the sulfides will be less time-consuming and more cost-effective.

Available literature indicates that microbial degradation of both sulfides and CM in a single-stage has thus far not achieved much success (Portier, 1991; Brierley and Kulpa, 1993; Amankwah and Yen, 2006; Yen et al., 2008). The limitation to single-stage processes has been attributed to the inability of the heterotrophic carbon deactivating microorganisms which work at relatively higher pH range (4-8) to coexist and function effectively at the optimum
conditions within which the acidophilic (pH, 1-2) sulfide oxidizing bacteria work (Amankwah et al., 2005; Madigan and Martinko, 2006).

Most of the investigations have recorded substantial degradation for bituminous-grade CM with little success in reducing preg-robbing for anthracite-grade CM, which incidentally constitutes about 50% of CM in refractory gold ores (Osseo-Asare et al., 1984; Sibrell et al., 1990; Pyke et al., 1999; Stenebraten et al., 2000). In addition, anthracite grade CM has a far higher capacity to adsorb gold than bituminous and lignite coals due to its maturity and hence well-developed graphitic planes (Jones et al, 1989; Ibrado and Feurstenau, 1992; Van Vuuren et al., 2000; Amankwah and Yen, 2006; Afidenyo, 2008). Consequently, using a microorganism that can decompose metal sulfides, and simultaneously deactivate CM and reduce its preg-robbing nature significantly, will be of immense benefit in the treatment of double refractory gold ores.

1.1.3 Phanerochaete chrysosporium

The white rot basideomycete, *Phanerochaete chrysosporium*, is a prominent lignin-degrading fungus from which the first lignin-degrading enzyme, lignin peroxidase (LiP), was isolated and characterized by Tien and Kirk (1983; 1984). The fungus has hence been studied in many laboratories as a model organism as it grows quickly and degrades lignin rapidly. *P. chrysosporium* also secretes manganese peroxidase (MnP), and these oxidative enzymes catalyze the biodegradation of lignin and a variety of aromatic carbonaceous materials including low rank coals, wood chips and environmental pollutants (Glenn et al., 1983; Bumpus and Aust, 1986; Ralph and Catcheside, 1997; Tunde and Tien, 2000; Martin and Petersen, 2001). Since the carbonaceous matter (CM) in refractory gold ores is characterized to be similar to coal, it is expected that the oxidative enzymes can biotransform the CM.

*P. chrysosporium* also secretes a H$_2$O$_2$-generating enzyme, glyoxal oxidase (Kersten and Kirk, 1987), and hydrogen peroxide is known to solubilize pyrite and arsenopyrite (McKimben and
Barnes, 1986; Antonijevic, et al., 1997; Jennings et al., 2000). The strong oxidizing environment produced by the oxidative enzymes of *P. chrysosporium* is also known to be responsible for the oxidative removal of sulfur from coal (Schreiner et al., 1988; Gonsalvesh et al., 2008; Aranda et al., 2009). It is therefore possible that *P. chrysosporium* can catalyze the biotransformation of carbonaceous matter and sulfides, and hence enhance gold extraction in the subsequent cyanidation step.

This dissertation thus describes research aimed at utilizing a single-stage fungal process to achieve simultaneous deactivation of carbonaceous matter and decomposition of metal sulfides in double refractory gold ores. Analysis of the data obtained from this study would contribute to the pool of scientific knowledge regarding processing of refractory gold ores and hence be of immense benefit to academia. In addition, the results could also find application in gold production communities.

1.2 Hypothesis

On the basis of the above considerations, the following hypotheses were built:

1. The extra-cellular oxidative enzymes and hydrogen peroxide generated by *P. chrysosporium* can;
   a. biotransform the native carbonaceous matter in double refractory gold ores and thus reduce its activity in gold adsorption, and
   b. solubilize sulfides and hence liberate the locked-up gold for direct contact with cyanide solution,
2. The fungal-treatment will ultimately increase gold extraction via cyanidation.
1.3  Specific research objectives

This study sought to investigate the simultaneous fungal-degradation of carbonaceous matter (CM) and sulfides in double refractory gold ores (DRGO) with the following specific interests:

- Investigate the extent of biotransformation of CM by *P. chrysosporium* with subsequent reduction in gold adsorption capabilities;
- Investigate the extent of biotransformation of sulfides ultimately to sulfate by *P. chrysosporium*;
- Assess the effect of process variables (time, pH, pulp density, temperature, and level of agitation) on biomodification of CM and sulfides;
- Examine the simultaneous degradation of CM and sulfides in DRGO by *P. chrysosporium*; and
- Provide understanding of the mechanism for biotransformation of DRGO by *P. chrysosporium*.

1.4  Approach to accomplish experimental objectives

The objectives set out for this study were realized by obtaining the dependent variables as a function of the independent variables using the techniques listed in Table 1.1 as a guide.
Table 1.1. Summary of the methodology required to accomplish the objectives

<table>
<thead>
<tr>
<th>Material</th>
<th>Independent variables</th>
<th>Dependent variables</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Carbonaceous</td>
<td>Coal type (lignite, sub-bituminous, bituminous and anthracite)</td>
<td>Gold adsorption test of treated solids</td>
<td>ICP-AES</td>
</tr>
<tr>
<td>matter</td>
<td>Media (glucose, millet and wheat bran)</td>
<td>Degree of graphitization</td>
<td>Raman</td>
</tr>
<tr>
<td></td>
<td>Time (1-21 days)</td>
<td>Degree of surface oxidation</td>
<td>Infrared, XANES</td>
</tr>
<tr>
<td></td>
<td>Pulp density (15-75 % solids)</td>
<td>Micro-porosity</td>
<td>BET</td>
</tr>
<tr>
<td></td>
<td>Temperature (25 °C and 37 °C)</td>
<td>Structural analysis</td>
<td>TEM</td>
</tr>
<tr>
<td></td>
<td>pH (4 and 6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2. Sulfides</td>
<td>Sulfides (pyrite and arsenopyrite)</td>
<td>Measurement of dissolved constituents in incubation solution</td>
<td>ICP-AES</td>
</tr>
<tr>
<td></td>
<td>Time (1-21 days),</td>
<td>Sulfur speciation and surface oxidation products of treated solids</td>
<td>XRD, LECO</td>
</tr>
<tr>
<td></td>
<td>Pulp density (15-60 %)</td>
<td></td>
<td>combustion methods</td>
</tr>
<tr>
<td></td>
<td>Temperature (37 °C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>pH (4 and 6.5)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3. Gold ores</td>
<td>Gold concentrate (flotation concentrate (FC) and bacterial-oxidized FC)</td>
<td>Maturity of CM</td>
<td>Petrographic analysis</td>
</tr>
<tr>
<td></td>
<td>Time (1-21 days)</td>
<td>Measurement of dissolved constituents in incubation solution</td>
<td>ICP-AES</td>
</tr>
<tr>
<td></td>
<td>Pulp density (15-60 %)</td>
<td>Sulfur speciation and surface oxidation products of treated solids</td>
<td>XRD, LECO</td>
</tr>
<tr>
<td></td>
<td>Temperature (37 °C)</td>
<td>Gold preg-robbing</td>
<td>combustion methods</td>
</tr>
<tr>
<td></td>
<td>pH (4 and 6.5)</td>
<td>Gold cyanidation</td>
<td>ICP-AES</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ICP-AES, fire assay</td>
</tr>
</tbody>
</table>

1.5 Scope of research

This dissertation is presented in nine chapters including the introductory and final concluding chapters. The various titles with brief descriptions of the chapters are shown in Table 1.2.
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Brief description</th>
</tr>
</thead>
<tbody>
<tr>
<td>One</td>
<td>General introduction</td>
<td>Problem statement, motivation, hypothesis and objectives for the work</td>
</tr>
<tr>
<td>Two</td>
<td>Mycohydrometallurgy: application of fungi in hydrometallurgy</td>
<td>Literature review of the diversity and application of fungi biosorption, bioleaching and degradation of organic matter</td>
</tr>
<tr>
<td>Three</td>
<td>Mycohydrometallurgy: Coal model for potential reduction of preg-robbing capacity of carbonaceous gold ores using the fungus, <em>Phanerochaete chrysosporium</em></td>
<td>Experiments establishing the potential of <em>P. chrysosporium</em> to reduce gold adsorption (preg-robbing) capacity of carbonaceous matter in refractory gold ores</td>
</tr>
<tr>
<td>Four</td>
<td>Fungal biotransformation of anthracite-grade carbonaceous matter: effect on gold cyanide uptake</td>
<td>Establishment of a set of incubation parameters that leads to maximum reduction in preg-robbing of anthracite when using <em>P. chrysosporium</em></td>
</tr>
<tr>
<td>Five</td>
<td>Gold adsorption on carbonaceous matter following fungal treatment: comparison of cyanide and non-cyanide gold complexes</td>
<td>Ability of carbonaceous matter of different maturity to adsorb different gold complexes (i.e., cyanide, thiourea, and thiosulfate), and reduction in adsorption due to fungal-biotransformation</td>
</tr>
<tr>
<td>Six</td>
<td>Biotransformation of sulfides using the fungus, <em>Phanerochaete chrysosporium</em>: A potential pretreatment process for refractory sulfidic gold ores</td>
<td>Experiments establishing the ability of <em>P. chrysosporium</em> to oxidize pyrite and arsenopyrite in refractory sulfidic gold ores</td>
</tr>
<tr>
<td>Seven</td>
<td>Biotransformation of double refractory gold ores by the fungus, <em>Phanerochaete chrysosporium</em></td>
<td>Experiments to establish the ability of <em>P. chrysosporium</em> to simultaneously deactivate carbonaceous matter and decompose sulfides in double refractory gold ores (DRGO), thus increasing gold extraction via cyanidation</td>
</tr>
<tr>
<td>Eight</td>
<td><em>In vitro</em> biotransformation of sulfides and carbonaceous matter in refractory gold ores using cell-free extracts of <em>Phanerochaete chrysosporium</em></td>
<td>Preliminary investigations to assess the possibility of simultaneous biotransformation of DRGO with cell-free extracts of <em>P. chrysosporium</em>.</td>
</tr>
<tr>
<td>Nine</td>
<td>Conclusions and recommendations for future research</td>
<td>General conclusions, recommendations for future work and processing proposals</td>
</tr>
</tbody>
</table>
References


Hausen, D. M., 2000. Characterizing the textural features of gold ores for optimizing gold extraction. JOM., 52, 14-16.


Chapter 2

Mycohydrometallurgy: Application of fungi in hydrometallurgy

Abstract

Mycology is the study of the properties of fungi as well as their beneficial and adverse effects on humans and the environment, whereas hydrometallurgy is a branch of extractive metallurgy that focuses on the use of aqueous chemistry to recover metals from materials such as ores, concentrates, scrap and residues. “Mycohydrometallurgy” is therefore adopted to define the connection between mycology and hydrometallurgy, hence, the application of fungi in the field of metal extraction.

For decades, bacterial-based systems have been the focus of research efforts in biohydrometallurgy whereas fungal-mediated hydrometallurgy has received relatively little attention. Bacteria are useful in the oxidation of inorganic substances such as sulfide minerals and some organic materials like low rank coals, while fungi are capable of biotransforming recalcitrant (difficult to degrade) organic matter. Different types of fungi have been utilized in many areas where such organic matter is encountered, including the paper and pulp industry, breweries, coal solubilization, environmental clean-up and nutrient recycling in soils. In this study, the literature on the application of fungi in hydrometallurgy is reviewed so as to provide a general background with respect to diversity, versatility and hydrometallurgical applications in the areas of biosorption, bioleaching and biodegradation of recalcitrant organic matter. The biotransforming activities of the fungus of interest, Phanerochaete chrysosporium, are also highlighted.

Keywords: Mycohydrometallurgy; Mycology; Hydrometallurgy; Fungi; Biosorption, Bioleaching; Biodegradation
Microorganisms fall into five major groups which are bacteria, viruses, fungi, algae and protozoa, and biotechnology exploits the spectacular metabolic versatility of these microorganisms in the environment (Shuler and Kargi, 1992; Glazer and Nikaido, 1995). The contributions of bacteria and fungi to microbial biotechnology far exceed those of the other microorganisms, and these have been studied extensively (Stanier et al., 1986; Tortora et al., 2004; James et al., 2006; Madigan and Marting, 2006). Biohydrometallurgy, an extension of biotechnology, is the branch of extractive metallurgy which focuses on the use of aqueous chemistry and biological systems to recover metals from ores and metallurgical products (Gupta and Mukherjee, 1990; Glazer and Nikaido, 1995; Madigan and Martin, 2006). Biohydrometallurgy has focused on bacterial-based systems for decades whereas fungal-mediated hydrometallurgy has received relatively little attention (Sterflinger, 2000). Bacteria are useful in the oxidation of inorganic substances such as sulfide minerals and some organic materials like low rank coals (lignite) whereas fungi are capable of biotransforming recalcitrant organic matter.

Fungi are classified as a kingdom that is separate from plants, animals and bacteria (Tortora et al., 2004; James et al., 2006; Madigan and Marting, 2006; Anon, 2009a). Several types of fungi have been used in the degradation of organic matter in the biopulping industry, breweries, coal solubilization, environmental clean-up and nutrient recycling in soils (Bumpus and Aust, 1986; Glazer and Nikaido, 1995; Sterflinger, 2000). The metabolic versatility of fungi and their application in hydrometallurgy is currently being recognized and “mycohydrometallurgy” is adopted to define the link between mycology and hydrometallurgy, thus the application of fungi in metal recovery.

Fungi are particularly effective in colonizing and degrading wood and other organic carbon-containing materials such as coal, by secreting powerful extracellular enzymes and non-enzymatic agents (Crawford et al., 1983; Cohen et al., 1987; Tien and Kirk, 1988; Quigley et al., 1989; Hofrichter et al., 1997; Holker et al., 1999; Fakoussa and Hofrichter, 1999). The oxidative enzymes include lignin peroxidase, manganese peroxidase and laccase, and
hydrolytic enzymes, mainly esterases, whereas the non-enzymatic agents include alkaline metabolites and natural chelaters such as biogene amines and ammonium oxalate (Fakoussa and Hofrichter, 1999). In this chapter, the literature on the application of fungi in hydrometallurgy is reviewed so as to provide a general background with respect to diversity, versatility and hydrometallurgical applications such as biosorption, bioleaching and degradation of recalcitrant organic matter.

2.2 Fungi

Fungi are neither plants nor animals and exist as a different group of living organisms (Martin, 2002). Mycology is the discipline of biology devoted to the study of fungi and is often regarded as a branch of botany, even though genetic studies have shown that fungi are more closely related to animals than to plants (Tortora et al., 2004; James et al., 2006; Madigan and Martingo, 2006; Anon, 2009a).

The main difference between plants and fungi is that fungi lack chlorophyll and hence cannot synthesize food from sunlight through the process of photosynthesis. Like animals, fungi obtain food from other organisms, and as fruiting bodies the large ones may be used for food. The fruit is supported by a vast network of extremely fine, hair-like filaments known as hyphae with which they access soil, wood and leaf litter, breaking it down and feeding on the nutrients. A mass of hyphae is known as the mycelium. Fungi reproduce by releasing large numbers of small spores estimated at 200 million per hour (Martin, 2002; Madigan and Martinko, 2006).

Fungi may be saprophytic (feed on dead plants and animal materials), parasitic (feed off a living host) or symbiotic (share a mutually beneficial relationship with another organism). Saprophytic fungi generate enzymes to soften the dead plant or animal. These enzymes increase the decomposition rate of the dead material and the fungi thus digest food externally, and reabsorb the nutrients. These fungi are recyclers and are responsible for the release of 85% of carbon in dead plant and animal material, and free up the nutrients making them
available to other living organisms. *Phanerochaete chrysosporium*, *Ceratocystis fagacearum* and *Mycorrhizal* are examples of saprophytic, parasitic and symbiotic fungi respectively.

Parasitic fungi are harmful to hosts while symbiotic fungi penetrate the roots of trees and feed on the nutrients produced during photosynthesis. In response to the symbiotic relation, the fungal hyphae acts as an extension of the roots of the plant, collecting essential resources such as water, phosphorus and trace elements which are fed back into the host plant. In addition, symbiotic fungi protect the host plant by attacking parasitic fungi (Martin, 2002; Madigan and Martinko, 2006).

### 2.3 Sorption of metals by fungi

The need for cheaper and more efficient systems for the removal of heavy metals from effluents and from contaminated environments has generated interest in the use of biological systems, and these systems have been studied considerably in recent times. Available literature indicates that the use of plant biomass and that of lower animals in the sorption of heavy and toxic metals have several advantages over chemical techniques due to their efficient removal of metal ions at lower concentrations (Brierley, 1990; Glazer and Nikaido, 1995; Volesky, 2001; Yalcinkaya et al., 2001).

Several fungi have been investigated for their metal sorption/bioaccumulation activities, and the complexity of the fungal structure implies that there are many ways by which dissolved metals can be captured by the cells. The sorption mechanisms are therefore diverse and in some cases they are still not very well understood (Brierley, 1990; Glazer and Nikaido, 1995; Volesky, 2001; Yalcinkaya et al., 2001; Madigan and Martinko, 2006). In general, however, fungal sorption mechanisms can be divided into (1) metabolism dependent and (2) non-metabolism dependent. Relative to where the metal removed from solution is found, sorption may be classified as (a) extracellular accumulation, (b) cell surface sorption and (c) intracellular accumulation (Volesky, 2001).
Biosorption can be accomplished either with only fungal biomass or fungal biomass immobilized in other materials such as carboxymethyl cellulose (Lakshmanan et al., 1989; Alibhai et al., 1993; Yetis et al., 2000; Blaudez et al., 2000; Yalcinkaya et al., 2001; Baldi et al., 2007). Baldrian (2003) noted that the two white rot basidiomycetous fungi, *Phanerochaete chrysosphorium* and *Trametes versicolor* have the ability to bioaccumulate Cd, Cu, Hg, Ni, Pb, Cr and Zn. Investigations by Pryfogle et al. (1989) shows that the fungi *Rhizopus cohnii* and *Rhizopus arrhizus* bioaccumulate chromium, and *Aspergillus niger*, *Pleurotus pulmonarius* and *Schizophyllum commune* sorb copper. The microorganisms, *Aspergillus niger*, *Penicillium simplicissimum*, *Aspergillus foetidus* and *Aspergillus carbonarius* are known to survive in environments with high metal ion concentrations and exhibit high metal tolerance in the presence of Ni, Co, Fe, Mg and Mn up to metal ion concentrations of 2000 mg/L (Valix et al., 2001; Valix and Loon, 2003).

A summarized compilation of biosorption of heavy metals by fungi in the literature is presented in Table 2.1. As shown in the table, the two most tested fungi are *Phanerochaete chrysosphorium* and *Trametes versicolor* which demonstrated the ability to sorb Cr$^{3+}$, Cd$^{2+}$, Ni$^{2+}$, Pb$^{2+}$, Cu$^{2+}$, Zn$^{2+}$ and Hg$^{2+}$ from both wastewater and contaminated soils. From initial metal ion concentrations of between 10 and 700 mg/L, sorption values of up to 100% were recorded utilizing *Phanerochaete chrysosphorium* in the sorption of Pb$^{2+}$, Cu$^{2+}$ and Zn$^{2+}$.
Table 2.1a. Heavy metal biosorption by fungi

<table>
<thead>
<tr>
<th>Fungus name and mode of contact</th>
<th>Metal sorbed</th>
<th>Experimental techniques</th>
<th>Quantitative estimates per gram of biosorbents</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Phanerochaete chrysosporium</em></td>
<td>Cr³⁺, Cd²⁺, Ni²⁺, Pb²⁺, Cu²⁺ and Hg²⁺</td>
<td>Review of sorption of metals by fungal biomass</td>
<td>N/A</td>
<td>Baldrian (2003)</td>
</tr>
<tr>
<td><em>Trametes versicolor</em>, mycelia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Phanerochaete chrysosporium</em>, immobilized biomass</td>
<td>Pb²⁺, Cu²⁺ and Zn²⁺</td>
<td>pH 6.0 10–50 °C 60 mins Initial concentration 10-400 mg/L</td>
<td>135.3, 102.8, 50.9 mg/g of Pb, Cu and Zn 20-100 % removal</td>
<td>Iqbal and Edyvean (2004)</td>
</tr>
<tr>
<td><em>Phanerochaete chrysosporium</em>, immobilized biomass</td>
<td>Pb²⁺ and Zn²⁺</td>
<td>pH 5.0 and 6.0 25 °C Initial concentration 30–600 mg/L</td>
<td>230, 282 and 355 mg/g of Pb and 30, 37 and 48 mg/g for Zn</td>
<td>Arica et al. (2003; 2004)</td>
</tr>
<tr>
<td><em>Phanerochaete chrysosporium, Polyporus versicolor</em>, mycelium</td>
<td>Cu²⁺, Cr³⁺, Cd²⁺, Ni²⁺ and Pb²⁺</td>
<td>N/A</td>
<td>57.5 and 110 mg Pb/g</td>
<td>Yetis et al. (2000)</td>
</tr>
<tr>
<td><em>Trametes versicolor</em>, immobilized biomass</td>
<td>Cd²⁺</td>
<td>15 and 45 °C Initial concentration 30–700 mg/L</td>
<td>124 and 153 mg Cd/g 68-84 % removal</td>
<td>Yalcınkaya et al. (2002)</td>
</tr>
<tr>
<td><em>Trametes versicolor</em>, immobilized biomass</td>
<td>Cu²⁺, Pb²⁺ and Zn²⁺</td>
<td>pH 4.0 and 6.0 15 and 45 °C 1 hr 1.0 g/L stock solution</td>
<td>1.51 -1.84 mmol/g Cu, 0.85 -1.11 mmol/g Pb and 1.33-1.67 mmol/g Zn</td>
<td>Bayramoglu et al. (2003)</td>
</tr>
</tbody>
</table>
Table 2.1b. Heavy metal biosorption by fungi (continued)

<table>
<thead>
<tr>
<th>Fungus name and mode of contact</th>
<th>Application</th>
<th>Experimental techniques</th>
<th>Metal sorbed</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sphaerotilus natans</em>, biomass</td>
<td>Cd(^{2+}), Cu(^{2+})</td>
<td>pH 3, 4, 5 and 6.0 biomass concentration 0.5, 1.0 and 2 g/L</td>
<td>N/A</td>
<td>Esposito et al. (2001)</td>
</tr>
<tr>
<td><em>Funalia trogii</em>, immobilized biomass</td>
<td>Hg(^{2+}), Cd(^{2+}) and Zn(^{2+})</td>
<td>pH range 3.0–7.0 15 and 45 °C Initial concentration 30–600 mg/L</td>
<td>Hg, Cd and Zn were 403.2, 191.6, and 54.0 mg/g</td>
<td>Arıca et al. (2004)</td>
</tr>
<tr>
<td><em>Pleurotus pulmonarius</em> and <em>Schizophyllum commune</em>, dead fungal biomass</td>
<td>Cu(^{2+})</td>
<td>pH 2.0 and 4.0 30 and 50 °C, 12 h Initial concentration 4 and 100 mg/L biomass concentration 0.5 and 3. g /L</td>
<td>1.6-5 mg/g</td>
<td>Veit et al. (2005)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em>, biomass</td>
<td>Cu(^{2+})</td>
<td>pH 6.0 30 min - 24 hr Initial concentration 100, 50, 25, 10 mg/L 100 mg biomass</td>
<td>70 %; 23.62 mg/g</td>
<td>Mukhopadhyay et al. (2007)</td>
</tr>
</tbody>
</table>
Table 2.1c. Heavy metal biosorption by fungi (continued)

<table>
<thead>
<tr>
<th>Fungus name and mode of contact</th>
<th>Application</th>
<th>Experimental techniques</th>
<th>Metal sorbed</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penicillium</em> sp., biomass</td>
<td>Zn$^{2+}$</td>
<td>pH 3-7</td>
<td>70 % in 5 min</td>
<td>Mishra and Choudhuri (1996)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30-70 °C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Initial concentration 4-20 mg/L ions</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1-0.5 g biomass</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus cohnii</em>, immobilized living and dead biomass</td>
<td>Cr$^{6+}$</td>
<td>28 °C</td>
<td>150-300 mg/g</td>
<td>Li et al. (2008)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10 min</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Initial concentration 50 mg/L Cr$^{6+}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Rhizopus arrhizus</em>, biomass</td>
<td>Cr$^{6+}$ and Fe$^{3+}$</td>
<td>25 °C, 50 and 500 mg/ L stock solution, 1 g cell wt /L,</td>
<td>50-500 mg/L</td>
<td>Sag et al. (1998), Sag (2001)</td>
</tr>
<tr>
<td><em>Streptomyces rimosus</em>, biomass</td>
<td>Cu$^{2+}$, Zn$^{2+}$ and Cr$^{6+}$</td>
<td>pH 5, 25°C, Initial concentration 0–300 mg/L, biomass dry weight 3 g/L</td>
<td>30 mg/g Cu 27.4 mg/g Zn and 26.7 mg/g Cr</td>
<td>Chergui et al. (2007)</td>
</tr>
</tbody>
</table>
2.4 Leaching of metals by fungi

Several fungi have been identified for their abilities in metal leaching (Dave and Natarajan, 1981; Baglin et al., 1992; Sukla and Panchanadikar, 1993; Torma et al., 1993; Tzeferis and Agatzini-Leonardou, 1994; Castro et al., 2000; Brandl et al., 2001; Valix and Loon, 2003; Le et al., 2006; Santhiya and Ting, 2005; 2006; Thangavelu et al., 2006; Mohapatra et al., 2007; Ren et al., 2009; Simate et al., 2010). The microorganisms may be utilized either in vivo (whole cells) or in vitro (cell free extracts); with the former mostly used.

The metals leached and the conditions utilized are presented in Tables 2.2. It can be seen from the table that the most utilized fungal genus is *Aspergillus* (about 70% of the time), with *Aspergillus niger* being the most versatile species. *A. niger* has been investigated for leaching of ores and metallurgical products, and is capable of secreting organic components such as oxalic and citric acids that dissolve the metals at temperatures between 30 and 40 °C. The metals dissolved include Cu, Sn, Al, Ni, Pb, Mn and Zn, and extraction efficiencies are very high up to 99%. For about 90% of the time, shake culturing is employed.
<table>
<thead>
<tr>
<th>Fungus name and mode of contact</th>
<th>Application</th>
<th>Experimental techniques</th>
<th>Metal leached</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus</em> and <em>Penicillium</em>, whole cells and supernatants</td>
<td>Leaching of metals from siliceous ores</td>
<td>Shake at pH 7.5 at 30 °C for 4 hr to 5 days for leaching of various heavy metals from calamine, garnierite</td>
<td>Zn and Ni</td>
<td>Castro et al., (2000)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em>, whole cells</td>
<td>Leaching of the metals from municipal solid wastes (MSW) incineration fly ash</td>
<td>Shake at pH 6.7 at 28-35 °C for 5-25 days for leaching of various heavy metals from fly ash,</td>
<td>11-87.4 % recovery of Al, Fe, Pb, Cd, Mn, and Zn</td>
<td>Torma et al (1993), Wu and Ting (2006), Xu and Ting (2009), Yang et al. (2009)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em>, extra-cellular components</td>
<td>Clean up of soil contaminated by passage of wastewater</td>
<td>Shake at near-neutral pH 7.8 at 30 °C for 15 days for leaching of various heavy metals from contaminated soil</td>
<td>15-100 % of Cu, Cd, Pb and Zn</td>
<td>Ren et al., (2009)</td>
</tr>
<tr>
<td><em>Aspergillus sp.</em>, whole cell</td>
<td>Nickeliferous ore leaching</td>
<td>Shake at pH 6.5 at 37 °C, Nickeliferous lateritic ore</td>
<td>0.1-92 % of Ni, Fe, Co, Mn, Mg</td>
<td>Sukla and Panchanadikar, (1993)</td>
</tr>
</tbody>
</table>
### Table 2.2b. Heavy metal leaching with fungi (continued)

<table>
<thead>
<tr>
<th>Fungus name and mode of contact</th>
<th>Application</th>
<th>Experimental techniques</th>
<th>Findings</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aspergillus niger,</em> <em>Penicillium simplicissimum,</em> <em>Aspergillus foetidus</em> and <em>Aspergillus carbonarius</em> strains, Whole cell</td>
<td>Adaptation of fungal strains to nickel toxicity in nickel laterite leaching, Tolerance to Ni, Co, Fe, Mg and Mn</td>
<td>25-32 °C for 9-18 days</td>
<td>Up to 2000 ppm of Ni, Co, Fe, Mg and Mn</td>
<td>Valix et al. (2001) Valix and Loon (2003)</td>
</tr>
<tr>
<td><em>Penicillia</em> and <em>Aspergilli,</em> whole cell</td>
<td>In situ leaching of nickeliferous laterite ores</td>
<td>Shake at pH 3.3-4 at 25 °C-30 °C for 48 days</td>
<td>25-60 % of Ni, Co, Fe</td>
<td>Alibhai et al., (1993)</td>
</tr>
<tr>
<td><em>Aspergillus foetidus</em> whole cell</td>
<td>Salt tolerance development</td>
<td>N/A</td>
<td>Up to 2000 ppm of salt concentration</td>
<td>Thangavelu et al. (2006)</td>
</tr>
<tr>
<td><em>Aspergillus foetidus</em> whole cell</td>
<td>Weathered saprolite ore</td>
<td>Shake at pH 3.8–5.7 at 30 °C, for 12-14 days</td>
<td></td>
<td>Le et al. (2006)</td>
</tr>
<tr>
<td><em>Aspergillus niger,</em> CMI 31821 whole cell and cell-free</td>
<td>Batch and semi-continuous leaching of nepheline bioleaching from fluid catalytic cracking catalyst</td>
<td>Shake at pH 1.8-3.7 at 30 °C for 30-60 days</td>
<td>9-64 % Ni, Fe, Al, V, Mo, and Sb</td>
<td>King et al. (1987) Aung and Ting (2005) Santhiya and Ting (2005)</td>
</tr>
</tbody>
</table>
Table 2.2c. Heavy metal leaching with fungi (continued)

<table>
<thead>
<tr>
<th>Fungus name and mode of contact</th>
<th>Application</th>
<th>Experimental techniques</th>
<th>Findings</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Cladosporium oxysporum</em>&lt;br&gt;Aspergillus flavus and&lt;br&gt;<em>Curvularia clavata</em></td>
<td><em>In situ</em> leaching of mainly low grade oxide uranium ore</td>
<td></td>
<td>50-71 % U dissolution</td>
<td>Mishra et al. (2009)</td>
</tr>
<tr>
<td><em>Candida krissii</em>, <em>Penicillium expansum</em> and <em>Mucor ramosissimus</em></td>
<td>TCP, aluminium phosphate (AlPO₄)</td>
<td>Shake at pH 7 at 30-32 °C at 140-160 rpm for 7 days</td>
<td>P dissolution</td>
<td>Xiao et al. (2008)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>Manganese ore</td>
<td>4-57 weeks</td>
<td>10-99 %</td>
<td>Baglin et al (1992)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em>, <em>Penicillium simplicissimum</em></td>
<td>Electronic scrap</td>
<td></td>
<td>65-95 % Cu, Sn, Al, Ni, Pb, and Zn</td>
<td>Brandl et al. (2001)</td>
</tr>
<tr>
<td><em>Penicillium sp</em> and <em>Aspergillus sp</em></td>
<td>Nickeliferous laterites</td>
<td>30 °C for 50 days</td>
<td>55-60 % of Ni</td>
<td>Tzeferis (1994)</td>
</tr>
<tr>
<td><em>Aspergillus niger</em> and <em>Aspergillus fumigates</em></td>
<td>Raw or roasted low-grade chromite overburden</td>
<td>30 °C for 28 days</td>
<td>14-34 % extraction of Ni</td>
<td>Mohapatra et al. (2007)</td>
</tr>
</tbody>
</table>
2.5 Biotransformation of sulfur compounds by fungi

Different fungi are involved in the oxidation or reduction of inorganic/organic sulfur compounds, and biotransformation activities may involve the use of whole cell, cell-free extracts and enzymes (Wainwright, 1978; Wainwright and Killham, 1980; Killham et al., 1981; Schreiner et al., 1988; Faison et al. 1991; Ray et al, 1991; Van Hamme et al., 2003). Various species of fungi have been employed in bio-desulfurization of coals with organic and inorganic sulfur content of up to 6%. Removal of up to 90% of inorganic sulfur and 30% of organic sulfur has been reported (Faison et al., 1991; Acharya et al., 2005; Gonsalvesh et al., 2008). The fungal-decomposition of sulfur has been attributed to the secretion of oxidative enzymes (Schreiner et al., 1988; Aranda et al., 2009). Some fungi, for example *Fusarium oxysporium*, are found to adapt to anaerobic conditions by replacing the energy-producing mechanism of oxygen respiration with sulfur reduction, and in the process sulfur is converted to hydrogen sulfide (Abe, 2007).

Table 2.3 presents fungal biotransformation of some sulfur compounds. It can be seen that fungi are mainly utilized in the desulfurization of coal and degradation of sulfur in soils. In coal, organic sulfur was the target while in soils, $S_2O_3^{2-}$, $S_4O_6^{2-}$ and $SO_4^{2-}$ were tested with varying degrees of success.
<table>
<thead>
<tr>
<th>Fungus name and mode of contact</th>
<th>Application</th>
<th>Experimental techniques</th>
<th>Findings</th>
<th>Authors</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aureobasidium pullulans</em>, cell-free extracts and enzyme</td>
<td>Sulfur oxidation in soil</td>
<td>Oxidation of S, S$_2$O$_3^{2-}$ and S$_4$O$_6^{2-}$ at 25 °C, for 24 h under shake culturing</td>
<td>Enzymatic oxidation; 141 mg/L of dissolved S$_2$O$_3^{2-}$ and S$_4$O$_6^{2-}$, and SO$_4^{2-}$</td>
<td>Killham et al. (1981)</td>
</tr>
<tr>
<td><em>Rhizopus oryzae</em> whole cell</td>
<td>Sulfur oxidation in soil</td>
<td>Oxidation of S, S$_2$O$_3^{2-}$ and S$_4$O$_6^{2-}$. S$_2$O$_5^{2-}$, CN$^-$ at 28-32 °C at pH 6 for 7, 10 days, under stationary culturing</td>
<td>52-290 mg/L of S$_2$O$_3^{2-}$ S$_4$O$_6^{2-}$ and SO$_4^{2-}$</td>
<td>Ray et al. (1991)</td>
</tr>
<tr>
<td><em>Paecilomyces sp.</em> whole cell</td>
<td>Desulfurization of coal-related compounds</td>
<td>Oxidation of dibenzo thiophene, ethyl phenyl sulfide and diphenyl sulfide</td>
<td>2,2-dihydroxybiphenyl sulfones produced</td>
<td>Faison et al. (1991)</td>
</tr>
<tr>
<td><em>Aspergillus sp.</em> whole cell</td>
<td>Desulfurization of coal</td>
<td>Incubation of 2 wt% of -74 µm coal with 2.13 % inorganic and 1.21 % organic for 10 days</td>
<td>Removal of 70–80 % total sulfur</td>
<td>Acharya et al. (2005)</td>
</tr>
<tr>
<td><em>Agrocybe aegerita</em> and <em>Coprinellus radians</em>, whole cells and purified extracellular peroxygenases</td>
<td>Desulfurization of fossil fuels</td>
<td>Incubation of dibenzo thiophene in vivo and in vitro at pH 7 at 100 rpm at 24 °C for up to 16 days under shake culturing</td>
<td>oxidized dibenzo thiophene (DBT) (110 µM) by 60-100 % into DBT sulfoxide, DBT sulfone</td>
<td>Aranda et al. (2009)</td>
</tr>
</tbody>
</table>
2.6 Biotransformation of organic carbon-containing materials

Several investigations have shown that there are some microorganisms involved in the degradation and/or mineralization of organic carbon-containing materials such as coal, humic acid, lignin, plastics and rubber. Others are automobile tires, diesel oil and sewage (Glazer and Nikaido, 1995; Fakoussa and Hofrichter, 1999; Tsuchii and Tokiwa, 2002). Mineralization converts the organic substances into inorganic substances and water (Wilson et al., 1987; Pyne et al., 1987; Strandberg and Lewis, 1987; Quigley and Dugan, 1989). The biotransformation of these compounds impact positively on metal extraction and/or environmental clean-up (Kergomard and Renard, 1982; Bumpus et al., 1985; Bumpus and Aust 1986; Sterflinger, 2000; Tunde and Tien, 2000).

2.6.1 Biotransformation of rubber and rubber products

Rubber and various kinds of rubber products have been used as substrates by fungi. *Fusarium solani* and *Spicaria violacea* have been found to biotransform automobile tires and latex. Biodegradation of rubber by the fungi generally leads to mineralization of the rubber but the carbon black found in automobile tires may be left as residue as the components responsible for degradation are not able to attack carbon black (Tsuchii and Tokiwa, 2002). This indicates that these microorganisms fall short in complete degradation of the substrates. Other fungal species that are known to biotransform natural rubber and rubber processing wastes include *Mucor racemous, Mucor sp.* and *Aspergillus niger* (Atagana et al, 1999).

2.6.2 Biotransformation of coal

Many fungi species have been reported to possess the ability to solubilize and/or degrade coal. Such fungi are either isolated from coal seams or tested in the laboratory on coal. The well known species include *Phanerochaete chrysosporium, Trametes versicolor, Penicillium*
waksmanii, Penicillium sp., Aspergillus sp., Candida sp., Poria placenta, Mucor sp, Paecilomyces sp, Fusarium oxysporum, Trichodemul atroviride, Nematoloma frowardii, Clitocybula dusenii, Auricularia sp., Coprinus sclerotigenis and Punus tigrinus (Cohen and Gabriele, 1982; Ward, 1985; 1993; Wilson et al., 1987; Stewart et al., 1990; Catcheside and Mallet, 1991; Hölker et al., 1999). The prominent fungi involved in the transformation of humic acids and different ranks of coal include the two white rot basidiomycetes, *Phanerochaete chrysosporium* and *Trametes versicolor* (Cohen et al., 1987; 1990; Fredrickson et al., 1990; Perez and Jeffries, 1992; Catcheside and Ralph, 1999; Ralph and Catcheside, 1994; 1997; Fakoussa and Hofrichter, 1999; Afidenyo, 2008).

### 2.6.3 Microbial transformation of carbonaceous matter in gold ores

Based on their constituents, the formation of carbonaceous matter (CM) has been likened to that of coals. For example, many of the organic compounds in CM associated with gold ores such as poly aromatic hydrocarbons and humic acids are present in lignite, while the more matured graphitic carbon is also present in bituminous and anthracite coals. In addition, CM and coal, which is also known as carbonaceous rock (Faison, 1992; Anon, 2009c), have similar carbon-to-hydrogen ratios (Sibrell et al., 1990, Stenebraten et al., 2000). Consequently, most microorganisms that attack coal may be able to attack CM.

Several investigations on the microbial transformation of CM in gold ores, with the aim of increasing gold extraction are available in the literature. Brierley and Kulpa (1992; 1993) showed that a consortium of heterotrophic bacteria, consisting of *Pseudomonas maltophilia*, *Pseudomonas oryzihabitans*, *Achromobacter sp.* and *Arthrobacter sp.* could reduce gold uptake by a carbonaceous ore leading to increase in gold recovery during cyanidation. Portier (1991) used a mixture of heterotrophic fungal and bacterial species including *Aspergillus bruneio-uniseriatus* and *Penicillium citrinum* fungi, followed by sulfide biooxidation using *Acidithiobacillus* and *Leptospirillum* bacteria. The microbial action enhanced cyanidation gold recovery from bituminous grade carbon-containing gold-bearing sulfur-containing ores.
Increase in gold extraction as a result of carbon degrading microbial pretreatment with *Streptomyces setonii*, following sulfide oxidation of double refractory gold ores has also been observed by other workers (Amankwah et al., 2005; Amankwah and Yen, 2006; Yen et al., 2008).

Using *S. setonii*, Amankwah and Yen (2006) observed a more extensive biodegradation in lignite and bituminous coal than in anthracite. In a solution of 5 mg/L Au, biotreatment reduced the gold adsorption capacity of bituminous, lignite and anthracite coals respectively from an average of about 19 % to 0.3 %, 22 % to 9 % and 45 % to 20 %. A white-rot fungus, *Trametes versicolor*, has also been reported to reduce the preg-robbing effect of bituminous coal from 40.3 % to 8.3 % and anthracite from 99.5 % to 27.7 % (Afidenyo, 2008). Most of the treatments mentioned above, though effective with bituminous grade CM, could not reduce the gold adsorption ability of anthracite-grade CM drastically. Anthracite-grade CM, however, comprises about 50 % of the entire CM in refractory gold ores (Hausen and Bucknam, 1984; Osseo-Asare et al., 1984; Sibrell et al., 1990; Pyke et al., 1999; Stenebraten et al, 2000; Schmitz et al., 2001; Vaughan and Kyin, 2004). Thus, further investigations into anthracite degrading microorganisms are required.

### 2.7 Biotransforming action of white rot fungi

White rot fungi are the main microorganisms involved in the biotransformation of organic carbon-containing materials. Though many members make up the family of white rot fungi, the two that have been most researched are *Phanerochaete chrysosporium* and *Trametes versicolor* (Cohen et al., 1987; Tien and Kirk, 1988; Fredrickson et al., 1990; Perez and Jeffries, 1992; Ralph and Catcheisde, 1994; 1997; Afidenyo, 2008). Of the two, *Phanerochaete chrysosporium* is the most studied, and the general characteristics of white rot fungi are inferred from this fungus.
*P. chrysosporium* belongs to the basidiomycetous family; a family of fungi whose filaments are characterized by an extensive network of cross-walls, and produce mushrooms as fruiting bodies (Sachs et al., 1989; Tortora et al., 2004; Madigan and Martinko, 2006; Anon, 2009a; 2009b). The fungus secretes enzymes that digest wood and allow its filaments to penetrate wood and degrade lignin (the most recalcitrant component of wood) with the aim of getting access to cellulose and hemicellulose (Tien and Kirk, 1983; Sachs et al., 1989). This leaves specks of white delignified areas amidst thin areas of firm wood, a phenomenon called white rotting. A network of fungal hyphae of *P. chrysosporium* growing on aspen wood chips is presented in Fig. 2.1 (Sachs et al., 1989). The authors reported the hyphae to be of 1-3 µm in diameter.

![Fig. 2.1. Photomicrograph of Web-like hyphal network of Phanerochaete chrysosporium (Sachs et al., 1989)](image_url)

*P. chrysosporium* is a prominent lignin-degrading fungus, from which the first lignin-degrading enzyme, lignin peroxidase, was isolated and characterized by Tien and Kirk (1983; 1984). *P. chrysosporium* has hence been studied in many laboratories as a model organism as it grows quickly, degrades lignin rapidly, and has a relatively high temperature optimum (37-
39°C) and low pH optimum (3.5-5.0) though it can grow over a wider range of temperature and pH (Tien et al., 1986; Andrawis et al., 1988).

Aside from lignin peroxidase (LiP), *P. chrysosporium* secrete manganese peroxidase (MnP) (Glenn et al., 1983) and H$_2$O$_2$-generating enzyme, glyoxal oxidase (Kersten and Kirk, 1987) under secondary metabolism in response to nutrient starvation (Kirk et al., 1986). The strong oxidizing environment created by the extra-cellular components of the fungus catalyzes the biodegradation of lignin and a variety of lignin-containing substrates such as low rank coals (Tien and Kirk, 1984; Faison and Kirk, 1992; Bumpus and Aust, 1986; Hammel et al., 1986; Kirk and Farrel, 1987; Ralph and Catcheside, 1997; Fakoussa and Hofrichter, 1999; Tunde and Tien, 2000). The structure of lignin peroxidase produced by *P. chrysosporium* is presented in Fig. 2.2. As shown in the figure, the enzymes of *P. chrysosporium* are heme-containing glycoproteins possessing one ferric heme per molecule of enzyme (Tien and Kirk, 1988) and can be represented simply as P[Fe(III)].

![Fig. 2.2. Structure of lignin peroxidase (A), and manganese peroxidase (B); enzymes generated by *P. chrysosporium* (Banci et al., 1992)](image-url)
When activated by hydrogen peroxide, lignin peroxidase (LiP) and manganese peroxidase (MnP) catalyse one-electron oxidation of lignin’s aromatic substructure leading to formation of cation free radicals and bond cleavage (Tien and Kirk, 1984, 1988; Glenn et al., 1983; Hammel et al., 1986; Kersten et al., 1985; Kirk et al., 1986; Gold and Alic, 1993). The general mechanism is shown in Equations 2.1 to 2.3 (modified after Andrawis et al, 1988; Banci et al., 1992; Edwards et al., 1992; Gold and Alic, 1993; Fakoussa and Hofrichter, 1999). The radicals may also react with the lignin polymer to depolymerize it, and the products may undergo further modification to form carbon dioxide (Bumpus and Aust, 1986).

\[
P[\text{Fe(III)}](\text{native enzyme}) + H_2O_2 \rightarrow P[O=\text{Fe(IV)}]^*(\text{comp I}) + H_2O \tag{2.1}
\]

\[
P[O=\text{Fe(IV)}]^*(\text{comp I}) + R \rightarrow P[O=\text{Fe(IV)}](\text{comp II}) + R^* \tag{2.2}
\]

\[
P[O=\text{Fe(IV)}](\text{comp II}) + R + 2H^+ \rightarrow P[\text{Fe(III)}](\text{native enzyme}) + R^{**} + H_2O \tag{2.3}
\]

Compounds I and II are the two free radical intermediates involved in the catalysis of the enzymes (Tien and Kirk, 1984; Kersten et al., 1985; Hammel et al., 1986; Kirk et al., 1986; Fakoussa and Hofrichter, 1999). At the initial stage of the catalysis, the enzymes utilize \(H_2O_2\) to get oxidized first to iron (IV) and then to a cation radical (Tien and Kirk, 1988; Kurek and Kersten, 1995) called compound I (Equation 2.1), which is 2 oxidation jumps above the native enzyme. Compound I subsequently reacts with a substrate molecule, R (Equation 2.2), to form compound II, which is one-electron oxidized. LiP Compound II returns to the resting enzyme state by reacting with the same or another molecule of substrate (Equation 2.3). The substrate cation radical (\(R^{**}\)) produced, can undergo a variety of non-enzymatic reactions and spontaneous rearrangement leading to further degradation and formation of a wide range of final products including oxygen-containing compounds as shown in Equation 2.4 (Paszczynski et al., 1985; Pease et al., 1989; Wariishi et al., 1991; Gold and Alic, 1993; Catcheside and Mallett, 1991; Fakoussa and Hofrichter, 1999).
MnP Compound II, on the other hand, exhibits an absolute requirement for Mn\(^{2+}\) as the reducing agent to get reduced to the resting enzyme state (Glenn et al., 1986; Pasczynski et al., 1985; Wariishi et al., 1991; Gold and Alic, 1993). The Mn\(^{3+}\) ions, so produced, are stabilized to high redox potentials by forming complexes with organic acids such as oxalate ((COO))\(^{-}\), malonate (CH\(_2\)(COO))\(^{-}\), malate (CH\(_3\)CHO(COO))\(^{-}\), tartrate (CH\(_3\)CHO\(_2\)(COO))\(^{-}\), and lactate (CH\(_3\)CH(OH)COO\(^{-}\)), all secreted by \textit{P. chrysosporium} (Catcheside and Ralph, 1999; Fakoussa and Hofrichter, 1999). The chelated Mn\(^{3+}\) diffuses from the surface of the enzyme and acts as diffusible redox mediator that oxidizes insoluble terminal substrates, cleaving chemical bonds such as non-phenolic and polycyclic aromatic hydrocarbons that are normally not accessible to attack by MnP directly (Catcheside and Ralph, 1999; Fakoussa and Hofrichter, 1999). By oxidizing the substrates, Mn\(^{3+}\) gets reduced thus recycling Mn\(^{2+}\).

It can be deduced from the above mechanism that the oxidation of carbon by the fungus will lead to reduction in carbon to oxygen ratios in the carbonaceous materials undergoing fungal treatment (Equation 2.4). This can happen via complete loss of carbon through the formation of carbon dioxide or the introduction of oxygen groups on the carbon which disrupts the continuous nature of the graphitic planes.

As mentioned earlier, \textit{P. chrysosporium} also produces hydrogen peroxide which is known to solubilize sulfides such as pyrite and arsenopyrite as shown in Equations 2.5 and 2.6 (McKibben and Barnes, 1986; Antonijevic, et al., 1997; Jennings et al., 2000; Aydogan, 2006).
\[
2\text{FeS}_2 + 15\text{H}_2\text{O}_2 \rightarrow 2\text{Fe}^{3+} + 4\text{SO}_4^{2-} + 2\text{H}^+ + 14\text{H}_2\text{O} \quad [2.5]
\]
\[
\text{FeAsS} + 7\text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{AsO}_4^{3-} + \text{SO}_4^{2-} + 2\text{H}^+ + 6\text{H}_2\text{O} \quad [2.6]
\]

The iron (III) and sulfuric acid produced constitute reagents that can be used for oxidation of sulfides (Bennett and Tributsch, 1978; Brierley, 1978; 1995; Hackl, 1997; Keller and Murr, 1982; Sand et al., 2001; Rawlings et al., 2003; Marsden and House, 2006). The strong oxidizing environment produced by the oxidative enzymes of \textit{P. chrysosporium} is also known to be responsible for the oxidative removal of sulfur from coal (Schreiner et al., 1988; Aranda et al., 2009). Furthermore, \textit{P. chrysosporium} has been used to degrade gold-bearing wood chips, produced from underground timber support, to liberate gold for cyanide leaching (Martin and Petersen, 2001). The authors observed a 10 % increase beyond the original 60 % cyanidation gold recovery of untreated wood chips. It is therefore believed that \textit{P. chrysosporium} can catalyze the biotransformation of carbonaceous matter and sulfides present in double refractory gold ores, and hence enhance gold extraction in the subsequent cyanidation step.

\section*{2.8 Conclusions}

Fungi are utilized extensively in the biotransformation of organic matter in paper and pulp industries, breweries, coal solubilization, environmental clean-up, nutrient recycling in soils and more recently in hydrometallurgy. “Mycohydrometallurgy” has been adopted to define the application of fungi in hydrometallurgy. This review considered the literature on the application of fungi in hydrometallurgy and expounded on the diversity, versatility and hydrometallurgical applications such as biosorption, transformation of sulfur compounds, leaching of metals and degradation of recalcitrant organic matter. The oxidative action of the versatile fungus, \textit{P. chrysosporium}, was discussed and the microorganism has the potential to biodecompose metal sulfides and carbonaceous matter in refractory gold ores.
References


Coherent references to relevant research include:


Chapter 3

Mycohydrometallurgy: Coal model for potential reduction of preg-robbing capacity of carbonaceous gold ores using the fungus, *Phanerochaete chrysosporium*

Abstract

During the cyanidation process for gold extraction, natural carbonaceous matter (CM) present in refractory gold ores adsorbs dissolved aurocyanide complexes, thereby reducing gold extraction - a phenomenon known as preg-robbing. The natural CM consists mainly of elemental carbon, humic acids and hydrocarbons, and coal can be used to model the behavior of CM. In this study, samples of lignite, subbituminous, bituminous and anthracite coals were used as surrogate materials to test the capability of the fungus, *Phanerochaete chrysosporium*, to reduce the preg-robbing capacity of CM.

By utilizing several growth media including glucose, millet and wheat bran, it was established that millet and wheat bran provided the best environment for fungal growth and biomodification of coal. As-received, control and biotreated coal samples were subjected to gold adsorption tests and the results indicate that *P. chrysosporium* can decrease the gold adsorption ability of coal by over 90% depending on the growth media used. Reduction in gold adsorption was more pronounced in anthracite due to its high ability to adsorb gold in the as-received form. The results demonstrate a potentially effective alternative pretreatment process that utilizes the fungus, *P. chrysosporium*, in reducing preg-robbing in gold extraction.

Keywords: Mycohydrometallurgy; Fungi; *Phanerochaete chrysosporium*; Biomodification; Culture media; Controls; Coals; Carbonaceous matter; Preg-robbing; Gold adsorption; Cyanidation.
3.1 Introduction

3.1.1 Mycohydrometallurgy

Mycology is the study of the properties of fungi; their beneficial and adverse effects on humans and the environment (Madigan and Martinko, 2006), whereas hydrometallurgy is a branch of extractive metallurgy which focuses on the use of aqueous chemistry to recover metals from materials such as ores, concentrates, scrap and residues (Gupta and Mukherjee, 1990; Osseo-Asare, 2009). ‘Mycohydrometallurgy’ is therefore adopted to define the connection between mycology and hydrometallurgy, thus the application of fungi in the field of metal recovery (Chapter 2; Osseo-Asare et al., 2009).

3.1.2 Preg-robbing effect of carbonaceous matter on gold extraction

The presence of carbonaceous matter (CM) in gold ores leads to two main deleterious effects: (a) confinement of gold with attendant difficult release from the CM matrix and (b) loss of dissolved metal via the ability of CM to adsorb gold from the pregnant solution (Osseo-Asare et al., 1984; Hutchins et al., 1988; Portier, 1991; Brierley and Kulpa, 1992; 1993; Amankwah et al., 2005). Adsorption of gold by CM leads to gold losses due to the fineness of the carbon (0.002 – 2 µm) (Afena, 1991) which does not permit it to be screen-separated for gold recovery, and this phenomenon is termed as preg-robbing (Osseo-Asare et al., 1984; Hausen and Bucknam, 1985) The most serious cause of preg-robbing is the presence of CM, though other minerals such as silicates and sulfides in gold ores have also been implicated (McDougall and Hancock, 1981; Hausen and Bucknam, 1985; Afena, 1991; Pyke et al., 1999; Rees and van Deventer, 2000a; Schmitz et al., 2001; Vaughan and Kyin, 2004; Tan et al., 2005). CM is composed mainly of hydrocarbons, humic acids, and graphitic/amorphous elemental carbon. The elemental carbon is the component that behaves like activated carbon and thus adsorbs gold, and the humic acid may form complexes with gold while the hydrocarbon fraction has negligible interaction with gold (Radtke and Scheiner, 1970; Osseo-
Asare et al., 1984; Kohr, 1994; Adams and Burger, 1998; Stenebraten et al., 1999; Pyke et al., 1999; Schmitz et al., 2001).

The extent to which CM preg-robs gold generally increases with its maturity. The graphitic elemental carbon has maturity similar to anthracitic grade coal, which is the most matured form of coal, whereas the amorphous elemental carbon may have maturity similar to the lower rank coals (Osseo-Asare et al., 1984; Hausen and Bucknam, 1985; Abotsi and Osseo-Asare, 1986; Sibrell et al., 1990; Pyke et al., 1999; Stenebraten et al., 2000; Van Vuuren et al., 2000; Vaughan, 2004; Schobert, 2007). Due to the complexity of CM, coal has been used as surrogate in its characterization as many of the components in CM such as kerogens and humic acids are present in lignite (the youngest form of coal) while the more matured graphitic carbon is also present in anthracite (Ibrado and Fuerstenau, 1992; Stenebraten et al., 1999; Van Vuuren et al., 2000; Amankwah and Yen, 2006). Indeed, kerogen and graphite respectively span the beginning and end of the coalification process (Van Krevelen, 1993; Schobert, 2007).

3.1.3 Biomodification of carbonaceous matter

Carbonaceous matter (CM) has to be eliminated or passivated before cyanidation in order to reduce preg-robbing, and some of the available techniques include roasting, chlorination and treatment with carbon-blanking agents such as kerosene and other organic reagents (Arriagada and Osseo-Asare, 1984; Abotsi and Osseo-Asare, 1987; Hutchins et al., 1988; Adams and Burger, 1998). The use of activated carbon to simultaneously adsorb gold during cyanidation has also been applied commercially to out-compete the natural CM in refractory gold ores in the carbon-in-leach technology (Matson and Fisher, 1981; Hutchins et al., 1988; Rees and van Deventer, 2000b; Marsden and House, 2006).

Currently there are studies into biomodification of carbon, considered more attractive for economic and environmental reasons as they occur at relatively low temperatures (25-40°C)
and at atmospheric pressure, and the microorganisms can easily be regenerated (Portier, 1991; Brierley and Kulpa, 1992; 1993; Amankwah et al., 2005; Yen et al., 2008). Portier (1991) used a mixture of heterotrophic fungal and bacterial species including *Aspergillus bruneio-uniseriatus* and *Penicillium citrinum* fungi, followed by sulfide biooxidation using *Acidithiobacillus* and *Leptospirillum* bacteria. The microbial action progressively enhanced cyanidation gold recovery from bituminous grade carbon-containing gold-bearing sulfur-containing ores. Brierley and Kulpa (1992, 1993), who used a carbon de-activating microbial consortium comprising of different combinations of species of *Pseudomonas, Achromobacter, Arthrobacter and Rhodococcus*, observed an increase in overall gold extraction which they attributed to partial deactivation as a result of blinding of active sites on carbon. Increase in gold extraction as a result of microbial pretreatment of CM has also been observed by other workers (Amankwah et al., 2005; Amankwah and Yen, 2006; Afidenyo, 2008; Yen et al., 2008). Amankwah and Yen (2006) investigated the effect of bacterial-treatment on different carbonaceous materials with varying degrees of maturity (lignite, bituminous and anthracite coals). The authors observed degradation with some degree of carbon dioxide formation when the coals were incubated with the actinomycete, *Streptomyces setonii*, and they reported a more extensive biotransformation in lignite and bituminous than in anthracite. It has also been reported that a white-rot fungus, *Trametes versicolor*, is able to passivate bituminous coal but only passivates anthracite to a lesser extent (Afidenyo, 2008).

In this study, the white-rot basidiomycetous fungus, *P. chrysosporium*, was investigated for its effectiveness in deactivating the active sites of CM for gold adsorption. The fungus was selected based on its ability to degrade a wide range of organic carbonaceous materials (Tien and Kirk, 1984; Faison and Kirk, 1985; Bumpus and Aust, 1986; Hammel et al., 1985; 1986; Kirk et al., 1986; Kirk and Farrel, 1987; Ralph and Catcheside, 1994, 1997; Tunde and Tien, 2000). Various ranks of coal were used as surrogates to represent CM in gold ores so as to monitor the changes in the preg-robbing behavior as a result of biomodification. The results reported here indicate that *P. chrysosporium* could reduce the adsorption behavior of anthracite by more than 90% depending on the growth media used.
3.2 Phanerochaete chrysosporium

*P. chrysosporium* is a white rot fungus which belongs to the basidiomycetous family; a family of filamentous fungi that exhibits mushroom-like growth with extensive network of cross-walled filaments (Madigan and Martinko, 2006; Anon, 2009a; 2009b). The filaments are able to penetrate wood and degrade lignin (the most recalcitrant component of wood) with the aim of getting access to cellulose and hemicellulose (Tien and Kirk, 1983), thus leaving specks of white delignified areas amidst thin areas of firm wood, a phenomenon referred to as white rotting. *P. chrysosporium* has been studied as a model organism as it grows quickly, degrades lignin rapidly, and has a relatively medium temperature optimum (37-39 °C) and low pH optimum (3.5-4.5), though it can grow over a wider range of temperature and pH (Tien et al., 1986; Andrawis et al., 1988). The fungus secretes the enzymes, lignin peroxidase (Tien and Kirk, 1983; 1984), manganese peroxidase (Glenn et al., 1983) and a hydrogen peroxide generating enzyme, glyoxal oxidase (Kersten and Kirk, 1987). These oxidative enzymes are known to catalyze the biodegradation of lignin and a variety of lignin-containing substrates including some low rank coals and persistent environmental pollutants (Tien and Kirk, 1984; Faison and Kirk, 1985; Bumpus and Aust, 1986; Hammel et al., 1985; 1986; Kirk et al., 1986; Kirk and Farrel, 1987; Ralph and Catcheside, 1994, 1997; Fakoussa and Hofrichter, 1999; Tunde and Tien, 2000). *P. chrysosporium* has also been used to degrade gold-bearing wood chips, produced from underground timber support, to liberate gold for cyanide leaching (Martin and Petersen, 2001). The authors observed a 10 % increase beyond the original 60 % cyanidation gold recovery of untreated wood chips.

3.3 Experimental investigations

3.3.1 Materials

Coal (anthracite, bituminous, sub-bituminous and lignite) samples with particle size less than 850 μm were provided by the Coal Bank of the EMS Energy Institute and these were crushed
and sieved to all passing 250 µm before incubation. Fungal spores of \textit{P. chrysosporium} ME446, were kindly obtained from Prof. Ming Tien of the Department of Biochemistry and Molecular Biology all of the Pennsylvania State University. Standard gold solution (50 µg/mL in 0.1 % sodium hydroxide and 0.05 % sodium cyanide) was supplied by High Purity Standards, and all other chemicals used were of reagent grade.

### 3.3.2 Media preparation and incubation

Biotransformation was explored by incubating coal samples with the fungus, \textit{P. chrysosporium}, cultivated in different media at 37 \(^\circ\)C for growth periods of up to 4 weeks, and within pH of 4.0-7.0 depending on the growth medium. The coal samples were autoclave-sterilized at 121 \(^\circ\)C, and the different media were either liquid or solid substrates as described in the subsections below.

#### Glucose broth and glucose agar media

Glucose broth and glucose agar media were prepared as described by Tien and Kirk (1988). The media without thiamin were autoclaved for 30 minutes and cooled down before addition of thiamin, which is heat-sensitive. Moist culturing with the fungus was done in agar plates with the glucose agar whilst liquid-submerged culturing was done in Erlenmeyer flasks with the glucose broth. The sterilized coal samples were added 7 days after inoculation of fungal spores in the media. Oxygen was introduced into the liquid cultures every three days as described by Tien and Kirk (1988).

#### Millet and wheat bran media

The use of solid substrates such as millet alone, and millet + wheat bran (MWB) obtained from Nature’s Pantry, State College, was also investigated. The media were prepared by using
10 g of millet for millet alone medium, and 8 g millet plus 2 g wheat bran for MWB medium. The media, in Erlenmeyer flasks, were moistened with water in the ratio of 1 g media: 1 mL double-distilled (dd) H$_2$O, and autoclaved at 121 °C for 30 minutes. On cooling, the media were inoculated with 1 mL each of spore suspension of *P. chrysosporium* (made by suspending 1 vial of spores in 25 mL of dd H$_2$O). The optical density of the cell suspension, used for inoculation, as measured with a Pharmacia LKB Ultraspec II UV-visible spectrometer at 600 nm was 0.3 relative to water. After a growth period of one week, 10 g of sterilized moist samples of the various coals were introduced into the cultures. After one week of incubation, the fungus grew over all of the coal samples in the millet, and MWB, and sandwiched the coals within two weeks. Control experiments were also conducted to establish the exclusive effect of *P. chrysosporium* on preg-robbbing capacity of coal. Fig. 3.1 shows the autoclaved media (3.1a and 3.1b), after one week incubation with *P. chrysosporium* (3.1c and 3.1d), and after two weeks incubation with coal (3.1e and 3.1f). A closer look showing the hyphal growth of *P. chrysosporium* on anthracite coal after 2-week incubation in MWB medium is presented in Fig. 3.2

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**Fig. 3.1.** Incubation of *P. chrysosporium* in millet and wheat bran. Autoclaved millet (A), autoclaved MWB (B), 1-week culture of fungus and millet (C), 1-week culture of fungus and MWB (D), 2-week incubated culture of *P. chrysosporium* with anthracite and millet (E), and 2-week incubated culture of *P. chrysosporium* with anthracite and MWB (F)
Fig. 3.2. Growth of *P. chrysosporium* on anthracite coal after 2-week incubation in MWB medium

### 3.3.3 Incubation and harvesting of carbonaceous samples

All experiments with the fungus were done under aseptic conditions to avoid introduction of other organisms. The coals were introduced into 1-week pre-grown cultures and incubated in triplicates within the given contact time at 37 °C. At the end of the period the samples were washed with water to get rid of the media and fungal biomass. Complete removal of the fungal biomass in the case of glucose media was not possible, especially with the lower rank coal samples, as the biomass adhered tenaciously to the carbon samples. The washed samples were dried at 37 °C for 7 days. In general, visual observation indicated that growth of the fungus was very impressive for the incubation conditions and the substrates investigated, confirming the broad substrate utilization by *P. chrysosporium* (Tien and Kirk, 1988).
3.3.4 Gold adsorption experiments

Gold solution (50 µg/mL = 254 µM) was diluted with 1 g/L (25 mM) NaOH at pH 12 to obtain a 5 µg/mL (25.4 µM) gold solution which was used for the adsorption tests. Triplicate samples of biomodified, control and as-received coals of various weights (0.1 g - 1 g) were contacted with 25 mL of 5 µg/mL gold solution and agitated at 150 rpm on a New Brunswick Series 25 incubator shaker for 24 hours. At the end of the contact time the residual solution was filtered and gold in the filtrate determined using Perkin-Elmer Optima 5300 inductively coupled plasma – atomic emission spectrometer (ICP-AES). The difference between the concentration of gold in solution before and after the adsorption test was computed with respect to the amount of carbon-containing material used for the adsorption, as shown in Equation 3.1, to obtain the preg-robbing effect of carbon (PEC) in µmol/g.

\[
PEC = 25 mL \times \left( \frac{C - FC \frac{25 \mu g}{mL}}{197 \frac{g}{mol}} \right) \left( \frac{1}{WC \ g} \right)
\]

In Equation 3.1, IC and FC are the initial and final concentrations of gold in solution, WC is the weight of carbon material used in the adsorption test, 25 is the volume of gold solution used and 197 is the molar mass of gold. The difference in PEC values between as-received and treated carbonaceous material gives an indication of the effect of fungal-biomodification on preg-robbing of gold by the carbon-containing materials.

3.4 Results and Discussion

*P. chrysosporium* was incubated with various carbonaceous materials; lignite, subbituminous, bituminous and anthracite coals in liquid and solid substrate media. The response to fungal biomodification was determined by the reduction in the ability of carbon materials to adsorb gold which can be translated into a reduction in preg-robbing ability of carbonaceous matter. Equation 3.1 was used to compute the preg-robbing effect and the fungal-treatment variables.
considered were coal ranks, contact time and growth media as demonstrated in the ensuing figures. Control experiments were set up to monitor the direct contribution of the media utilized. The results are presented and then discussed afterwards.

3.4.1 Effect of carbonaceous characteristics on biotransformation

For tests performed in submerged glucose media, Fig. 3.3 shows the effect of incubation time on the adsorption of gold by the various coals. The gold adsorption capabilities of as-received lignite, subbituminous and bituminous coals were very low, below 0.5 \( \mu \text{mol} \) of gold per gram of carbon while that of anthracite was very high, at 4.5 \( \mu \text{mol/g} \).

![Figure 3.3](image)

Fig. 3.3. Effect of fungal-treatment time on gold adsorption by lignite, subbituminous, bituminous and anthracite coals. The growth medium was glucose broth

As indicated in Fig. 3.3, within four weeks of incubation in submerged glucose medium, the adsorption capacities of lignite, subbituminous and bituminous coals did not change significantly owing to the fact that the as-received did not adsorb much gold (less than 0.5
µmol/g). However, the adsorption capacity of anthracite was greatly reduced from about 4.5 µmol/g to 0.4 µmol/g after incubation with *P. chrysosporium* for one week, and described a plateau at that value as the contact time was extended beyond one week up to four weeks.

Figure 3.4 presents the effect of 2-week fungal-incubation in moist glucose agar medium on the preg-robbing ability of various coals. The diagram depicts about the same level of gold adsorption before and after fungal incubation for all the coal materials except for anthracite. Figure 3.5 illustrates the effect of 2-week fungal-incubation in millet and MWB on the gold adsorption ability of various coals. The trend in Fig. 3.5 confirms those observed in Figs. 3.3 and 3.4, with anthracite demonstrating significant adsorption in the as-received state, and significant reduction in gold adsorption (over 90%) after fungal-treatment.

![Diagram showing gold adsorption on various coals](image)

Fig. 3.4. Effect of two-week fungal-treatment on the preg-robbing ability of lignite, subbituminous, bituminous and anthracite coals. The growth medium was moist glucose agar
Fig. 3.5. Effect of two-week fungal-treatment on the preg-robbing ability of lignite, subbituminous, bituminous and anthracite coals. The media used were millet and MWB

Since the as-received samples of lignite, subbituminous and bituminous coals did not show significant gold adsorption capabilities in the cyanide medium, it might not be appropriate to measure the degree to which these coals have been biotransformed using aurocyanide adsorption test. Gold adsorption test in cyanide medium, on the other hand, can be used to predict the extent of biotransformation in anthracite by *P. chrysosporium*. The low gold adsorption for both the as-received and biomodified lignite, sub-bituminous and bituminous coals was observed throughout this work. Bio-treated samples of both lignite and subbituminous coals showed slightly higher gold adsorption when treated in glucose agar medium and this may be attributed to the entrained biomass, which was found in this research to possess some minor degree of adsorption capacity.
In contrast, higher gold adsorption in as-received lignite and bituminous coals, and a more intense bacterial-biodegradation in lignite and bituminous than anthracite coals was observed by Amankwah and Yen (2006) using S. setonii. The literature contains varied reports on adsorption of gold onto different coal materials from different origins and of different maturity. These observations are due to the wide variations in coal deposits resulting, in part, from different plant inputs and coal generating conditions (Faison, 1992; Fakoussa and Hofrichter, 1999; Schobert, 2007), which precludes the direct transfer of the characteristics of coals in one area to coals of other regions. The trend, however, is always very high gold adsorption on anthracite relative to lignite, subbituminous and bituminous coals (Ibrado and Fuerstenau, 1992; Van Vuuren et al., 2000; Amankwah and Yen, 2006).

The atomic ratio of carbon to oxygen in coal decreases with coal maturation or extent of coalification from lignite to anthracite. Lignite, the geologically youngest coal, is formed from the decomposition of kerogen, which occurs at the ‘halfway point’ across the conversion of organic matter to fossil fuel in the coalification process. The process beyond lignite is marked initially by demethoxylation of lignin, decarboxylation of resins and coupling of phenolic compounds liberated by lignin decomposition to form subbituminous coal. This is followed by partial dealkylation of aromatic ring systems and resins, and aromatization of resin structures to form bituminous coal. The final coalification jump to anthracite occurs through substantial demethanation of aromatic systems, thermal breakdown of aliphatic structures to poly-enes, and cyclization of poly-enes to aromatics, stacking and growth of aromatic ring system and structural ordering (Faison, 1992; Van Krevelen, 1993; Schobert, 2007; Anon, 2009c).

Using data from the Coal Bank of the EMS Energy Institute, the Pennsylvania State University, atomic ratios of carbon to oxygen in the coal samples were estimated to be between 5 and 11 for lignite, subbituminous and bituminous coals, whereas it was 42 for anthracite. This might explain the relatively higher aurocyanide adsorption on anthracite due to higher degree of graphitization as against the other coal ranks (Fig. 3.6).
The two most important properties of carbon for gold adsorption are large specific surface area and highly developed graphitic structure (Jones et al., 1989; Ibrado and Fuerstenau; 1992). It has been reported that for low rank coals, micro-porosity is more important while for high rank coals, the importance is more on active sites for adsorption (Van Vuuren et al., 2000). Though bituminous is more matured than lignite and sub-bituminous, it is the least porous among all the coal ranks. The porosity of anthracite is higher than that of bituminous coal but lower than that of lignite. Nevertheless, anthracite has far more active sites for gold adsorption than lignite owing to its higher degree of graphitization for the reason that increased graphitization is known to boost gold adsorption than porosity (McDougall and Hancock, 1981; Jones et al, 1989; Van Krevelen, 1993).
3.4.2 Effect of growth media

Visual inspection indicated that the fungus preferred millet and wheat bran over the glucose media. This gives a technical advantage since, as compared to the glucose media, millet and wheat bran are simpler and more cost effective in terms of components and expertise required for preparation. Millet and wheat bran constitute complete food containing the ingredients required by the fungus, including carbohydrates, sugar, proteins, iron, calcium, manganese, magnesium, phosphorus, potassium, selenium, sodium, zinc, copper, boron, vitamins, fatty acids and amino acids (Anon, 2009d; 2009e). The moist millet and wheat bran media possess higher surface area for microbial attachment and have better aeration as a result of air pockets created due to the interstices between the grains. Increased aeration has been shown to enhance the lignin-degrading activity of *P. chrysosporium* (Tien and Kirk, 1988). In contrast, the glucose media which is either liquid or continuous solid may be deficient of oxygen. Figure 3.7 compares the effect of different media on 2-week biotransformation of anthracite.

It is evident from Fig. 3.7 that both millet and MWB media offered a better system for fungal growth leading to a more pronounced reduction in preg-robbing (more than 95 %) whereas glucose media reduced preg-robbing to about 90 %. On the basis of cost and nutritional values, supplementing millet with wheat bran is advantageous since in general millet is more expensive than wheat bran which is a discard from wheat processing. The use of wheat bran alone is however not preferred due to its very low density which requires a large volume for the process.
Fig. 3.7. Effect of different media utilized on gold adsorption behavior of anthracite; as-received (AR), glucose broth (GB), glucose agar (GA), millet + wheat bran (MWB), millet (M). The biotransformation period was 2 weeks.

The possible contribution of the MWB medium to the biotransformation action of coal by *P. chrysosporium* was investigated by means of control experiments which were set up without fungal addition. The gold adsorption behavior of coals used in the control experiments was compared with the fungal-treated coals in Fig. 3.8. It is evident from the figure that MWB medium can reduce the preg-robbing capacity of anthracite coal by about 25% within 14 days of treatment as compared to over 95% reduction in gold adsorption by anthracite in the case of MWB cultured with *P. chrysosporium*. The slight decline in preg-robbing due to media alone may be attributed to fine suspended particulates that might have blinded some of the pores on the carbonaceous material.
3.5 Biotransforming action of *P. chrysosporium*

*P. chrysosporium* is known to produce oxidative enzymes that catalyze oxidation of lignin’s aromatic substructure leading to formation of cation free radicals and bond cleavage (Tien and Kirk, 1984, 1988; Glenn et al., 1986; Hammel et al., 1985; Kersten et al., 1985; Kirk et al., 1986; Bumpus and Aust, 1986; Andrawis et al, 1988; Banci et al., 1992). The radicals may also react with the lignin polymer to depolymerize it and the products may undergo further modification to form carbon dioxide (Bumpus and Aust, 1986). The general mechanism was presented in Chapter 2 and is represented below in Equations 2.1-2.3 (modified after Andrawis et al, 1988; Banci et al., 1992; Edwards et al., 1992).
The equations present enzymes and free radical intermediates referred to as compounds I and II, which are involved in the catalysis (Tien and Kirk, 1984; Kersten et al., 1985; Hammel et al., 1985; Kirk et al., 1986; Fakoussa and Hofrichter, 1999). During the initial stages of the catalysis, $H_2O_2$ generated by the fungus is utilized by the enzymes (Kurek and Kersten, 1995), to get oxidized first to iron (IV) and then to a cation radical (Tien and Kirk, 1988) called compound I (Equation 2.1), which is 2 oxidation levels above the native enzyme (Gold and Alic, 1993). Compound I subsequently reacts (Equation 2.2) with a substrate molecule, $R$, to form Compound II, which is one-electron oxidized, and Compound II returns to the resting enzyme state by reacting (Equation 2.3) with another molecule of substrate (Andrawis et al., 1988; Banci et al., 1992; Gold and Alic, 1993). The substrate cation radical ($R^+$) produced in Equation 2.3, can undergo a variety of non-enzymatic reactions and spontaneous rearrangement leading to further degradation and formation of a wide range of final products (Paszynski et al., 1985; Glenn et al., 1986; Pease et al., 1989; Wariishi et al., 1991; Gold and Alic, 1993; Catcheside and Mallett, 1991; Fakoussa and Hofrichter, 1999).

From the above mechanism, it can be inferred that oxidation of carbon by the fungus may lead to an increase in oxygen to carbon ratios through formation of carbon dioxide or introduction of oxygen groups on the carbon which disrupts the continuous nature of the graphitic planes required for gold adsorption (Jones et al., 1989; Klauber, 1991; Ibrado and Feurstenau, 1992; Van Krevelen, 1993). Infrared analysis of bacterial treated coal samples by Amankwah and Yen (2006) revealed an increase in oxygen groups on the coal and a subsequent reduction in gold adsorption ability. It is therefore possible that biotransformation of anthracite leading to a reduction in gold adsorption was as a result of introduction of oxygen groups and/or loss of carbon following oxidation. Another possibility is the blocking of carbon pores by the spores,

\[
P[Fe(III)](\text{native enzyme}) + H_2O_2 \rightarrow P[O-Fe(IV)^*](\text{comp I}) + H_2O \quad [2.1]
\]
\[
P[O-Fe(IV)^*](\text{comp I}) + R \rightarrow P[O-Fe(IV)](\text{comp II}) + R^* \quad [2.2]
\]
\[
P[O-Fe(IV)](\text{comp II}) + R + 2H^+ \rightarrow P[Fe(III)](\text{native enzyme}) + R^* + H_2O \quad [2.3]
\]
hyphae and/or biofilms generated by *P. chrysosporium* which obviously will lead to passivation and a decrease in active sites for adsorption.

The findings suggest that *P. chrysosporium* can potentially reduce the preg-robbing ability of carbonaceous matter in gold ores. Other aspects of this on-going research focus on establishing the optimum parameters for incubation and the effect of fungal treatment on refractory gold ores among others. Preliminary results are promising though the process is far slower compared to the existing high temperature and chemical methods used in deactivating carbonaceous matter. Nevertheless, biological processes are normally slower than chemical processes but once tested to work within engineering design capabilities, biological processes such as biooxidation of sulfides are accepted as alternatives on the basis of cost and environmental issues (Hutchins et al., 1988; Brierley, 1995). In addition, in a batch culture such as that used in this study, microbial growth and metabolic activity increase to a maximum and then reduce with further increase in processing time. Conversely, in a continuous system, growth phase is sustained indefinitely (Shuler and Kargi, 1992). Thus it is expected that in a continuous system processing time will be shorter without sacrificing biotransformation of the carbonaceous matter. The initial findings reported here make this process a potential alternative to other processes that can modify carbonaceous matter, and thus merits further investigations into the engineering possibilities.

### 3.6 Conclusions

The ability of *P. chrysosporium* to interact with, and reduce the gold adsorption capabilities of carbonaceous matter (CM) has been investigated and reported for the first time by this work. Various ranks of coal were used as surrogate materials to predict the response of CM to biotransformation by *P. chrysosporium*. Reduction in gold adsorption capabilities of the surrogates was used to estimate declining preg-robbing ability. Various culture media used under different incubation conditions affirmed that *P. chrysosporium* can grow on all the coal ranks. The results, however, show that of the coal samples tested, only as-received anthracite
coal exhibited significant gold-cyanide adsorption ability, which was used as a means of analyzing the extent of biotransformation. After contact with the fungus, preg-robbing ability of anthracite reduced by between 90 and 97% depending on the media.

By comparing submerged culturing using glucose broth medium and moist culturing using glucose agar, solid millet and wheat bran, it was established that millet and wheat bran constitute the best media for incubation, resulting in more than 95% reduction in preg-robbing. The findings demonstrate a potentially effective alternative pretreatment process for reducing preg-robbing and require further investigations into possible commercial application.
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Chapter 4

Fungal biotransformation of anthracite-grade carbonaceous matter: Effect on gold cyanide uptake

Abstract

Refractory carbonaceous gold ores contain carbonaceous matter that adsorbs gold during cyanidation leading to reduction in gold recovery. In a related investigation (Chapter 3), the white rot fungus, Phanerochaete chrysosporium was shown to reduce the gold adsorption ability of anthracite coal by more than 90% in a mixture of millet and wheat bran medium. This study focused on establishing the incubation parameters that led to maximum reduction in gold adsorption by anthracite. The results indicate that P. chrysosporium deactivates anthracite within a wide range of time, pulp density, temperature, pH, and level of agitation. A processing time of 5-7 days, temperature and pH of 37 °C and 4 respectively were the best conditions. The best pulp densities for stationary and shake culturing respectively were 60% and 25%.

Analysis of the nature of anthracite using Raman, FTIR and XANES spectroscopies indicated that fungal-treatment led to a reduction in carbon content and a boost of oxygen-containing groups such as carboxyls and carbonyls. In addition, BET surface area and micro pore volume reduced by 76 % and 80% respectively whereas average pore diameter increased by 65%. The results suggest that P. chrysosporium biotransforms anthracite by surface oxidation, which leads to a decrease in C/O ratio and disruption of the continuous graphitic structure, thus decreasing the active sites necessary for adsorption. Another factor is reduction in surface area via plugging of pores possibly by slimy substance formed through fungal interaction with the growth medium, and hence decreasing accessibility of gold to the adsorption sites. Fungal action also led to pore enlargement, thus rendering the pores unsuitable for adsorption of the aurocyanide ion.

Keywords: Phanerochaete chrysosporium; Surface modification; Biotransformation; Preg-robbing, pH; Pulp density; Temperature; Anthracite; Porosity; Aurocyanide; Adsorption; Characterization.
4.1 Introduction

Refractory carbonaceous gold ores are those that contain carbonaceous components, which adsorb dissolved (or preg-robbing) gold during cyanidation, resulting in reduced gold extraction. Carbonaceous matter (CM) presents a serious and challenging problem in the extraction of gold from refractory carbonaceous gold ores, and several techniques have been investigated to deactivate CM and prevent preg-robbing. Among these processes are roasting, chemical treatment using organic reagents and microbial treatment (Arriagada and Osseo-Asare, 1984; Abotsi and Osseo-Asare, 1987; Hutchins et al., 1988; Afenya, 1991; Portier, 1991; Brierley and Kulpa, 1993; Kohr, 1994; Adams and Burger, 1998; Rees and Van Deventer, 2000; Amankwah and Yen, 2006).

Microbial pretreatment processes are gaining importance as potential alternatives on the basis of cost and environmental issues, due to the relatively lower operating temperatures and the fact that microorganisms can rejuvenate themselves to recycle the reagents needed for the reaction. A number of bacteria and fungi have been tested for deactivation of carbonaceous matter having various degrees of maturity, and there are reports of increase in overall gold extraction. Among the microorganisms used are the bacteria *Streptomyces setonii*, *species of Pseudomonas*, *Achromobacter*, *Arthrobacter* and *Rhodococcus*, and fungi *Trametes versicolor*, *Aspergillus bruneio-uniseriatus* and *Penicillium citrinum* (Portier, 1991; Brierley and Kulpa, 1993; Amankwah et al., 2005; Afidenyo, 2008; Yen et al., 2008).

In an investigation of biodegradation of lignite, bituminous and anthracite coals using *S. setonii*, Amankwah and Yen (2006) observed a more extensive biodegradation in lignite and bituminous coal than in anthracite. In a solution of 5 mg/L Au, biotreatment reduced the gold adsorption capacity of bituminous, lignite and anthracite coals respectively from an average of about 19 % to 0.3 %, 22 % to 9 % and 45 % to 20 %. A white-rot fungus, *Trametes*
versicolor, has also been reported to reduce the preg-robbing effect of bituminous coal from 40.3 % to 8.3 % and anthracite from 99.5 % to 27.7 % (Afidenyo, 2008).

Anthracite grade CM comprises more than fifty percent of the components of CM in refractory gold ores (Hausen and Bucknam, 1984; Osseo-Asare et al., 1984; Sibrell et al., 1990; Pyke et al., 1999; Stenebraten et al, 2000; Schmitz et al., 2001; Vaughan and Kyin, 2004). In addition, anthracite grade CM has a far higher capacity to adsorb gold than bituminous and lignite coals due to the maturity and thus well-developed graphitic structure of anthracite (Jones et al, 1989; Klauber, 1991; Ibrado and Feurstenau, 1992; Van Vuuren et al., 2000; Amankwah and Yen, 2006; Ofori-Sarpong et al., 2010). Consequently, identifying a microorganism that can deactivate anthracite and reduce its gold adsorption significantly will be of immense benefit in the treatment of carbonaceous gold ores.

In Chapter 3 and Ofori-Sarpong et al. (2010), it was shown that anthracite interacted the strongest with dissolved gold and also gold adsorption reduced the most after fungal treatment. In this chapter, the interaction of P. chrysosporium with anthracite and its effect on aurocyanide adsorption is further explored to ascertain the response to changes in pH, pulp density, temperature and agitation.

### 4.2 Experimental investigations

#### 4.2.1 Materials, medium preparation and incubation

Anthracite coal samples, the fungus P. chrysosporium and the MWB medium were obtained and incubated as presented in Chapter 3, Section 3.3 and Ofori-Sarpong et al. (2010). The coal was incubated under different conditions including processing time, pulp density, temperature, pH, agitation and sterile versus non-sterile culturing. The pH was buffered to 4 and 6 respectively with succinic acid and potassium phosphate. Reagent grade potassium phosphate, succinic acid and sodium hydroxide were obtained from VWR.
4.2.2 Characterization of anthracite

In order to better understand the adsorption results, characterization was performed on both as-received and 14-day fungal treated anthracite using various techniques. The changes in surface characteristics of anthracite were observed by measuring surface area, pore volume and size by BET analysis, and the presence of oxygen groups and aliphatics by Fourier Transform Infrared (FT-IR) and X-ray absorption near edge structure (XANES) spectroscopies.

BET and pore size analyses were conducted using a Micromeritics ASAP 2000 Multi-Point BET analyzer and samples were analyzed for pore size by high pressure nitrogen absorption. FT-IR analysis was conducted by diluting milled samples of both as-received and biotreated anthracite with spectroscopic grade KBr in the ratio of 1:100 (Miller and Stace, 1972). Each mixture was ground in an agate mortar to homogenize the two components and reduce the scattering effect of the relatively larger particles. Analysis was carried out in a Bruker IFS 66/S FT-IR Spectrometer. For XANES analysis, the samples were dried and ground with a mortar and pestle to achieve homogeneous thickness (Szulczewski et al., 2001; Seiter et al., 2008). The sample was then mounted on adhesive tape attached to the sample holder and analyzed with the National Synchrotron Light Source in Brookhaven National Laboratory, NY.

Raman scattering is a spectroscopic technique based on inelastic scattering of a laser source monochromatic light, used to diagnose the internal structure of molecules and crystals (Potgieter-Vermaak, 2000). In this chapter, Raman spectroscopy was used for semi-quantitative analysis of the changes in graphitic nature of carbonaceous samples following fungal-treatment by *P. chrysosporium*. Samples were mounted on adhesive sample holder and the spectra were collected via the Confocal WITec XY Raman spectrometer, with a polarized laser light of 488 nm wavelength using a 40X objective lens. The spectra were collected at 5 min interval with 1 s integration time.
4.2.3 Photomicrographs of *P. chrysosporium*

*P. chrysosporium* maintained on millet and wheat bran medium for 2 weeks was observed with a Nicon Elipse 80i Microscope and the photomicrograph taken with the attached camera. The NIS-Element AR 3.0 software was used to process the image. The photomicrographs were taken at 100, 400 and 1000 magnification.

4.2.4 Analysis of data

The preg-robbing effect of carbon (PEC) which gives an indication of the effect of fungal-biomodification on adsorption of gold by carbon was computed as described in Chapter 3, Section 3.4. Comparing to the amount (µmol/g) adsorbed by the as-received anthracite (AAR), percentage reduction in preg-robbing (PRP) as a result of fungal-treatment was computed as in Equation 4.1. All experiments were carried out in triplicates and the data reported in the figures are mean values of the 3 samples, with the error bars representing the standard error (SE) of the means. In Equation 4.2, SD is the sample standard deviation (Equation 4.3) where n is the sample size, \( x_i \) and \( x \)-bar are the \( i \)th value and the mean respectively.

\[
\text{PRP}(\%) = \left( \frac{\text{PEC}}{\text{AAR}} \right) \times 100\% \tag{4.1}
\]

\[
\text{SE}_x = \frac{\text{SD}}{\sqrt{n}} \tag{4.2}
\]

\[
\text{SD}_x = \sqrt{\frac{\sum_{i=1}^{n} (x_i - \bar{x})^2}{n-1}} \tag{4.3}
\]
4.3 Results and discussions

4.3.1 Reduction in gold adsorption following fungal-treatment

The reduction in gold adsorption (preg-robbing) ability of anthracite following fungal treatment is presented in Fig. 4.1. Fungal-treatment was conducted at 60 % solids for 14 days at 37 °C and pH 6.5. From an initial adsorption value of 4.5 µmol/g for the as-received sample, gold adsorption reduced to 0.25 µmol/g after 14 days of treatment representing a reduction of about 95 %.

![Fig. 4.1. Reduction in gold adsorption by anthracite after fungal treatment at 60 % solids density for 14 days at 37 °C and pH 6.5. For adsorption tests, 0.1 g of anthracite was contacted with 25 mL of 5 µg/mL gold solution and agitated at 150 rpm for 24 hours](image_url)
4.3.2 Effect of pulp density

The effect of pulp density on biotranformation of anthracite by *P. chrysosporium* is shown in Fig. 4.2. The incubation conditions used were 14 days, 37 °C and pH 6.5. After stationary incubation for 7 days at a pulp density of 25 % solid, reduction in preg-robbing was 88 % from a solution of concentration 5 mg/L Au. By using the same initial fungal dosage for stationary incubation with pulp densities of 25 %, 40 %, 60 %, and 75 %, the average percentage reduction of gold adsorption were 88 %, 92.5 %, 95.5 % and 95 % respectively.

![Bar graph showing the effect of pulp density on biotransformation of anthracite by *P. chrysosporium* in stationary culturing (37 °C, pH 6.5; 14 days)](image)

From Fig. 4.2, pulp densities of 60 % and 75 % led to the highest reduction in preg-robbing of treated anthracite. The higher the pulp density the greater the mass available for fungal growth, hence the higher biotransformation in the 60 % and 75 % pulp densities as against the 25 % and 40 % pulp densities. At solid densities of 60 % and 75 %, the media and sample were moistened but did not form slurry. The discontinuous nature of the moist substrate
allowed for the formation of air pockets which provided the oxygen necessary for growth and activity of *P. chrysosporium* which is an aerobe (Tien and Kirk, 1988).

For shake cultures, as shown in Fig. 4.3, lower pulp densities were utilized, and there was 95% reduction in average gold adsorption for materials incubated at 15% and 25% solids while that of the sample treated at 40% solids reduced by 91%. From the figure, pulp densities of 15% and 25% for shake culturing led to higher reduction in preg-robbing of treated anthracite than 40% pulp density. At the lower pulp densities there is improved diffusion of oxygen into the slurry as the sample is disturbed by agitation (Marsden and House, 2006), providing the dissolved oxygen required for fungal-growth and activity. In addition, the ratio of oxidants to material being treated is higher with the lower pulp densities. However, 25% pulp density was chosen over 15% due to the mass of material that can be treated per unit time.

![Fig. 4.3. Effects of pulp density on biotransformation of anthracite by *P. chrysosporium* in shake culturing (37°C, pH 6.5; 14 days)](image-url)
Since the reduction in gold adsorption achieved at pulp densities of 60 % and 75 % after 7 days of fungal treatment were equally good, further investigations were carried out to monitor the effect of fungal-treatment time (Fig. 4.4) on reduction in preg-robbing. After an incubation period of 1 day, the gold adsorption abilities reduced by 78 % for the sample cultured at 60 % solids and 68 % for that cultured at 75 % solids. After the 5th day, preg-robbing by both samples reduced by more than 90 %, and over 95 % by the 7th day. It can be deduced from Fig. 4.4 that a processing time of 5-7 days is suitable for the process. Due to the reduced presence of water in the 75 % solids material, the fungus grew and formed a matrix that covered the anthracite making separation between biomass and sample more difficult. Consequently, a pulp density of 60 % may be preferred on the basis of reduced material losses during post incubation harvesting of treated materials.

![Fig. 4.4. Effect fungal-treatment time on biotransformation of anthracite at pulp densities of 60 % and 75 % solids under stationary culturing (37 °C, pH 6.5)](image-url)
The choice between stationary and shake culturing will depend on a balance between volume of material available for treatment and the time and technique available for harvesting treated material. Whereas per unit time, much more material can be treated via stationary than shake culturing, washing of treated material from shake culturing is less cumbersome and can be accomplished at a relatively shorter time with much less material losses than in stationary culturing where fungal biomass and ore particles become entangled making separation difficult.

4.3.3 Effect of temperature

The effect of incubation temperature as a function of time for stationary culturing at 60% solids and pH of 6.5 is demonstrated in Fig. 4.5.

![Graph showing the effect of incubation temperature on fungal treatment.](image)

**Fig. 4.5.** The effects of incubation temperature as a function of time in stationary culturing (60% solid, pH 6.5)
At processing temperature of 37 °C, gold adsorption ability reduced by 80 % after the 1\textsuperscript{st} day and further to about 96 % after the 7\textsuperscript{th} day. Beyond this day, the gold adsorption capabilities remained the same with increasing incubation time to the 21\textsuperscript{st} day. The sample incubated at 25 °C followed a similar trend though the reduction was much slower as after an incubation period of 1 day, the gold adsorption ability was reduced by 66 %. Sorption reduced further to a final value of 96 % after 21 days.

Shake culturing was conducted at 25 % solids, pH 6.5, and at temperatures of 25 °C and 37 °C over a period of 21 days. At 37 °C, the gold adsorbed reduced by 78 % after the 1\textsuperscript{st} day, and followed a plateau at 95 % reduction after the 7\textsuperscript{th} day as shown in Fig. 4.6. The trend is similar to that observed for stationary culturing (Fig. 4.5). For incubation at 25 °C, gold adsorption was halved after the 1\textsuperscript{st} day and reduced by 92 % after 21 days.

Fig. 4.6. The effects of incubation temperature as a function of time in shake culturing (25 % solid, pH 6.5)
It is clear from Fig. 4.5 and 4.6 that incubation at 37 °C gives a higher reduction in preg-robbing ability of anthracite than at 25 °C for both stationary and shake culturing. A processing time of 5 to 7 days is also more favorable.

Though the temperature for optimum growth of *P. chrysosporium* is 37 °C (Tien and Kirk, 1988) the study at 25 °C was done to investigate changes in preg-robbing behavior at ambient temperature. Work done by Kirk and Farrel (1987) shows that 37 °C provides the microorganism the right temperature for growth and production of oxidative enzymes. This is thus responsible for the higher performance at 37 °C compared to 25 °C. The challenge would be settling for lower preg-robbing reduction under ambient temperature as against extra heating cost for a higher preg-robbing reduction. Nevertheless, the catalysis of *P. chrysosporium* leads to the generation of heat which can sustain reactions above ambient temperature.

### 4.3.4 Effect of pH

The effect of pH on biotransformation of anthracite by *P. chrysosporium* was investigated at pH 4 and 6.5. Incubation was conducted at 25 % solids and 37 °C for 7 days under shake culturing. A pH of 4 was obtained by buffering the pulp with succinic acid and using NaOH to adjust the pH, whereas pH of 6.5 was obtained with potassium phosphate buffer. Fig. 4.7 reveals that after one day of incubation about 85 % reduction was realized at pH of 4 as against 78 % reduction at near neutral pH. By the 7th day, there was near parity in sorption reduction after fungal-treatment for the two pH values at 95 % and 97 %.
Investigations by Tien and Kirk (1984) indicate that the growth and ability of the fungus to secret oxidative enzymes peak around a pH of 4, and this may be responsible for the higher reduction in gold adsorption at that pH compared to the near neutral condition. The pH of the fresh culture media was around 6.5 and for tests where pH was not controlled, it was observed that pH reduced over time to between 4 and 5.5. This is because *P. chrysosporium* has the tendency to buffer itself by producing organic acids such as oxalic acid ((COOH)$_2$) malonic acid (CH$_2$(COOH)$_2$) and tartaric acid (CH$_3$CHO$_2$(COO)$_2$) that enables it to function around its optimum pH (Tien and Kirk, 1984; Catcheside and Ralph, 1999; Fakoussa and Hofrichter, 1999). At both pH values, a residence time of 5-7 days was found to be suitable.
4.3.5 Effect of sterilization

The effect of sterilizing the medium and material before fungal inoculation is shown in Fig. 4.8. When incubation was conducted for 3 days, adsorption onto the non-sterile sample decreased by 90 % as against the sterile system that recorded 92 %.

![Graph showing effect of sterilization](image)

**Fig. 4.8.** Effect of sterilizing on the time dependence of the biotransformation action of *P. chrysosporium* (37 °C, pH 6.5; 60 % solids; stationary)

Sorption reduction for the 5th and 7th days were 97 % for the non-sterile while for a similar period the sterile averaged 96 %. Judging from the average values, the non-sterile appeared to offer a slight advantage over the sterile. The main aim of sterilization is to get rid of resident microorganisms that are naturally present in the media and/or substrate to be treated. Thus, in the unsterilized culture, there will be other microorganisms aside the one specifically used for inoculation. Consequently, the seemingly slight advantage might be due to positive
contribution of native microorganisms in the medium and/or the anthracite to the biotransformation action of *P. chrysosporium*. It might also be the result of excess production of oxidative enzymes by *P. chrysosporium* in response to competitive and antagonistic survival against other possible native microorganisms (Waksman, 1941; Mille-Lindblom and Tranvik, 2003; Madigan and Martinko, 2006; Mille-Lindblom, 2006).

4.3.6 Effect of growth medium

Figure 4.9 depicts the contribution of the growth medium (MWB) to the biotransformation action of *P. chrysosporium*. The lower portions of the bars represent percentages of the total biotransformation attributed to the medium alone whereas the upper portions show the additional reduction in preg-robbing capacity of anthracite when the medium is cultured with *P. chrysosporium*.

![Diagram showing combined effect of MWB and fungus in the biotransformation of anthracite](image)

Fig. 4.9. Combined effect of MWB and fungus in the biotransformation of anthracite (37 °C, pH 6.5; 60 % solids; stationary; up to 14 days)
In the presence of the fungus, there was 4-8 fold decrease in gold adsorption in comparison to the media alone. The slight decline in preg-robbing due to the medium alone may be attributed to fine suspended particulates that might have blinded some of the pores on the carbonaceous material (Chapter 3; Ofori-Sarpong et al., 2010).

4.4 Investigations of surface biomodification of anthracite

From the above section, it is clear that *P. chrysosporium* possesses the ability to biotransform anthracite-grade carbonaceous matter under various incubation conditions and reduce its gold adsorption activity substantially. The following section investigated the nature of surface biomodification imparted onto anthracite by the fungus.

4.4.1 Surface area and pore characterization of anthracite

The adsorption sites for gold are located in the pores of the adsorbent and hence it was necessary to know how fungal transformation changes the nature of these pores. The surface area and porosity measurements are presented in Fig. 4.10. BET surface area of the as-received anthracite was found to be 4.08 m$^2$/g, a number similar to that of anthracite samples studied by Miller and Sibrell (1991) who obtained 4.0 m$^2$/g. After fungal treatment, the BET surface area reduced by 76 % to 0.98 m$^2$/g.

The micro pore volume and average pore diameter of the as-received sample were 15.09 x 10$^{-4}$ cm$^3$/g and 5.7 nm respectively and after treatment, the micro pore volume reduced to 2.82 x 10$^{-4}$ cm$^3$/g while the average pore diameter increased to 9.5 nm respectively. These translate into micro pore volume reduction of 80 % and increase of 65 % in average pore diameter (Fig. 4.10). The micropore surface area on the other hand reduced by about 80% following fungal treatment to 0.61 m$^2$/g.
All these changes potentially reduce gold adsorption onto carbon. A reduction in both pore volume and surface area led to subsequent decrease in adsorption as both parameters influence adsorption of the aurocyanide complex (McDougall and Hancock, 1981).

### 4.4.2 Photomicrograph of *P. chrysosporium*

The photomicrograph of *P. chrysosporium* maintained on MWB medium for 2 weeks was taken with Nicon Elipse 80i Microscopic camera, and is presented in Fig. 4.11. The picture shows hyphae and vegetative spores of the fungus, and diameter of the hyphae was 1-3 µm.
which is similar to that obtained by Sachs et al. (1989) when *P. chrysosporium* was cultured on wood within a week. This number is within the range (0.5-10 µm) reported by other researchers, and the size is known to depend on the substrate and the culturing time (Kirk et al., 1986; Sachs et al., 1989; Friese and Allen, 1991; Lyford, 1966; Steinberg and Rillig, 2003). The spores were estimated to be 0.4-2 µm in diameter. Though the sizes are bigger than the micropores and mesopores (< 0.2 µm) of carbon, the tiny spores may be able to access the over 0.2 µm sized macropores which are the admission pores to the smaller pores where gold adsorption usually takes place (McDougall and Hancock, 1981). It is important to investigate the nature of the material that causes blockage of the pores and accounts for the sharp reduction in the surface area and volume of the smaller pores (Fig. 4.10). However, TEM analysis did not reveal information in that direction.

![Photomicrograph of *P. chrysosporium*](image_url)

**Fig. 4.11.** Photomicrograph of *P. chrysosporium*. The fungus was maintained on MWB medium for 2 weeks.
4.4.3 Modification of graphitic structure

*Raman Spectroscopy*

The degree of graphitization of carbonaceous materials such as anthracite can be studied by identifying the G and D lines under Raman spectroscopy (Sadezky et al., 2005; Potgieter-Vermaak et al., 2010). The G-line indicates the graphitic or ordered nature of the structure whereas the D-line, disordered nature of the structure due to edge defects or lattice disorder. There are various peaks that have been assigned to graphitic and disordered structures in various ways by different researchers but the two most distinct ones are the peaks around wavelengths of 1580 cm\(^{-1}\) known as the G line, and 1370 cm\(^{-1}\), the D line (Sadezky et al., 2005; Potgieter-Vermaak et al., 2010). Figure 4.12 presents the Raman spectrograms of as-received and treated anthracite.

![Raman Spectrograms](image)

**Fig. 4.12.** Raman spectra of anthracite; as received and 14-day fungal treated at 37 °C and pH 4.
Changes in intensities of the peaks signal a change in degree of orderliness or disorderliness (Leventhal an Hofstra, 1990; Bustin et al., 1995). It can be seen from the figure that all the peak intensities reduced following fungal-treatment. Considering the two major peaks (1580 cm\(^{-1}\) and 1370 cm\(^{-1}\)), reduction was more apparent in the disordered line (1370 cm\(^{-1}\)) than the graphitic line (1580 cm\(^{-1}\)). This is because, like many processes in nature, microbial attack initiates at points of weakness and thus the less structured zones (Wackett and Ellis, 1999). Altogether, reduction in carbon content possibly due to complete oxidation to carbon dioxide will result in lower carbon-to-oxygen ratio, known to be unfavorable to gold adsorption (Ibrado and Fuerstenau, 1992; Ofori-Sarpong et al., 2010).

4.4.4 Modification of surface functional groups

Infrared analysis

Infrared spectroscopy measures the ability of matter to absorb, transmit or reflect infrared radiation and relates it to the chemical composition of that substance (Miller and Stace, 1972). Crystals and molecules absorb infrared radiation depending on the inter-atomic vibrations. Certain vibrations are related to specific groups and these are termed characteristic frequencies. The absorption/transmission of radiation plotted as a function of the wavelength gives information about the structure and functional groups present on materials (Dyke et al, 1971). For a given substance, changes in the functional groups due to a pretreatment process may be registered by a change in the spectral configuration. Figure 4.13 shows the infrared spectra of as-received anthracite and a 14-day biotreated sample. The peak assignments on the samples were compared with earlier work by Rao (1963), Silverstein et al. (1981) and Painter (1983). The OH peak between 3200 and 3000 cm\(^{-1}\) on both the as-received and biotreated anthracite samples is generally attributed to the presence of water while the peak between 2400 and 2200 cm\(^{-1}\) is due to carbon dioxide. The C=C band at 1582 cm\(^{-1}\) is considered to be characteristic of the hexagonal ring structure of graphite (Painter et al., 1983).
By comparing the two spectra, it is realized that the major changes occurred at 2929 cm$^{-1}$, 2856 cm$^{-1}$, and 1715 cm$^{-1}$. The peaks at 2929 cm$^{-1}$ and 2856 cm$^{-1}$ are due to the presence of aliphatic hydrocarbons while carbonyl (C=O) groups are responsible for the peak at 1715 cm$^{-1}$ (Rao, 1963; Silverstein et al., 1981; Painter, 1983). The C=O group which is present in the treated sample indicates that there was formation of more oxygen containing groups on the surface of anthracite. With the increase in the aliphatic groups and the carbonyl groups on anthracite, it can be inferred that the degree of aromaticity was reduced due to fungal treatment. These changes will result in reduced adsorption as aurocyanide adsorption, which requires large continuous areas of graphitic plates, not interrupted by edges terminating with C-H or C-O groups (Jones et al., 1989; Ibrado and Fuerstenau, 1992; Jia et al., 1998).

![Infrared spectra of both as-received and biotreated anthracite. Anthracite was treated for 14 days at 60 % solids, 37 °C and pH 4.](image_url)
**XANES Spectroscopy**

In this study, x-ray absorption near edge structure (XANES) spectroscopy was utilized in observing changes in the surface functional groups on anthracite. Figure 4.14 compares the as-received and 14-day biotreated anthracite, and the spectra shows an increase in intensity of the oxygen-containing groups. Compared with studies by Braun et al (2005), the peaks between 285 and 286 eV are associated with carbonyls and phenols while the hump at 287 eV is for aliphatics and the peak between 288 to 289 eV signifies the presence of carboxylic acids. Though these peaks were on the as-received sample, they became more pronounced following fungal treatment. This observation confirms that of the infrared analysis which also showed an increase in oxygen-containing groups.

![Graph showing XANES spectra comparison](image)

Fig. 4.14. A comparison of the as-received and biotreated anthracite spectra from oxygen XANES. Anthracite was treated for 14 days, at 60 % solids density, 37 °C and pH 4.
4.5 Proposed mechanism responsible for reduction in gold adsorption after biotransformation of anthracite by \textit{P. chrysosporium}

The current theory of gold adsorption from alkaline cyanide solution favors adsorption of the gold dicyano complex on the surface of graphitic planes of carbon via donation of delocalized pi electrons from the graphitic planes to the empty 6s shell of gold ion (McDougall et al., 1980). Though the surface of carbon is negatively charged due to sp$^2$ hybridization, this interaction outweighs the electrostatic repulsion between the negatively charged surface of carbon and the Au(CN)$_2^-$ complex (Jones et al, 1989; Klauber, 1991; Ibrado and Feurstenau, 1992; 1995; Jia et al., 1998).

In a review by Osseo-Asare et al. (1984), it was noted that modification of the surface of carbon by organic additives such as kerosene and heavy oils, rather than complete oxidation to carbon dioxide, can substantially reduce gold uptake by carbon. Similar results have been reported by several other investigators (Abotsi and Osseo-Asare, 1987; Adams and Burger, 1998; Pyke et al., 1999). The reduction in gold adsorption has been interpreted to be the consequence of preferential attachment of the organic compounds to the hydrophobic graphitic planes (Jia et al., 1998). Destruction of graphitic planes, complete and/or partial oxidation of carbon and thus increase in oxygen-to-carbon ratios are also known to decrease the gold adsorption capacity of carbon (Osseo-Asare et al, 1984; Sibrell et al. 1990; Ibrado and Feurstenau, 1992; Amankwah and Yen, 2006; Ofori-Sarpong et al., 2010).

Based on information provided by Klauber (1991) and Poinen and Thurgate (2003) as shown in Fig. 4.15, the radius of the gold cyanide ion, Au(CN)$_2^-$, can be estimated as 0.733 nm, and it can lie approximately on 3 adjacent graphitic planes (3 x 0.2456 = 0.7368 nm). The continuous nature of the graphene layers is thus required for the adsorption of aurocyanide complex from alkaline solutions (Jones et al., 1989; Klauber, 1991; Ibrado and Feurstenau, 1992, 1995).
In this study, Raman, infrared and XANES spectra indicated that the biotransformation of anthracite by *P. chrysosporium* led to interruptions in the graphitic layers and subsequent introduction of oxygen/hydrogen groups which can partly explain the reduction in gold adsorption following fungal biotransformation.

The hyphae of *P. chrysosporium*, which are known to penetrate wood and soil, is estimated to be between 0.5-10 µm in diameter (Kirk et al., 1986; Sachs et al., 1989; Friese and Allen, 1991; Lyford, 1966; Steinberg and Rillig, 2003) depending on the substrate and the culturing time. In this study, the diameters of the hyphae and spores produced by *P. chrysosporium* taken under high magnification were 1-3 µm and 0.4-2 µm respectively. These sizes are bigger than micropores and mesopores in anthracite but may be able to access macropores (>0.2 µm) (Marsden and House, 2006) and reduce gold adsorption by decreasing the ease of access of aurocyanide ion to the adsorption sites (McDougall et al., 1980; Jia et al., 1998; Van Vuuren et al., 2000; Marsden and House, 2006).

Surface area and pore size measurements as shown in Fig. 4.10 signify a drastic reduction in surface area and micro pore volume with increase in average pore size diameter following
fungal-treatment. Microbial transformation of solids begins with the formation of a slime layer around the area of attack. This layer is composed of polysaccharides (Glazer and Nikaido, 1995; Madigan and Martinko, 2006) and it is possible that these organic substances penetrated and coated more of the smaller pores. As discussed by Osseo-Asare et al. (1984), coating of the surface by organic matter will lead to reduction in gold adsorption. Since gold adsorption is reported to occur mostly in the micropores (McDougall and Hancock, 1981) a reduction in available micropores and hence increase in available pore diameter contributed to the reduction in gold adsorption. It is important to investigate the nature of the material that caused blockage of the pores and accounted for the sharp reduction in surface area and volume of the smaller pores. Surface examination using TEM has so far not provided answers in this direction.

4.6 Conclusions

The white rot basidiomycetous fungus, *Phanerochaete chrysosporium* was utilized in reducing gold adsorption by anthracite coal which served as surrogate for carbonaceous matter in refractory gold ores. Fungal treatment resulted in reduction of the gold adsorption ability of anthracite by more than 90 % in a mixture of millet and wheat bran medium.

Fungal modification with *P. chrysosporium* was found to take place in a wide range of time, pulp density, temperature, pH and level of agitation. After contact with the fungus, gold adsorption ability of anthracite reduced by about 90-97 %, depending on the incubation conditions. A pH of 4 and temperature of 37 °C were found to enhance biotransformation. Stationary culturing permitted the use of higher pulp densities and the best for stationary and shake culturing respectively were 60 % and 25 % solids. Altogether, strict choices of the parameters will depend on materials required to be treated, techniques available for treatment and post-incubation harvesting. Other considerations are a balance between capacity/tonnage, extra cost on reagents and processing time. Firm decisions can be taken when cost analyses are conducted.
Raman, infrared and XANES analyses showed that fungal treatment led to a reduction in carbon content and introduction of oxygen containing groups such as carbonyls and an increase in aliphatic groups. Further investigations showed that BET surface area and micro pore volume decreased by 76 % and 80 % respectively whereas average pore diameter increased from 5.7 nm to 9.5 nm. The combined characterization results from Raman, XANES, IR and BET suggest that *P. chrysosporium* biotransforms anthracite in two main ways. In one way there is loss of carbon through complete oxidation to carbon dioxide and surface oxidation by introduction of oxygen groups which disrupts the continuous graphitic structure thus decreasing the active sites necessary for adsorption. The second route is reduction in available surface area via plugging of pores possibly by slimy substances formed by interaction of the fungus with the medium, hence decreasing accessibility of gold to the adsorption sites.
References


Chapter 5

Gold adsorption on carbonaceous matter following fungal treatment: Comparison of cyanide and non-cyanide gold complexes

Abstract

Though cyanide has been the universal reagent for gold leaching on counts of higher stability, lower cost and better understood chemistry, its high toxicity and slower leaching kinetics have called for research into alternative leaching reagents, of which thiosulfate and thiourea appear to be promising. Unlike the cyanide system, interaction of thiourea and thiosulfate systems with carbonaceous matter (CM) has not received much research attention. As non-cyanide reagents become important alternatives for gold leaching, it is imperative to assess the degree of gold adsorption (preg-robbing) in these systems and find ways of reducing its effect. As part of on-going research, the effectiveness of the fungus, *Phanerochaete chrysosporium*, to biotransform anthracite-grade CM and reduce preg-robbing in cyanide solution has been established.

This chapter presents the results of a study on reduction in gold uptake by CM in non-cyanide systems due to fungal-treatment. Comparison is made between cyanide and non-cyanide systems on the bases of changes in gold adsorption by surrogate CM and CM in refractory gold ores. The results indicate that gold adsorption by as-received surrogates is in the order of cyanide > thiourea > thiosulfate, whereas after fungal-transformation, reduction in gold adsorption is more pronounced in cyanide than in thiourea and thiosulfate solutions. The average reduction in gold adsorption on the CM is about 70-97 % in cyanide, 60-92 % in thiourea and 50-91 % in thiosulfate. In general, the results exhibit a potential technique for reducing preg-robbing in the processing of carbonaceous gold ores with thiourea and thiosulfate solutions.

Keywords: *Phanerochaete chrysosporium*; Biotransformation; Carbonaceous matter; Preg-robbing; Gold adsorption; Cyanide; Thiourea; Thiosulfate.
5.1 Introduction

5.1.1 Lixivants in gold extraction

Cyanide has remained the preferred complexing agent in the leaching of gold for over a century though many other complexing agents exist. The major reasons for the acceptance of cyanide over the other lixivants include higher chemical stability and lower cost (Groenewald, 1976; Osseo-Asare et al., 1984; Nicol et al., 1987; Hiskey and Atluri, 1988; Smith and Martell, 1989; Meng and Han, 1993; Wan et al., 1993; La Brooy et al., 1994; Ritchie et al., 2001; Muir and Aylmore, 2004; Senanayake, 2004). In addition, adsorption and desorption of gold cyanide complex onto and from the most widely used adsorbent, activated carbon, has been studied the most with great success (McDougall et al., 1980; Adams and Flemings, 1989; Jones et al., 1989; Ibrado and Fuerstenau, 1995; Rees and van Deventer, 2000).

In spite of the good attributes, the high toxicity of cyanide, the need for faster leaching kinetics and hence shorter processing times, and an environmentally friendly recovery process for gold ores have necessitated research into alternative leaching reagents. The reagents include thiourea, thiosulfate, iodine, bromine and thiocyanate (Wan et al., 1994; Sparrow and Woodcock, 1995; Yen et al., 1998; Schmitz et al., 2001; Fleming et al., 2002; 2003; Navorro et al., 2002; 2006). Of the non-cyanide reagents, those that have the potential to replace cyanide, and have been researched most in the past 30 years include thiourea and thiosulfate (Groenewald, 1976; 1977; Hiskey., 1981; 1984; Mensah-Biney et al., 1994; Grosse et al., 2003; Muir and Aylmore, 2004; Gonen et al., 2007; Osseo-Asare, 1988).

5.1.2 Adsorption of gold complexes on carbon

The dissimilarities in complexing reagents present significant differences in their interactions with carbon. Gold thiourea is cationic with a +1 charge while cyanide and thiosulfate are
anionic but with charges of -1 and -3 respectively (McDougal and Hancock, 1981; Osseo-Asare et al., 1988; Muir and Aylmore, 2004; Marsden and House, 2006). The ability of activated carbon to adsorb these three gold complexes is in the order of Au(CN)₂⁻ > Au(CS(NH₂)₂)₂⁺ > Au(S₂O₃)²⁻ (Gallagher et al., 1989; Brown and Deschenes, 1993; Arriagada and Gardia, 1997; Grosse et al., 2003; Muir and Aylmore, 2004; Marsden and House, 2006).

The mechanism of adsorption of these complexes has been discussed extensively in the literature (McDougal et al., 1980; Jones et al., 1989; Adams and Fleming, 1989; Gallagher et al., 1989; Kongolo et al., 1990; Brown and Deschenes, 1993; Yen et al., 1994; Arriagada and Gardia, 1997; Zhang et al., 2004; Muir and Aylmore, 2004; Navarro et al., 2006). Several theories have been proposed for gold cyanide adsorption but currently there seems to be a consensus that adsorption of aurocyanide ion (Au(CN)₂⁻) onto carbon occurs without chemical change and that the graphitic structure of carbon is of great importance. There is disparity though as to where the adsorbed gold resides, and the two major theories are predominant adsorption on the planes of graphitic layers, and adsorption at the edges of graphitic layers where there are oxygen functional groups (Jones et al., 1989; Klauber, 1991; Sibrell and Miller, 1992; Ibrado and Fuerstenau, 1995; Poinen and Thurgate, 2003).

Gold thiourea complex, Au(CS(NH₂)₂)₂⁺ has been reported to adsorb onto activated carbon without undergoing any chemical change (Fleming, 1987; Schmidt et al., 1988). Using activated carbons of different precursors, Arriagada and Gardia (1997) noted that the carbons with oxygenated surface functional groups; carboxylic-like and phenolic or quinonic-like, had higher affinity for gold thiourea complex than gold cyanide complex. In a study by Zhang et al. (2004), the authors proposed that gold thiourea is generally adsorbed onto activated carbon in the form of Au(I)–thiourea complex and partial decomposition into metallic gold may occur at locally concentrated high gold loadings.

Gold thiosulfate complex, Au(S₂O₃)₂³⁻ has been shown by many investigators to have significantly less affinity for carbon compared to gold cyanide and thiourea complexes (Gallagher et al., 1989; Aylmore and Muir, 2001; Kononova et al., 2001; Schmitz et al.,
Gold thiosulfate complex has a high anionic charge resulting from the interaction between gold cation and large thiosulfate anions. The low affinity of activated carbon for $\text{Au(S}_2\text{O}_3\text{)}_{2}^{3-}$ complex is thus ascribed to the long pathway between the central gold atom in the complex and the adsorbent surface, as created by the large thiosulfate ions (Grosse et al., 2003; Aylmore and Muir, 2001; Muir and Aylmore, 2004).

5.1.3 Application of *P. chrysosporium* in reducing adsorption of gold complexes on carbon

Loss of gold due to preg-robbing is a major setback in the cyanide system and has been of great interest to researchers for many years in finding ways to mitigate the problem (McDougal et al., 1980; McDougall and Hancock, 1981; Osseo-Asare, 1988; Miller and Sibrell, 1991; Sibrell and Miller, 1992; Klauber, 1991; Ibrado and Fuerstenau, 1995). Considering the potential of thiourea and thiosulfate to replace cyanide or be used as alternatives, it is essential to assess the status of preg-robbing in these systems and to find possible ways of reducing it.

The application of the fungus, *Phanerochaete chrysosporium*, in the biotransformation of carbonaceous matter (CM) in gold ores has been discussed by Chapter 4 and Ofori-Sarpong et al. (2010) of this dissertation. In the study, biotransformation led to a reduction in aurocyanide uptake by CM and hence preg-robbing in cyanide solutions. The present study therefore focused on assessing the ability of *P. chrysosporium* to alter the gold adsorption ability of CM in thiourea and thiosulfate solutions.
5.2 Experimental investigations

5.2.1 Materials, medium preparation and incubation

Anthracite coal samples, *P. chrysosporium* and the millet and wheat bran (MWB) medium were obtained and incubated as presented in Chapter 3, Section 3.3 and Ofori-Sarpong et al. (2010). The gold-containing samples utilized were flotation concentrate (FC) and bacterial oxidized FC (BFC). Samples FC and BFC, which had been milled to all passing 75 µm, were obtained from the sulfide treatment plant at Bogoso Mine of Golden Star Prestea-Bogoso Resources, Ghana. Standard gold cyanide solution (50 µg/ml in 0.1 % sodium hydroxide and 0.05 % sodium cyanide) was supplied by High Purity Standards. Powdered gold (I) sodium thiosulfate (Na$_3$Au(S$_2$O$_3$)$_2$.xH$_2$O hydrate, 99.96 % metal basis), gold flakes (APS 1.5-3.0 µm, 99.96 % metal basis), thiourea, sodium thiosulfate, sodium hydroxide and sulfuric acid were supplied by Alfa Aesar. Biotransformation was explored by incubating coal and gold ore samples with *P. chrysosporium*, cultivated in MWB medium at 37 °C for different growth periods of up to 3 weeks, and at pH of 6.5. At the end of the incubation period the samples were washed with water in the ratio of about 1 g sample to 200 ml H$_2$O to get rid of the media and fungal biomass, and then dried at 37 °C for 7 days.

5.2.2 Gold adsorption experiments

Gold-cyanide solution of concentration 50 µg/mL Au was diluted with 1 g/L NaOH at pH 12.8 to obtain 5 and 10 µg/mL gold solutions which were used for the adsorption analysis. Gold thiosulfate solution was prepared by dissolving 130 µg of gold (I) sodium thiosulfate in 500 ml of 0.1 M thiosulfate solution at pH 9-11 to obtain 50 µg/mL Au. To obtain 50 µg/mL Au in thiourea solution, 50 µg of gold flakes was dissolved in 500 mL of 0.001 M thiourea solution at pH of 1-2 at 40 °C. Both thiosulfate and thiourea gold stock solutions were diluted appropriately to obtain 5 and 10 µg/mL solutions, which were used for the adsorption test. Triplicate samples of biomodified, control and as-received coals of various weights (0.1 g – 1
g) and gold ores of 2 g - 5 g each were contacted with 25 mL of 5 µg/mL and/or 10 µg/mL gold solutions and agitated at 150 rpm for 24 hours. At the end of the contact time the residual solution was filtered and gold in the filtrate determined using Perkin-Elmer Optima 5300 inductively coupled plasma – atomic emission spectrometer (ICP-AES).

5.2.3 Petrographic analysis of gold concentrate

Petrographic evaluation and thermal maturity analysis on the organic carbon in the gold concentrate was conducted using Zeiss Universal research microscope. The gold concentrate was mounted in epoxy, ground and polished with 600-grit emery paper before analysis.

5.3 Results and Discussion

5.3.1 Analysis of gold concentrates

Results of analysis of the as-received gold concentrates are presented in Table 5.1. The grade of gold in both samples is very high at above 30 g/t with total sulfur and sulfide sulfur in FC at 19.3 % and 14.9 % respectively. BFC has sulfide sulfur content of 3.0 % which is due to unoxidized sulfide minerals after biooxidation. Other constituents are as shown in Table 5.1.
Table 5.1. Partial chemical analysis of as-received gold concentrates

<table>
<thead>
<tr>
<th>Constituent</th>
<th>Grade, %</th>
<th>Constituent</th>
<th>Grade, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>FC</td>
<td>BFC</td>
<td>FC</td>
</tr>
<tr>
<td>Gold</td>
<td>30.2 g/t</td>
<td>39.3 g/t</td>
<td>Iron</td>
</tr>
<tr>
<td>Silver</td>
<td>2.2 g/t</td>
<td>3.1 g/t</td>
<td>Arsenic</td>
</tr>
<tr>
<td>Organic carbon</td>
<td>3.6</td>
<td>4.0</td>
<td>Copper</td>
</tr>
<tr>
<td>Total carbon</td>
<td>6.1</td>
<td>4.0</td>
<td>Zinc</td>
</tr>
<tr>
<td>Sulfide sulfur</td>
<td>14.9</td>
<td>3.0</td>
<td>Lead</td>
</tr>
<tr>
<td>Sulfate sulfur</td>
<td>4.6</td>
<td>4.5</td>
<td>Nickel</td>
</tr>
<tr>
<td>Total sulfur</td>
<td>19.3</td>
<td>7.5</td>
<td></td>
</tr>
</tbody>
</table>

5.3.2 Effect of carbonaceous characteristics on gold adsorption from cyanide, thiourea and thiosulfate solutions onto surrogate CM

The gold adsorption behavior of anthracite, bituminous, sub-bituminous and lignite coals from cyanide, thiourea and thiosulfate solutions before and after biotransformation by *P. chrysosporium* are presented in Figs. 5.1, 5.2 and 5.3 respectively. The maturity of coal, and thus, the extent of graphitization decreases in the order of anthracite > bituminous > sub-bituminous > lignite. Conversely, the percentage of oxygen and hydrogen and thus, the ratio of oxygen to carbon, and hydrogen to carbon decrease in the opposite direction (Van Kreveren et al., 1993). It has been shown that gold cyanide adsorbs preferentially on anthracite relative to the other coals due to better developed graphitic structure in anthracite (Ibrado and Feurstenau, 1992; Amankwah and Yen, 2006). Correlating the carbon-to-oxygen ratios with gold adsorption, it was inferred in Chapter 3 and Ofori-Sarpong et al. (2010) that the adsorption of gold cyanide on anthracite is due to its high carbon-to-oxygen ratio of 42 compared to 5-11 for bituminous, sub-bituminous and lignite.
Fig. 5.1. Effect of one-week fungal-treatment on the gold adsorption ability of lignite, sub-bituminous, bituminous and anthracite coals in cyanide (CN) solution (37 °C; pH 6.5; 60 % solids)

It can be seen from Figs. 5.2 and 5.3 that gold adsorption from thiourea and thiosulfate solutions follow similar trends as that observed in the cyanide solution (Fig. 5.1) with anthracite adsorbing about 5-20 times as much as the other coals. Comparing the gold complexes though, it is clear that there is very low adsorption from thiosulfate solution on all the coals, and this trend is in agreement with what is reported in literature (Gallagher et al., 1989; Grosse et al., 2003; Schmitz et al., 2001; Marsden and House, 2006). The low affinity of activated carbon for \( \text{Au(S}_2\text{O}_3)_2^{3-} \) complex is ascribed to the long pathway between the central gold atom in the complex and the adsorbent surface, as created by the large thiosulfate ions. In addition, the negatively charged carbon surface exerts a stronger electrostatic repulsion with the highly negative thiosulfate complex compared to the other complexes (Kononova et al., 2001; Grosse et al., 2003; Aylmore and Muir, 2001; Muir and Aylmore, 2004).
Fig. 5.2. Effect of one-week fungal-treatment on the gold adsorption ability of lignite, sub-bituminous, bituminous and anthracite coals in thiourea (TU) solution. (37 °C; pH 6.5; 60 % solids)

Whereas anthracite adsorbed much more in the cyanide solution than the thiourea solution, the opposite was true for lignite, sub-bituminous and bituminous coals. The positively-charged thiourea gold complex is reported to have better interaction with oxygen groups than aurocyanide complex, and this may explain the higher adsorption of gold thiourea by the lower coal ranks which contain more oxygen groups (Arriagada and Gardia, 1997; Nakbanpote et al., 2000). As mentioned earlier, adsorption of gold thiosulfate by all coal ranks was relatively very low.
Fig. 5.3. Effect of one-week fungal-treatment on the gold adsorption ability of lignite, subbituminous, bituminous and anthracite coals in thiosulfate (TS) solution. (37 °C; pH 6.5; 60 % solids)

Figure 5.4 compares the percentage reduction in gold adsorption due to fungal-treatment of the coals in all the gold solutions. After treatment of the coals with *P. chrysosporium*, the adsorption capacity for the gold complexes generally decreased. For all the solutions tested, the average reduction in gold adsorption was much more significant on anthracite than the other coals. Average reduction in gold adsorption for all coals was highest in cyanide solution as depicted in Fig. 5.4.
Fig. 5.4. Reduction in cyanide (CN), thiourea (TU) and thiosulfate (TS) gold adsorption on lignite, subbituminous, bituminous and anthracite due to surface biotransformation by \textit{P. chrysosporium} (37°C; pH 6.5; 60 % solids; 7 days)

The comparison of gold adsorption on anthracite from cyanide, thiourea and thiosulfate solutions is presented in Fig. 5.5, whereas Fig. 5.6 shows the effect of the incubation medium on gold adsorption on anthracite. Gold adsorption onto anthracite was highest in cyanide solution at 4.5 µmol/g followed by thiourea at about 2.2 µmol/g and thiosulfate at 0.4 µmol/g as shown in Fig. 5.5. Fungal treatment reduced sorption in all solutions to below 0.3 µmol/g. It is clear from Fig. 5.6 that gold adsorption for the controls were very close to that of the as-received samples, and that the reduction in gold adsorption is essentially due to biotransformation by \textit{P. chrysosporium}. 

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Fig. 5.5. Comparison of gold adsorption on anthracite from cyanide, thiourea and thiosulfate solutions as a function of fungal-treatment time. (37 °C; pH 6.5; 60 % solids; up to 21 days)

Fig. 5.6. Gold adsorption on as-received, control and treated anthracite from cyanide, thiourea and thiosulfate solutions (37 °C; pH 6.5; 60 % solids; 7 days).
5.3.3 Effect of fungal biotransformation on the adsorption of gold complexes onto gold concentrates

Having established the effect of fungal biotransformation of the surrogate carbonaceous materials on the adsorption of the gold complexes, the flotation concentrate (FC), and FC biooxidized with iron and sulfur oxidizing bacteria (BFC) were also tested to ascertain the extent to which their preg-robbing abilities change following fungal-transformation. These results are presented in Figs. 5.7 and 5.8. Within the boundaries established by the error bars the levels of gold adsorption for the as-received FC was slightly lower than BFC for each of the complexing solutions utilized. The above statement is also true for adsorption after fungal treatment. This observation is based on the fact that the bacteria treatment of FC to produce BFC does not inhibit the ability of CM to preg-rob gold, and the reduction in mass of concentrate due to the treatment process boosts up the effective concentration of CM making it a higher preg-robber.

![Fig. 5.7. Comparison of gold adsorption on flotation concentrate (FC) from cyanide, thiourea and thiosulfate solutions before and after fungal-treatment. (37 °C; pH 6.5; 60 % solids; 7 days)](image-url)
Fig. 5.8. Comparison of gold adsorption on bacterial-oxidized flotation concentrate (BFC) from cyanide, thiourea and thiosulfate solutions before and after fungal-treatment. (37 °C; pH 6.5; 60 % solids; 7 days)

Petrographic evaluation and thermal maturity analysis on the organic carbon in the gold concentrate using Zeiss Universal research microscope as shown in Fig 5.9, revealed that the organic carbon is fine with particle size in the range of 10-40 µm. The mean maximum reflectance was 3.7 %, which puts it in the anthracite-range maturity (Van Krevelen, 1993). These carbon particles are coarser than those in similar refractory materials that were analyzed by Afenya (1991) in which he obtained sizes of minus 2 µm. The figure also shows sulfide particles in the double refractory gold ore.

Though the CM was characterized to have anthracite grade maturity it is clear from both Figs. 5.7 and 5.8 that the level of reduction in preg-robbing does not compare well with that of anthracite, which was used as surrogate material. This observation may be due to the complex nature of the refractory gold ores with 3-4 % organic carbon and many other minerals composing about 96-97 % (Table 5.1) in contrast to anthracite which has more than 90%
organic carbon. It is not clear at this point whether there was concurrent gold leaching during the gold adsorption test and/or if fungal-treatment activated some other components in the ore to sorb gold. Nevertheless, preg-robbing reduction of 50-70 % in refractory gold ores is promising.

Fig. 5.9. Petrographic analysis of double refractory flotation gold concentrate

5.4 Fungal biotransforming action on the adsorption of gold thiourea and thiosulfate on carbonaceous materials

In contrast to the cyanide system, extensive research in gold adsorption by carbonaceous matter within the thiourea and thiosulfate systems is yet to be realized. This study is therefore important as it reports for the first time, the extent of reduction in gold up-take in these systems as a result of fungal-biotransformation of carbonaceous matter. The adsorption of gold thiosulfate complex by activated carbon is low and not considered a practical way of recovering gold from thiosulfate solutions, and thus not much research has been done regarding the mechanisms of gold adsorption (Hu and Gong, 1989; Marsden and House, 2006).
The proposed mechanisms by which *P. chrysosporium* biotransforms the surface of carbon are through surface oxidation and plugging or blockage of adsorption pores. The above was confirmed through characterization using such techniques as Raman, Fourier Transform Infrared (FT-IR) and X-ray absorption near edge structure (XANES) spectroscopies, and BET pore volume and size analysis (Chapter 4). Figs. 5.1-5.3 demonstrate that, in like manner to the cyanide system, the graphitic structure of carbon is important for gold adsorption from thiourea and thiosulfate systems (McDougal et al., 1980; Jones et al., 1989; Miller and Sibrell, 1991; Ibrado and Fuerstenau, 1992). The reduction in gold adsorption from the thiourea and thiosulfate media subsequent to fungal treatment can thus be explained on one hand by the destruction in the graphitic structure. On the other hand, reduction in gold adsorption is due to cutback in surface area and pore volume of carbon due to blockage possibly by fungal hyphae or jelly-like substances generated by fungal-interaction with the medium. Despite the fact that free growing fungal hyphae are bigger (>100 nm) than the micropore size, it can be postulated that like all other organisms when constrained, the hyphae may grow and penetrate smaller openings (<100). This requires further investigations.

Though adsorption on as-received carbon is highest in cyanide solution, it can be seen in Fig. 5.4 that the percentage reduction in adsorption after fungal treatment is also higher in cyanide than thiosulfate and thiourea. This observation could be due to the larger ionic sizes of the thiosulfate and thiourea which make it possible for them to adsorb in relatively bigger pores available after the fungal treatment (Nakbanpote et al., 2000; Syna and Valix, 2003; Miur and Allymore, 2004). Another possibility in the case of thiourea, in preference to cyanide, might be the ability to interact with oxygen groups (Arriagada and Gardia, 1997; Nakbanpote et al., 2000).
5.5 Conclusions

This chapter reports, for the first time, preg-robbing of gold thiourea and gold thiosulfate complexes by different grades of carbonaceous matter (CM), and the extent of reduction in preg-robbing in these systems as a result of biotransformation of CM by the fungus, *Phanerochaete chrysosporium*. The adsorption of gold on the as-received CM occurred in the order of cyanide > thiourea > thiosulfate, agreeing with the trend reported in literature. After fungal biotransformation, reduction in gold adsorption was more evident in cyanide (70-97 %) than in thiourea (60-92 %) and thiosulfate (50-91 %) solutions depending on the type of CM. Biotransformation of CM led to reduction in micropore volume and increase in oxygen content all of which militated against uptake of gold cyanide by CM. Comparing the gold concentrates (FC and BFC) to the surrogate anthracite coal, gold uptake and reduction in gold uptake by anthracite were far more than FC and BFC. This observation may be due to the complex nature of the refractory gold ores having 3-4 % organic carbon as against 90 % organic carbon in anthracite. The above notwithstanding, preg-robbing reduction of 50-70 % in refractory gold ores is promising.
References


Chapter 6

Biotransformation of sulfides using the fungus, Phanerochaete chrysosporium: A potential pretreatment process for refractory sulfidic gold ores

Abstract

This study assessed the capability of the fungus, Phanerochaete chrysosporium, to decompose pyrite, arsenopyrite and a sulfide-containing flotation concentrate in an effort to develop a microbial process for pretreating refractory gold ores. The extent of biotransformation was monitored by analyzing for iron, sulfur and arsenic in incubation solutions, and for sulfide sulfur in the residual solids. For arsenopyrite, 1.5 wt%, 7.2 wt% and 10.3 wt% of iron, arsenic and sulfur respectively were present in the incubation solution after 21 days of fungal treatment, whereas for pyrite, there was 1.2 wt% iron and 6.0 wt% sulfur. For the same processing period in the case of the flotation concentrate, 1.8 wt%, 6.1 wt% and 10.7 wt% respectively of iron, arsenic and sulfur remained in solution. Overall, the decomposition of sulfide sulfur in the samples was 15 wt%, 35 wt% and 57 wt% respectively for pyrite, arsenopyrite and the flotation concentrate. Changes in sulfide sulfur concentration and the formation of oxide phases during fungal treatment were confirmed using Raman spectroscopy and X-ray diffraction analysis. These results suggest that P. chrysosporium is a promising microorganism for oxidative decomposition of metal sulfides associated with refractory gold ores.

Keywords: Phanerochaete chrysosporium; Biotransformation; Sulfides; Pyrite; Arsenopyrite; Flotation concentrate, Refractory gold ores
6.1 Introduction

6.1.1 Refractory sulfidic gold ores and bacteria oxidation

In sulfidic refractory gold ores, tiny gold particles typically < 1 µm (Marsden and House, 2006) may be highly disseminated and locked up within the grain boundaries or fractures of sulfide minerals such as pyrite and arsenopyrite. Thus, decomposition of the sulfides is required to liberate the gold (Baako, 1972; Boyle, 1979; Cabri et al., 1989; Komnitsas and Pooley, 1989; Benzaazoua et al, 2007). Commercially, roasting, pressure oxidation and bacteria oxidation are among the processes employed in sulfide oxidation (Bennett and Tributsch, 1978; Brierley, 1978; Arriagada and Osseo-Asare, 1984; Hutchins, 1988; Berezowsky et al., 1988; Yannopoulos, 1991; Nyavor and Egiebor, 1992; Brierley, 1995; Rawlings et al., 2003).

Biooxidation makes use of iron and sulfur chemolithoautotrophic bacteria, mainly Leptospirillum ferrooxidans, Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans to catalyze the oxidation of sulfides, liberating gold for subsequent cyanidation (Lundgren and Silver, 1980; Livesey-Goldblatt et al, 1983; Brierley, 1997; Hackl, 1997; Kelly and Wood, 2000). The bacteria gain energy by oxidizing ferrous iron and elemental sulfur which generate in situ, ferric ions and sulfuric acid, the lixivants responsible for leaching the sulfides (Keller and Murr, 1982; Sand et al., 1995; Rawlings et al., 1999; Rawlings, 2002; Madigan and Martinko, 2006). The main reactions involved in pyrite oxidation can be summarized in Equations 6.1 to 6.5 (Bennett and Tributsch, 1978; Lundgren and Tano, 1978; Sand et al., 2001; Rohwerder et al., 2003; Holmes, and Bonnefoy, 2006; Marsden and House, 2006).

\begin{align}
2\text{FeS}_2 + 7\text{O}_2 + 2\text{H}_2\text{O} &\rightarrow 2\text{Fe}^{2+} + 4\text{SO}_4^{2-} + 4\text{H}^+ \tag{6.1} \\
4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ &\rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O} \tag{6.2} \\
\text{FeS}_2 + 14\text{Fe}^{3+} + 8\text{H}_2\text{O} &\rightarrow 15\text{Fe}^{2+} + 16\text{H}^+ + 2\text{SO}_4^{2-} \tag{6.3} \\
\text{FeS}_2 + 2\text{Fe}^{3+} &\rightarrow 3\text{Fe}^{2+} + 2\text{S}^0 \tag{6.4}
\end{align}
Equation 6.1 is reported to occur much faster in an environment of suitable bacteria whereas 6.2 and 6.5 do not proceed to any appreciable limit under atmospheric conditions and are said to rely entirely on bacterial catalysis (Holmes, and Bonnefoy, 2006; Marsden and House, 2006). Though Equations 6.3 and 6.4 can proceed without microbial involvement, their products, $\text{Fe}^{2+}$ and $S^0$, have to be oxidized by the bacteria to regenerate the lixiviants for ongoing oxidation of pyrite (Bennett and Tributsch, 1978; Lundgren and Tano, 1978; Murr, 1980; Rawlings et al., 2003; Sand et al., 2001; Holmes, and Bonnefoy, 2006; Marsden and House, 2006).

At the end of biooxidation, gold may be totally liberated or associated with relatively more porous iron oxides and thus amenable to cyanidation (Marsden and House, 2006). Unfortunately, because the bacteria are autotrophs they synthesize carbon from carbon dioxide (Silver, 1970; Glazer and Nikaido, 1995; Madigan and Martinko, 2006) and do not use organic carbon. Hence, carbonaceous matter (CM) is not degraded in the case of double refractory gold ores (DRGO). The CM is consequently routed into downstream cyanidation circuits where it adsorbs (preg-robs) dissolved gold. It is therefore imperative to deal with this second part of double refractoriness even after the biooxidation process. Current research efforts have been focused on two-stage processes to oxidize sulfides and deactivate CM (Brierley and Kulpa, 1993; Amankwah et al., 2005; Afidenyo, 2008; Yen et al., 2008). However, using a “one-pot” process that can simultaneously oxidize sulfides and deactivate CM will be of immense benefit in the treatment of DRGO as it will shorten process time, and thus cut down cost.
6.1.2 Fungal-biotransformation of sulfides and carbonaceous matter

The fungus, *Phanerochaete chrysosporium*, secretes the oxidative enzymes, lignin peroxidase and manganese peroxidase (Glenn et al., 1983; Tien and Kirk, 1983; 1988), which are capable of degrading aromatic carbonaceous materials. Ofori-Sarpong et al. (2010a) used the fungus to deactivate anthracite-grade carbonaceous matter and reduce its gold adsorption ability by more than 90%. *P. chrysosporium* also secretes a H$_2$O$_2$-generating enzyme, glyoxal oxidase (Kersten and Kirk, 1987), and hydrogen peroxide is known to solubilize pyrite and arsenopyrite as shown in Equations 6.6 and 6.7 (McKibben and Barnes, 1986; Antonijevic, et al., 1997; Jennings et al., 2000; Antonijevic, et al., 2004; Aydogan, 2006; Aydogan et al., 2007).

\[
2\text{FeS}_2 + 15\text{H}_2\text{O}_2 \rightarrow 2\text{Fe}^{3+} + 4\text{SO}_4^{2-} + 2\text{H}^+ + 14\text{H}_2\text{O} \quad [6.6]
\]

\[
\text{FeAsS} + 7\text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{AsO}_4^{3-} + \text{SO}_4^{2-} + 2\text{H}^+ + 6\text{H}_2\text{O} \quad [6.7]
\]

The iron (III) and sulfuric acid produced constitute the reagents created by the chemolithotrophic bacteria for the indirect biooxidation of sulfides, as discussed in section 6.1.1 above (Brierley, 1978; Hackl, 1997; Keller and Murr, 1982; Sand et al., 1995; 2001; Holmes and Bonnefoy, 2006).

Various species of bacteria and fungi have been employed in bio-desulfurization of coals with sulfur (organic and inorganic) content of up to 6%. Removal of up to 90% of inorganic sulfur and 30% of organic sulfur has been reported (Faison et al., 1991; Acharya et al., 2005; Gonsalvesh et al., 2008). The fungal-decomposition of sulfur has been attributed to secretion of oxidative enzymes (Schreiner et al., 1988; Van Hamme et al., 2003; Aranda et al., 2009), such as those produced by *P. chrysosporium*. This study therefore aims at investigating the ability of the CM-deactivating fungus, *P. chrysosporium*, to also solubilize/oxidize sulfides found in refractory gold ores in order to develop a single-stage process for the pretreatment of double refractory gold ores.
6.2 Experimental investigations

6.2.1 Materials

Fungal spores of *P. chrysosporium* ME446, were obtained from the Ming Tien lab of the Department of Biochemistry and Molecular Biology, Penn State University. Pyrite and arsenopyrite samples were supplied by VWR and Ward’s Natural Science Establishment respectively. The sulfide-containing flotation concentrate (FC) already milled to all passing 75 µm was obtained from the sulfide treatment plant at Bogoso Mine of Golden Star Prestea-Bogoso Resources, Ghana. The growth media for the fungus, millet and wheat bran (MWB) were obtained from Nature’s Pantry, State College, PA, whereas reagent grades succinic acid and sodium hydroxide were obtained from Alfa Aesar.

6.2.2 Medium preparation, incubation and harvesting of treated material

The millet and wheat bran (MWB) medium was prepared by using 8 g millet plus 2 g wheat bran. Double-distilled (dd) H₂O used for the incubation was buffered with succinic acid and the pH adjusted to 4 using sodium hydroxide. The medium, in Erlenmeyer flasks, was moistened with water in the ratio of 1 g medium: 1 mL of dd H₂O, and autoclaved at 121°C for 30 min. On cooling, the medium was inoculated with 1 mL of spore suspension of *P. chrysosporium* (made by suspending 1 vial of spores in 25 mL of dd H₂O). The optical density of the cell suspension, used for inoculation, as measured with a Pharmacia LKB Ultraspec II UV-visible spectrometer at 600 nm was 0.3 relative to water.

Pyrite and arsenopyrite samples were ground to all passing 75 µm, and 10 g each was moistened with 5 mL of water and autoclave-sterilized for 30 min. Sterilized dd H₂O was added to each sample to achieve 30% solids, and the samples were incubated in triplicates with the culture medium at 37 °C, for up 21 days on a New Brunswick Series 25 Incubator shaker at 150 rpm. The potential was monitored throughout the experiments with a VWR
Scientific meter model 3000. Control experiments were also set up for 14 days under similar conditions as described above, except that there was no addition of fungus. This was necessary in order to establish the exclusive effect of the fungus on biotransformation of sulfides. At the end of the incubation period the samples were washed with water in the ratio of about 1 g sample to 200 ml H₂O to get rid of the media and fungal biomass, and then dried at 37 °C for 7 days.

6.2.3 Determination of dissolved components and sulfide sulfur analysis of fungal-treated material

Inductively coupled plasma – atomic emission spectroscopy (ICP-AES) is a technique used routinely for the qualitative and quantitative determination of elements in solution. ICP-AES has also proven to be a quick and invaluable technique for the determination of total dissolved sulfur and has found tremendous application in many fields including soil science and environmental engineering. Soluble sulfur constituents determined by this technique include Na₂SO₄, FeSO₄, CuSO₄, S₂O₃²⁻, SO₃²⁻ and Na₂S (Zhao et al., 1994; Sarudi et al., 1998; Wang et al., 1999; McGuire et al., 2001; Dewil et al., 2006). The liquid samples obtained after fungal-treatment were tested for total dissolved arsenic, iron and sulfur using ICP-AES.

Before determining the sulfide sulfur in both the as-received and processed solids, sulfate sulfur and elemental sulfur, if any, had to be removed. For elemental sulfur (S⁰), samples were digested in the ratio of 2 g solid sample to 25 mL tetrachloroethylene for 60 min in a boiling water bath, after which the sample was filtered. The dried filter cake was then contacted with 3 M HCl in the ratio of 1g solid to 50 mL solution (Ciminelli and Osseo-Asare, 1995; Caldeira et al., 2003; Rait and Aruscavage, 2006). The mixture was heated at 80 °C for 90 min and then filtered. The filter cake was washed with 400 ml of water and dried at 70 °C. Sulfide sulfur in the filter cake was then determined by the volumetric combustion technique using the LECO Sulfur determinater SC-4444DR.
6.2.4 Characterization of flotation concentrate

Both X-ray diffraction and Raman spectroscopy were used to evaluate phase changes in the flotation concentrate before and after treatment with *P. chrysosporium*. Raman scattering is a spectroscopic technique based on inelastic scattering of a laser source monochromatic light, used to diagnose the internal structure of molecules and crystals. Raman spectra was collected using the Confocal WITec XY Raman spectrometer, with a polarized laser light of 488 nm wavelength using a 40X objective lens. The spectra were collected at 5 min interval with 1 s integration time.

For X-ray diffraction (XRD) studies, the samples were ground to fine powder in an agate mortar and then sprinkled on the surface of a quartz zero-background sample holder. The analysis was carried out using PANalytical X’Pert Pro powder diffractometer with X’celerator detector. A Ni-filtered CuKα radiation produced at 45 KV and 40 mA was used for the analysis. The scan was run from 10 to 70 degrees for 2-theta at a scan speed of 2 deg/min. Data acquisition was done using MDI Jade 9 software.

6.2.5 Analysis of data

The extent of biotransformation of sulfides by *P. chrysosporium* was evaluated by analyzing the incubation solution for total dissolved iron, sulfur and arsenic, and by determining sulfide sulfur in the residual solids. Fungal dissolution of constituents (FDC) was estimated in wt% as shown in Equation 6.8, where V is the volume of solution used in fungal incubation, C is the concentration of dissolved constituent as measured by ICP-AES, Pm is the percentage of the constituent in the sulfide material and W is the mass of sulfide material used. Equation 6.9 also depicts the percentage conversion of sulfide sulfur (CoS) in the residual solids, where SI and SF are respective sulfide sulfur contents before and after fungal treatment.
\[
FDC \text{ (wt\%)} = \frac{V \text{ (mL)} \times C\left(\frac{\mu g}{\text{mL}}\right)}{P_{\text{m}} \times \bar{x} \times W} \times 100\%
\]  
[6.8]

\[
\text{CoS} \text{ (wt\%)} = \frac{(\text{SI} - \text{SF}) \text{ (g)}}{\text{SI}} \times 100\%
\]  
[6.9]

All experiments were carried out in triplicate, and the data reported in the figures are mean values, with the error bars representing the standard error (SE) of the means. In Equation 6.10, SD is the sample standard deviation (Equation 6.11) where \( n \) is the sample size, \( x_i \) and \( \bar{x} \) are the \( i \)th value and the mean respectively.

\[
\text{SE}_x = \frac{\text{SD}}{\sqrt{n}}
\]  
[6.10]

\[
\text{SD} = \sqrt{\frac{\sum_{i=1}^{n} x_i - \bar{x}^2}{n - 1}}
\]  
[6.11]

### 6.3 Results and discussion

#### 6.3.1 Transformation of pyrite by *P. chrysosporium*

To discuss the direct effect of *P. chrysosporium* on the biotransformation of sulfides it was necessary to examine the effect of the growth media, and this was done by running control experiments. The results obtained for pyrite samples incubated for two weeks both in the absence and presence of *P. chrysosporium* are illustrated in Fig. 6.1. The figure shows that though there is some amount of dissolution of pyrite in the absence of fungal biomass, the presence of the fungus led to 3-6 fold increase in dissolution of iron and sulfur. The minor solubilization in the control experiment might be due to natural oxidation of sulfides
according to the reaction shown in Equation 6.1. These are, however, slow reactions and cannot lead to any appreciable level of dissolution under atmospheric conditions. In a strong oxidizing environment containing hydrogen peroxide and oxidative enzymes, such as that generated by *P. chrysosporium*, the conversion of ferrous to ferric ion as shown in Equation 6.12 is possible (Brierley, 1978; Keller and Murr, 1982; Sand et al., 1995; Hackl, 1997; Rawlings, 2002; Rohwerder et al., 2003; Marsden and House, 2006).

\[
\text{Fe}^{2+} \rightarrow \text{Fe}^{3+} + e^{-} \quad \text{[6.12]}
\]

![Bar chart showing constituents in solution (% of initial weight) for control and treated samples.](image)

**Fig. 6.1.** Two-week biotransformation of pyrite in the absence and presence of *P. chrysosporium*. The experiment was conducted at 37 °C for 14 days at pH 4 and 30 % solids

The fungal-dissolution of pyrite as a function of time is depicted in Fig. 6.2. After 21 days of fungal treatment there was 1.2 wt% iron and 6.0 wt% sulfur in the incubation solution. It can be seen from the figure that the rate of dissolution of pyrite is higher at the initial stages (up to 7 days) but decreases afterwards. In a batch culture such as the one utilized in this study,
microbial growth and hence activity increases exponentially in the initial stages of growth, and retardation takes place afterwards due to exhaustion of growth media and generation of waste products of metabolism (Shuler and Kargi, 1992). The reduction in microbial activity and availability of oxidants may account for the trend observed.

Fig. 6.2. Accumulation of dissolved sulfur and iron during the incubation of pyrite with *P. chrysosporium*. The experiment was conducted at 37 °C for up to 21 days at pH and 30 % solids

The pH for optimum operation of *P. chrysosporium* is 4 (Tien and Kirk, 1988) and it is at this pH ± 0.3 that the experiment was carried out. It is known that in oxidizing leaching of pyrite above pH of 3 there is production of insoluble iron oxides/hydroxides/sulfates such as Fe$_2$O$_3$, FeOOH and FeSO$_4$, which precipitate on the surface of unreacted material (Osseo-Asare et al., 1984; Marsden and House, 2006). Other components that may be present in the system
include sulfate, sulfite, thiosulfate, and elemental sulfur (Ciminelli, 1987; Ciminelli and Osseo-Asare, 1995; Wei and Osseo-Asare, 1997; Shahverdi et al., 2001; Bhakta and Arthur, 2002; Caldeira et al., 2003). Accumulation of elemental sulfur in the system has the potential to passivate the unreacted surfaces and thus obstruct movement of reactants and products to and from the reaction sites leading to reduction in the overall rate of reaction (Lindstrom et al., 1992; Marsden and House, 2006; Mousavi et al., 2006). Stoichiometrically, the molar ratio of sulfur to iron in pyrite is 2:1. However, this ratio does not tally with the measurements made as the ratio is about 5 moles of sulfur to 1 mole of iron. The deficiency of iron in the solution is likely due to precipitation as explained above. The progressive decomposition of pyrite with time during treatment with the fungus is shown in Fig. 6.3.

Fig. 6.3. Biotransformation of sulfide sulfur in pyrite as a function of fungal-treatment. The experiment was conducted at 37 °C for up to 21 days at pH 4 and 30 % solids

The mass of unconverted pyrite was estimated from the percentage of sulfide sulfur as measured by the LECO technique. From initial sulfide sulfur content of 52 %, there was a
reduction in sulfide sulfur to 43% within 21 days of incubation, representing an oxidation of 15%. About 90% of the conversion was achieved within the initial 10 days of fungal-treatment. The generally low level of oxidation of pure sulfides having initial sulfide sulfur content of about 52% is likely to translate into higher percentage oxidation with refractory sulfdic gold concentrates with lower sulfide sulfur content.

6.3.2 Transformation of arsenopyrite by *P. chrysosporium*

The dissolution of the constituents of arsenopyrite in the absence and presence of the fungus, *P. chrysosporium* is presented in Fig. 6.4. In the absence of the fungus, dissolution of arsenic, iron and sulfur was marginal at 0.9 wt%, 0.16 wt% and 2.6 wt% respectively.

![Fig. 6.4. Two-week biotransformation of arsenopyrite in the absence and presence of *P. chrysosporium*. The experiment was conducted at 37 °C for 14 days at pH 4 and 30 % solids](image-url)
When the sample was incubated with *P. chrysosporium*, dissolution increased to relatively high values of 6.3 wt%, 1.3 wt% and 9.2 wt% for arsenic, iron and sulfur respectively. The minor solubilization in the control experiment might be due to natural oxidation of sulfides according to the reactions shown in Equation 6.13 (Marsden and House, 2006). In a strong oxidizing environment the conversion of arsenite to arsenate is possible as shown in Equation 6.14. The progressive increments of iron, sulfur and arsenic in incubation solution during the fungal-treatment of arsenopyrite over different incubation periods are presented in Fig. 6.5.

\[
4\text{FeAsS} + 11\text{O}_2 + 6\text{H}_2\text{O} \rightarrow 4\text{Fe}^{2+} + 4\text{SO}_4^{2-} + 12\text{H}^+ + 4\text{AsO}_3^{3-} \quad [6.13]
\]

\[
2\text{H}_3\text{AsO}_3 + \text{O}_2 \rightarrow 2\text{H}_2\text{AsO}_4^- \quad [6.14]
\]

**Fig. 6.5.** Dissolution of iron, sulfur and arsenic from arsenopyrite as a function of incubation time. The experiment was conducted at 37 °C for up to 21 days at pH 4 and 30 % solids.
Over a period of 21 days, the concentrations of iron, sulfur and arsenic in solution increased consistently ending at 1.5 wt%, 7.2 wt% and 10.3 wt% respectively. The trends in Fig. 6.5 indicate that the concentrations of the constituents in solution are yet to reach their respective plateaus, and the processing time for maximum degradation extends beyond 21 days. The stoichiometric ratio of iron, arsenic and sulfur in 1 mole of arsenopyrite is 1:1:1. This ratio changed based on the amount of these constituents in incubation solution as the ratio in solution was about 0.14 mol of Fe, 0.7 mol of As and 1 mol of sulfur after 21 days of treatment. This implies a deficiency of 0.86 mol of Fe and 0.3 mol of As in solution, due to precipitation.

According to the Eh-pH diagram for Fe-As-S-H_2O system in Fig. 6.6, at pH 4 as was used in this study, the formation of Fe^{2+} and FeOOH is possible above Eh of 0.3 V (SHE). Goethite (FeOOH) exists as the most dominant solid species of iron (Osseo-Asare et al., 1984). In the present study, measured potentials were in the range 0.4 to 0.5 V (SHE) indicating the possibility of goethite precipitation which may partly explain the very low values of total iron (0.9 wt%) in solution.

In addition, precipitation of ferric arsenate at pH above 2 has been observed, with concomitant decrease in reaction rate (Escobar et al., 2000; Brierley, 2003; Lindstrom et al., 2003; Marsden and House, 2006). Overall the conversion of sulfide sulfur in arsenopyrite as determined by the LECO combustion technique was 35 wt%.

The higher overall decomposition of arsenopyrite as compared with 15% in the case of pyrite is because pyrite is more noble than arsenopyrite and the As-S bond is weaker than the Fe-S bond (Marsden and House, 2006). Microbial attack is naturally initiated from weaker zones in a material (Wackett and Ellis, 1999).
6.3.3 Transformation of flotation (sulfidic gold) concentrates by *P. chrysosporium*

Figure 6.7 presents the direct dissolution of iron, sulfur and arsenic from sulfidic gold concentrates by *P. chrysosporium* after 14-day contact with MWB medium with and without fungal biomass. As explained in section 6.3.2, the slight dissolution obtained in the control experiment may be due to natural dissolution of sulfides in the presence of water and oxygen, which may cease in a limited oxidizing environment. However, in the presence of the fungus and the associated oxidizing environment generated, about 2-5 fold increase in dissolution was observed. Figure 6.8 depicts the dissolution of sulfides from FC by *P. chrysosporium* as a function of incubation time in weight percent (Equation 6.9).
Fig. 6.7. Two-week biotransformation of flotation concentrate in the absence and presence of \textit{P. chrysosporium}. The experiment was conducted at 37 °C for 14 days at pH 4 and 30 % solids.

The dissolution of sulfide begins after the initiation of incubation and rises rapidly within the first week. Though the rate of dissolution declines after a week of incubation, Fig. 6.8 shows a consistently increasing dissolution as the incubation period increases. This signifies that given more time for incubation, dissolution will continue.
Fig. 6.8. Dissolution of sulfides in flotation concentrate by *P. chrysosporium* as a function of time for 21 days. The experiment was conducted at 37 °C for up to 21 days at pH 4 and 30 % solids.

Figure 6.9 presents the transformation of sulfide sulfur (Equation 6.10) in the residual solid materials after incubation. The left vertical axis represents the actual mass of sulfide sulfur in the flotation concentrate, and the mass was estimated from sulfide sulfur determined via the LECO combustion technique. From an initial mass of about 5 g of sulfide sulfur in the flotation concentrate, there was 48 % reduction within the initial 10 days of processing. Beyond this period, biotransformation stalled leading to a further 9% decrease bringing the total reduction in sulfide sulfur to 57% after 21 days.
Fig. 6.9. Biotransformation of sulfide sulfur in the flotation concentrate (FC) as a function of incubation time. The experiment was conducted at 37 °C for up to 21 days at pH 4 and 30 % solids.

6.3.4 Characterization of flotation concentrate

Many metal sulfides show Raman peaks between 300 and 400 wavelength (Ushioda, 1972; Mernagh and Trudu, 1993; Sasaki et al., 1995; Edwards et al., 1999) and this was confirmed in the current investigation. Raman spectroscopy of the as-received and treated flotation concentrate indicated a reduction in the intensity of sulfide peaks after fungal-biotransformation as presented in Fig. 6.10. These observations authenticate the decrease in sulfide sulfur as determined by the LECO combustion technique. Further analysis by XRD shows other products of sulfide sulfur oxidation (Fig. 6.11).
By comparing the as-received and fungal-treated samples in Fig. 6.11, it was observed that the treated material had two new oxygen containing phases; hematite (Fe$_2$O$_3$) and ferric arsenate (FeAsO$_4$.H$_2$O). In similar systems, ferric arsenate has been observed earlier by researchers such as Escobar et al. (2000), Brierley (2003) and Lindstrom et al. (2003). Though according to Fig. 6.6 goethite (FeOOH) is generally expected to be the dominant phase, it was not detected in the present study. It is likely that other oxides were precipitated but due to lower resolutions, the peaks might have been masked by the higher intensity peaks. Also, the diffractometer used for the study, has a detection limit of 5 % and thus any phase with less than 5 wt% composition may not show a peak on the diffractogram.
Fig. 6.11. X-ray diffractogram of as-received and fungal-treated flotation concentrate. Treatment was conducted for 14 days at 37 °C and pH 4

The presence of the oxidation products indicates clearly that *P. chrysosporium* has the ability to transform sulfides in refractory gold ores. These results compare well with those obtained by Brierley, (2003) in the biooxidation of refractory gold ores of about 2 % sulfide-sulfur content at 20-60 °C for 10 days using mesophilic and moderate-thermophilic bacteria, and hyper-thermophilic archea. The author achieved over 50 % increase in the subsequent gold recovery by cyanidation. In a related study, gold recovery increased appreciably following fungal treatment (Ofori-Sarpong et al., 2010b).
6.4 Conclusions

The fungus, *Phanerochaete chrysosporium*, which has proven its ability to reduce the preg-robbing capacity of anthracite-grade carbonaceous matter, was investigated for its ability to also transform sulfides, in an effort to develop a microbial process for pretreating refractory gold ores. The results show that *P. chrysosporium* is capable of solubilizing sulfides. For an incubation period of 21 days, sulfide sulfur decreased by 35 % in the treated arsenopyrite sample and 15 % in the pyrite sample. These materials had initial sulfide sulfur content of 19.6 and 52 % respectively. For refractory sulfidic gold concentrate with about 15 % initial sulfide sulfur content, a higher oxidation level of 57 % was realized. Changes in sulfide sulfur concentration and the formation of oxide phases during fungal treatment were confirmed using Raman spectroscopy and X-ray diffraction analysis. For all the samples, there was a strong correlation between the decomposition of sulfides and incubation period. These results indicate that the fungus, *P. chrysosporium*, is a potential microorganism for oxidative decomposition of metal sulfides associated with refractory gold ores.
References


Chapter 7

Biotransformation of double refractory gold ores by the fungus, Phanerochaete chrysosporium

Abstract

Double refractory gold ores (DRGO) contain organic carbonaceous matter (CM) and metal sulfides, and thus require pretreatment before cyanidation for gold extraction. Though there have been several studies into microbial pretreatment of DRGO, available literature indicates that microbial degradation of both sulfides and CM in a single-stage process has not achieved much success. In an on-going research, the fungus Phanerochaete chrysosporium, has been used to effectively reduce the gold adsorption (preg-robbing) nature of CM on one hand, and decompose sulfides on the other.

The present research reports for the first time; the ability of P. chrysosporium to simultaneously decompose sulfides in refractory gold ores, liberating locked-up gold, and deactivating carbonaceous matter, thus reducing preg-robbing. Flotation concentrate (FC) of DRGO and bacterial-oxidized FC (BFC) were utilized in this investigation. Sulfur speciation of the biotreated solids indicated progressive decomposition of pyritic sulfur with time, and incubation solution showed increase in dissolved iron, sulfur and arsenic. Cyanidation of fungal-treated concentrates yielded an increase in recovery of 37 % and 13 % for FC and BFC respectively over the as-received concentrates. The results demonstrate a potential alternative pretreatment process for DRGO.

Keywords: Phanerochaete chrysosporium; Double refractory gold ores; Carbonaceous matter; Flotation concentrate; sulfidic minerals; Bacteria; biooxidation; Fungi; Biotransformation; Cyanidation; Gold recovery.
7.1 Introduction

Gold ores are termed double refractory when they contain organic carbonaceous matter (CM) and sulfide minerals (Nyavor and Egiebor, 1992; Amankwah et al., 2005). Sulfide minerals encapsulate fine gold particles and prevent direct contact between gold particles and leaching reagents, thus interfering with gold dissolution, whereas CM adsorbs dissolved aurocyanide complexes, thereby reducing gold extraction - a phenomenon known as preg-robbing (Osseo-Asare et al., 1984a; Hausen and Bucknam, 1985; Schmitz et al., 2001).

For gold locked up in double refractory ore (DRGO) to be amenable to cyanidation, pretreatment is required to decompose the shielding sulfidic minerals and in consequence liberate the gold. Processes such as roasting, pressure oxidation and bacterial oxidation have been used commercially to pretreat refractory gold ores (Bennett and Tributsch, 1978; Arriagada and Osseo-Asare, 1984; Hutchins, 1988; Weir and Berezowsky, 1986; Berezowsky et al., 1988; Afenya, 1991; Brierley, 1995; Rawlings et al., 2003). Roasting involves the use of high temperature (450-700 °C) to decompose sulfidic and carbonaceous materials in the presence of oxygen/air. The combustion reaction leads to generation of harmful gases such as sulfur dioxide and arsenic trioxide, which cause environmental problems (Komnitsas and Pooley, 1989; Marsden and House, 2006). Pressure oxidation makes use of high temperature and pressure, typically 180-225 °C and 1500-3200 kPa, to decompose metal sulfides in the presence of water. Though this process eliminates the harmful gaseous products, it is associated with operational difficulties such as safety due to high operating temperatures and pressures, higher rates of material corrosion, and higher equipment cost (Weir and Berezowsky, 1986; Marsden and House, 2006). In view of the high cost and environmental concerns associated with the above processes, bacteria oxidation has become a method of choice (Brierley, 1997; Marsden and House, 2006).

For the past two decades, the use of biooxidation, which can be seen as the biological counterpart of pressure oxidation, has become more prominent in part to curtail high cost and also to improve upon gold recovery and environmental/safety aspects of processing (Livesey-
Goldblatt et al., 1983; Komnitsas and Pooley, 1989; Yannopoulos, 1991; Hackl, 1997; Rawlings, 1998). According to Brierley (1995), biooxidation can generally reduce capital costs of roasting by 12-20%, operating costs by 10% in some cases, and construction time by 25% (Brierley, 1997). Several bacteria are known to oxidize sulfides but the ones commonly used in commercial biooxidation of refractory gold ores are *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* (Lundgren and Silver, 1980; Livesey-Goldblatt et al., 1983; Brierley, 1997; Hackl, 1997; Kelly and Wood, 2000; Holmes and Bonnefoy, 2006). Unfortunately, because the bacteria are autotrophs, they synthesize carbon from carbon dioxide and do not use organic carbon, and hence, the CM is not degraded in the case of DRGO (Rohwerder et al., 2003; Madigan and Martinko, 2006).

Apart from roasting that aims at eliminating the carbon, passivation has been employed using carbon-blanking agents such as kerosene and other organic reagents (Abotsi and Osseo-Asare, 1987; Hutchins et al., 1988; Adams and Burger, 1998). Though yet to be applied commercially, biomodification of carbon appears more attractive from economical and environmental points of view, as it occurs at relatively low temperatures (25-40 °C) and at atmospheric pressure. In addition, lixiviants are regenerated *in situ* due to the presence of the microorganisms. Microorganisms employed in this manner include the bacteria; *Pseudomonas sp.*, *Achromobacter sp.*, *Streptomyces setonii*, and the fungi; *Aspergillus bruneio*, *Penicillium citrinum*, *Trametes versicolor* and *Phanerochaete chrysosporium* (Portier, 1991; Brierley and Kulpa, 1992; 1993; Amankwah and Yen, 2006; Afidenyo, 2008; Yen et al., 2008; Ofori-Sarpong et al., 2010a).

Available literature indicates that microbial degradation of both sulfides and CM in a single-stage has not yet been realized with great success (Brierley and Kulpa, 1993; Amankwah and Yen, 2006; Afidenyo, 2008; Portier, 1991; Yen et al., 2008). The limitation to single-stage processes has been attributed to the inability of the heterotrophic carbon deactivating microorganisms, which work at relatively higher pH values (4-8), to coexist and function effectively at the optimum conditions within which the acidophilic (pH: 1-2) sulfide oxidizing bacteria work (Tortora et al., 2004; Amankwah et al., 2005; Madigan and Martinko, 2006).
In on-going studies, the white rot lignin-degrading fungus, *Phanerochaete chrysosporium*, which secretes oxidative enzymes, has been utilized to deactivate anthracite-grade carbonaceous matter and reduce gold preg-robbing by more than 90% (Ofori-Sarpong et al., 2010a). In a related study using pyrite, arsenopyrite and flotation concentrate of DRGO, *P. chrysosporium* was able to decompose the sulfides progressively over time. For a processing time of 21 days, conversions of sulfides in pyrite, arsenopyrite and flotation concentrate respectively were 15 wt%, 35 wt% and 57 wt% (Ofori-Sarpong et al., 2010b). *P. chrysosporium* has also been used to degrade gold-bearing wood chips to liberate gold for cyanide leaching, and the authors observed a 10% increase beyond the original 60% cyanidation gold recovery of untreated wood chips (Martin and Petersen, 2001). This study investigated the simultaneous carbon passivating and sulfur oxidizing ability of *P. chrysosporium* in a single stage process using double-refractory gold ores.

### 7.2 Experimental investigations

#### 7.2.1 Materials, medium preparation and incubation

Gold-containing samples, *P. chrysosporium* and the millet and wheat bran (MWB) medium were obtained and incubated as presented in Chapter 6, Section 6.2 and Ofori-Sarpong et al. (2010b). Analysis of the incubation solution for dissolved iron, arsenic and sulfur, and sulfide sulfur determination in residual solids were conducted as detailed in the same chapter. Sigma flux used for fire assaying of gold was obtained from Sigma Chemicals.
7.2.2 Cyanidation

Cyanidation was conducted at 25 °C on 25 g of as-received and fungal treated materials at 30% pulp density and 10 g/L cyanide concentration. The pH was maintained between 11 and 12 by the addition of sodium hydroxide. The samples were agitated on a New Brunswick Series 25 incubator shaker at 150 rpm for 24 hours in partially covered Erlenmeyer flasks to allow oxygen into the system as required by the fundamental equation for cyanidation (Equation 7.1).

\[
4\text{Au} + 8\text{CN}^- + 2\text{H}_2\text{O} + \text{O}_2 \rightarrow 4\text{Au}^\text{CN}_\text{2}^- + 4\text{OH}^- \quad [7.1]
\]

At the end of the leaching period the pulp was filtered, and the solution obtained was analyzed for gold by ICP-AES whereas the residual solids (tails) were assayed for residual gold by fire assaying.

7.2.3 Fire Assaying

The gold in solid samples was determined by fire assaying with atomic absorption spectrometric finish. In this method, a known mass of gold-containing sample is mixed with three times its mass of Sigma flux made up of lead oxide, sodium tetraborate, wheat flour and sodium carbonate. The mixture was heated to 1100 °C in a fireclay crucible and kept at the temperature for 45 min. During the process, lead oxide is reduced by the carbon-containing flour and the lead produced collects the precious metal to form the lead button. The other fluxes react with unwanted materials in the gold ore to form a slag that separates from the lead on pouring, due to its lower density. The lead was hammered into a rough cube and cupelled at 950 °C on a shallow dish made of magnesium oxide, known as a cupel. During cupellation lead is absorbed by the cupel, leaving the precious metal bead, which is digested with aqua-regia and the gold determined by ICP-AES.
7.2.4 Analysis of data

After incubating the concentrates with and without *P. chrysosporium*, the solutions were filtered for determination of arsenic, iron and sulfur. Fungal dissolution of constituents (FDC) and percentage conversion of sulfide sulfur (CoS) were calculated as defined in Chapter 6, section 6.2.

The cyanidation test was conducted to evaluate the extent of liberation of gold, and thus cyanide amenability following fungal-transformation of FC and BFC. Equation 7.2 was used in calculating the percentage gold recovery (PGR), where W is the mass of gold concentrate and V is the volume of water used in the cyanidation experiment, C is the concentration of gold in the leachate after cyanidation as determined by ICP-AES whereas HG is the head grade of the gold concentrate before cyanidation as determined by conventional fire assaying.

\[
PGR(\text{wt}%) = \frac{1}{W} (g^{-1})xV(mL)xC(\frac{\mu g/mL}{mL}) \cdot \frac{HG(g/t)}{HG(g/t)} \times 100\%
\]

[7.2]

7.3 Results and discussion

The double refractory gold ores mined by GSPBR contain about 2% sulfur and this is lower than about 10% sulfur required for biooxidation. For this reason, the ore is ground and froth flotation is used to concentrate sulfides which serve as feed for biooxidation. The biooxidation process does not destroy carbonaceous matter and it continues to adsorb gold in the subsequent cyanidation step. Consequently, it was necessary to subject both FC and BFC to fungal treatment to ascertain the ability of the fungus to decompose sulfides and deactivate carbonaceous matter. BFC was selected for testing to also allow comparison between bacteria-treated and fungi-treated concentrates.
7.3.1 Fungal-biotransformation of sulfides in flotation concentrate (FC)

The concentration of dissolved iron, arsenic and sulfur in incubation solution was determined, presented and discussed in Chapter 6 under Figs. 6.7 and 6.8. Changes in the concentration of sulfide sulfur is an important parameter in the biotransformation of sulfide minerals, and the transformation of pyritic sulfur in the residual solids as a result of fungal-treatment of FC is depicted in Fig. 7.1.

![Graph showing biotransformation of sulfide sulfur](image)

**Fig. 7.1.** Biotransformation of sulfide sulfur in the flotation concentrate (FC) as a function of incubation time. The experiment was conducted at 37 °C for up to 21 days at pH 4 and 30 % solids

It can be seen from the figure that the sulfide sulfur content of the residual solids reduced from an initial value of 15 % of FC to about 6 %, representing a percentage decomposition of 57 %. About 48 % decomposition occurred within the initial 10 days of fungal-treatment as
shown in Fig 7.1, after which the decomposition rate reduced leading to additional 9% decomposition within the next 11 days. As explained in Chapter 6, precipitation of products such as goethite, ferrous sulfate, ferric arsenate and elemental sulfur on the surface of unreacted material can slow down the reaction (Osseo-Asare et al., 1984b; Ciminelli and Osseo-Asare, 1995; Wei and Osseo-Asare, 1997; Shahverdi et al., 2001; Bhakta and Arthur, 2002; Caldeira et al., 2003; Marsden and House, 2006).

7.3.2 Fungal-biotransformation of sulfides in bacterial-treated FC (BFC)

The effect of *P. chrysosporium* on the biotransformation of BFC is presented in Fig 7.2. The assessment of the direct effect became possible by running control experiments with the growth media alone, under similar conditions as those used in the fungal experiment. It is clear from the figure that there was a sharp increase in decomposition of the major constituents in BFC by 3-5 folds in the presence of the fungus. Analysis of the residual solids revealed an overall oxidation of about 85% sulfide sulfur within 14 days of fungal-treatment. The results obtained after biotreating both FC and BFC demonstrate the potential of *P. chrysosporium* to oxidize sulfides in DRGO and thus liberate gold for cyanidation. The fungal-decomposition of sulfides in BFC allowed for extra liberation of gold beyond the bacterial oxidation of FC, as discussed in Section 7.3.3.
Fig. 7.2. Two-week fungal-treatment of BFC in the absence and presence of *P. chrysosporium*. The experiment was conducted at 37 °C for 14 days at pH 4 and 30 % solids.

### 7.3.3 Effect of fungal-treatment on cyanide amenability of FC and BFC

The recoveries of gold from FC and BFC are presented in Fig. 7.3 for 14-day control and fungal-treated materials. The figure shows an increase in gold extraction as a result of fungal-treatment. The recoveries of gold from the respective control experiments of samples FC and BFC were 47 % and 84 %, whereas after treatment with *P. chrysosporium*, gold recovery increased to 75 % and 93.5 % correspondingly. Changes in cyanide-amenability of sample FC as a function of fungal treatment time is presented in Fig. 7.4.
Fig. 7.3. Gold recovery from fungal-treated and control FC and BFC. Cyanidation was conducted at 30% pulp density for 24 h at pH 11 and cyanide strength of 10 g/L.

Fig. 7.4. The effect of fungal treatment time on gold extraction from Sample FC. Cyanidation was conducted at 30% pulp density for 24 h at pH 11 and cyanide strength of 10 g/L.
After 24-h cyanidation, the percentage of gold extracted from the as-received flotation concentrate was 40%. This value increased to 67% within 7 days of incubation, and there was progressive improvement over time to 78% at the 21st day of fungal-treatment. The sequence in Fig. 7.4 shows that extraction has not yet reached a plateau and it is possible to extract some more gold if fungal treatment time is extended. This is expressed in the correlation between fungal-decomposition of sulfide sulfur in FC over time, and gold recovery by cyanidation (Fig. 7.5).

![Graph showing variation of sulfur transformation and gold extraction as a function of fungal-treatment time.](image)

**Fig. 7.5.** Variation of sulfur transformation and gold extraction as a function of fungal-treatment time. Cyanidation was conducted at 30% pulp density for 24 h at pH 11 and cyanide strength of 10 g/L.

The trends of both sulfide-sulfur conversion and gold extraction show increments, and consequently, it is expected that with increase in fungal-treatment time more sulfides will be decomposed, more gold will be liberated for cyanidation, and gold extraction will increase. The limitation in the action of the fungus on the flotation concentrate to achieve higher gold
recovery values could be due to the amount of refractory materials present in excess of the oxidizing enzymes secreted by the fungus, thus leading to incomplete oxidation (Tien and Kirk, 1983; 1988). With the same amount of gold concentrate, growth media and fungus, treatment of BFC (Fig. 7.2) realized over 3 times percentage decomposition of sulfide as compared to FC (Fig. 6.7 in Chapter 6). This disparity is due to the difference in initial sulfide content; 3 % in BFC as against 15 % in FC, making the available oxidizer to sulfide ratio higher in the case of BFC. Changes in cyanide-amenability of sample BFC as a function of fungal treatment time is presented in Fig. 7.6.

Fig 7.6. Gold extraction from BFC as a function of fungal incubation time. Cyanidation was conducted at 30 % pulp density for 24 h at pH 11 and cyanide strength of 10 g/L.

In a manner similar to gold extraction from the flotation concentrate, recovery from sample BFC also increased after fungal treatment (Fig. 7.6). From an initial recovery of 81 %, gold extraction increased to 94 % within 10 days of incubation, beyond which the extraction stagnated up to 21 days of treatment. This is because sulfide decomposition peaked at 85 % within 14 days of fungal-treatment of BFC. In addition, the high levels of dissolved gold concentrations in solution (17 mg/L) achieved during cyanidation prevented more gold from being dissolved from the solids (Marsden and House, 2006).
7.4 Assessment of fungal- and bacterial- treatment of gold concentrates: Single-stage against two-stage processes

To evaluate the effectiveness of the fungal process as a potential single-stage pretreatment method for double refractory gold ores, it was necessary to compare the extent of gold extraction from as-received flotation concentrate (FC), fungal-treated FC (FFC), as-received bacterial-treated FC (BFC) and fungal-treated BFC (FBFC). The comparison is visually accessible in Fig. 7.7, which shows increase in gold extraction in the order of FC (40 %) < FFC (78 %) < BFC (81 %) < FBFC (94 %).

![Gold recovery comparison](image)

**Gold recovery (%)**

**Gold concentrates:**
FC-flotation concentrate; FFC-fungal-treated FC; BFC-bacterial-treated FC; FBFC-fungal-treated BFC

BFC and FFC are from single stage processes while FBFC is from a two-stage process. The gold recovery from FBFC was 13 % and 16 % above BFC and FFC respectively. The average gold recoveries from FC after fungal-treatment alone (FFC), and after commercial bacterial-
treatment process alone (BFC), compare favorably with respective values of 78 % and 81 % (Fig 7.7). The closeness of the two values highlights the potential of *P. chrysosporium* in the biotransformation of refractory gold ores.

*P. chrysosporium* has an added advantage of reducing gold adsorption, and Fig. 7.8 shows the decomposition of sulfides, reduction in gold adsorption following deactivation of carbonaceous matter (CM), and associated increase in gold extraction after fungal treatment of double refractory gold ores. From Fig 7.8, a reduction in sulfide sulfur and gold adsorption by CM which were 57 % and 64 % respectively, translated into an increase in gold extraction of 37 %.

![Graph showing changes in sulfide sulfur content, gold adsorption, and cyanidation gold recovery from fungal treatment.](image)

**Fig. 7.8.** Changes in sulfide sulfur content, gold adsorption by carbonaceous matter and gold extraction from FC following 14-day fungal treatment. Cyanidation was conducted at 30 % pulp density for 24 h at pH 11 and cyanide strength of 10 g/L.

In an independent but related study, Yen et al. (2008) utilized the carbon deactivating fungus, *Trametes versicolor*, bacterium, *Streptomyces setonii*, the sulfide oxidizing bacteria *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, and abiotic processes to treat different types of double refractory gold ores. The
authors used different combinations of the microorganisms mentioned to treat the ores in two stages, and reported increase in overall gold recoveries. To compare with the findings being reported in this study, their recoveries for FC, FFC, BFC and FBFC (as defined above) were in the order of 15-22% for FC; 54-65% for FFC; 71-81% for BFC and 87-92% for FBFC. A first-stage fungal process from the various two-stage combinations by Yen et al. (2008) can be viewed as equivalent to the single-stage fungal process (FFC) utilized in the current study. Comparing the two studies therefore, it can be concluded that for fungal treatment alone (single-stage) this study achieved a higher overall recovery (78%), which is much higher than the (54-65%) achieved by Yen et al. (2008), an overall increase of 20-44% in favor of the present study.

The ability of *P. chrysosporium* to reduce the gold adsorption activity of carbonaceous matter (CM) in addition to decomposing sulfides suggests that the recovery of FFC should technically outweigh that of BFC, since the bacteria treatment does not deactivate CM. Notwithstanding the above, the fungal-process is at its infant stage of just about 3-year laboratory batch research. On the other hand, the bacteria process has undergone over 4 decades of research spanning from laboratory batch/continuous through pilot to commercial scale. It therefore stands to reason that the fungal-process has yet to overcome teething problems, such as optimization of kinetics and quantification of biomodifiers and materials balance. When the experiments are conducted in a continuous system instead of the batch units used, the processes could be faster due to continuous feeding of reactants and removal of products.

### 7.5 Conclusions

This research examined the capability of *Phanerochaete chrysosporium* to simultaneously biotransform sulfides and carbonaceous matter in double refractory gold ores (DRGO). It is comprehensible from the investigations that *P. chrysosporium* has the ability to solubilize and oxidize sulfide minerals and deactivate carbonaceous matter in DRGO. The results show that
at pH 4, substantial sulfide dissolution takes place in the presence of the fungus leading to relatively high solution concentrations of iron, sulfur and arsenic with overall sulfide sulfur decomposition of 57 % and 75 % of FC and BFC after 21 days of fungal-treatment. Within the same period, gold extraction increased appreciably from 40 % to 78 % for sample FC, and 81 % to 94 % for sample BFC, signifying increase in recovery of 38 % and 13 % over the as-received FC and BFC respectively. For sample FC which has residual sulfide sulfur content of about 6 % after 21-day fungal treatment, there is a great potential for more sulfides to dissolve leading to further increase in gold extraction. The single stage fungal-treatment of FC which led to an overall recovery of 78 % compares well with the recovery (81 %) of the bacterial-treated FC. Given the infant stage of the fungal process, the closeness of its gold recovery value to that of the commercial bacterial process highlights the potential of *P. chrysosporium* in the biotransformation of refractory gold ores.
References


Chapter 8

In vitro transformation of carbonaceous matter and sulfides in refractory gold ores using cell-free extracts of Phanerochaete chrysosporium

Abstract

This chapter reports the findings of preliminary investigations aimed at assessing the use of hydrogen peroxide and cell-free extracts from the fungus, Phanerochaete chrysosporium, to effect biotransformation of sulfides and carbonaceous matter (CM) in double refractory gold ores (DRGO) to increase gold extraction. The cell-free components were extracted from already grown fungal culture by filtering through cheesecloth and fiberglass. After 72 hrs of treatment with the cell-free extracts (in vitro), total dissolved iron and sulfur from pyrite stood at 2.6 wt% and 3.7 wt% respectively. For the same period, total dissolved arsenic, iron and sulfur in solution were up to 5.2 wt%, 0.9 wt% and 6.0 wt% respectively from flotation concentrate. Analysis for sulfide sulfur in the residual solids of the gold concentrate indicated about 25 wt% oxidation within 24 hours of cell-free treatment. Gold adsorption capacity of anthracite (used as surrogate for CM) reduced by 30 % after the cell-free treatment for 24 hr. Using cyanide-leaching, gold recovery increased by 24 % after 24-hr treatment with the cell-free extracts as against 15% for treatment with 2 mM hydrogen peroxide alone. The in vitro treatment had a slight advantage over treatment with whole cells of the fungus (in vivo) with gold recovery of 66 % as against 62 % after 72 hr-treatment in vivo. In general, cell-free decomposition of the samples did not increase beyond 24 hours of contact time, possibly due to exhaustion of the oxidative components of the extracts. These initial results indicate clearly that in vitro processing is a promising alternative to in vivo processing of DRGO using P. chrysosporium.

Keywords: Phanerochaete chrysosporium; Refractory gold ores; Flotation concentrate; Pyrite; Anthracite; Fungi; Cell-free extracts; Enzymes; Hydrogen peroxide; Biotransformation; Gold adsorption; Cyanidation; Gold recovery; In vitro; In vivo.
8.1 Introduction

Double refractory gold ores (DRGO) contain sulfide minerals and carbonaceous matter (CM) which require pretreatment before cyanidation to decompose sulfide minerals and liberate encapsulated gold particles, and deactivate CM to prevent gold uptake during cyanidation (Osseo-Asare et al., 1984a; Hausen and Bucknam, 1985; Hutchins, 1988; Weir and Berezowsky, 1986; Afenya, 1991). Biological pretreatment processes are gaining acceptance on the bases of cost, safety and environmental issues (Livesey-Goldblatt et al, 1983; Komnitsas and Pooley, 1989; Brierley, 1997; Hackl, 1997; Rawlings et al., 2003). Several bacteria and fungi have been studied to assess their ability to decompose sulfides and/or deactivate carbonaceous matter in refractory gold ores (Lundgren and Silver, 1980; Portier, 1991; Brierley and Kulpa, 1992; Amankwah et al., 2005; Afidenyo, 2008; Yen et al., 2008).

By utilizing whole cells of *P. chrysosporium*, the white rot lignin-degrading fungus demonstrated its ability to deactivate anthracite-grade coal used as a model for CM in refractory gold ores. The fungal-treatment led to reduction in the gold adsorption capacity of anthracite by more than 90 % (Chapter 3; Ofori-Sarpong et al., 2010a). When the fungus was used in the treatment of refractory gold concentrates for up to 2 weeks, gold adsorption reduced by 64-70 % (Chapter 5). In a related study using pyrite, arsenopyrite and flotation concentrate (FC), *P. chrysosporium* was able to decompose the sulfides progressively over time. Conversions of sulfides in pyrite, arsenopyrite and FC respectively were 15 wt%, 35 wt% and 57 wt% after 21 days of fungal treatment (Chapter 6; Ofori-Sarpong et al., 2010b). Subsequent cyanidation of the treated material led to an increase in gold recovery of 38 % over the untreated material, bringing the overall recovery to 78 % (Chapter 7).

*Phanerochaete chrysosporium* produces the enzymes lignin peroxidase (LiP), manganese peroxidase (MnP), and hydrogen peroxide, which are known to be responsible for the degradation of a host of materials including carbon- and sulfur-containing substrates (Glenn et
al., 1983; Tien and Kirk, 1983; Kersten and Kirk, 1987; Schreiner et al., 1988; Gold and Alic, 1993; Ralph and Catcheside, 1997; Martin and Petersen, 2001). As explained by Tien and Kirk (1984; 1988), these cell-free components can be extracted and utilized in vitro (absence of fungus) for biotransformation (Couto et al., 2006). It was therefore necessary to investigate the possibility of using the fungus as a ‘factory’ to produce the oxidative enzymes, and extracting the enzymes for use in biotransformation, instead of the biomass. Also, as detailed in Chapter 3, Fig 3.2 and Ofori-Sarpong et al. (2010), the biomass of the fungus is fluffy. Because of this, it is a daunting task to separate the biomass from ore particles after processing due to entanglement. Consequently, the use of cell-free extracts may offset this separation difficulty and avoid the possibility of materials loss during washing. In addition, the use of cell-free components has the potential to facilitate better understanding of the process chemistry and kinetics.

In this study, crude enzymes were extracted from already grown *P. chrysosporium*, and the potency of the cell-free extracts in deactivating carbon and decomposing sulfides in DRGO was examined. The degree of in vitro transformation of the materials was determined by investigating the gold adsorption ability of CM, reduction in sulfide sulfur content of sulfidic materials and cyanide amenability of the gold concentrates. A processing time comparison between in vitro and in vivo treatments was also made.

### 8.2 Experimental investigations

#### 8.2.1 Materials

Gold-containing samples, pyrite, anthracite grade coal, *P. chrysosporium* and the millet and wheat bran (MWB) medium were obtained as presented in Chapters 3 and 6. Reagent grades of dextrose powder, ammonium tartrate, succinic acid, thiamin, manganese sulfate, sodium tartrate, hydrogen peroxide, hydrochloric acid, sodium hydroxide and sodium cyanide were supplied by VWR. Cheesecloth and fiber glass filter were also supplied by VWR.
8.2.2 Preparation of cultures and extraction of cell-free components

Glucose broth (liquid) medium was prepared with dextrose powder (carbon source) and ammonium tartrate (nitrogen source) as described by Tien and Kirk (1988). Succinic acid was used to buffer the solution, and the pH adjusted to 4.0 with hydrochloric acid or sodium hydroxide. The medium without thiamin (protein source) was autoclaved for 30 minutes and cooled down before addition of thiamin, which is heat-sensitive. The medium was inoculated with 1 vial of spore suspension of *P. chrysosporium* per 1000 mL of medium. The inoculated medium was distributed as 10 mL solution into 125 mL Erlenmeyer flasks, and allowed to incubate for 14 days under stationary culturing at 37 °C. Oxygen was introduced into the cultures on the first day of incubation and every three days as described by Tien and Kirk (1988). Development of a brown coloration on the mycelia signifies appearance of ligninase activity (formation of enzyme) in the extracellular fluid (Tien and Kirk, 1988). By the 14th day about 75% of the incubation flasks had the brown coloration and so the extract was harvested on day 14. At the end of the incubation period, the cell-free liquid (supernatant) was extracted from the culture by filtration through quadruple-layered cheesecloth and fiber glass. The cell-free extract (crude enzymes) was buffered with sodium tartrate to pH of 4 and used immediately.

8.2.3 Preparation of reagents for the treatment of materials

Aside from using the cell-free extracts, it was necessary to assess the use of hydrogen peroxide alone in the treatment of the substrates. This is because *P. chrysosporium* produces hydrogen peroxide, in addition to secreting oxidative enzymes. In the catalytic action of the fungus, hydrogen peroxide is required to activate the enzymes, lignin peroxidase (LiP) and manganese peroxidase (MnP) (Glenn et al., 1983; Tien and Kirk, 1984; Kersten and Kirk, 1987), and this is detailed in Chapter 3 and Ofori-Sarpong et al. (2010a). Hydrogen peroxide is produced in the range of 2-30 µM by the fungus (Tonon and Odier, 1988) depending on the amount of enzyme secreted (Kelly and Reddy, 1986). In abiotic sulfide dissolution however,
higher concentrations of 1-5 M have been used (McKibben and Barnes, 1986; Antonijevic, et al., 1997; Jennings et al., 2000; Aydogan, 2006). In this research, 2 mM of hydrogen peroxide which is about a hundred times that secreted by the microbes, and far less than that utilized in abiotic processes was used to assess the extent to which hydrogen peroxide can transform sulfides and carbonaceous matter. The concentration utilized in this study, thus mimics a microbial system with excessive hydrogen peroxide production. In addition, MnP requires Mn(II) ions in its oxidative cycle as detailed in Andrawis et al. (1988), Banci et al. (1992) and Chapter 2, and concentrations in the range of 0.1-20 mM have been used (Gold and Alic, 1993). Overall, concentration used in this chapter was 100 µM prepared from manganese sulfate solution. The solutions were buffered using 20 mM sodium tartrate at pH 4 to ensure high enzymatic activity (Tien and Kirk, 1988). The substrates (anthracite, pyrite, gold concentrate) were treated with the hydrogen peroxide and the cell-free fungal extracts at 15 % solids and pH 4 for up to 72 hrs at an agitation speed of 150 rpm on a New Brunswick Series 25 Incubator shaker. After treatment, the treated material was filtered and the residue dried at 37 °C for 7 days.

8.2.4 Post-incubation experiment

Total dissolved arsenic, iron and sulfur in the incubation solution was determined using inductively coupled plasma – atomic emission spectroscopy (ICP-AES), whereas sulfide sulfur in the residual solids was determined using LECO volumetric combustion technique as described in Chapter 6 and Ofori-Sarpong et al. (2010b). Gold adsorption experiments were also conducted as described in Chapter 3 and Ofori-Sarpong et al. (2010a), whilst cyanidation and fire assaying of the residual solids were conducted as detailed in Chapter 7. Experiments used for the treatment of the substrates were carried out in duplicates, and the data are reported as mean values.
8.2.5 Surface area and porosity analysis

Surface area and pore analysis was conducted using Micromeritics Gas adsorption Analyzer ASAP 2020 by high pressure nitrogen adsorption. In this method, nitrogen is admitted to a solid material in controlled increments, and after each adsorptive dose the pressure is allowed to equilibrate and the quantity of gas adsorbed calculated. The gas volume adsorbed at each pressure (at constant temperature) defines an adsorption isotherm, from which the quantity of gas required to form a monolayer over the surface of the solid and its pores is determined. The area covered by each adsorbed gas molecule is used to estimate the surface area whereas the isotherms of adsorption and desorption are used to estimate the pore size, pore volume and area of the material (Van Krevelen, 1993).

8.2.6 Transmission electron microscopy (TEM)

The structural features of the as-received and treated anthracite were examined using the JEOL 2010 LaB6 transmission electron microscope (TEM). The sample was dispersed on a Holey carbon-coated TEM support grid and placed on a sample holder in the sample chamber of the TEM. TEM is a technique employed by irradiating a thin specimen with a high-energy electron beam typically 100-200 keV. It can assess the structural features of materials in the nanometer range (Van Krevelen, 1993). The electron beam is focused by a number of magnetic lenses situated before and after the position of the specimen being analysed. These help in controlling illumination, magnification and focusing of the specimen and the image formed. As the beam interacts with the specimen, an image is formed and recorded by a digital CCD camera.
8.3 Results and discussion

8.3.1 Effect of cell-free extracts processing on gold adsorption behavior and surface modification of anthracite

The amount of gold adsorbed on anthracite treated variously, is depicted in Fig. 8.1. The diagram shows reduced gold adsorption of 13 wt% and 30 wt% respectively for hydrogen peroxide and the cell-free extracts. *P. chrysosporium* secretes both peroxide and oxidative enzymes, and thus the higher level of biotransformation observed with the cell-free extracts may be attributed to the combined effects of hydrogen peroxide and the enzymes. The 30% reduction in gold uptake is very low, compared to 65-85% achieved when whole cells of the fungus was used in treating anthracite for the same period (Chapter 4).

Fig. 8.1. A comparison of the gold adsorption behavior of treated anthracite (15 % solids, 37 °C, 24 hr, pH 4)
BET surface area, micropore area and micropore volume of the as-received anthracite were 4.07, 2.84 and $1.51 \times 10^{-3}$ cm$^3$/g. After treatment with the cell-free extracts, these values reduced by 82 %, 85 % and 86 % respectively as shown in Fig. 8.2. The reduction in pore volume (86 %) and surface area (82 %) after enzyme treatment compares well with the whole cell-treated carbon, which registered 81 % and 78 % respectively (Chapter 4). Though the crude enzyme-treated carbon showed slightly higher reduction in pore volume and surface area, these did not translate into higher reduction in gold up-take.

Fig. 8.2. Surface area and porosity analysis of anthracite samples before and after treatment using whole cell and cell-free extracts of *P. chrysosporium* (15 % solids, 37 °C, 24 hr, pH 4)

Further analysis of the structural nature of anthracite using TEM indicated a more amorphous surface following both the whole cell and cell-free treatments as compared to the as-received anthracite (Fig. 8.3). The figure shows varying shades of grey with the lighter sections...
indicating less dense material and the darker sections, denser material. Figure 8.3a shows a well-ordered structure of as-received anthracite and this structure is similar to that obtained by Pappano and Schobert (2009) for studies on graphitizability of a Pennsylvania anthracite. The amorphous nature shown in Figs 8.3b and 8.3c, coupled with the extensive reduction in surface area (Fig. 8.2), is due to surface oxidation (Chapter 4) and surface coating with organic fungal metabolites (Glazer and Nikaido, 1995), which leads to blinding of adsorption sites (Osseo-Asare et al., 1984a; Abotsi and Osseo-Asare, 1987; Adams and Burger, 1998). Gold cyanide adsorption is known to be more favorable on structured carbon due to interaction of the gold central ion with pi electrons of the graphitic planes (Jones et al., 1989; Klauber et al., 1991; Ibrado and Fuerstenau, 1992).

Fig. 8.3a. TEM analysis of as-received anthracite samples showing a very structured surface
Fig. 8.3b. TEM analysis of anthracite samples after treatment using whole cells of *P. chrysosporium* showing relatively more amorphous surface (37 °C, pH 4, 60 % solids, 7 days)

Fig. 8.3c. TEM analysis of anthracite samples after treatment using cell-free extracts of *P. chrysosporium* showing very amorphous surface (37 °C, pH 4, 15 % solids, 3 days)

Similar adsorption levels of the aurocyanide ion was expected on both *in vivo* and *in vitro* treated carbons as the carbon structures were similar and quantitative changes in pore volume...
and surface area were equally high. However, the \textit{in vitro} adsorbed more gold. The much higher reduction in gold adsorption after \textit{in vivo} treatment indicates that the presence of the fungus contributes extra components that enhance deactivation of the active sites on carbon (Chapter 4). Since the results of the cell-free (\textit{in vitro}) treatment are only preliminary, further investigations are required to confirm the trends observed.

\textbf{8.3.2 Effect of cell-free extracts and hydrogen peroxide processing on biotransformation of sulfides}

The results obtained for pyrite constituents in solution after being treated for up to 72 hr in the cell-free extracts is presented in Fig 8.4 whilst comparison of 24-hr treatment in hydrogen peroxide alone, and in extracts is illustrated in Fig. 8.5. It is clear from Fig. 8.4 that the concentration of constituents in solution increased beyond 4 hr of treatment until 24 hr, after which there was no further dissolution. However, the reaction was not complete as analysis of the residual solids for sulfide sulfur indicated only about 10 wt% oxidation. The observed trends can thus be due to depletion of oxidative enzymes in the solution after 24 hr of treatment. Compared with Fig. 8.1 on gold adsorption, Fig. 8.5 shows a similar trend of the extracts giving better results than the hydrogen peroxide alone. Hydrogen peroxide produced by the fungus is in the range of 2-30 \(\mu\)M depending on the amount of enzyme secreted (Kelly and Reddy, 1986; Tonon and Odier, 1988). In this research, a much higher concentration of 2 mM was used to establish the effect a 100-fold increase in hydrogen peroxide concentration. Yet, even at that concentration, the presence of the enzymes gave a more pronounced effect. This is because the extract contains the oxidative enzymes and hydrogen peroxide both of which possess oxidizing ability.
Fig. 8.4. *In vitro* dissolution of constituents during pyrite transformation as a function of time (15% solids, 37 °C, up to 72 hr)

Fig. 8.5. Comparison of constituents in solution after pyrite treatment with H$_2$O$_2$ and fungal extract (15% solids, 37 °C for 24 hr)
Stoichiometrically, the molar ratio of sulfur to iron in pyrite is 2:1. However, this ratio does not tally with the observed solution concentrations in this study as the ratio is about 3 mol of sulfur to 1 mol of iron. The deficiency of iron in the treatment solution is due to production of insoluble iron oxides/hydroxides/sulfates such as Fe₂O₃, FeOOH and FeSO₄ which precipitate above pH of 2 (Osseo-Asare et al., 1984b; Ciminelli and Osseo-Asare, 1995; Shahverdi et al., 2001; Bhakta and Arthur, 2002; Caldeira et al., 2003; Marsden and House, 2006; Chapter 6).

8.3.3 Biotransformation of flotation concentrate by cell-free extracts

The extent of decomposition of flotation concentrate (FC) using cell-free extracts was assessed partly by analyzing for constituents in solution, and for sulfide sulfur in the residual solids after treatment. The corresponding results are presented in Figs. 8.6 and 8.7. Figure 8.6 shows an increase in dissolution of arsenic, iron and sulfur between the processing periods of 4 and 24 hr. Not much dissolution occurred after 24 hr and this is due to depletion of oxidizing components in solution.

In agreement with the trends observed for the constituents in solution (Fig. 8.6), sulfide sulfur analysis of the residual solids (Fig. 8.7) indicated an increase in decomposition from a value of 17 wt% within 4 hours of treatment to 25 wt% in 24 hr. Only 2 % extra decomposition was observed between 24 and 72 hr bringing the final value to 27 wt%. The two major possibilities for reduction in decomposition rate are exhaustion of oxidizing reagents and formation of precipitates at the pH of 4 utilized in the process. The precipitates account for the difference between the higher value (27 %) of overall decomposition of sulfide sulfur as compared to the lower value of only 6 wt% total sulfur present in the treatment solution (Fig. 8.6).
Fig. 8.6. *In vitro* dissolution of constituents during transformation of flotation concentrate as a function of time (15 % solids, 37 °C, up to 72 hr)

![Dissolved constituents graph](image)

Fig. 8.7. Decomposition of sulfide sulfur in flotation concentrate with fungal extracts (15 % solids, 37 °C, up to 72 hr)

![Sulfide sulfur converted graph](image)
8.3.4 Cyanidation of flotation concentrate

The aim of using *P. chrysosporium* to biotransform double refractory gold ores is to reduce uptake of gold by carbonaceous matter and to decompose sulfides to liberate locked up gold for dissolution. Ultimately therefore, gold recovery is expected to increase after the treatment. In the previous chapter (Chapter 7), *in vivo* treatment using whole cells of the fungus led to an increase in gold recovery of 38 % over that of the as-received flotation gold concentrate. Comparison of cyanide leaching of as-received and FC treated with H$_2$O$_2$, and cell-free extracts of *P. chrysosporium* is depicted in Fig. 8.8.

![Bar chart](image)

**Fig. 8.8.** Cyanide amenability of flotation concentrate (as-received) and after 24-hr treatment with H$_2$O$_2$, and cell-free extracts of *P. chrysosporium*. Both samples were processed at 15 % solids and 37 ºC at pH 4.
Following the common trend observed, the fungal extract gave better results (64 %) than treatment with hydrogen peroxide alone (55 %) within 24 hrs. Though both hydrogen peroxide and the enzymes generated by the fungus possess oxidizing ability, the combined effect as exhibited in the performance of the cell-free extracts is better.

The oxidation of pyrite and arsenopyrite with hydrogen peroxide is well established and the reactions are illustrated in Equations 8.1 and 8.2 (McKibben and Barnes, 1986; Antonijevic, et al., 1997; Jennings et al., 2000; Aydogan, 2006).

\[
2\text{FeS}_4 + 15\text{H}_2\text{O}_2 \rightarrow 2\text{Fe}^{3+} + 4\text{SO}_4^{2-} + 2\text{H}^+ + 14\text{H}_2\text{O} \quad [8.1]
\]
\[
\text{FeAsS} + 7\text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{AsO}_4^{3-} + \text{SO}_4^{2-} + 2\text{H}^+ + 6\text{H}_2\text{O} \quad [8.2]
\]

The oxidative enzymes, LiP and MnP, secreted by \textit{P. chrysosporium} are so called because they require peroxide to get activated for the oxidative action on substrates. The fungus therefore produces hydrogen peroxide for this process (Glenn et al., 1983; Tien and Kirk, 1983; Bumpus and Aust, 1986; Kersten and Kirk, 1987; Gold and Alic, 1993; Ralph and Catcheside, 1997; Chapter 3). However, since hydrogen peroxide is a strong oxidant it will, apart from activating the enzymes, complement the oxidative action. This in consequence explains the higher levels of decomposition and gold recovery obtained with the experiments in which the fungal extracts were used.

The comparison of FC treated with whole cell and cell-free extracts is presented in Fig. 8.9. The diagram shows that after 72 hr of treating the flotation concentrate with whole cells and cell-free extracts of \textit{P. chrysosporium}, cyanidation led to gold recoveries of 62 % and 66 % respectively, giving the cell-free solution a slight edge over the whole cells. For a better understanding of the kinetics, systematic quantification and purification of the extracts are required to obtain the pure enzyme which may then be used in estimating the actual amount of
enzyme required to decompose a given amount of sulfides and carbonaceous matter in gold ores.

Fig. 8.9. Cyanide amenability of flotation concentrate (as-received) and after treatment with whole cell and cell-free extracts of \textit{P. chrysosporium} (15\% solids, 37 \degree C, 72 hr).

\textbf{8.4 Proposed mechanism of biotransformation of sulfides by \textit{P. chrysosporium}}

The enzymes of \textit{P. chrysosporium} are heme-containing glycoproteins possessing one ferric heme per molecule of enzyme (Tien and Kirk, 1988) and can be represented as P[Fe(III)]. The mechanism of lignin degradation using the enzyme, lignin peroxidase, is established and has been used to predict the degradation of a host of other aromatic substrates, including coals and carbonaceous matter (CM) in gold ores (Andrawis et al., 1988; Banci et al., 1992; Edwards et al., 1993; Gold and Alic, 1993; Fakoussa and Hofrichter, 1999; Ofori-Sarpong et al., 2010a). The mechanism of sulfide decomposition in the bacteria oxidation system has been discussed extensively by several researchers (Bennett and Tributsch, 1978; Lundgren and Tano, 1978; Sand et al., 2001; Rohwerder et al., 2003; Holmes, and Bonnefoy, 2006; Marsden and House,
2006; Ofori-Sarpong et al., 2010b). The ideas of fungal decomposition of CM and bacteria oxidation of sulfides are adopted in predicting possible reactions in the fungal decomposition of inorganic sulfides.

The initiation of enzymatic attack on substrates is triggered by activation of the enzyme via reduction of hydrogen peroxide to water as shown in Equation 8.3. Having donated two electrons, the enzyme forms a two-electron oxidized intermediate called compound I. Reduction of Compound I back to the native enzyme state involves two steps of receiving one electron at a time, with compound II as the one-electron oxidized intermediate (Equations 8.4 and 8.5), where SC represents sulfide or carbon used as substrate for degradation. The oxidative degradation of pyrite is thus proposed as represented in Equations 8.6 and 8.7.

\[
P[\text{Fe(III)}] \text{(native enzyme)} + H_2O_2 \rightarrow P[O=\text{Fe(IV)}^{**}] \text{(Comp I)} + H_2O \quad [8.3]
\]

\[
P[O=\text{Fe(IV)}^{**}]\text{(Comp I)} + SC \rightarrow P[O=\text{Fe(IV)}] \text{(Comp II)} + SC^{**} \quad [8.4]
\]

\[
P[O=\text{Fe(IV)}] \text{(Comp II)} + SC + 2H^+ \rightarrow P[\text{Fe(III)}] \text{(native enzyme)} + SC^{**} + H_2O \quad [8.5]
\]

\[
2P[O=\text{Fe(IV)}^{**}] + \text{FeS}_2 \rightarrow 2P[O=\text{Fe(IV)}] + \text{Fe}^{2+} + 2S^0 \quad [8.6]
\]

\[
2P[O=\text{Fe(IV)}] + \text{FeS}_2 + 4H^+ \rightarrow 2P[\text{Fe(III)}] + \text{Fe}^{2+} + 2S^0 + 2H_2O \quad [8.7]
\]

Being a catalyst, the enzyme is not consumed, and the overall reaction which is an addition of Equations 8.3, 8.6 and 8.7 is given by Equation 8.8, and thus the enzyme is recycled.

\[
2\text{FeS}_2 + 2H_2O_2 + 4H^+ \rightarrow 2\text{Fe}^{2+} + 4S^0 + 4H_2O \quad [8.8]
\]

Using ferrous ion in place of pyrite as the substrate, Equations 8.9-8.11 can be written in like manner as Equations 8.6-8.8. Compound II may also react with elemental sulfur as shown in Equation 8.12. The reactions in Equations 8.9-8.12 lead to the production of ferric ions and sulfate, and these are responsible for indirect decomposition of pyrites in the biooxidation system.
P[O-Fe(IV)]^+] + Fe^{2+} \rightarrow P[O-Fe(IV)] + Fe^{3+} \quad [8.9]

P[O-Fe(IV)] + Fe^{2+} + 2H^+ \rightarrow P[Fe(III)] + Fe^{3+} + H_2O \quad [8.10]

H_2O_2 + 2Fe^{2+} + 2H^+ \rightarrow 2Fe^{3+} + 2H_2O \quad [8.11]

3P[O-Fe(IV)] + S^0 + H_2O \rightarrow 3P[Fe(III)] + SO_4^{2-} + 2H^+ \quad [8.12]

The hydrogen peroxide reaction with pyrite also leads to the formation of Fe^{3+} and SO_4^{2-} (McKibben and Barnes, 1986; Antonijevic, et al., 1997; Jennings et al., 2000; Aydogan, 2006). Non-enzymatic reactions that can occur in the fungal degradation system may therefore include Equations 8.13 – 8.16. All these would contribute to the oxidative decomposition of pyrite, the dissolved constituents in solution and the solid products reported in this study.

Fe^{2+} + SO_4^{2-} \rightarrow FeSO_4 \quad [8.13]

FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2S^0 \quad [8.14]

2FeS + 7O_2 + 2H_2O \rightarrow 2Fe^{2+} + 4SO_4^{2-} + 4H \quad [8.15]

2Fe^{3+} + H^+ + O_2 \rightarrow FeOOH \quad [8.16]

### 8.5 Conclusions

In this chapter, the cell-free extracts from the fungus, *Phanerochaete chrysosporium*, were examined for their ability to biotransform sulfides and carbonaceous matter in double refractory gold ores (DRGO) *in vitro*. The conditions of treatment included up to 72 hr of contact time under agitation at 150 rpm, at 15 % solids, and pH of 4. Within 24 hr of treatment, total dissolved iron and sulfur from pyrite stood at 2.6 wt% and 3.7 wt% respectively, whereas arsenic, iron and sulfur in incubation solution were up to 5.2 wt%, 0.9 wt% and 6.0 wt% respectively from flotation concentrate. Analysis of sulfide sulfur in the residual solids of the gold concentrate indicated about 25 wt% oxidation within 24 hr of
treatment. Within the same treatment period, gold recovery by cyanide-leaching was 64 %, an increase of 24 % over the as-received material.

Comparing treatment by the cell-free extracts with that by hydrogen peroxide alone, gold recovery was 9 % more for the extract-treated (64 %) as the hydrogen peroxide-treated yielded 55 % within 24 hr of treatment. Comparison with whole cell treatment gives a slight advantage in favor of the cell-free treatment, which recorded gold recovery of 66 % after 72 hr of treatment as against 62 % for the whole cell treatment. These preliminary findings indicate clearly that in vitro processing is a promising alternative to in vivo or whole cell processing of DRGO using P. chrysosporium.
References


Chapter 9
General conclusions, recommendations and processing proposal

9.1 General Conclusions

‘Mycohydrometallurgy’ has been adopted in this research to define the application of fungi in hydrometallurgy. The ability of the white rot basidiomycetous fungus, Phanerochaete chrysosporium, to biotransform sulfides and carbonaceous matter has been studied. Carbonaceous matter (CM) was tested for reduction in gold adsorption ability while sulfides were evaluated to ascertain the oxidation capabilities of the fungus. The biotransforming action of the fungus was assessed on various ranks of coal (lignite, subbituminous, bituminous and anthracite) that served as surrogates for CM, and also on pyrite and arsenopyrite, major metal sulfides associated with gold ores. Actual gold concentrates; flotation concentrate (FC) and bacteria-treated flotation concentrate (BFC) containing sulfides and CM were utilized to confirm the fungal action on the surrogates.

For the various coals utilized, reduction in gold adsorption capabilities of the surrogates was used to estimate declining preg-robbing ability. Various culture media used under different incubation conditions affirmed that P. chrysosporium could grow on all the coal ranks. The results, however, showed that of the coal samples tested; only anthracite coal exhibited significant gold adsorption ability which was used as a means of analyzing the extent of biotransformation. Fungal treatment resulted in reduction in gold adsorption ability of anthracite by more than 95% in a mixture of millet and wheat bran medium.

Fungal treatment with P. chrysosporium was found to take place in a wide range of processing time, pulp density, temperature, pH and level of agitation. After contact with the fungus, gold adsorption ability of anthracite reduced by about 90-97 %, depending on the incubation
conditions. A pH of 4 and temperature of 37 °C were found to enhance biotransformation. Stationary culturing permitted the use of higher pulp densities and the best for stationary and shake culturing respectively were 60 % and 25 % solids.

When tested in different complexing systems, the adsorption of gold on the as-received CM occurred in the order of cyanide > thiourea > thiosulfate. After fungal biotransformation, average reduction in gold adsorption was more evident in cyanide (70-97 %) than in thiourea (60-92 %) and thiosulfate (50-91 %) depending on the type of CM. In addition, there was a reduction of 50-70 % in preg-robbing activity of the gold concentrates, FC and BFC.

Aside from deactivating the active sites on CM, P. chrysosporium showed the capability to decompose sulfide minerals. For an incubation period of 21 days, sulfide sulfur decreased by 35 % in treated arsenopyrite and 15 % in pyrite. These materials had initial sulfide sulfur content of 19.6 % and 52 % respectively. For refractory sulfidic gold concentrate with about 15 % initial sulfide sulfur content, a higher oxidation level of 57 % was realized. For all the samples, there was a strong correlation between the decomposition of sulfides and incubation period.

When gold concentrates were contacted with the fungus, the investigations showed that P. chrysosporium could simultaneously biotransform sulfides and CM in double refractory gold ores (DRGO). The results showed that at pH 4, substantial sulfide dissolution took place in the presence of the fungus leading to relatively high solution concentrations of iron, sulfur and arsenic. Overall, sulfide sulfur decomposition of 57 % from FC and 75 % from BFC was realized after 21 days of fungal-treatment. Within the same period, gold extraction increased appreciably from 40 % to 78 % for sample FC, and from 81 % to 94 % for sample BFC. This signifies increase in recovery of 38 % and 13 % over the as-received FC and BFC respectively. The single stage fungal-treatment of FC which led to an overall recovery of 78 % compares very well with the recovery (81 %) of the bacterial-treated FC.
The investigations also revealed that both the whole cells of *P. chrysosporium* and cell-free extracts were capable of transforming both surrogates and gold concentrates leading to deactivation of CM, decomposition of sulfides and increase in gold extraction. The cell-free extracts exhibited a slight advantage over the whole cell processing with 4% increase in gold recovery over a similar processing period.

During fungal degradation of sulfide minerals, changes in sulfide sulfur concentration and the formation of oxide phases were confirmed using Raman spectroscopy and X-ray diffraction analysis. For carbonaceous matter, Raman, infrared and XANES spectroscopies showed that fungal treatment led to reduction in carbon, introduction of oxygen containing groups such as carboxylic and carbonyls. Further investigations showed that BET surface area and micro pore volume reduced by 76% and 80% respectively whereas average pore diameter increased from 5.75 nm to 9.48 nm. TEM studies revealed an increase in the amorphous nature of the surface of carbon after microbial action. The characterization results suggest that *P. chrysosporium* biotransforms anthracite in two main ways. In one way, there is surface oxidation by introduction of oxygen groups which disrupts the continuous graphitic structure thus decreasing the active sites necessary for adsorption. The second route is reduction in surface area via plugging of pores possibly by hyphae or biofilms produced through fungal interaction with the substrate. These led to reduced accessibility of gold to the adsorption sites.

**9.2 Recommendations**

The following recommendations are made based on experimental data and visual observations made during the investigations. In the present study, the reduction in gold adsorption on CM was due mainly to inaccessibility of the aurocyanide to the micropores. Further characterization studies, probably using XRD mapping, should be conducted to verify the cause of this blockage.
The fungus *P. chrysosporium* has the potential to grow and form a matrix that entangles ore particles making separation difficult. It is recommended that a counter-current decantation technique be investigated to aid washing and complete separation of fungal biomass from ore particles. The entanglement caused by fungal biomass may be avoided when *in vitro* biotransformation is adopted. Thus the study on *in vitro* treatment should be pursued further so cell-free extracts instead of whole cells are employed. Quantification and purification of the extracts to obtain the pure enzyme will help in estimating the actual amount of enzyme required to decompose a given amount of sulfides and carbonaceous matter in gold ores.

The data obtained for sulfide processing indicated that further dissolution of sulfur is possible when processing time is extended beyond 21 days. Further dissolution will promote release of encapsulated gold and increase metal recovery. It is recommended that further experiments be conducted to allow complete oxidation of sulfides and also at a faster rate.

*P. chrysosporium* is known to function best at a relatively low acidic pH of 4. This pH poses a challenge regarding the ability of elemental sulfur and ferric arsenate to precipitate on the unreacted sulfide particles leading to reduction in reaction and problems with downstream gold recovery processes. To address this problem, it is suggested that *P. chrysosporium* be adapted to function well at lower pH. If this becomes possible the precipitation reactions may be reduced. In addition, *P. chrysosporium* may be tested in a consortium with the acidophilic sulfide oxidizing bacteria to investigate a possible synergistic degradation within a shorter processing time.

In the cyanidation experiments using BFC, recoveries did not rise beyond 94% due in part to the very high solution gold concentration which prevented more gold from being dissolved from the solids. For such high values, a carbon-in-leach (CIL) system, where activated carbon is introduced into the system for simultaneous leaching and gold adsorption may be better. Further studies should be conducted using the CIL system to increase gold extraction.
All the experiments using *P. chrysosporium* were conducted under batch conditions. It is recommended that these reactions be conducted under continuous/semi-continuous conditions to ascertain the implications on processing time. By using the continuous system, a scale-up operation may be conducted to monitor how a pilot plant would perform.

### 9.3 Processing proposal

The results of the biodegradation of both sulfides and carbonaceous matter (CM) in a single stage by the fungus *P. chrysosporium* constitute a novel technical process for microbial pretreatment of refractory gold ores. In its application, flotation concentrate (FC) containing sulfides and CM may be subjected to fungal treatment in stirred tanks and also in bioheaps as the fungus has proven to work well under both stationary and shake conditions.

In bioheap application, the FC may be loaded onto support rocks of size between 10 and 20 mm in an agglomerator using fungal culture as wetting agent. The coated support rocks may be stacked onto already prepared impervious pads where fungal culture is sprinkled for the microorganisms to deactivate CM and decompose sulfides (Fig 9.1). The support rocks will allow easy aeration which will enhance the process as the fungus is an aerobe. At the end of the processing period, the biooxidized concentrate may be removed by a hydraulic system after which washing and solid-liquid separation is performed in counter-current decantation thickeners. The thickened slurry may then be conditioned, and gold in the solid residue leached using the well-known process of cyanidation.

In its application in a continuously stirred tank reactor (CSTR), slurry of FC may be conditioned with growth media and mixed with *P. chrysosporium*. With agitation and sparging of air, the microorganism will biotransform sulfides and CM, and release gold for subsequent cyanidation. After fungal pretreatment, solid-liquid separation may be performed in counter-current decantation thickeners and the thickened slurry conditioned for cyanidation as shown in Fig 9.2. In the investigations, *in vitro* studies showed comparable results to those
of whole cell treatment. Hence in its application, the fungus may be grown, the oxidative enzymes extracted and fed into the CSTRs to be utilized in biotransformation.

Fig. 9.1. Proposed flow sheet for bioheap leaching of flotation concentrate using *P. chrysosporium*
The operating pH was deduced from this study to be 4.0. To reduce energy consumption, temperature may be maintained at ambient conditions. The processing time may, however, be confirmed from pilot studies. The technical advantages of this proposal compared with the current biooxidation process are that the pretreatment process leads to reduction in preg-robbing behavior of the ore and an increase in gold recovery during cyanidation. *P. chrysosporium* has a biosafety of one and is therefore neither pathogenic nor harmful to humans, which makes the process environmentally compatible.
VITA
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