Recovery Of Impregnated Gold From Waste Mine Timber Through Biological Degradation

W. Marais

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Recovery Of Impregnated Gold From Waste Mine Timber Through Biological Degradation

By

W. Martin

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Supervisor: Prof. FW Petersen

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DECLARATION

I hereby certify that this dissertation is my own original work, except where specifically acknowledged in the text. Neither the present dissertation nor any part thereof has previously been submitted at any other university or technikon.

W. Martin

-- September 2000

ABSTRACT

The large quantities of wood chips produced at mines from damaged underground timber contain gold that cannot be completely recovered by cyanidation. A fungus that can degrade a portion of the wood matrix will allow the gold that was previously locked up, to come into contact with the cyanide solution during beneficiation, thereby improving recoveries. The fungus Phanerochaete chrysosporium produces enzymes that use the organic compounds found in lignin as substrate. Consequently, the fungus is able to selectively break down lignin, which is one of the major components of wood.

Chips sampled from Vaal Reef Mine contained between 2 and 5 mg/kg gold. The main source of gold in the chips was determined to be impregnated gold-bearing ore and discrete gold particles. Direct cyanidation resulted in around 60 per cent recovery prior to biological treatment. Despite relatively high weight losses caused to the chips as a result of treatment with Phanerochaete chrysosporium gold recovery only increased 10 per cent after 4 weeks treatment compared to direct recovery without treatment.

Keywords

Wood chips; gold; cyanidation; *Phanerochaete chrysosporium*

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INTRODUCTION 1

Mines annually produce tons of refractory wood resulting from blasted-out timber packs and props. The ore together with any blasted-out timber is hauled to the surface and transferred to crushers and the milling circuit where it is further reduced in size. The now small pieces of waste timber are referred to as wood chips and unless removed will block pipelines, blind screens between adsorption tanks and take up space in the elution columns intended for carbon.

The chips and other trash material are removed with screens installed at strategic points in the circuit. The chips are easiest removed when the pulp is dilute and the first screens are installed between the milling plant and thickening stage with additional screens installed before the leaching stage and prior to the carbon-in-pulp plant. The refractory chips contain gold and are consequently stockpiled for future recycling. The gold in the chips originate from ore and discrete gold particles blasted, crushed and milled into the wood fibres and gold adsorbed from the pregnant liquor. Today, with the tremendous pressure on profit margins in the gold-mining industry, which is mining steadily declining grades at ever greater depths it becomes necessary to consider recovering the gold in the chip piles. Unfortunately, direct cyanidation yield unsatisfactory recoveries since the particles are physically blasted, crushed and pounded into the complex wood matrix. Roasting of refractory chips is carried out on a large scale, but not justified by the large capital outlay and operating costs involved. This study focuses on an environmentally friendly and potentially inexpensive method to biodegrade the chips with a fungal microorganism and cause a collapse of the wood matrix. Degrading the wood matrix can facilitate better contact of encapsulated gold-bearing ore and discrete gold particles with a cyanide solution and yield a higher gold recovery.

1.1 Modern Ore Processing And Gold Recovery

Most gold ore is processed, with the collected gold smelted to over 75 per cent purity at or near the mine site. While processing plants differ in their needs, the basic design includes crushing, grinding, leaching, adsorption, elution and electrowinning. Below follows a discussion of a typical processing and recovery plant:

1.1.1 Crushing and Transportation

Ore hauled to the surface is crushed in a primary crusher so that the fragments are suitable for transportation and milling. A rubber belted conveyor transports ore and waste to the mill and waste disposal area. A large electromagnet is located at the beginning of the conveyor to remove any steel debris from the old workings.

1.1.2 Grinding

Ore is stockpiled at the mill, and then in a 24-hour operation it is fed into the processing plant. Firstly, the ore is loaded into a continuous feed semi-autogenous grinding (SAG) mill along with lime, water and steel grinding balls. The result is a fine slurry. The mill is so named because the rock itself does some of the crushing. The material discharges from this mill and is size classified by a primary hydrocyclone. Large particles are returned to the SAG mill for further grinding. The finer particles are pumped to a set of smaller hydrocyclones. These classify the material to give a final product of 80 per cent smaller than 53 microns. The larger particles from these hydrocyclones are fed to a ball mill for further grinding. The material is now directed to a screen where trash and tramp oversize is removed. Trash screen undersize flows by gravity to a grinding thickener feed tank. Raw water is added to the grinding thickener feed tank to reduce the soluble chloride content in the thickener underflow. Thickener underflow is pumped to pre-oxidation tanks. The thickener underflow has a high solid content, usually more than 50 per cent.

A portion of the grinding thickener overflow may be recycled to the grinding circuit for mill water use and SAG mill feed dilution. Grinding thickener overflow can also be used as dilution water in the neutralisation circuit.

1.1.3 Pre-Oxidation and Autoclave Circuit

Grinding thickener underflow can be pumped into pre-oxidation tanks. Some acidic slurry from the autoclave flash tank is recycled back to the pre-oxidation tanks to remove contained carbon dioxide in the ore in the form of carbonates, and to control the overall sulphide content by recycling solids that have already been oxidised. Discharge from the pre-oxidation tanks is delivered to the autoclave circuit. Gaseous oxygen is sparged into the autoclave. High-pressure quench water is also injected to control the operating temperature at around 200°C throughout the length of the autoclave. Autoclave pressure is typically controlled at 2400 to 2700 kPa by controlled venting of free gases to the quench vessel. The super-heated slurry must be cooled. One method of cooling is by flashing steam as the pressure drops in passing through a choke. Slurry from the flash tank flows by gravity to thickeners where acid and soluble salts are washed from the slurry prior to neutralisation.

1.1.4 Neutralisation and Cyanidation

Underflow from the thickeners feeds the small, intensely agitated pre-mix tank, in which milk-of-lime is added to neutralise the residual acid, precipitate the solubilised metals, and raise the pH to 10.5, which is the alkalinity required for subsequent cyanidation. Final pH adjustment is made in the neutralisation tank by adding milk-of-lime. The neutralisation tank overflows to the leach tank, where the bulk of the sodium cyanide solution required for leaching is added. These two tanks, as well as the first CIP tank, are sparged with low-pressure air to ensure sufficient oxygen for the dissolution of contained gold.

1.1.5 Adsorption

Apart from taking up space in the elution column, wood chips present problems in the adsorption stage where the slurry of fine ore and water (the 'pulp') are treated with cyanide in large tanks that are stirred mechanically or by air-agitation. Activated carbon is used to adsorb the gold directly from the cyanided pulp which flows continually from one adsorption tank to the next while a stream of activated carbon is transferred intermittently in the opposite direction. The gold value of the solution is reduced at each stage by the carbon until almost barren at the final tank. A pre-screen prevents trash material from entering the adsorption tanks with the carbon and additional screens prevent the carbon moving between tanks. Pre-screening is especially important since oversize particles, mostly the mentioned wood chips blind interstage screens and induces poor flow characteristics in the carbon stream.

1.1.6 Washing

Carbon is advanced to gold recovery as a slurry. The slurry is removed from the carbon on a vibrating screen. Water sprays on the screen wash most of the adhering ore slurry from the loaded carbon, which is then discharged to an acid wash column. The gold recovery facility includes several unit operations to remove the gold from the carbon and produce gold bullion as a final product. Loaded carbon is washed with a 3 per cent hydrochloric acid solution, primarily to remove calcium scale, which precipitates during the leach process. Acid solution is circulated upward through the wash column from the acid wash recycle tank, and overflow from the column is returned to the same tank. This wash cycle is maintained for approximately 1 hour and is followed by draining of the residual acid and rinsing of the carbon with filtered fresh water for a period at least 3 times the washing cycle.

1.1.7 Elution

Desorption or elution (removal of the gold cyanide complex from the carbon) takes place in a continuous column using the Anglo American Research Laboratories (AARL) system. This system consists in soaking the loaded carbon in a hot caustic cyanide solution followed by stripping with clean, demineralised water. The carbon is transferred from the acid wash column and de-watered on a screen prior to discharge to the upper chamber of the column where the soaking takes place. A pre-soak solution, comprising caustic and sodium cyanide, circulates through the chamber continuously at excess of 90°C, with the temperature maintained by a steam-heated exchanger in the circulating loop. The carbon then moves into the desorption (elution) vessel. Water for desorption is heated to between 100 and 115°C by steam and washes the gold off the carbon. The water is injected near the bottom of the column and overflows from the top of the vessel to the pregnant solution tank. Pressure in the column is controlled to prevent flashing of the hot water. The pregnant solution can be routed through a recuperative heat exchange if necessary to reduce its temperature to below boiling point. Pregnant solution is collected in a tank, from where it is fed continuously to electrowinning.

Carbon is discharged from the bottom of the vessel. Cold water is added to the discharge pipe to cool the carbon prior to its discharge to the stripped carbon tank. Stripped carbon is transferred to a carbon regeneration kiln for reactivation. Carbon is first de-watered on a stationary dewatering screen, with supplemental dewatering occurring in the kiln feed bin. The kiln is heated indirectly by fuel oil. Residual moisture in the carbon provides sufficient steam to make the internal atmosphere relatively inert, thus preventing oxidation of the carbon as it is heated to about 675°C. The heating process volatilises any adsorbed hydrocarbons and reactivates the carbon. The hot reactivated carbon discharges into an agitated sump flooded with water, where the carbon is quenched. Make-up virgin carbon is added, as required, to a separate agitated tank for pre-attrition before use.

1.1.8 Electrowinning

The pregnant gold-concentrated solution is pumped to electrowinning. In electrowinning, the soluble gold is plated on to cathodes. Barren solution is pumped to leach for utilisation of the contained cyanide and recovery of any remaining gold values. Loaded cathodes are removed periodically and placed in cathode wash tanks where the gold, in the form of a sludge, together with wash-down from the cells themselves, flows to a conical bottom filter feed tank from where it is pumped to a plate and frame filter press. A portion of the filtrate is re-used as cell wash-down water and the rest reports to the barren solution tank. The gold sludge is recovered as a wet cake and placed in trays.

1.1.9 Smelting

Oxide ore contains some mercury and a mercury retort is included in the gold room equipment as a worker hygiene precaution. If required the trays of gold sludge are placed in the retort overnight for the removal of mercury and moisture prior to smelting. Dried slimes are mixed with flux consisting of borax, sodium nitrate, feldspar and soda ash, and placed in an induction furnace. The charge is melted, the slag poured off, and the metal poured into bars. Assay samples from each bar are taken with vacuum tubes before solidification. The bars are cleaned, weighed, stamped and stored in a safe inside the vault until shipment. The product is gold bullion containing mostly gold and some silver, with minor impurities. Slag is crushed and screened inside the gold room to recover gold prills, with the clean slag recycled to the grinding circuit. Assuming that 100 g of slag are produced for each kilogram of gold, approximately 250 kg of slag are produced each month.

1.2 Objectives

The objectives of this study were to investigate the characteristics of gold found in waste timber as a result of mechanical impregnation, adsorption and absorption. It was also undertaken to determine if pre-treatment of the chips via degradation with the white rot fungus *Phanerochaete chrysosporium* could assist in improving gold yield beyond that attained by direct cyanidation.

LITERATURE REVIEW 2

2.1 The Motivation for Recovering Gold from Waste Timber

The normal practise up until recently was to throw away waste timber. Perhaps the motivation for recovering gold from waste timber can best be understood by reading the following excerpt written by Martin Creamer (Creamer, 1996)) for Engineering News.

"*Gold recovered from waste timber which is usually thrown away funded half of the recent Harmony gold mine dividend, Randgold chairperson Peter Flack tells The Engineering News At Top Level series. The discovery of millions of rands in blasted-out timber packs and props was the result of a chance question. 'What happens to all this wood?' Flack asked. 'It's thrown away,' he was told. 'Do you think there could be any gold in it?' That was the key question. To find out, three incinerators were fabricated from cast-off scrap iron at a cost of R150 000. 'They incinerated the wood, reduced it to ash, beneficiated the ash and produced more than R6,8 million worth of gold,' says Flack.*"

Reclaiming the gold from ashes is the most direct way of recovery and is economically favourable. However, since the ashed material is carbonaceous in nature it is not completely amenable to direct cyanidation. Recoveries are actually as low as 43 per cent (Johns and Matthew, 1990). The carbonaceous materials resulting from burning adsorb the aurocyanide ion from the solution phase thereby effectively "pre-robbing" the gold from the cyanide solution. A second solution is to treat the waste timber chemically to try and open up the matrix and expose the contained gold-bearing ore to cyanide treatment.

Since chemical treatment requires the use of hazardous materials and can run into high operating costs a third option, that of degrading the chips biologically was proposed. In nature various microorganism do just this, by decomposing woody material that end up on the forest floor. Speeding up the natural degradation process of the waste timber by exposing it to aggressive microorganisms such as the families of wood-rotting fungi can prove to be viable and was the focus of this study.

2.2 Timber In The Mining Industry

Wood has remained one of the most important renewable natural resources. Because it is sparsely wooded, South Africa has since 1946 planted 1 million hectares compromising 1 per cent of the total land area with such species as eucalyptus. Eucalyptus presently compromise 34 per cent of the timber range in South Africa.

Eucalyptus is native to Australia, New Zealand, Tasmania and nearby islands. Most species have long, slender, leathery evergreen leaves that turn edgewise to the sun, thus retarding evaporation. The trees grow rapidly. This is one reason why they have been so widely planted in South Africa. They are used for timber and fuel, as windbreaks and avenue trees, and for such conservation purposes as slowing erosion. Eucalyptus is a very durable and tough wood, which along with its availability makes it the perfect structural support material for mines. Typical uses include columnar supports and packs (see Figure 2.1) used as permanent support mediums in the majority of stopes within South Africa.

2.3 Cell Wall Material of Wood

Plant cell wall material is composed of three important constituents: cellulose, hemicellulose and lignin. According to the species, wood contains on a dry basis between 40 and 55 per cent cellulose, 25 and 40 per cent hemicellulose and between 15 and 35 per cent lignin. The cell wall provides a rigid barrier that prevents the cell from bursting in its hypotonic environment and allows the protoplast to generate the outward hydrostatic pressure called turgor. Turgor is the

motive force of cell expansion and also provides much of the rigidity of unlignified tissues. Variations in the chemical composition and structure of the cell wall reflect its changing functions during cell development.

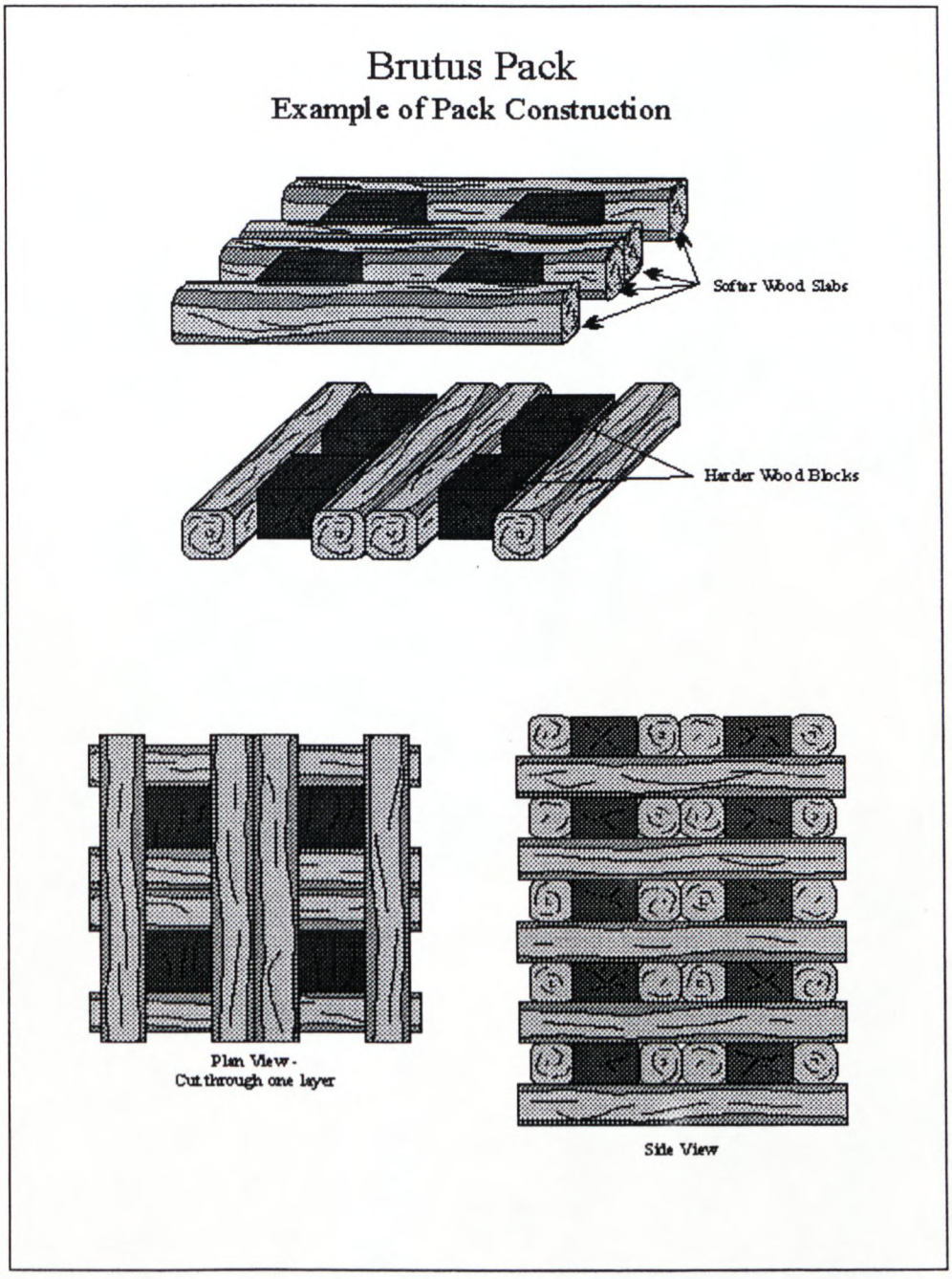


Figure 2.1 The Brutus Pack is a product of Mining Products Development. Printed with permission from Frans Pienaar, Director, Mining Products Development.

2.3.1 Cellulose

Cellulose is present as the main structural component in wood cell walls. It exists in the form of microfibrils, which are long chains of glucose molecules linked together. Cellulose microfibrils are generally thought to be more or less square in cross-section, but are of indeterminate length. The function of cellulose microfibrils is to impart strength to the cell wall, while hemicellulose and lignin exists as matrix materials.

2.3.2 Hemicellulose

Hemicelluloses are relatively short-branched polymers of xylose, arabinose, mannose and glucose. Hemicelluloses bind bundles of cellulose fibrils to form microfibrils, which enhance the stability of the cell wall. They also cross-link with lignin, creating a complex web of bonds, which provide structural strength, but also challenge microbial degradation (Ladisch, 1983; Lynch, 1992).

2.3.3 Lignin

The third major component of wood cell walls is lignin. Lignin is a complex three-dimensional polymer of phenylpropane units, which are cross-linked to each other with a variety of different chemical bonds. Lignin is completely amorphous and serves as an encrusting material surrounding microfibrils. This complexity has thus far proven as resistant to detailed biochemical characterization as it is to microbial degradation, which greatly impedes an understanding of its effects. Lignin affords considerable rigidity to the cell wall and because of its hydrophilic properties; it also influences the swelling characteristics of wood. Several models have been put forward to explain the association between the polysaccharide and lignin components in wood cell walls. It is accepted that cellulose microfibrils make up the backbone structure of the wall and the hemicelluloses and lignin exist as matrix materials around the microfibrils (Preston, 1962; Fengel, 1970; Fengel and Wegener, 1989).

2.4 Agents for Wood Decay

Many different biological agents decompose wood if adequate environmental conditions are available. Fungi, bacteria, and insects may all attack wood and degrade cell wall components or cause mechanical disintegration. Microorganisms and insects usually colonize wood in natural environments quickly, and the process of decomposition begins. Structural polymers in wood are gradually reduced to simpler molecules, and finally to CO_2 and water. Although various tree species have wood that is structurally and chemically distinct, all wood is susceptible to biological degradation.

2.5 Fundamental Mycology

Fungi live in a wide range of natural and man-made habitats. They are found in terrestrial and aquatic environments and occur as parasites growing on living plant and animal tissues, or as saprophytes growing on dead organic matter. In this latter respect they have a very important role to play in the processes of natural degradation and recycling of waste materials in soil, water and compost situations. Fungi derive their energy and carbon requirements from organic materials, although some fungi are also known to absorb carbon dioxide but may not necessarily use it as a carbon source (Eaton and Hale, 1993). Fungi are eukaryotic organisms which means that their cells have membrane-bound nuclei, mitochondria, endoplasmic reticulum, Golgi apparatus, vesicles, vacuoles, lipid bodies and storage granules. In general the fungi show greater morphological complexity than bacteria, the majority of which are unicellular. Fungi are often described as microorganisms, but many form large fruit bodies that serve as spore-bearing structures. These fungi are the more advanced members of the fungal kingdom and include many of the wood decay fungi.

2.5.1 Fungal Nutrition

Fungi have a requirement for organic compounds as a source of energy and carbon, which are used in normal cell metabolism. Organic and inorganic compounds are available to wood-inhabiting fungi in several forms. They are found in wood itself as structural polysaccharides i.e. insoluble cellulose and hemicelluloses, soluble sugars, minerals etc., and the immediate external environment which hyphae may colonize, particularly soil, or other microorganisms may supply them. Many wood-inhabiting fungi do not cause decay. The staining fungi and the moulds are able to colonize wood without causing significant damage to cell walls. Their carbon source requirements are met mainly by the available soluble sugars in the wood cell lumina.

It is important to consider the significance of the very low nitrogen levels found in wood. The carbon to nitrogen ration (C:N) in wood ranges from 300-1200:1. In fungal mycelium the nitrogen content can be up to 100 times greater. It is clear that wood-decay fungi must actively conserve and recycle their cellular nitrogen and that the nitrogen resources available to hyphae can be supplemented. Besides the major organic components in wood, fungi are also dependent on inorganic nutrients for growth. Growth media for routinely cultivating fungi in the laboratory are chemically non-defined and many are prepared from various plant extracts such as potato, carrot and malt. Many wood-destroying Basidiomycotina are grown on 2 per cent or 5 per cent malt extract agar, which is rich in sugars. This type of medium is not necessarily suitable for inducing cellulotic activity by some decay fungi and a chemically defined medium is often used.

A defined medium specifies not only the chemical components but also the amount of each component in the medium. The inorganic ingredients must include the major elements nitrogen, phosphorus, potassium, sulphur, magnesium, etc. and also micronutrients such as iron, copper, zinc, boron, etc. Some of these micronutrients can be incorporated into media trace quantities, as impurities of the macronutrients, but they are essential elements for the maintenance and growth of fungi. When present in higher concentration several of these elements are fungitoxic and are indeed the active ingredients of many commercial wood preservatives. In addition to the

requirement for a basic carbon source and inorganic nutrients, some fungi will only grow on media that are supplemented with vitamins, notably biotin and thiamin, which they are unable to synthesize themselves. Certain members of the Basidiomycotina have special needs and are grown on media containing thiamin hydrochloride; others require NH_4^+ as an inorganic nitrogen source because they are unable to utilize NO_3^-.

2.6 The White Rot Fungus *Phanerochaete chrysosporium*

White rot is a form of wood decay that results in bleaching of the wood. The term is commonly used to describe the appearance of the decayed wood. The white rot fungi are able to degrade lignin extensively and may do so while degrading other wood cell wall components. The cellulosic portion of wood is attacked to a lesser extend, resulting in the characteristic white colour of degraded wood.

Originally described as *Chrysosporium lignorum* due to its *Chrysosporium* imperfect state and has also been described as *Sporotrichum pulverulentum*. The basidiomycete *Phanerochaete chrysosporium* (the scientific name means "visible hair, golden spore") is a fungus with unusual degradative capabilities. To date 59 strains of this fungus have been isolated from various sources in South Africa, including our indigenous forests (De Koker et al, 1996).

This organism degrades a truly amazing range of xenobiotic compounds (nonbiological foreign chemicals) using both intracellular and extracellular enzymes. As examples, the fungus degrades benzene, toluene, ethylbenzene, and xylenes (the so-called BTEX compounds), chlorinated compounds such as 2, 4, 5-trichloroethylene (TCE), and trichlorophenols. The latter is present as contaminants in wood preservatives and also other pesticides. In addition, other chlorinated benzynes can be degraded with or without toluenes being present. Apparently, this microorganisms carries out such amazing feats after active growth, during the secondary metabolic lignin degradation phase. Degradation of some compounds involves important extracellular enzymes including lignin peroxidase and manganese dependent peroxidase. A critical

enzyme is pyranose oxidase, which releases H_2O_2 for use by the manganese dependent peroxidase enzyme. The H_2O_2 is also a precursor of the highly reactive hydroxyl radical, which participated in wood degradation. Apparently the pyranose oxidase is located in the interperiplasmic space of the fungal cell wall, where it can function either as a part of the fungus or be released from the fungus and penetrate into the wood substrate. It appears that the non-specific enzymatic system that releases these oxidizing products degrades many cyclic, aromatic, and chlorinated compounds related to lignins.

2.6.1 Mechanisms Of Lignin Biodegradation

Because lignin is an insoluble polymer, the initial steps in its biodegradation must be extracellular. The final steps in lignin mineralisation, culminating in the release of CO_2, are likely to take place inside the fungal hyphae. Therefore, the extracellular reactions must break lignin into fragments that are able to diffuse to the hyphae and cross the cell membranes. Unlike other biopolymers, the monomers in lignin are joined by ether and C-C bonds that are not readily hydrolysed. Chemical and physiological evidence shows that lignin degradation, like its biosynthesis, is predominantly oxidative, although reductive reactions may also participate (Haemmerli et al, 1986). Oxidation of lignins that contain free phenolic groups would be expected to cause further polymerisation. However, some low molecular weight fragments are also released (Ishihara and Miyazaki, 1972; Kurek et al, 1990). Lignolytic fungi seem to be able to tip the balance between polymerisations and depolymerisation in favour of fragmentation, possibly by removing the low molecular weight pieces from the reaction mixture (Hammel et al, 1993). Because lignin is formed by a random polymerisation process it has a complex and irregular structure. The diversity of the inter-unit linkages and the irregularity of their arrangement make it difficult for a ligninolytic fungus to produce enzymes that could recognize and cleave all of them. The solution that has evolved in the white-rot fungi is to produce enzymes of low specificity that initiate, but do not direct, oxidative reactions in lignin. Kirk and Farrel (1987) have termed this process enzymatic combustion: the enzyme activates the lignin to overcome an energy barrier and begin a thermodynamically favoured oxidative fragmentation without further control of the reaction pathway by the enzyme.

Lignin biodegradation does not proceed by an orderly removal of the peripheral subunits as single ring compounds; it also involves oxidation of the aromatic rings and side chains in the interior of the polymer, increasing the hydrophilicity and solubility of the polymer core at the same time as fragments of varying size are set free. The disorderly nature of this degradation agrees with the concept of enzymatic combustion.

2.6.2 Lignin Degrading Enzymes of *P. chrysosporium*

Two enzymes produced by the white rot fungus *Phanerochaete chrysosporium* account for its lignin degrading properties. These two enzymes are respectively known as lignin- and manganese peroxidase.

2.6.2.1 Lignin Peroxidase

Once called ligninase, this enzyme is a heme peroxidase with an unusually high redox potential and low optimum pH (Umezawa and Higuchi, 1991; Gold and Alic, 1993). It shows little substrate specificity, reacting with a wide variety of lignin model compounds and even unrelated molecules (Barr and Aust, 1994). The enzyme is susceptible to inactivation by H_2O_2 (Wariishi and Gold, 1990), and to inhibition or masking of its actions by components of lignocellulosic media (Bonnen et al, 1994). Veratryl alcohol (VA) enhances its action on many substrates, including lignin (Hammel et al, 1993; Kurek et al, 1990); the effect of veratryl alcohol has been attributed to acting as mediator (Harvey et al, 1986), protecting against inactivation (Wariishi and Gold, 1990), or acting as a preferred substrate for the second one-electron reduction in the enzyme's catalytic cycle (Koduri and Tien, 1994).

2.6.2.2 Manganese Peroxidase

This is another heme peroxidase, but it shows a strong preference for Mn(II) as its reducing substrate (Glen and Gold, 1985). The product Mn(III) forms a complex with organic acids and

diffuses away from the enzyme to oxidize other materials, such as lignin. The redox potential of the Mn peroxidase – Mn system is lower than that of lignin peroxidase and it does not oxidize non-phenolic lignin models.

2.6.3 Enzyme Access to Lignin

Native lignin is hydrophobic, insoluble, and sequestered in a dense matrix of polysaccharides, which restricts access of enzymes to only the surface of the wood cell wall. Physical contact between the enzymes and lignin might be a rate-limiting factor in lignin biodegradation. For manganese peroxidase this may be less of a problem; complexed Mn(III) ions can more readily diffuse into the lignocellulose complex than proteins can. For lignin peroxidase, the problem seems to resolve in two ways, depending on the type of decay. During simultaneous decay, the porosity of the cell wall does not increase enough to allow enzyme penetration into the lignin-polysaccharide matrix. However, in this type of decay, polysaccharides are removed from the wall at the same rate as lignin, so that the zone of active lignin degradation remains directly accessible at the surface. In selective delignification, the cellulosic matrix of the secondary wall remains in place and lignin degradation takes place at a considerable distance from the surface of the fibre lumen. Immunochemical labelling shows those lignin peroxidases can penetrate the selectively delignified cell walls, indicating that removal of lignin and hemicellulose creates channels wide enough to allow access of the enzymes to the sites of lignin degradation. Thus, in either case, the fungal enzymes can reach their substrate.

2.7 Specific Objectives

The impregnated portion of gold in refractory wood chips is thought to be locked up in the complex wood matrix. The correctness of this assumption will be a point for investigation.

Literature identifies lignin as the matrix material around microfibrils responsible for affording rigidity to prevent excessive cell expansion and possible rupture. Fungi exist that are capable of removing lignin from wood by producing lignin-degrading enzymes. One such fungus in particular, *Phanerochaete chrysosporium*, has been widely studied in the paper-and-pulping industry because of its amazing lignin degrading system. This capability of this fungus will be investigated under different conditions as a possible solution for "opening-up" the wood matrix of refractory chips and facilitating improved recovery of impregnated gold. Electron microscope studies will be undertaken to gain a better understanding of the lignin degradation processes and gold-recovery studies will be undertaken to investigate the successes of biological treatment on gold recovery.

MATERIALS AND EXPERIMENTAL METHODS 3

The research was divided into two parts. The objective of the first part was to gain a better understanding of the value, species and recovery of gold from refractory chips. The second part focuses on the fungus *Phanerochaete chrysosporium* and the degradation of wood chips.

3.1 Wood Chips

The first part of the research investigating characteristics of gold in refractory chips was conducted on wood chips donated by Vaal Reefs Mine, Klerksdorp. The refractory chips had paper, plastics and other trash material among it, which could not all be removed by hand, or sieving and was consequently substituted with laboratory prepared chips to standardize conditions in the degradation studies. Logs from a tree identified as a eucalypt by the Department of Forestry at Stellenbosch University were debarked and cut into 1 cm disks with a power-driven band saw and chipped in a hammer-type mill. Refractory and laboratory prepared chips were sieved to sort the chips according to size and stored at 80°C in an oven. The chips were kept in the oven until used to prevent the growth of contaminating microorganisms and to maintain a constant dry weight.

3.1.1 Preparing Wood for Chemical Analysis

The neutral solvents, ethanol and benzene, were used to remove materials which are not part of the wood substance or which may interfere with chemical analysis. The chips must be ground to a fine particle size to permit complete reaction of the wood with the reagents used in the analysis. The chips were ground to pass a 0.4 mm (40—mesh) screen. Wood extractives are materials soluble in neutral solvents and are not generally considered as part of the wood substance. These

materials should be removed before any chemical analysis of wood substance, except where the extraction process and subsequent washing could interfere with certain chemical analyses. Ethanol-benzene was used to extract waxes, fats, some resins, and possibly some portions of wood gums. Hot water was used to extract tannins, gums, sugars, and colouring matter.

The procedure of extraction was carried out in a chemical fume hood. The ground chips were placed in an extraction thimble and the extraction thimble placed in position in a Soxhlet apparatus. A small cone of fine mesh screen wire was placed on top of the thimble to prevent any loss of the specimen. The ground chips was extracted with 200 ml of the solvent consisting of 1 volume ethanol (95 per cent) and two volumes benzene (reagent grade). Extraction continued for 6 to 8 hours and the liquid was kept boiling briskly so that siphoning from the extractor was no less than four times per hour. After extraction with ethanol-benzene, the wood was transferred to a Büchner funnel, to remove the excess solvent with suction. Thereafter, the thimble and wood was washed with ethanol to remove the benzene. The wood was returned to the thimble and extracted with 95 per cent ethanol for at least 4 hours or until the alcohol siphoned over colourless. The sample was again transferred to a Büchner funnel to remove the excess solvent with suction. The thimble and wood sample was washed with distilled water to remove the ethanol and transferred to a 1000 ml Erlenmeyer flask to which 500 ml boiling distilled water was then added. The flask was heated for 1 hour in a hot water bath by keeping the water boiling and surrounding the flask with boiling water. After extraction the sample was filtered on a Büchner funnel and washed with 500 ml boiling distilled water. The treated wood was kept in an oven at 80°C until it was analysed.

3.1.2 Determining Lignin Content Of Wood Chips

The carbohydrates in wood were hydrolysed and solubilised by sulphuric acid; the acid-insoluble lignin was filtered off, dried and weighed. This method of lignin determination known as the Klason method defines lignin as a wood constituent insoluble in 72 per cent sulphuric acid. Sulphuric acid, 72 per cent H_2SO_4 was prepared by carefully pouring 665 ml of concentrated

H_2SO_4 (95 per cent) into 300 ml distilled deionised water, and after cooling, making the volume up to 1000 ml. The strength was adjusted to 24 N by titration with a standard alkali. The acid solution was cooled in a refrigerator to 15°C before use. Test specimens of the wood were prepared for chemical analysis in accordance with the procedure previously outlined. The oven dried prepared samples were allowed to cool down in a desiccator and 1 g placed in 100 ml beakers. Cold (15°C) 72 per cent sulphuric acid was added to the beakers containing the specimens. A total of 15 ml of acid was gradually added in small increments, while stirring the material. The beaker was kept in a bath at 2°C while the material disperses. After the specimen dispersed, the beaker was covered with a watch glass and kept in a bath at 20°C for 2 hr. After complete solution 300 ml water was added to a 1000 ml Erlenmeyer flask and the material from the beaker transferred to the flask. The solution was rinsed and diluted with water to 3 per cent concentration of sulphuric acid, to give a total volume of 575 ml. The solution was boiled for 4 hours and the volume maintained by frequent addition of hot water. The insoluble material (lignin) settles out. Taking care not to stir up the precipitate, the supernatant solution was decanted through a filtering crucible that has been tarred. Next the lignin was transferred quantitatively to the filter, using hot water and a rod. The crucible with lignin was dried in an oven at 105°C to a constant weight, cooled in a desiccator and weighed. In order to correct for the ash in lignin, an empty porcelain crucible, was heated with cover in a muffle furnace at 600°C for 15 min. The crucible was placed in a desiccator to cool for 45 minutes and weighed to the nearest 0.1 mg. The lignin was transferred to the crucible and, with the cover removed, placed in a muffle furnace at 100°C. The temperature was gradually raised to 600°C so that the material became carbonised without flaming. The material was allowed to ignite at 600°C for 4 hours, to burn away any carbon. Complete ignition is indicated by the absence of black particles. The crucible was covered, cooled in a desiccator and weighed to the nearest 0.1 mg.

The percentage lignin was calculated as follows:

Lignin, per cent = (L-A)/W

where:

L = weight of lignin in mg

A = weight of ash in mg

W = weight of test specimen in mg

The lignin content was reported as the average of two determinations, to the nearest 0.1 per cent.

3.2 Gold Studies

Adsorption, elution and cyanidation experiments with the wood chips were carried out in perspex batch reactors of height 15 cm, internal diameter 11 cm, and with three evenly spaced baffles of width 1 cm. The chips were kept in suspension by agitating the slurry with a flat blade impeller of width 6 cm and height 5 cm at a stirring speed of 300 rpm. Solution pH was adjusted and maintained as necessary by addition of 1M $Ca(OH)_2$.

3.2.1 Analysing Trace Amounts Of Gold In Wood Chips

The gold in the wood chips were leached from the encapsulated ore and wood matrix via acid attack and analysed on a Figureite Tube Atomizer (GTA). No less than 1 g (dry weight) of the sample to be analysed was placed in a porcelain crucible and heated in a muffle furnace at 100°C. The temperature was gradually raised to 600°C so that the material became carbonised without flaming. The material was allowed to ignite at 600°C for 4 hours, to burn away any carbon. Complete ignition is indicated by the absence of black particles. The sample was leached twice with freshly prepared aqua regia while stirring constantly. The sample was boiled to dryness after adding each aliquot. A third aliquot was added and the temperature of the mixture raised to near

boiling, then cooled slowly to room temperature, filtered, washed with 5 per cent (v/v) HCL and made up to 100 ml with distilled water. Standards of 0.01, 0.02 and 0.04 ppm were made from 1000 µg/ml gold solution and samples analysed on a Varian GTA coupled to a Varian Techtron AA-1275 atomic adsorption spectrophotometer. If the measured absorbance of a sample exceeded the upper limit of the GTA instrument the solution was diluted with a known amount of distilled water to bring the concentration within acceptable limits.

3.2.2 Analysing Gold Concentrations Higher Than 1 ppm

The procedure used to analyse gold concentrations higher than 1 ppm was the same as for trace amounts with the exception that higher concentration standards were made up and the measuring instrument used was a Perkin Elmer 3300 atomic absorption spectrophotometer (AAS).

3.3 Fungal Strains

Two different strains of *Phanerochaete chrysosporium* with the designations BKMF-1767 and ME-446 were obtained from the culture collection of the Department of Microbiology, Rhodes University, Grahamstown. Cultures were maintained on malt extract agar at 4°C until used. Malt extract plate cultures were inoculated from these slants and incubated at 39°C for 5 days prior to their use.

3.3.1 Preparing Agar For Maintenance Of Fungus

The following ingredients were added to a 1000 ml Schott bottle:

Glucose, 10 g

Malt Extract, 10 g

Peptone, 2 g

Yeast Extract, 2 g

Asparagine, 1 g

KH_2PO_4, 2 g

$MgSO_4.7H_2O$, 1 g

Thiamin-HCL, 1 mg

Agar, 20 g

The flask was filled to the 1000 ml mark with distilled water. The opening was plugged with non-adsorbent cotton wool and covered with foil. The medium was autoclaved for 15 minutes to sterilize the solution and dissolve solids. The hot agar was poured 1 cm thick into petri dishes so that the bottoms are evenly covered. Casting was done in a sterile operating laminar flow hood. After the agar cool down it becomes stiff with a consistency as jelly.

3.3.2 Inoculation Procedure

Inoculation was carried out in a sterile operating laminar flow hood. A platinum rod was used to transfer spores. The loop was flamed until red, allowed to cool and the mycelial surface of the inoculation plate was scraped and the spores transferred to fresh agar plates. The plates were stored upside down to reduce the risk of contamination and prevent dehydration. Spore production in the slants usually required 2 to 5 days of growth at 39°C.

3.3.3 Preparing Wood Chips For Inoculation

Laboratory prepared chips were removed from the oven and allowed to cool in a desiccator for at least 1 hour. The chips were soaked for 24 hours in a modified chemically defined medium proposed by Leatham (1983). A 250 ml Erlenmeyer flask was weighed and the chips (5 g dry weight per sample) transferred to the flask. The flask was covered with tin foil and sterilized in an autoclave for 15 minutes at 121°C. After cooling the foil was removed from the flask and the chips aseptically inoculated in an operating laminar flow hood with a 1 cm^2 plug taken from a 5 day old plate culture. The flask was sealed with a sterile rubber stopper. The stopper had two holes drilled through it with tubes pushed through each hole. The tubes were clamped shut and the

tube-openings on the outside of the flask were covered with sterile non-adsorbent cotton wool. If not stated otherwise; samples were incubated at 39°C and 65 per cent moisture. Humidity was not controlled; instead samples were re-hydrated as necessary with sterilized distilled water to the 65 per cent moisture level as measured against a control sample. The clamps on the tubes are released every third day and fresh air is pumped through the cotton wool into the flask through one of the tubes. The cotton wool filters the air and the second tube functions as an air outlet to prevent a build-up of pressure.

3.3.4 Modified Chemically Defined Medium (MCDM)

The modified chemically defined medium was added to all chips to help jump-start degradation and was made up as follows:

Contents per kilogram ovendry chips:

D-glucose, 37.5 g
L-glutamic acid, 0.38 g
KH_2PO_4, 3 g
$MgSO_4.7H_2O$, 3 g

Mineral stock solution, 15 ml (plus-minus 15 ml)
Trace element stock solution, 1.5 ml (plus-minus 1.5 ml)
Vitamin stock solution, 1.5 ml (plus-minus 1.5 ml)
Sterile water, 652 ml

Mineral stock solution was prepared by dissolving 3.67 g of $CaCl_2.2H_2O$, 3.60 g of $MnCl_2.4H_2O$, and 1.04 g of $ZnCl_2$ (anhydrous) in 1 L distilled water.

Trace element stock solution was prepared by dissolving 14.1 g of $Fe(NH_4)_2(SO_4).6H_2O$, 0.784 g of $CuSo_4.6H_2O$, 0.081 g of $CoCl_2.6H_2O$, 0.038 g of $SnCl_2.2H_2O$, and 2 ml of concentrated HCL in 1 L distilled water.

Vitamin stock solution was prepared by dissolving 1 g each of inositol and thiamine.HCL, 0.1 g each of pyriodoxine.HCl, nicotinic acid, sodium pantothenate and riboflavin, 0.03 g of biotin, and 0.01 g each of folic acid and cyanocobalomin in 1 L distilled water.

3.3.5 Measuring Spore Concentration

Spore numbers were determined by measuring absorbency of a spore suspension. If the spores to be counted were from a plate culture the surface was washed with sterile water and scraped. If the spores to be measured were from growth in a flask of chips then sterile water was poured into the flask and the flask shaken to loosen spores. The milky white spore suspension was filtered through sterile glass wool to free it of contaminating mycelia and the spore concentration determined by measuring absorbance at 650 nm. An absorbance of 1.0 cm^{-1} is approximately 5 x 10^6 spores/ml.

GOLD IN REFRACTORY WOOD CHIPS 4

4.1 Introduction

The value of the gold in the chips should exceed the costs of recovery. Although a cost analysis is outside the scope of this project the gold grade of the ore/chips should give a good indication whether or not recovery of the gold is feasible. The gold may be loaded onto the chips by a combination of three separate methods: adsorption and absorption of the gold complex and impregnation of gold-bearing ore and discrete gold particles. The gold complex may be recovered by elution, but the impregnated particles will first need to be dissolved in cyanide prior to recovery by elution. Biological treatment will be investigated as a tool to increase the efficiency of gold recovery by cyanidation. The modes of recovery will indicate the form of the loaded gold, for example a mass balance on the wood chips and the elution liquid after elution will give a good indication of how much gold is present in complex form. It will be assumed that the gold that could not be eluted from the chips are not present as a complex, but rather originate from impregnation.

4.2 Gold Grade Of Refractory Chips

The particles diameters of the refractory chips are between 850 μm and 4750 μm. Scanning electron microscopes and electron microprobes were used to perform energy dispersive (EDS) and wave length dispersive spectroscopy (WDS) on refractory chips samples. Polished sections were prepared and studied under an optical microscope and a scanning electron microscope. A large amount of dust consisting of pyrites, silicates and quarts was detected on the surface and in wood fibres, which confirm the presence of ore inside the matrix.

Figure 4.1

Backscattered SEM image of wood chip showing foreign particles in wood matrix.

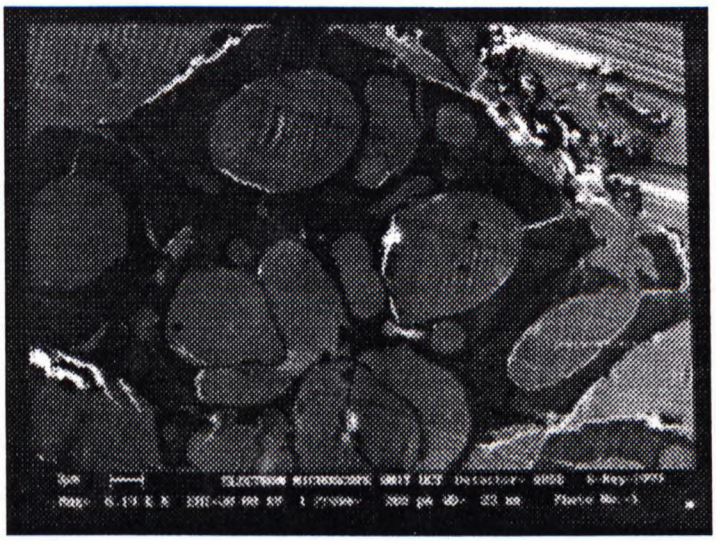


Figure 4.2

TEM of refractory wood chip showing foreign particles inside wood cells.

An automatic gold search of 12 polished sections encountered 4 gold particles. The studied sections were cut to be smaller than 3 mm and the encountered gold particles had surface areas of 40.9, 5.6, 13.6 and 7.5 square micron. Transmission electron microscopy (TEM) show that in most instances the ore particles are located inside cells, which suggest that any gold dissoluted from the ore would need to diffuse through several cell walls prior to recovery. Although it was determined that pregrobbing contributed very little to the gold value of waste timber (see section 4.3), some of the gold dissolved from the ore might still adsorb onto wood fibres and further contribute to the inefficiency of conventional cyanidation.

Refractory chips chemically analysed for trace amounts of gold contained between 2 and 5 mg(Au)/kg. The preliminary conclusion at this stage of the research is that the main sources of gold in the chips are discrete gold particles and gold-bearing ore.

(Accompanying tables: 4.1 ; Accompanying Figures: 4.3)

4.3 Chemical Determination Of Gold Species

The standard method to remove gold adsorbed onto activated carbon is by elution of the gold complex. In order to quantify the amount of gold adsorbed onto refractory chips from the pregnant liquor versus the gold associated with ore and present as discrete particles, the efficiency of elution when applied to wood chips was investigated. Laboratory prepared chips were stirred in 20 ppm(Au) gold cyanide as $KAu(CN)_2$. The contact time was 24 hours and pH was maintained at 11. Equilibrium loading was reached after approximately 4 hours and the final gold loading was 14 mg/kg. The gold was subsequently eluted from the chips with hot (90°C) water at pH 10. Equilibrium recovery was attained after approximately 3 hours. The final recovery after 4 hours was 90 per cent and indicate that elution also successfully recovers adsorbed gold from wood chips. During elution of refractory chips the gold concentration of the solution varied a great deal with time instead of increasing as expected. This prompted us to take into account the effect of activated carbon removed from the circuit with wood chips. On average there was more than 44 g

carbon per kilogram of refractory chips. The gold loading on the carbon was only 23 mg(Au)/kg. An adsorption experiment revealed the carbon had a remaining capacity of only 250 mg(Au)/kg. The normal capacity for activated carbon can be between 26 and 30 mg/g, and indicate the carbon particles were screened from the pulp after elution and prior to regeneration. The size of the carbon particles indicate the carbon may have been screened of as fines which would further contribute to a lower than normal capacity as a result of the decreased surface area. Because elution was carried out as a batch operation the carbon particles were able to re-adsorb some of the gold eluted from refractory chips resulting in the unusual recovery pattern. Samples of refractory chips were screened for carbon and the elution experiment was repeated. This time equilibrium was reached after 3 hours and 15 per cent of the gold was recovered. The results confirm that preg-robbing contributes only a small fraction of the gold value and that gold recovery should be focussed on the impregnated portion of gold.

(Accompanying tables: 4.2, 4.3, 4.4, 4.5 ; Accompanying Figures: 4.4, 4.5, 4.6, 4.7)

4.4 Gold Recovery By Cyanidation

Refractory chips stirred for 24 hours in 20 ppm free cyanide at pH 11 yielded a 64 per cent recovery prior to treatment with the fungus. After a 4 week treatment with strain BKMF-1767 at optimal conditions of 39°C and 65 per cent moisture the recovery increased to 74 per cent. Recovery proceeded more rapidly for untreated samples. It is possible that mycelium on the surface of treated samples initially inhibits recovery by blocking the pathways through which cyanide must penetrate the matrix and dissolved gold must be recovered. Eventually the shearing forces as a result of agitation provided by the impeller remove most of the remaining growth from the surface.

(Accompanying tables: 4.6, 4.7, 4.8, 4.9, 4.10, 4.11 ; Accompanying Figures: 4.8)

4.5 Summary Of Results

The gold grade of the refractory wood chips is between 3 and 5 mgAu/kg. Impregnation contributes 85 per cent of the total gold value of the refractory wood chips. The recovery process should proceed in the following order: biological treatment, cyanidation and elution. Biological treatment can 'open-up' the wood matrix allowing greater contact between the encapsulated gold and the cyanide solution and decrease the amount of woody material that can re-adsorb dissoluted gold during subsequent cyanidation. Elution should be the final step in the process to recover gold that re-adsorbed onto the wood fibers during cyanidation. It was found that biological treatment resulted in an improved recovery of 10 per cent compared to recovery without any treatment. The small improvement in gold recovery could suggest that the broken down fraction of the wood does not bear much gold or that some percentage of the released gold are re-adsorbed in the mycelia network of *Phanerochaete chrysosporium*.

GOLD IN REFRACTORY WOOD CHIPS - TABULATION OF RESULTS

4

Table 4.1

Au value of refractory wood chips taken from the general dump at Vaal Reefs Mine.

dp (μm)	q (mgAu/kg)
850 < dp < 1180	2.000
850 < dp < 1180	2.425
850 < dp < 1180	2.425
850 < dp < 1180	1.975
1180 < dp < 2360	5.875
1180 < dp < 2360	3.600
1180 < dp < 2360	4.075
1180 < dp < 2360	3.450
2360 < dp < 4750	4.175
2360 < dp < 4750	3.375
2360 < dp < 4750	4.075
2360 < dp < 4750	3.425

Table 4.2

Results for adsorption of gold cyanide onto laboratory prepared wood chips.

Gold cyanide as $KAu(CN)_2$; V=1.0 L; W=15 g; pH 11;
2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Co	q (mgAu/kg)
0	20.314	1.0000	0.00
10	20.268	0.9977	3.07
20	20.187	0.9937	8.47
30	20.140	0.9914	11.60
180	20.111	0.9900	13.53
240	20.106	0.9898	13.87
1440	20.108	0.9899	13.73

Table 4.3

Results for elution of laboratory prepared wood chips loaded with gold cyanide.

V=1.0 L; W=9 g; pH 10; T=90 °C; p = atmospheric;

2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)
0	0.000	0.000	10.35
10	0.052	0.619	4.57
20	0.067	0.798	2.91
30	0.071	0.845	2.46
180	0.082	0.976	1.24
240	0.084	1.000	1.02

Table 4.4

Results for elution of refractory wood chips and activated carbon.

V=1.0 L; W=20 g; pH 10; T=90 °C; p=atmospheric;
2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)
0	0.000
10	0.002
20	0.025
30	0.014
180	0.031
240	0.014

Table 4.5

Results for elution of refractory wood chips.

V=1 L; W= 40 g; pH 10; T=90 °C; p=atmospheric;
2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)
0	0.000	0.000	2.31
10	0.009	0.657	2.08
20	0.011	0.764	2.04
30	0.015	1.070	1.93
180	0.014	0.992	1.96
240	0.014	1.000	1.96

Table 4.6

Cyanidation results for untreated refractory wood chips: Run 1

C_{KCN}=20 ppmCN; V=1 L; W=15 g; pH 11;

2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)	q/q_o
0	0.000	0.000	4.470	1.000
5	0.016	0.381	3.403	0.761
10	0.020	0.473	3.147	0.704
20	0.022	0.522	3.008	0.673
30	0.022	0.530	2.986	0.668
50	0.028	0.671	2.593	0.580
90	0.034	0.807	2.213	0.495
180	0.041	0.977	1.737	0.389
240	0.043	1.024	1.603	0.359
1380	0.042	1.001	1.670	0.374
1440	0.042	1.000	1.672	0.374

Table 4.7

Cyanidation results for untreated refractory wood chips : Run 2

C_{KCN}=20 ppmCN; V=1 L; W=15 g; pH 11;
2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)	q/q_o
0	0.000	0.000	3.940	1.000
5	0.004	0.112	3.673	0.932
10	0.021	0.585	2.540	0.645
20	0.017	0.471	2.813	0.714
30	0.022	0.613	2.473	0.628
50	0.029	0.808	2.007	0.509
90	0.035	0.967	1.627	0.413
180	0.036	1.004	1.540	0.391
240	0.037	1.035	1.466	0.372
1380	0.036	1.008	1.529	0.388
1440	0.036	1.000	1.548	0.393

Table 4.8

Cyanidation results for untreated refractory wood chips : Run 3

C_{KCN}=20 ppmCN; V=1 L; W=15 g; pH 11;

2360 < dp < 4750 µm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)	q/q_o
0	0.000	0.000	3.190	1.000
5	0.019	0.581	1.904	0.597
10	0.016	0.482	2.123	0.666
20	0.022	0.669	1.710	0.536
30	0.017	0.507	2.067	0.648
50	0.028	0.836	1.340	0.420
90	0.031	0.941	1.107	0.347
180	0.033	0.986	1.008	0.316
240	0.033	1.000	0.976	0.306
1380	0.032	0.955	1.075	0.337
1440	0.033	1.000	0.976	0.306

Table 4.9

Cyanidation results for refractory wood chips after 4 weeks treatment with BKMF-1767: Run 1

C_{KCN}=20 ppmCN; V=1 L; W=15 g; pH 11;
2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)	q/q_o
0	0.000	0.000	2.970	1.000
5	0.005	0.162	2.637	0.888
10	0.002	0.065	2.837	0.955
20	0.009	0.291	2.370	0.798
30	0.019	0.615	1.703	0.574
50	0.020	0.647	1.637	0.551
90	0.020	0.647	1.637	0.551
180	0.021	0.679	1.570	0.529
240	0.025	0.809	1.303	0.439
1380	0.031	1.003	0.903	0.304
1440	0.031	1.000	0.909	0.306

Table 4.10

Cyanidation results for refractory wood chips after 4 weeks treatment with BKMF-1767: Run 2

C_{KCN}=20 ppmCN; V=1 L; W=15 g; pH 11;
2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)	q/q_o
0	0.000	0.000	6.090	1.000
5	0.011	0.157	5.357	0.880
10	0.022	0.314	4.623	0.759
20	0.023	0.329	4.557	0.748
30	0.028	0.400	4.223	0.693
50	0.034	0.486	3.823	0.628
90	0.054	0.771	2.490	0.409
180	0.058	0.829	2.223	0.365
240	0.061	0.871	2.023	0.332
1380	0.071	1.014	1.357	0.223
1440	0.070	1.000	1.423	0.234

Table 4.11

Cyanidation results for refractory wood chips after 4 weeks treatment with BKMF-1767: Run 3

C_{KCN}=20 ppmCN; V=1 L; W=15 g; pH 11;
2360 < dp < 4750 μm; 300 rpm

Time(min)	C (ppmAu)	C/Ce	q (mgAu/kg)	q/q_o
0	0.000	0.000	4.280	1.000
5	0.016	0.327	3.213	0.751
10	0.019	0.388	3.013	0.704
20	0.022	0.449	2.813	0.657
30	0.031	0.633	2.213	0.517
50	0.029	0.592	2.347	0.548
90	0.035	0.714	1.947	0.455
180	0.034	0.694	2.013	0.470
240	0.034	0.694	2.013	0.470
1380	0.049	1.000	1.013	0.237
1440	0.049	1.000	1.013	0.237

GOLD IN REFRACTORY WOOD CHIPS - FIGURES

4

(The solid lines with the figures present experimental trends and are not model predictions)

Figure 4.3

Au value of refractory wood chips taken from the general dump at Vaal Reefs Mine.

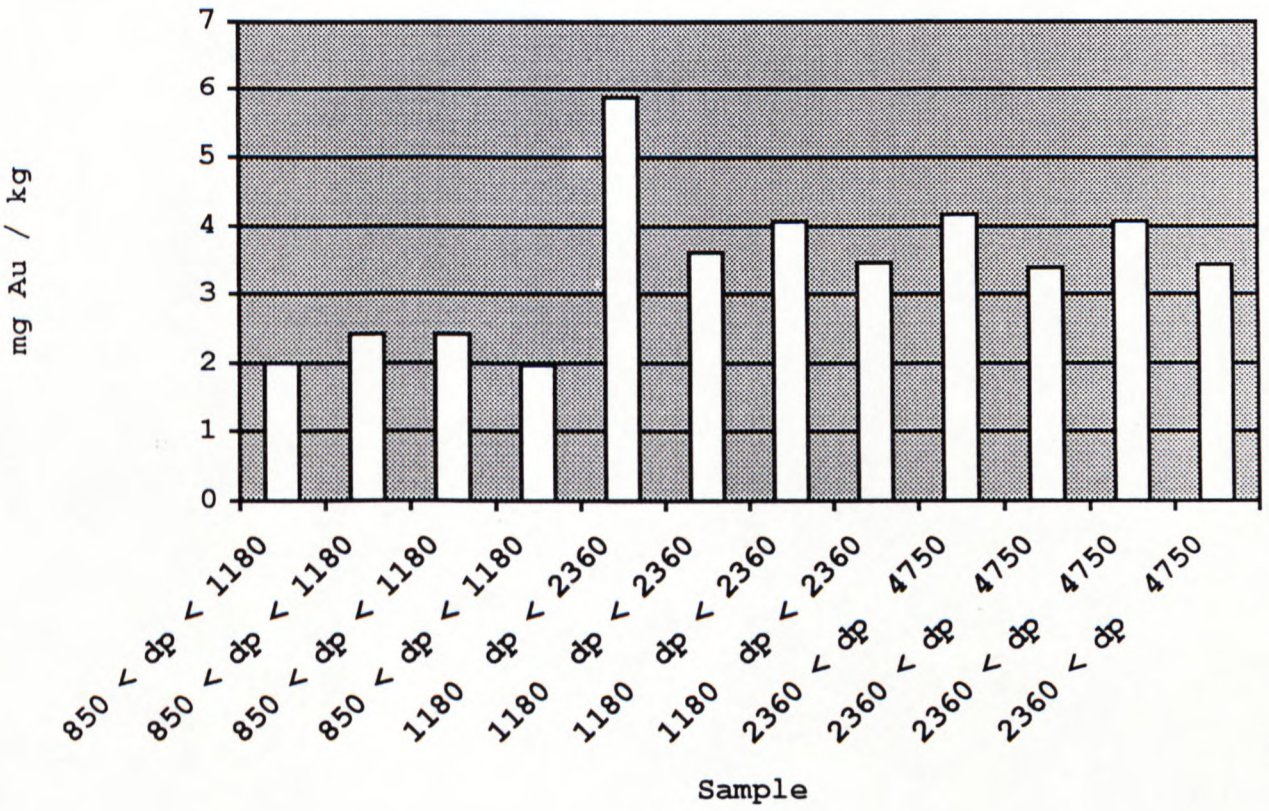


Figure 4.4

Results for adsorption of gold cyanide onto laboratory prepared wood chips.

gold cyanide as $KAu(CN)_2$; V=1.0 L; W=15 g; pH 11;
2360 < dp < 4750 μm; 300 rpm

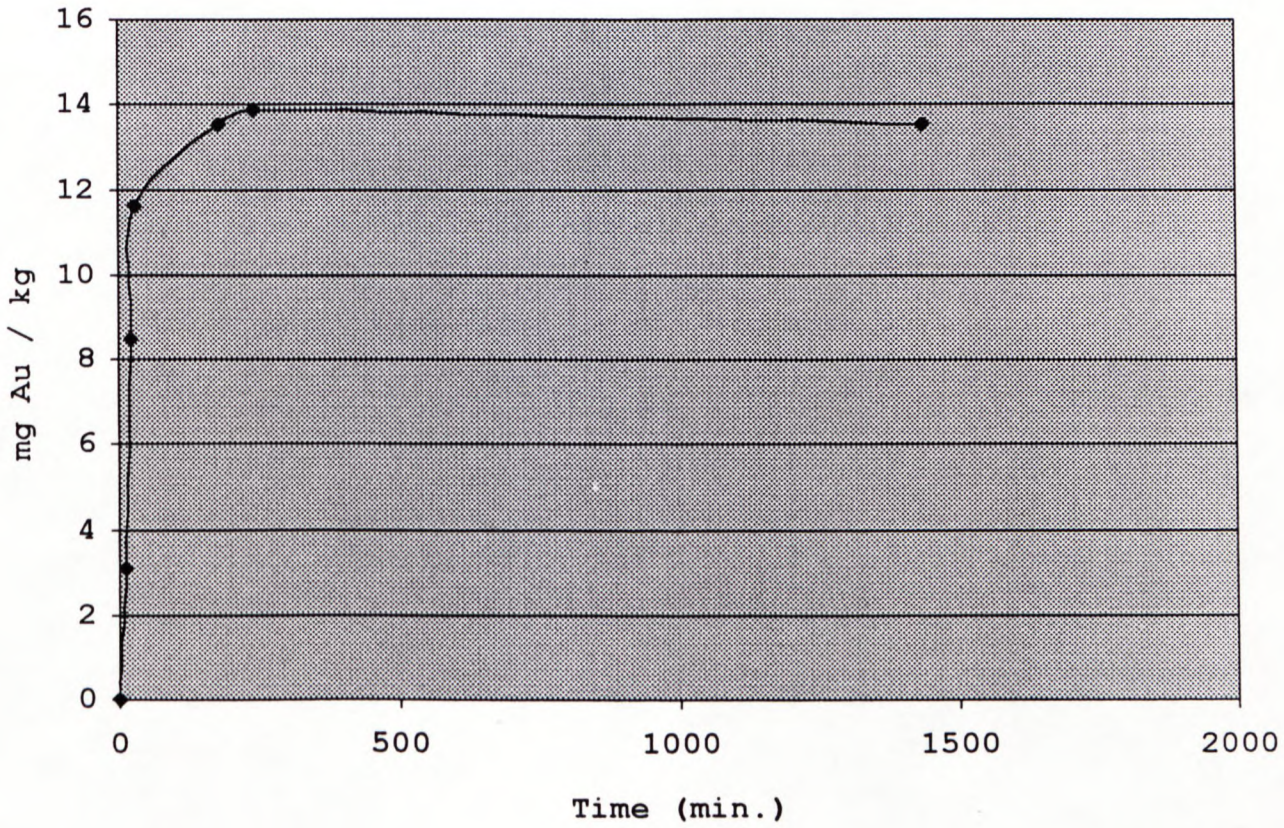


Figure 4.5

Results for elution of laboratory prepared wood chips loaded with gold cyanide.

V=1.0 L; W=9 g; pH 10; T=90°C; p=atmospheric;

2360 < dp < 4750 µm; 300 rpm

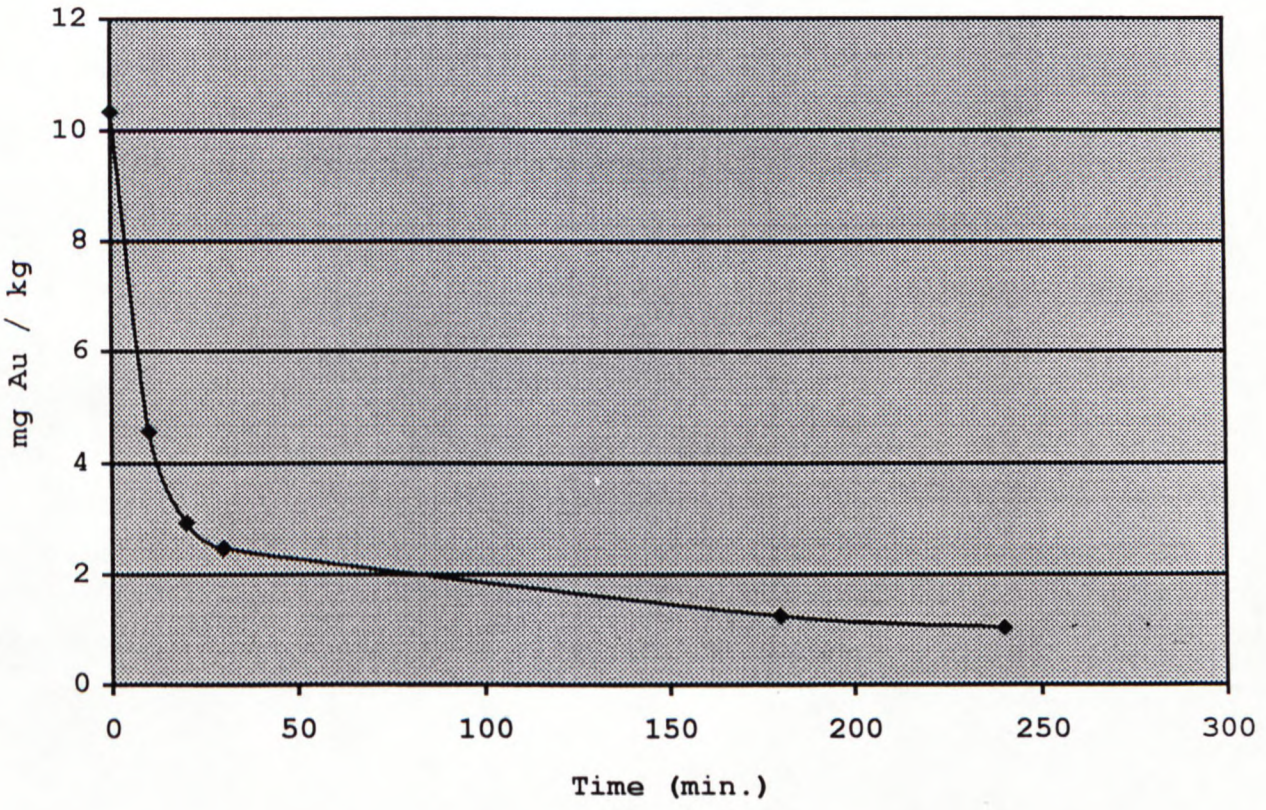


Figure 4.6

Results for elution of refractory wood chips and activated carbon.

V=1.0 L; W=20 g; pH 10; T=90°C; p=atmospheric;

2360 < dp < 4750 μm; 300 rpm

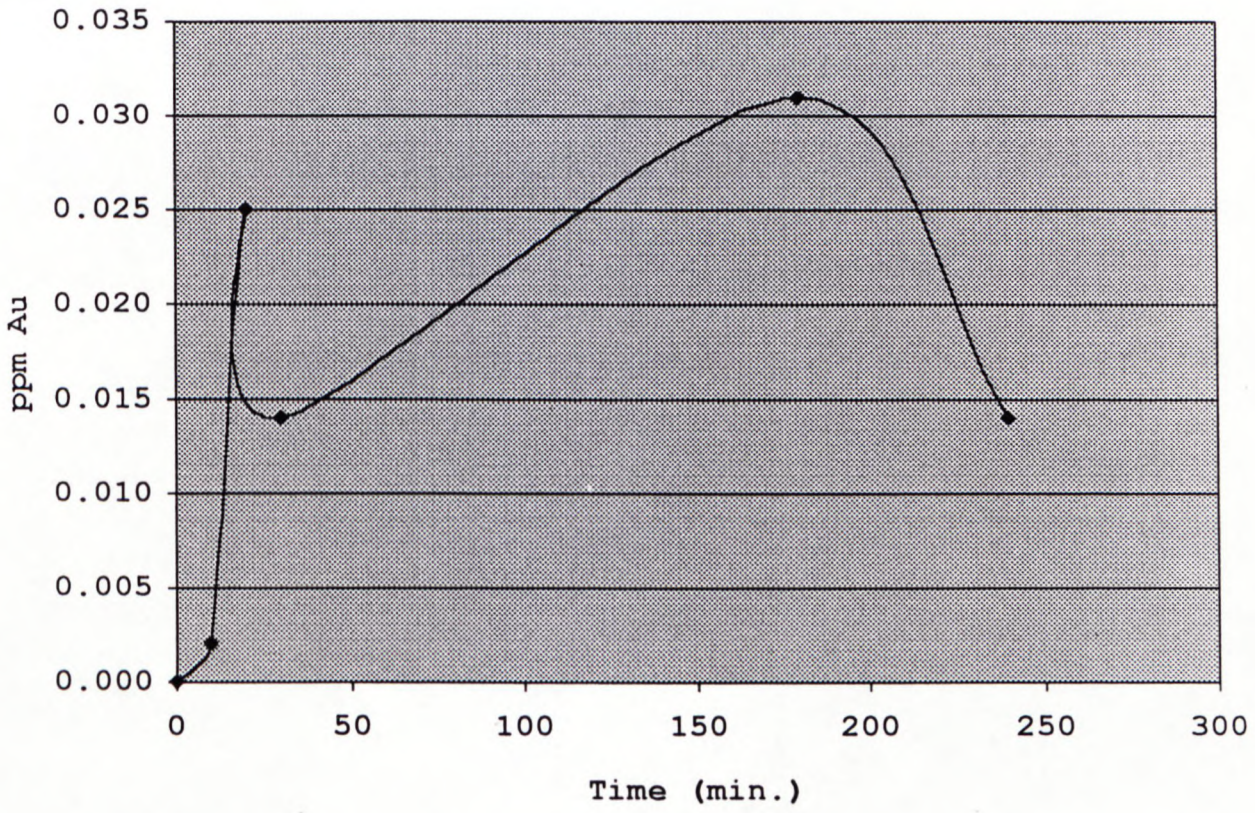


Figure 4.7

Results for elution of refractory wood chips.

V=1 L; W= 40 g; pH 10; T=90°C; p=atmospheric;

2360 < dp < 4750 µm; 300 rpm

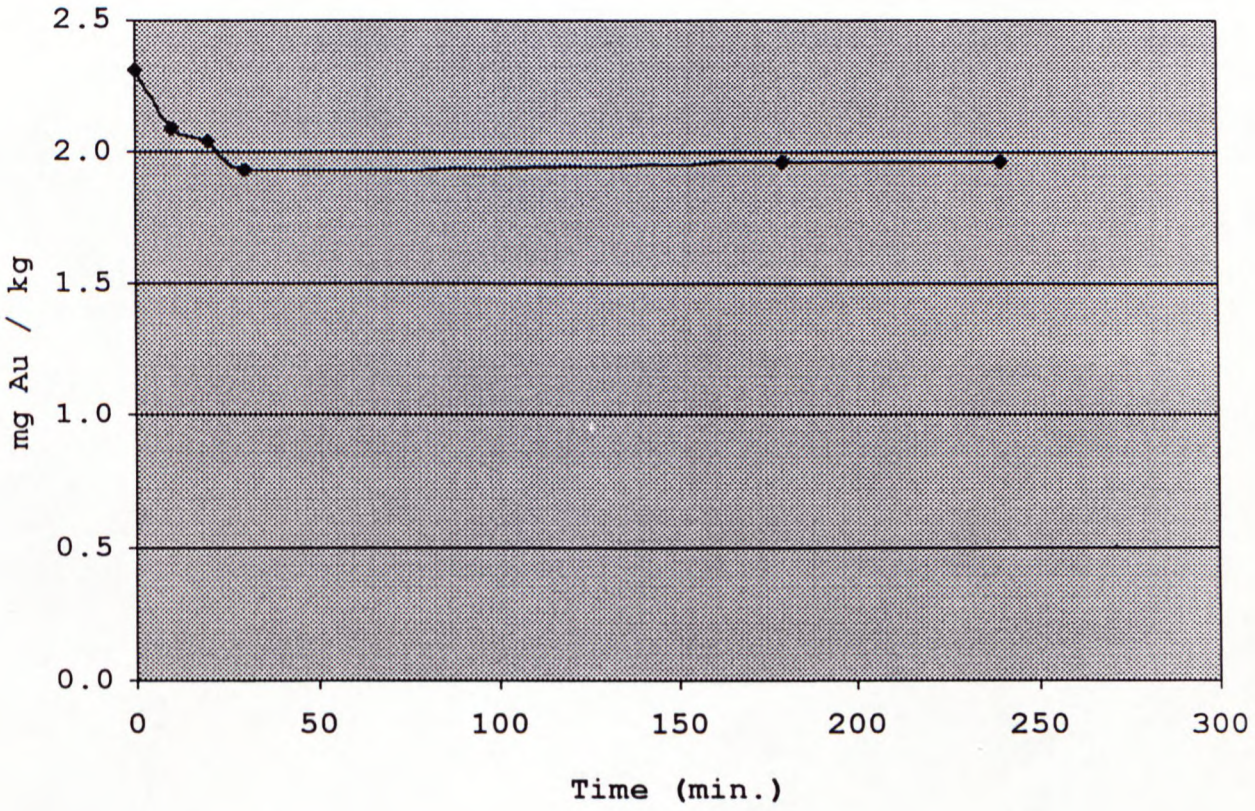
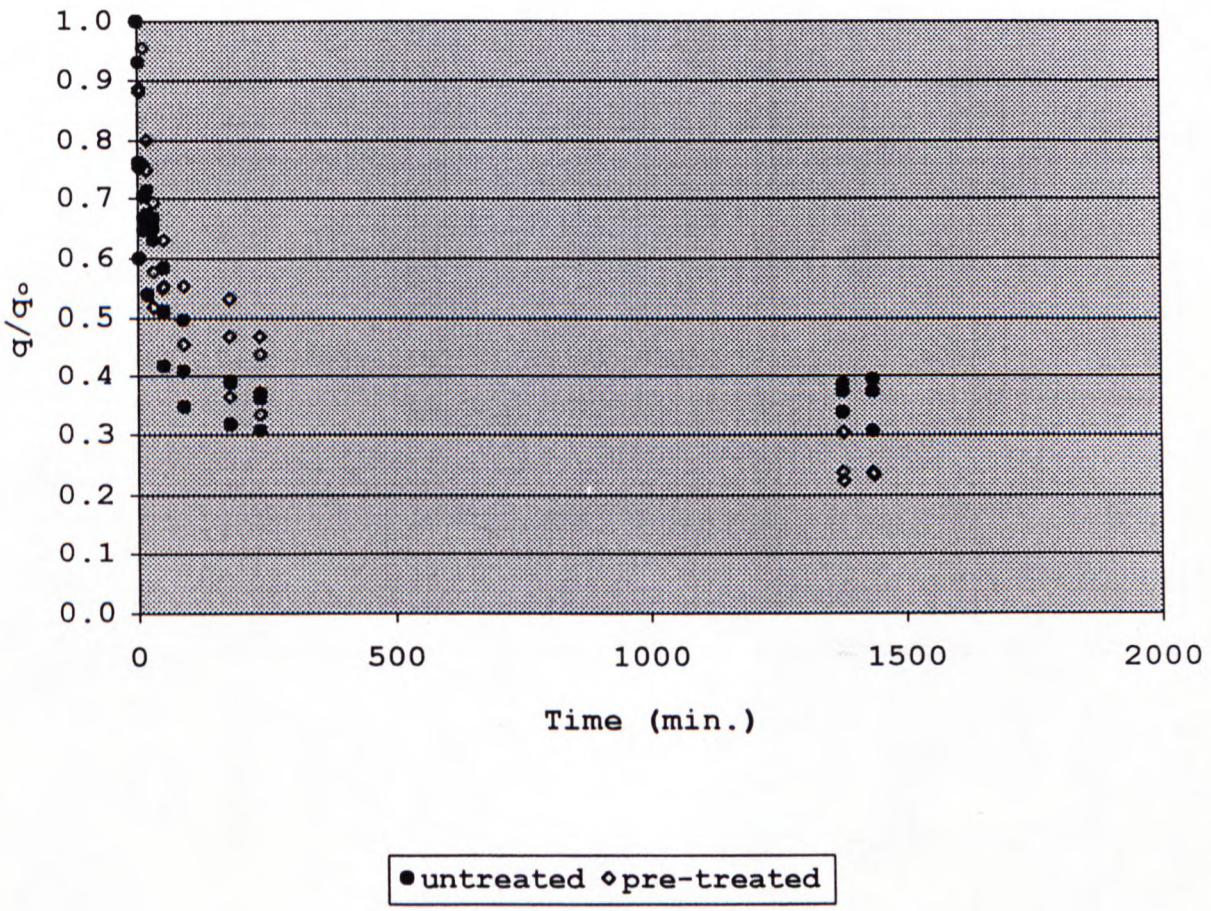


Figure 4.8

Cyanidation results for untreated and biologically pre-treated refractory wood chips

C_{KCN}=20 ppmCN; V=1 L; W=15 g; pH 11;

2360 < dp < 4750 µm; 300 rpm



DEGRADATION OF WOOD CHIPS 5

5.1 Introduction

To help optimise recovery of impregnated gold from refractory wood chips it is necessary to determine the conditions that will optimise the effectiveness of the biological treatment process. This involves determining the factors that will influence the lignin degrading process. *Phanerochaete chrysosporium* is a lignin-selective wood degrading fungi. Assuming that any reduction in weight after biological treatment is the result of lignin removal only, we are able to express the corresponding lignin loss as a function of weight loss after determining the initial lignin content.

5.2 Lignin Content Of Wood Chips

Samples of refractory and laboratory prepared chips were analysed for lignin. The normal lignin content for wood depending on the species is between 15 and 35 per cent, but the laboratory prepared and refractory chips measured 33 per cent and 40 per cent respectively. The values should be in the same range since all the chips are eucalyptus and the abnormally high value for the refractory chips is attributed to the method of lignin determination. Klason analysis defines lignin as a wood constituent insoluble in 72 per cent sulphuric acid. The gold- and ore particles inside the refractory chips were not dissolved by the acid and contribute the additional mass.

(Accompanying tables: 5.1 ; Accompanying Figures: 5.1)

5.3 Fungal Screening

Two strains of *Phanerochaete chrysosporium* with the designations BKMF-1767 and ME-446 were screened by inoculating laboratory prepared chips with either strain and incubating for 4 weeks at 39°C and 65 per cent moisture. BKMF-1767 caused 10 per cent greater weight loss of the chips than ME-446. The outcome prompted us to focus on BKMF-1767 in remaining investigations.

White delignified areas appear on the chips during the second week after inoculation and at this time a reduction in weight could also be measured. The enzymes causing lignin breakdown are produced during secondary metabolism and the weight loss indicate the fungus remained in primary metabolism throughout the first week and entered secondary metabolism in the weeks after.

(Accompanying tables: 5.2, 5.3 ; Accompanying Figures: 5.2, 5.3)

5.4 Spore Numbers

Fungal growth appears on inoculated chips during the first week of incubation. The growth usually became significantly visible 3 to 4 days after inoculation. Spore numbers increased 299 per cent and 260 per cent during the first and second week respectively, and slowed down to 5 per cent and 7 per cent during the third and fourth week respectively. Samples inoculated with greater spore numbers did not experience increased weight loss. Samples inoculated with 2.6×10^4, 1×10^5 and 2.1×10^5 spores experienced weight losses of 14 per cent, 15 per cent and 15 per cent respectively. White delignified areas appeared earlier on samples inoculated with higher spore numbers, but since the available nutrients are fixed degradation also ends earlier resulting in equal weight losses over extended periods.

Inoculating samples with larger spore numbers do however reduce the risk of other microorganisms growing on the chips by making it difficult for the other microorganism to compete for nutrients. Unidentified fungal growth was observed in earlier runs inoculated with the lower spore numbers. All contaminated runs were repeated.

(Accompanying tables: 5.4, 5.5, 5.6 ; Accompanying Figures: 5.4, 5.5, 5.6)

5.5 Effect Of Particle Size On Degradation

Particle size had a pronounced effect on the degree of degradation. Wood dust with a particles size less than 1 mm experienced a weight loss of 29 per cent and pieces larger than 2 cm suffered less than 1 per cent weigh loss. The larger exposed surface area of smaller chips favours faster degradation since growth start on the surface and continues to penetrate into the matrix.

(Accompanying tables: 5.7 ; Accompanying Figures: 5.7)

5.6 Temperature Studies

Highest weight losses were obtained for samples of chips incubated at 30°C and 40°C with no visible growth occurring at 50°C. The reported optimum for *Phanerochaete chrysosporium* is 39°C. Weight losses were low for samples incubated at 20° and although growth was observed at 10°C no measurable weight loss could be determined. This suggest the enzymatic processes responsible for degradation occur optimally between 30°C and 40°C and that the enzymes are not produced at 10°C or are produced and subsequently inactivated by the cold. Fungal contaminations were observed in the samples incubated at 10°C and 20°C and indicate a decreased capacity in *Phanerochaete chrysosporium* to compete against foreign microorganisms.

(Accompanying tables: 5.8 ; Accompanying Figures: 5.8)

5.7 Moisture Studies

All the chips were pre-treated with a chemically defined medium (Leatham, 1983) to jump start degradation. Because of this, the lowest moisture level that can be tested after addition of the medium was 65 cent on a dry basis. The fungus grew well at 65 per cent moisture, and even grew at 100 per cent moisture although weight loss could not be measured at 100 per cent. At more than 100 per cent moisture the chips were too wet and growth did not take place. Growth also did not take place on dry samples (no addition of chemically defined medium).

(Accompanying tables: 5.9 ; Accompanying Figures: 5.9)

5.8 Disrupting The Mycelial Mat

Samples of chips inoculated with the fungus were vigorously shaken by hand once a day during the week until all visible mycelial growth was removed from the surface. Control samples were kept stationary and the difference in extend of degradation between the shaken and stationary samples was less than 4 per cent. Although vigorous shaking help to better distribute spores the benefit was offset against disruption of growth that successfully attached to the wood surface,

(Accompanying tables: 5.10 ; Accompanying Figures: 5.10)

5.9 Degradation Of Refractory Wood Chips Inoculated With BKMF-1767

In order to help standardize conditions laboratory prepared chips were used in all previous degradation studies. The fungus also had no difficulty growing on refractory chips and the weight losses compared well with that measured for laboratory prepared chips treated under the same conditions.

(Accompanying tables: 5.11 ;Accompanying Figures: 5.11)

5.10 Summary Of Results

BKMF-1767 was identified as a more aggressive candidate for lignin removal than ME-446. In order to help optimise weight losses as a result of treatment with BKMF-1767 the samples should be incubated at 39°C and 65 per cent moisture (dry basis). The longer the period of treatment the higher the weight losses. Samples of the fungus grown on wood did not react negatively to movement and inoculating samples with higher spore numbers help reduce the risk of contaminating growth.

DEGRADATION OF WOOD CHIPS - TABULATION OF RESULTS

5

Measured lignin content of refractory and laboratory-prepared wood chips.

Table 5.1

Sample	Laboratory prepared chips	Refractory chips
1	36.1 per cent	38.3 per cent
2	32.4 per cent	46.8 per cent
3	31.8 per cent	44.7 per cent
4	31.1 per cent	34.1 per cent

Table 5.2

Degradation results for laboratory-prepared chips inoculated with ME-446.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent (dry basis)

	W (g)	-W	-Lignin
Control	5.01	-0.20 per cent	-0.61 per cent
Week 1	4.98	0.40 per cent	1.21 per cent
Week 2	4.93	1.40 per cent	4.24 per cent
Week 3	4.85	3.00 per cent	9.08 per cent
Week 4	4.83	3.40 per cent	10.29 per cent

Table 5.3

Degradation results for laboratory-prepared chips inoculated with BKMF-1767.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent (dry basis)

	W (g)	-W	-Lignin
Control	5.00	0.00 per cent	0.00 per cent
Week 1	5.00	0.00 per cent	0.00 per cent
Week 2	4.65	7.00 per cent	21.19 per cent
Week 3	4.62	7.60 per cent	23.00 per cent
Week 4	4.35	13.00 per cent	39.35 per cent

Table 5.4

Spore count of conidia on 5-day old plate cultures of malt extract agar inoculated with BKMF-1767.

Measured at absorbance of 650 nm.

Plate	Sample 1 (spores/cm^2)	Sample 2 (spores/cm^2)	Sample 3 (spores/cm^2)	Average (spores/cm^2)
1	2.4E+04	2.3E+04	2.3E+04	2.3E+04
2	2.2E+04	2.2E+04	2.1E+04	2.2E+04
3	2.7E+04	2.7E+04	2.7E+04	2.7E+04
4	3.6E+04	3.5E+04	3.4E+04	3.5E+04
5	2.6E+04	2.6E+04	2.6E+04	2.6E+04
6	2.9E+04	2.8E+04	2.8E+04	2.8E+04
7	2.4E+04	2.3E+04	2.2E+04	2.3E+04
8	2.6E+04	2.5E+04	2.5E+04	2.5E+04

Table 5.5

Spore count of conidia on laboratory-prepared chips inoculated with BKMF-1767.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent (dry basis)

Sample	Spores	Increase
Inoculation	2.6E+04	----------
Week 1	1.0E+05	298.72 per cent
Week 2	3.7E+05	259.81 per cent
Week 3	3.9E+05	5.41 per cent
Week 4	4.2E+05	7.25 per cent

Table 5.6

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Effect inoculation with greater spore numbers had on extend of weight loss.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent (dry basis)

Spores	W (g)	-W	-Lignin
2.60E+04	4.28	14.40 per cent	43.59 per cent
1.00E+05	4.24	15.20 per cent	46.01 per cent
2.10E+05	4.26	14.80 per cent	44.80 per cent

Table 5.7

Degradation results after 4 weeks for laboratory-prepared wood chips inoculated with BKMF-1767 : Effect of particle size.

W=5.00 g; T=39°C; M=65 per cent (dry basis)

dp (μm)	W (g)	-W	-Lignin
850 < dp < 1180	3.55	29.00 per cent	87.78 per cent
4750 < dp < 19200	4.33	13.40 per cent	40.56 per cent
dp > 19200	4.94	1.20 per cent	3.63 per cent

Table 5.8

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Temperature studies.

W=5.00 g; 2360 < dp < 4750 μm; M=65 per cent (dry basis)

Sample	W (g)	-W	-Lignin
10°C	5.04	-0.80 per cent	-2.42 per cent
20°C	4.84	3.20 per cent	9.69 per cent
30°C	4.73	5.40 per cent	16.34 per cent
40°C	4.36	12.80 per cent	38.74 per cent
50°C	5.01	-0.20 per cent	-0.61 per cent

Table 5.9

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Moisture studies.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C

Moisture (dry basis)	W (g)	-W	-Lignin
0 per cent	5.02	-0.40 per cent	-1.21 per cent
65 per cent	4.49	10.20 per cent	30.87 per cent
100 per cent	4.73	5.40 per cent	16.34 per cent
120 per cent	5.00	0.00 per cent	0.00 per cent
150 per cent	5.00	no growth	0.00 per cent
200 per cent	5.00	no growth	0.00 per cent

Table 5.10

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Effect disruption of mycelial mat had on degradation.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent (dry basis)

Sample	W (g)	-W	-Lignin
stationary	4.44	11.20 per cent	33.90 per cent
stationary	4.51	9.80 per cent	29.66 per cent
stationary	4.41	11.80 per cent	35.72 per cent
shaken	4.54	9.20 per cent	27.85 per cent
shaken	4.71	5.80 per cent	17.56 per cent
shaken	4.59	8.20 per cent	24.82 per cent

Table 5.11

Degradation results after 4 weeks for refractory chips inoculated with BKMF-1767.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent (dry basis)

	W (g)	-W	-Lignin
Control	4.98	0.40 per cent	1.21 per cent
Sample 1	4.44	11.20 per cent	33.90 per cent
Sample 2	4.56	8.80 per cent	26.64 per cent
Sample 3	4.48	10.40 per cent	31.48 per cent

DEGRADATION OF WOOD CHIPS - FIGURES

5

Measured lignin content of refractory and laboratory-prepared wood chips.

Figure 5.1

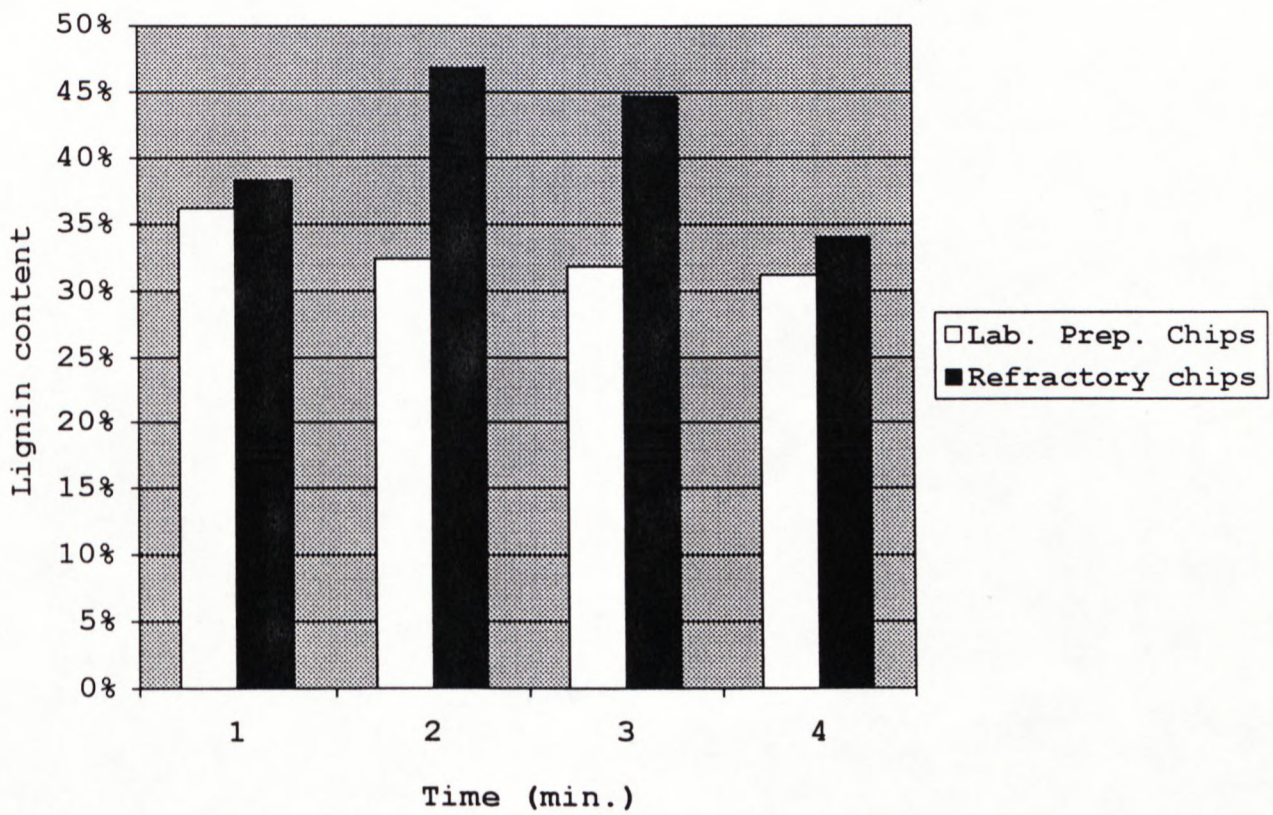


Figure 5.2

Degradation results for laboratory-prepared chips inoculated with ME-446.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent;

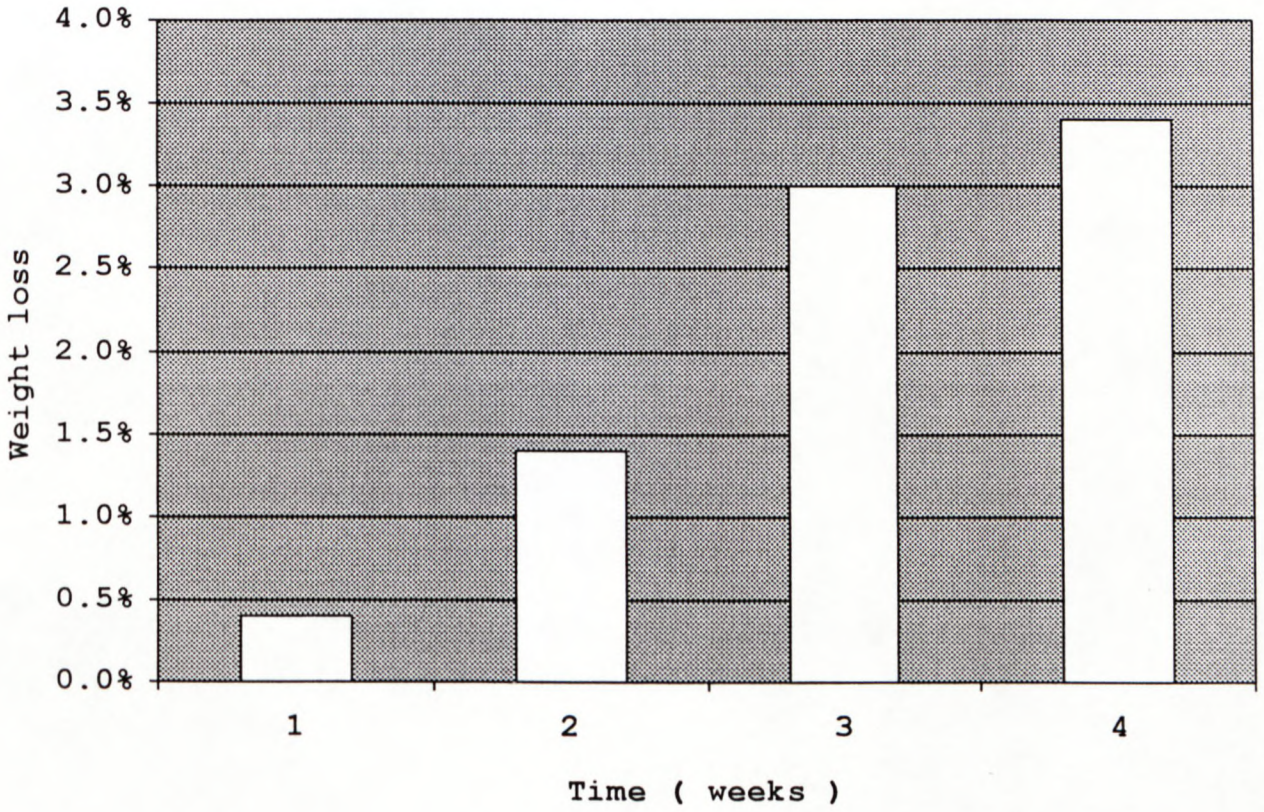


Figure 5.3

Degradation results for laboratory-prepared chips inoculated with BKMF-1767.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent;

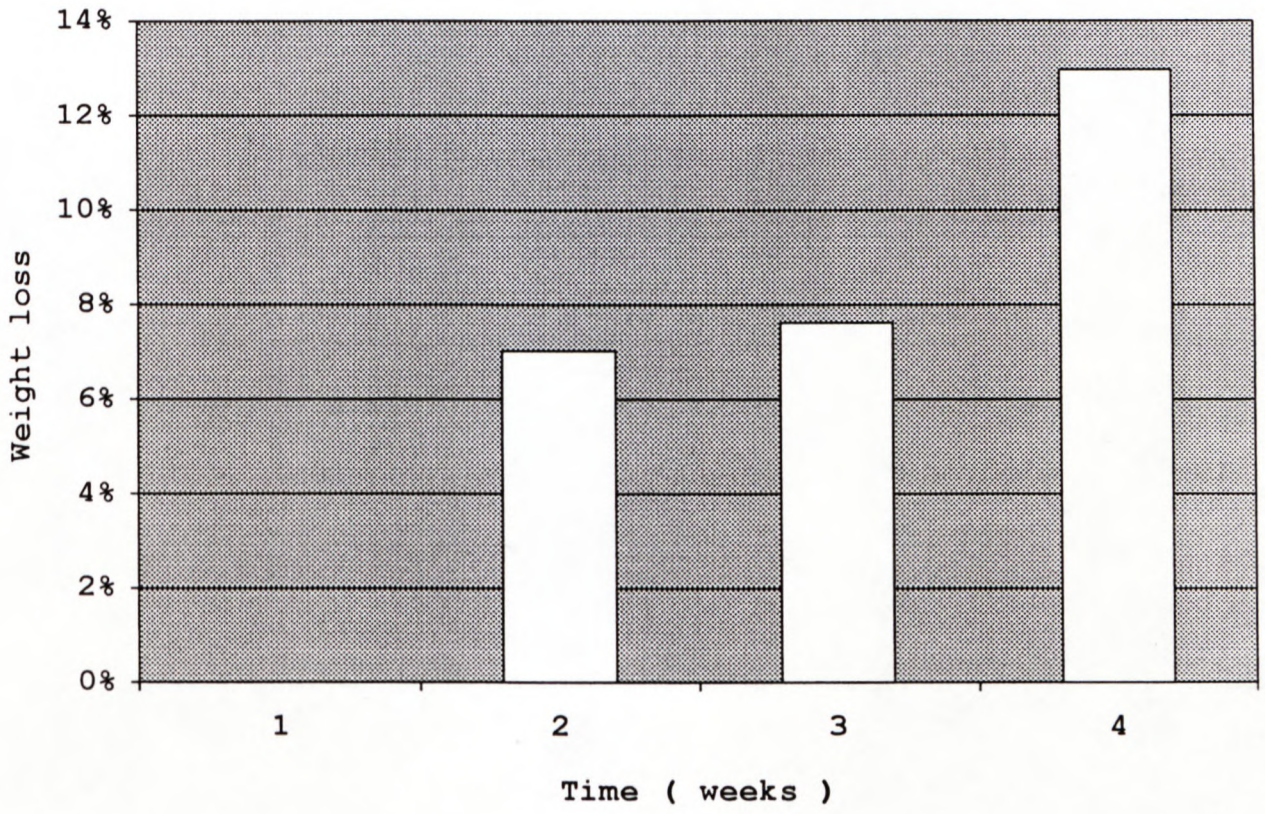


Figure 5.4

Spore count of conidia on 5-day old plate cultures of malt extract agar inoculated with BKMF-1767.

Measured at absorbance of 650 nm.

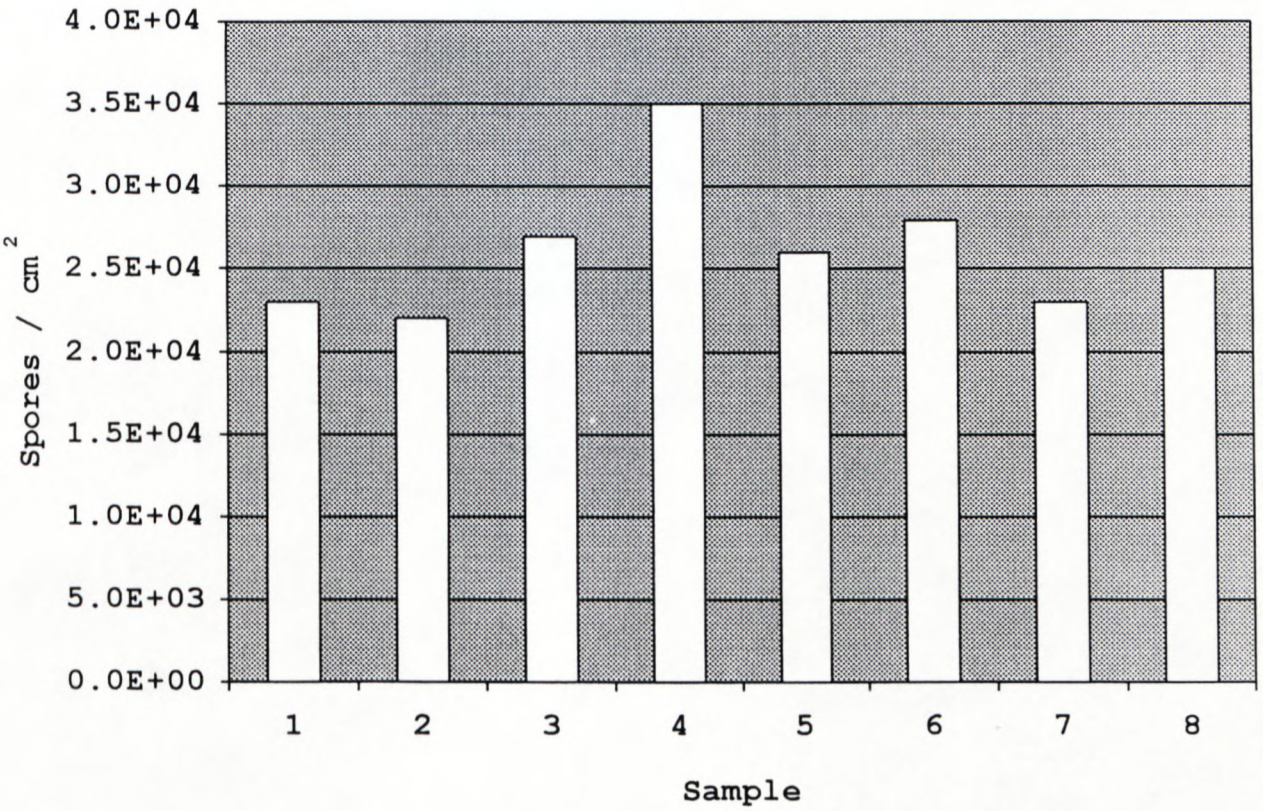


Figure 5.5

Spore count of conidia on laboratory-prepared chips inoculated with BKMF-1767.

W=5.00 g; 2360 < dp < 4750 µm; T=39°C; M=65 per cent;

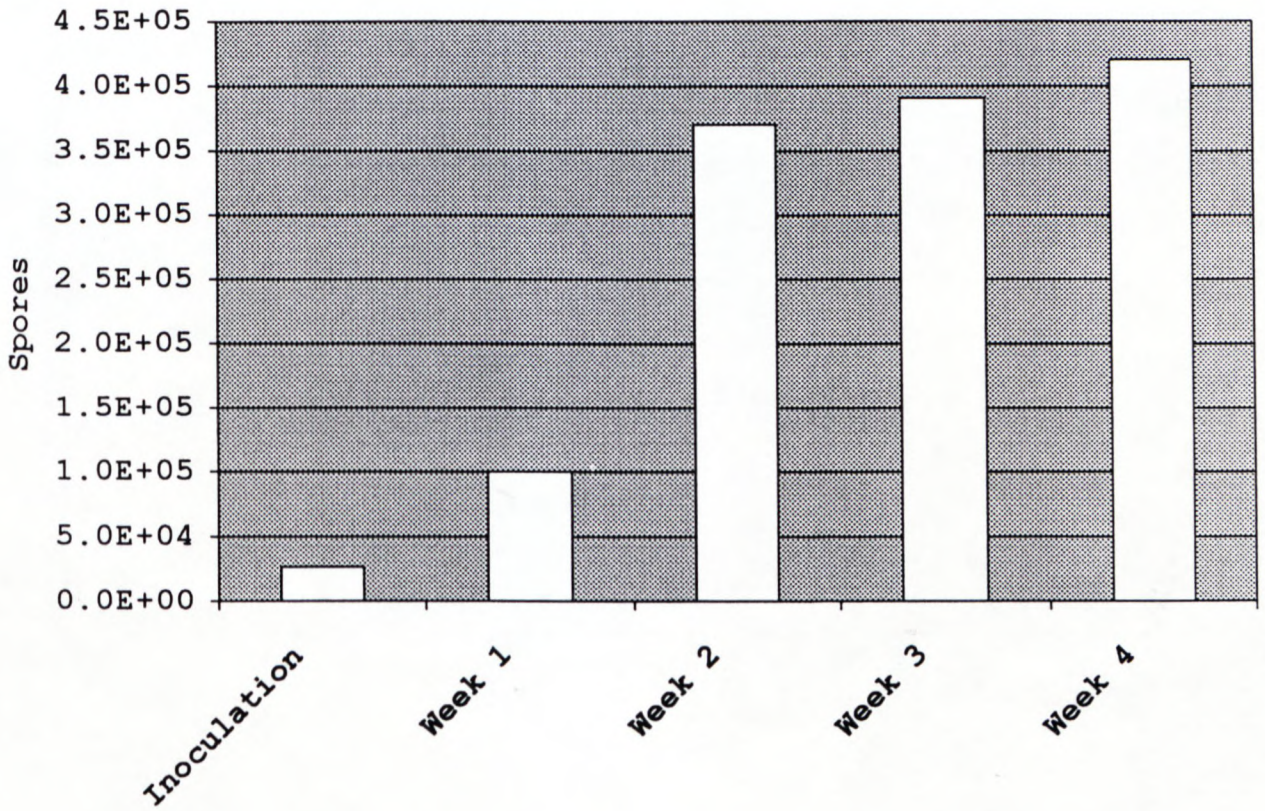


Figure 5.6

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Effect inoculation with greater spore numbers had on extend of weight loss.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent;

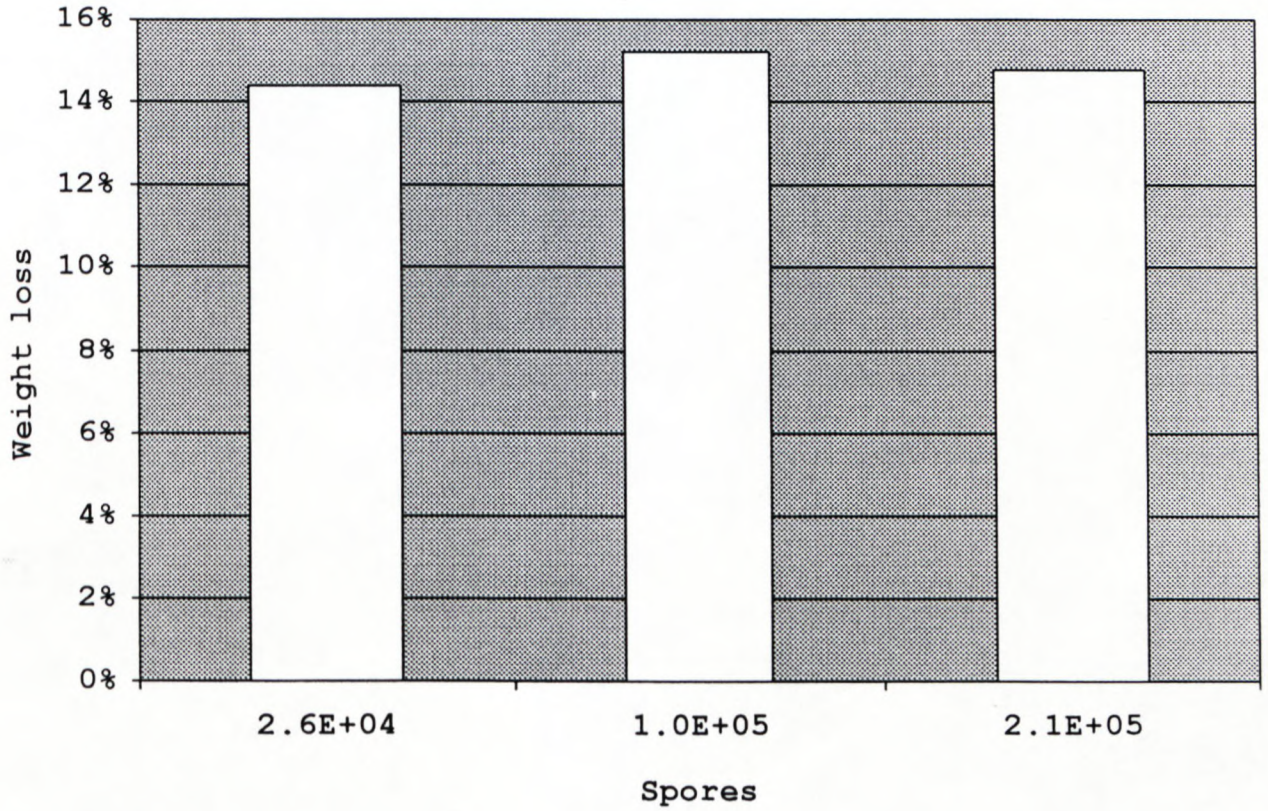


Figure 5.7

Degradation results after 4 weeks for laboratory-prepared wood chips inoculated with BKMF-1767 : Effect of particle size.

W=5.00 g; T=39°C; M=65 per cent;

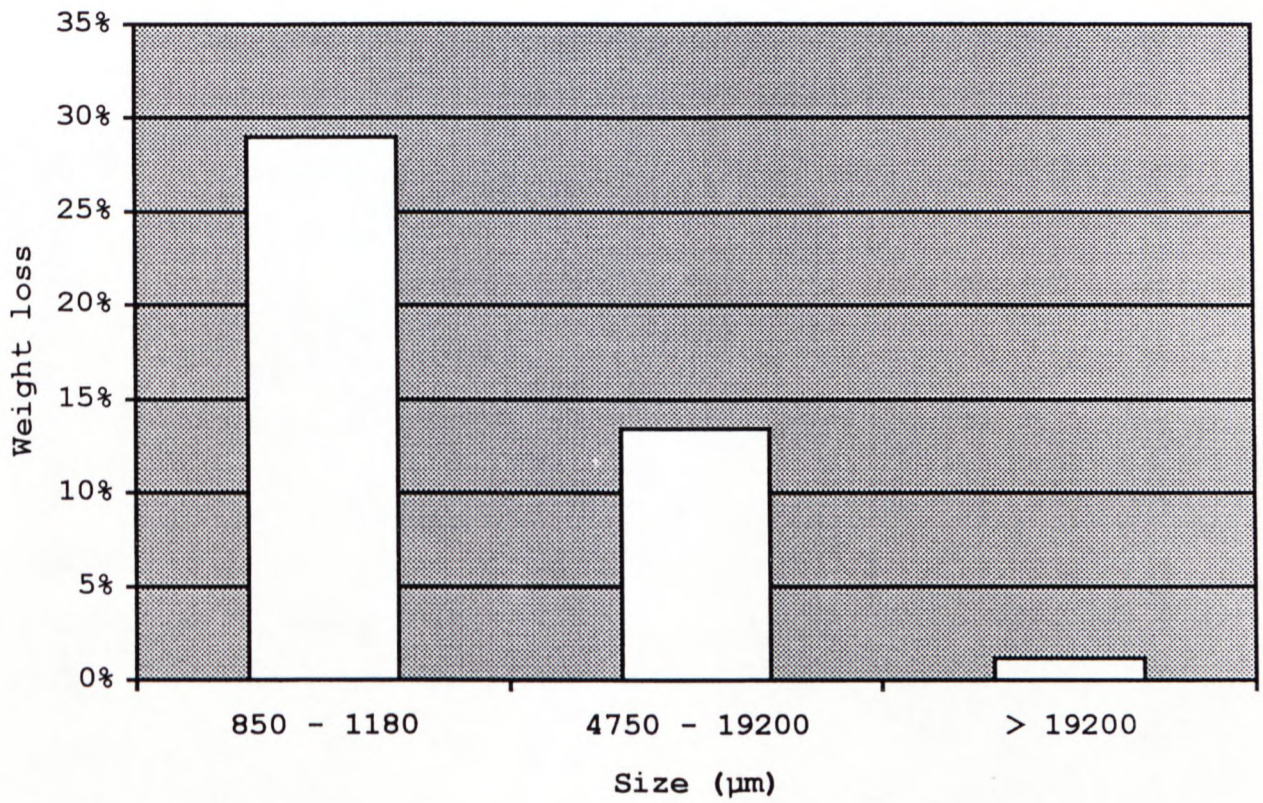


Figure 5.8

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Temperature studies.

W=5.00 g; 2360 < dp < 4750 μm; M=65 per cent;

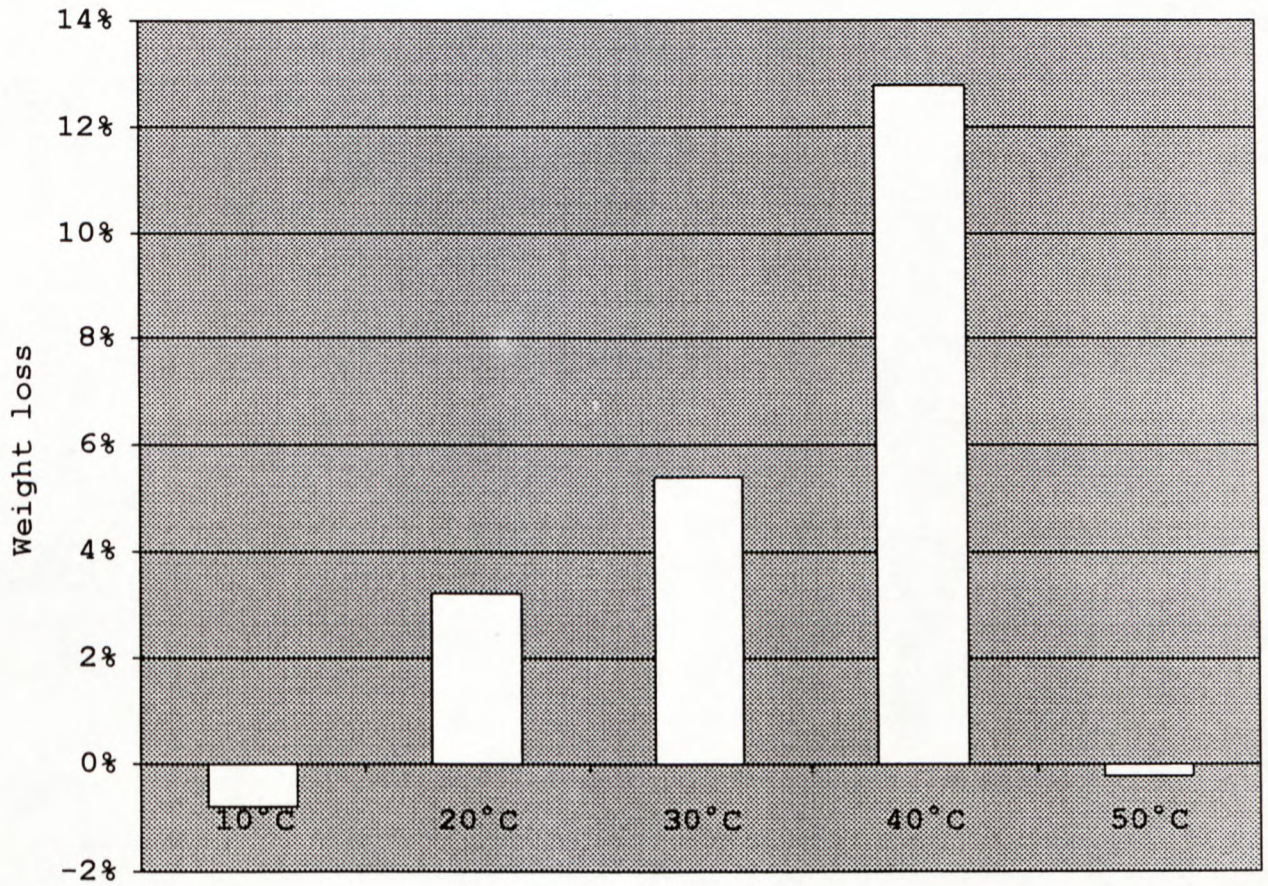


Figure 5.9

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Moisture studies.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C

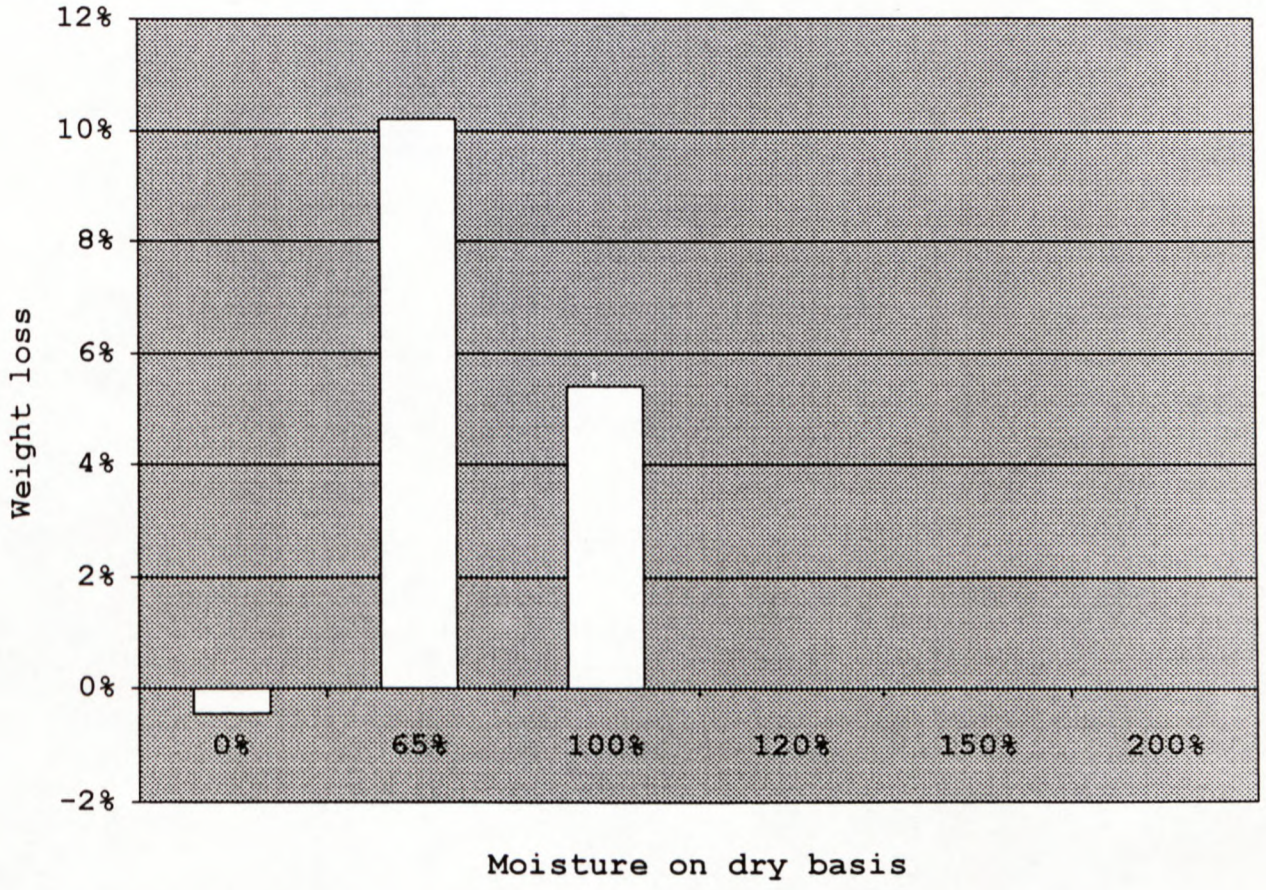


Figure 5.10

Degradation results after 4 weeks for laboratory-prepared chips inoculated with BKMF-1767 : Effect disruption of mycelial mat had on degradation.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent;

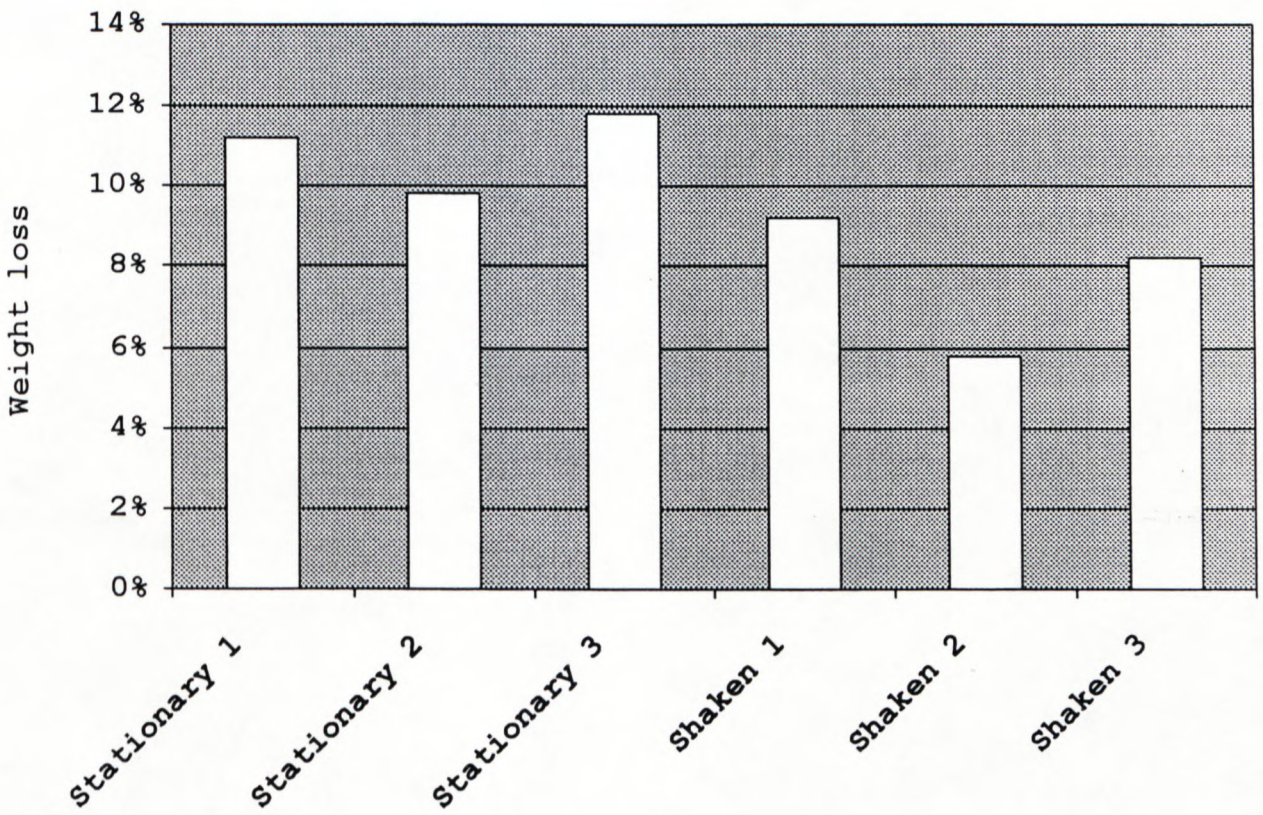
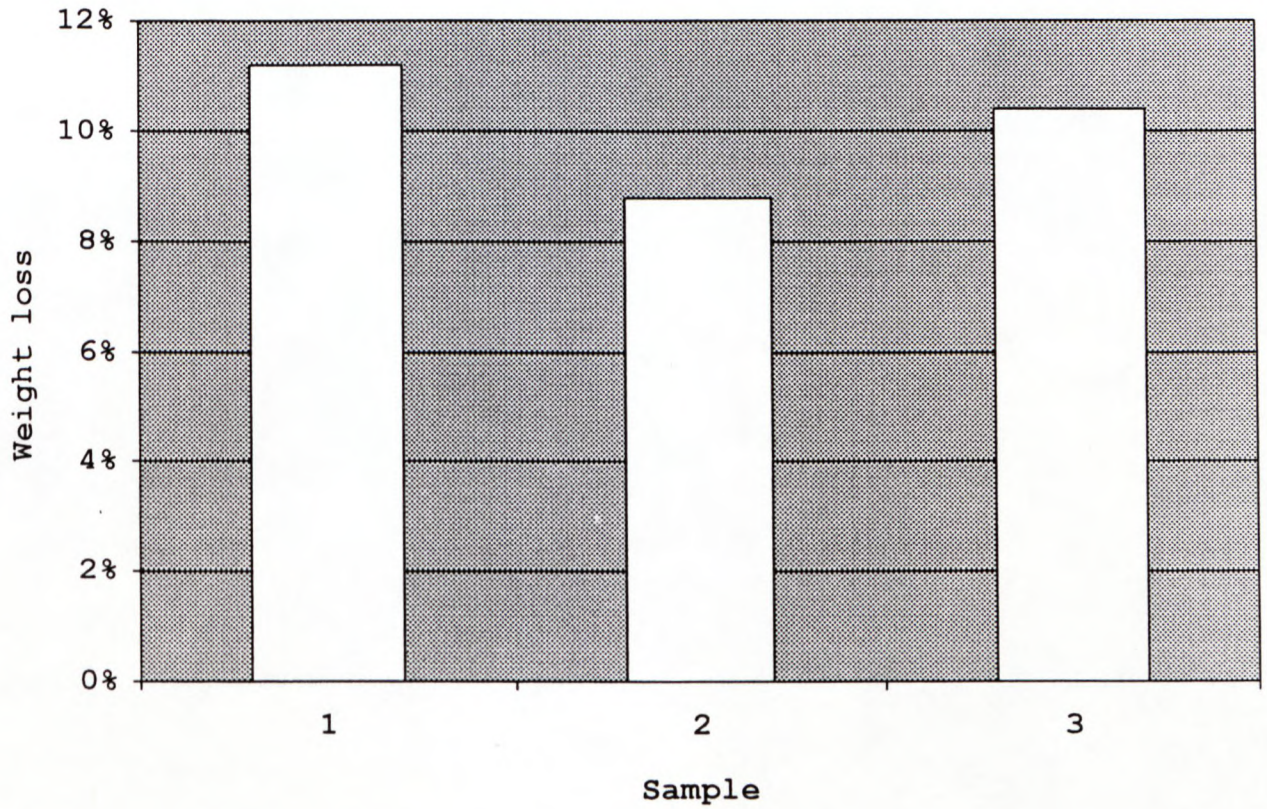


Figure 5.11

Degradation results after 4 weeks for refractory chips inoculated with BKMF-1767.

W=5.00 g; 2360 < dp < 4750 μm; T=39°C; M=65 per cent;



DEGRADATION OF WOOD CHIPS - A VISUAL INVESTIGATION 6

6.1 Introduction

Researchers have spend a lot of effort investigating the mechanisms by which the enzymes produced by *Phanerochaete chrysosporium* break down lignin. The focus of this paper has not so much been on the lignolytic system of *Phanerochaete chrysosporium* as it has been on the resulting effect caused by the enzymes to the wood structure. Already it was discovered that the particles caught in the matrix are in fact caught inside the wood cells and not lodged in the wood fibres. In order to improve an understanding of what the changes to the wood structure will be as a result of degradation and whether or not this will result in an 'opening-up' of the wood cells a visual investigation of the degradation process was launched.

6.2 Microscope Studies

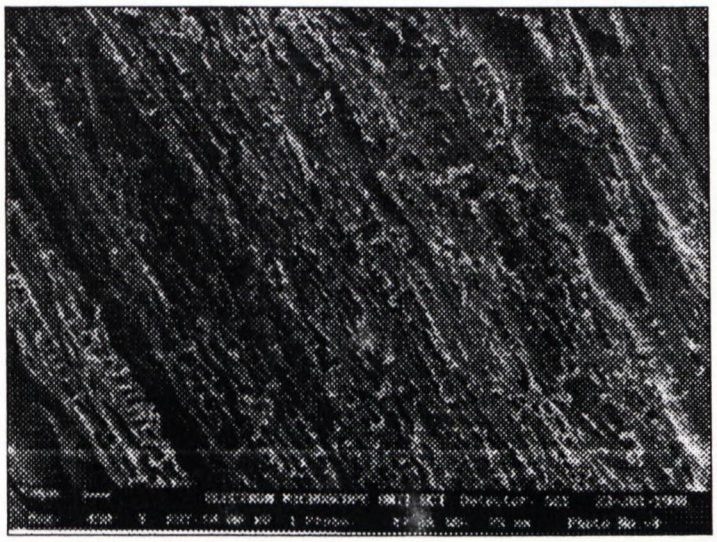


Figure 6.1

Throughout the course of our investigations all chips were kept in an oven to maintain a dry weight and prevent the growth of contaminating organisms before being removed for use. The unexposed chips were studied under an electron microscope and Figure 6.1 is one of the scanning electron microscope (SEM) images taken of a healthy wood surface. The surface appears regular and completely intact. The image will serve as a reference to compare other chips samples against that were exposed to the fungus.

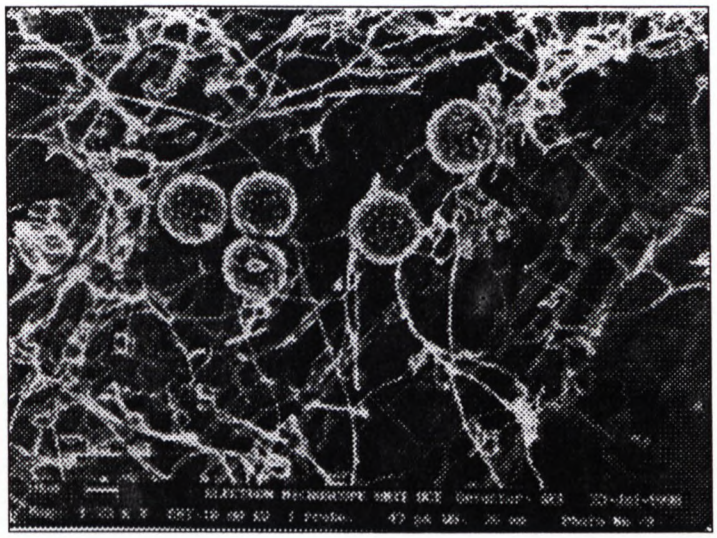


Figure 6.2

Figure 6.2 is another scanning electron image taken of the surface of a wood sample. This sample however was inoculated with spores from *Phanerochaete chrysosporium* strain BKMF-1767. The image was taken after the sample spend 1 week in incubation. By this stage fungal growth has spread over the wood surface and progressed to a thick mycelial web. The larger spherical shapes are sporangium the contents of which will be converted to an indefinite number of spores.

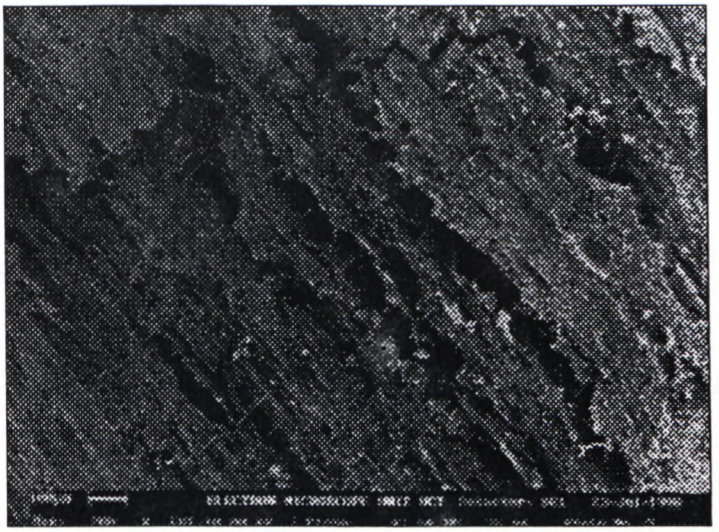


Figure 6.3

Figure 6.3 is an image taken of the surface of a wood chip removed from a sample that was incubated for 4 weeks following inoculation with spores from *Phanerochaete chrysosporium*. The sample was rinsed prior to taking the image to remove fungal growth from the surface and reveal the changes caused as a result of degradation. The irregular pockets and channels of rot are clearly visible when comparing the surface against that for the control shown as Figure 6.1.

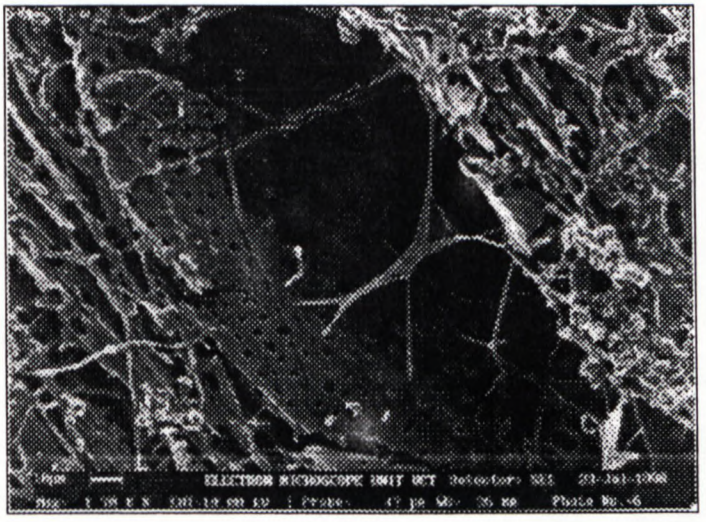


Figure 6.4

Figure 6.4 is a view into a pocket of rot showing fungal growth inside the pocket and strands of mycelium penetrating into the matrix through pits in the walls.

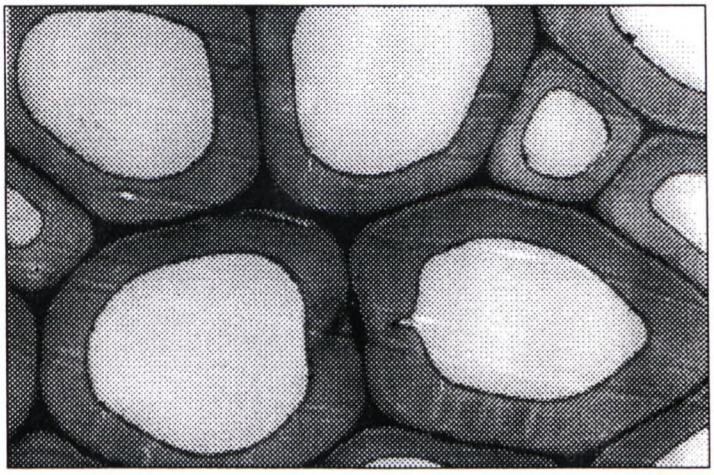


Figure 6.5

Figure 6.5 is a transmission electron image taken of healthy wood cells. The sample shown is of a control that was incubated for 4 weeks. It is clear from the state of the cells that no degradation took place.

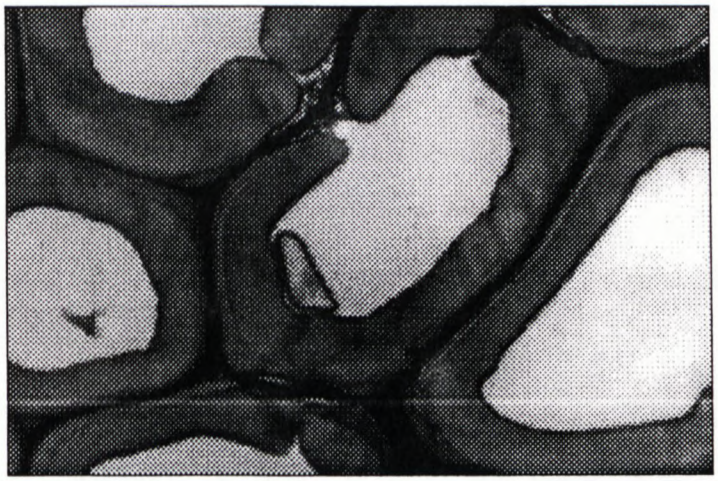


Figure 6.6

Figure 6.6 is an electron image taken of a sample that was inoculated and incubated for 1 week. A hyphae is seen where it penetrated into the lumen of a cell, but as yet degradation has not commenced in any of the cells shown.

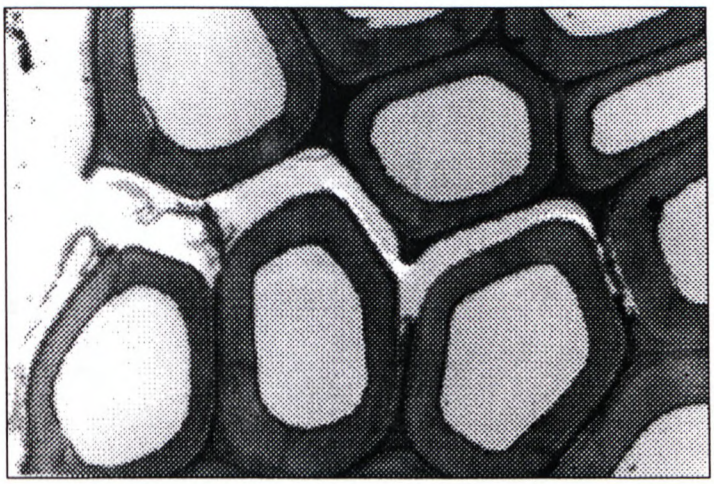


Figure 6.7

Preferential attack of lignin was observed with separation of adjacent cells after the lignin-rich middle lamella was degraded (Figure 6.7). Degradation seem to continue a distance from fungal penetration so that it seems physical contact between the hyphae and wood is not essential, but that the enzymes diffuse over a distance.

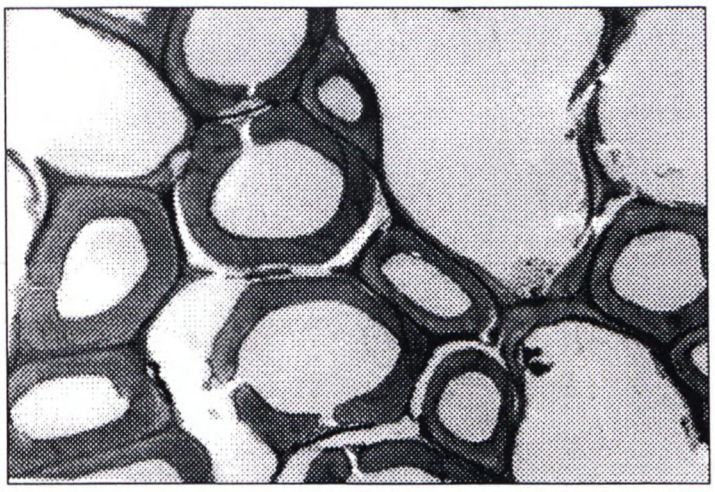


Figure 6.8

Figure 6.8 show advanced cell degradation, with many cells already completely degraded. Again note that while some cells remain intact their middle lamellas, which are lignin concentrated, have been removed.

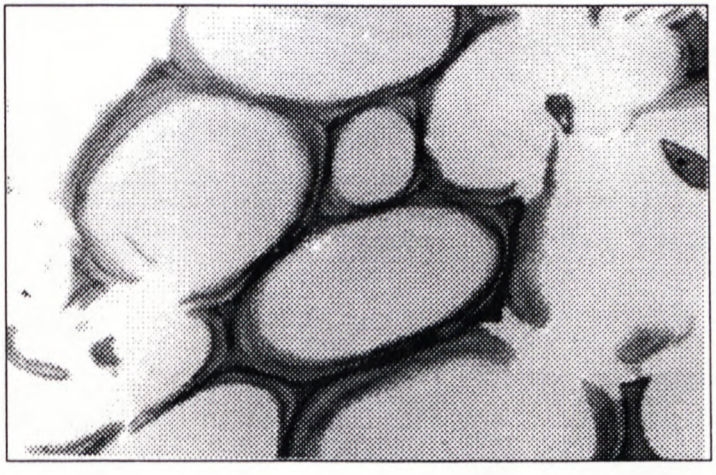


Figure 6.9

Figure 6.9 show wood cells in a very advanced state of degradation. Only what can be described as the "skeletal" parts of the cells remain.

CONCLUSIONS AND RECOMMENDATIONS 7

- The particle diameter of refractory chips from Vaal Reef Mine range from less than 850 μm to over 2 cm.

- EDS and WDS show a large amount of dust inside wood fibres and on the surface of refractory chips. The dust consists of quarts, pyrites and silicates, which confirm ore inside the wood matrix.

- Automated gold searches encountered discrete gold particles inside the wood matrix

- Pregrobbing contributes for less than 20 per cent of gold in refractory chips. The main source of gold is gold-bearing ore and discrete gold particles impregnated into the wood matrix by blasts, crushing and milling.

- TEM coupled with EDMA show encapsulated particles are mostly located inside wood cells. Gold dissolved from the ore can thus re-adsorb onto the surrounding fibres.

- There are a more than 44 mg/g carbon fines with refractory chips. The carbon is already loaded near capacity, but can still adsorb a significant amount of gold and contributes to the inefficiency of traditional cyanidation. The gold must be eluted from the carbon following cyanidation of the wood chips.

- Chemical analyses indicate the gold values of refractory chips to be between 2 and 5 mg/kg.

- Klason method of lignin determination gives the lignin content of fresh (virgin) laboratory prepared wood chips and refractory chips as 33 per cent and 40 per cent respectively. Ore and gold particles inside the wood matrix contributed the additional 7 per cent mass.

- Two strains of the white rot (lignin degrading) fungus *Phanerochaete chrysosporium* were screened. The strains are ME-446 and BKMF-1767. The latter was focused on since it caused the greater weight loss in samples of wood chips.

- Fungal growth appears on samples of wood chips 3 to 4 days after inoculation. White delignified areas appear the second week after inoculation and coincide with measurable reductions in weight.

- Inoculating samples with higher spore numbers do not necessarily result in increased weight losses over extended periods of treatment.

- The smaller the particle size of wood chips the larger the resulting weight loss as a result of fungal treatment. The particles should therefore be reduced to the smallest size possible prior to fungal treatment and subsequent gold recovery.

- Highest weight losses were obtained for samples incubated at 40°C and 65 per cent moisture.

- Despite the poor physical condition of refractory wood chips, weight losses compared favourable with that obtained for fresh (virgin) laboratory prepared chips treated under similar conditions.

- Microscope studies assisted in determining the growth and degradation patterns of the fungus. After mycelia attach to the surface it continues to penetrate through pits in the walls and can be seen to penetrate into wood cells. Lignin rich areas such as the middle lamella falls target to degradation first. Once the middle lamella that holds adjacent cells together has been removed the cells start to separate. Degradation was also observed to continue a distance away from where hyphae penetrated cells and indicates the enzymes diffuse over distances to break down lignin.

- Treatment with the white rot fungus *Phanerochaete chrysosporium* caused what is biologically considered to be an advanced state of degradation. However, treatment did not assist in what can be considered a significant improvement in gold yield.

- The final conclusion is that biological degradation with a white rot fungus does not provide a worthwhile alternative to conventional methods such as roasting. An aggressive cellulose degrading fungus might show more promise considering cellulose contributes almost 30 per cent more of the total woody mass than do lignin.

- At this time bio-degradation is not yet a feasible alternative to roasting. The recoveries after degrading the chips were only slightly better than it was for the untreated samples. Although lignin is the matrix material in wood, cellulose make up a larger percentage of the wood mass, around 40 to 55 per cent and a fungus that degrades cellulose might be needed more than one that removes lignin. If a cellulose degrading fungus does result in greater weight loss, it could be a better alternative in the pre-treatment of refractory chips as a means to improve gold recovery by cyanidation. For best degradation results, the use of *Phanerochaete chrysosporium* together with other organisms such as the before mentioned cellulose degrading fungi is worth further investigation.

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NOMENCLATURE

		Units
C	Liquid phase concentration	ppm
Ce	Equilibrium liquid phase concentration	ppm
Co	Initial liquid phase concentration	ppm
dp	Particle diameter	μm
M	Moisture on a dry basis	%
q	Gold loading	mg/kg
qe	Equilibrium loading	mg/kg
qo	Initial loading	mg/kg
T	Temperature	°C
V	Volume of solution	L
W	Weight (mass)	g