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Review

Bioremediation of textile effluent using *Phanerochaete chrysosporium*

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Enormous volumes of effluent are generated at different stages of textile manufacturing, as a result of the use of copious amounts of chemicals and dyes. Several tons of textiles required to meet up with societal demands are produced daily in this industry. Effluent derived from the textile and dyestuff activities can provoke serious environmental impact in the neighboring receptor water bodies because of the presence of toxic reactive dyes, chlorolignin residues and dark coloration. Nature has demonstrated its capacity to disperse, degrade, absorb or otherwise dispose of unwanted residues in the natural sinks of the atmosphere, waterways, ocean and soil. It is realized however that this ability is not finite. The discharge of these waste residues into the environment eventually poison, damage or affect one or more species in the environment, with resultant changes in the ecological balance. The biological breakdown of the chlorolignin residues and the chromophoric groups responsible for the dark coloration of the textile effluent can be accomplished by the use of enzymes from the white rot fungus, *Phanerochaete chrysosporium*. The siderophores detected from the culture of the organism have been found useful in the decolourization and remediation of the effluent. This review summarizes the available information in the use of this fungus for bioremediation purposes and also assesses the current status of the technology.

Key words: Bioremediation, textile effluent, *Phanerochaete chrysosporium*, white rot fungi, peroxidase enzymes.

INTRODUCTION

The world's ever increasing population and her progressive adoption of an industrial– based lifestyle has inevitably led to an increased anthropogenic impact on the biosphere. In textile production, opportunities exist for the release into the ecosystem of potentially hazardous compounds at various stages of the operation. These pollutants are produced in an effort to improve human standard of living and fashion but ironically, their unplanned intrusion into the environment can reverse the same standard of living by impacting negatively on the environment.

Textile effluents can seep into the aquifer and pollute the underground water, or where it is discharged without proper treatment into water bodies, the pollutants cannot be confined within specific boundaries. They can therefore affect aquatic life in enormous ways. Metallic effluents can have ecological impacts on water bodies leading to increased nutrient load especially if they are essential metals. These metals in effluent may increase fertility of the sediment and water column and consequently lead to euthrophication, which in open waters can progressively lead to oxygen deficiency, algal bloom and death of aquatic life (Purdom and Anderson, 1980). Water contaminated with metallic effluent can cause several health problems. Lead for instance, can

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interfere with enzyme activities and formation of red blood cells. It can affect nerves and brain at low concentration. Heavy metals such as mercury, cadmium, and chromium can bio-accumulate, and through the food chain, to toxic levels in man. Mercury can cause numbness, locomotor disorders, brain damage, convulsion, and nervous problems. Cadmium is responsible for kidney tubular impairment and osteomalacia. Cadmium, zinc and manganese are reported to affect ion regulation if present in sufficient concentrations (Howells, 1990). Cadmium and manganese are also known to affect calcium metabolism, development and skeletal calcification as well as long term effect on spawning and recruitment of fish and other aquatic lives. Any change in pH of water bodies as a result of influx of effluent, can cause serious change in water chemistry, which can affect resources especially around the coastal areas. These effects on water bodies can be very significant. Dyes in water bodies also affect photosynthetic organisms and consequently impact negatively on the food chain. The reason for this global effect also lies in the weather systems and the biogeochemical cycling of elements, which aid in rapid dispersal of the pollutants.

Traditional methods for the clean up of pollutants usually involve, the removal of unwanted materials through sedimentation and filtration, and subsequent chemical treatments such as flocculation, neutralization and electro-dialysis before disposal. These processes may not guarantee adequate treatment of the effluent. Moreover, they are often laborious and expensive, considering the volume of wastes released during the industrial production process.

The past two decades have seen a tremendous upsurge in the search for cost effective and environmentally sound alternatives to the conventional methods for dealing with wastes. The technologies that have emerged as most promising are those that closely mimics the time tested, natural system that have restored environments to their original status following undesirable perturbations. In fact the self-restoring process in nature is what has actually given birth to the concept that the self- cleansing ability of nature is infinite. Of all the technologies that have been investigated, bioremediation has emerged as the most desirable approach for cleaning up many environmental pollutants in effluents.

Bioremediation uses living systems especially microorganisms to catalyze the degradation of wastes without disruption of the environment. Fungal systems appear to be most appropriate in the treatment of colored and metallic effluents (Ezeronye and Okerentugba, 1999). In the world of fungi, *Phanerochaete chrysosporium* has emerged a model system in textile, polycyclic aromatic hydrocarbon (PAH), and pulp and paper mill effluent remediation. *P. chrysosporium* is a basidiomycete fungus able to degrade complex compounds such as starch, cellulose, pectin, lignin, lignocelluloses, which are characteristics of textile effluent. It can also decolorize *azo-tiphenyl* methane dyes by its lignin peroxidase enzyme. *P. chrysosporium* is capable of producing extracellular enzymes such as manganese peroxidase, effective in decolorization of textile effluent. This fungus appears to be the best candidate, so far studied, in textile effluent treatment *in situ*. There is no doubt therefore, that if properly harnessed, the fungus can be very useful in the treatment and disposal textile and related effluents.

GENERATION AND COMPOSITION OF TEXTILE EFFLUENTS

The basic raw materials in textile are cotton, flax, jute, wool, silk and synthetic polymers fibers such as nylon, polyesters, polyethylene and rayon (Dara, 1993). The cellulosic textiles are composed of pure cellulose and can be categorized as:

1. Natural cellulosic fibers, which include cotton, abaca, coir, flax hemp, henequen, jute and sisal.
2. Man- made cellulosic fibers, which are cuprammonium, polynosic and viscose.

Cotton (*Gossypium spp*) is a fiber crop, which belongs to the plant family, Malvaceae. It produces cotton lint, a white fiber used in the textile industries. Cotton fiber is a single plant cell. Its cross-section is oval, compared with the normal hexagonal plant cell. However, like small plant cells, cotton have a distinct cuticle, well-developed primary and secondary walls and lumen. The cuticle is the 'skin' of the cotton fiber. It is composed of a waxy layer (cotton wax) only a few molecules thick. The inert nature of this cotton wax protects the rest of the fiber against chemical and other degrading agents. Bleaching during cotton finishing removes much of the cuticle or wax (Gohl and Vilensky, 1987). This enables cotton to absorb moisture more quickly. Subsequently, laundering gradually removes most of the remaining cuticle. The primary cell wall, which is immediately underneath the cuticle, is composed of very fine threads of cellulose, called fibrils. The secondary cell wall forms the bulk of fiber. Concentric layers of the spiraling cellulosic fibrils make up the secondary wall. Much of the strength and stability of the cotton fiber and hence, of yarn and fabrics, are attributed to these spiral fibrils. The cotton polymer is a linear cellulose polymer with repeating units of cellobiose, which consists of glucose. The polymer consists of about 5000 cellobiose units (Gohl and Vilensky, 1987). The fibers are resistant to alkalis and relatively unaffected by normal laundering.

Flax fiber is classified as a natural cellulose multi-fiber. 'Linen' is the term applied to the yarn spun from flax

fibers and to the cloth or fabric woven from this yarn. Flax polymer is chemically the same as the cotton polymer both are cellulose polymers. Viscose is a man-made natural polymer of cellulose or regenerated cellulose filament. Thus cotton, flax and viscose are all cellulosic in nature. Other textile fibers composed of natural protein include wool, silk, mohair and cashmere. Synthetic textile fibers on the other hand are composed of synthesized polymers not found in nature such as acrylic, nylon, polyester, and polyethylene.

The various operations involved in cotton textile mill are combing, spinning, sizing, weaving and knitting. The seeds are removed from cotton (combing) before the cotton is carded (i.e. made fluffy) and before they are spinned. The spinning wheel makes the cotton into thread (yarn) before they are dyed into different colors for weaving different patterns to make clothing, curtains, carpet and many other products. All these are dry processes except sizing. The 'grey cloth' obtained after the above operations is subjected to the various wet treatment processes such as desizing, scouring, bleaching, mercerizing, dyeing or printing and finishing. All these processes generate considerable volumes of effluents, which contain such chemical substances as dyes, alkalis, chromium, phenol, oils and waxes.

TREATMENT OPTIONS FOR TEXTILES WASTES

The dyes present in textile effluent impart persistent color to the receiving streams and interfere with photosynthesis of the phytoplankton (Cunningham and Saigo, 2001). Other physical characteristics of the wastewater include odor, change in dissolved oxygen, presence of insoluble substances and corrosive properties. The colloidal and suspended purities cause turbidity in the receiving streams. The dissolved minerals may increase salinity of the water and thus may render it unfit for irrigation or consumption. Toxic chemicals such as chromium and sulphites may destroy fishes and microorganisms responsible for self-purification of water in streams. Immediate oxygen demand due to the impurities such as starch, sulphites, nitrites, deplete the dissolve oxygen content of water. Starch cotton debris constitute organic wastes which are oxygen demanding. They can undergo decomposition/degradation by bacterial activity. The chemicals use in the processes may change pH of the effluent and once disposed into the water body affects aquatic lives. Dissolved solids can also form incrustations on the surfaces of sewers and chemicals may cause corrosion of the metallic parts of the sewage treatment plants.

Thus, effluents that emanate from the production process of textiles, if not properly disposed, can cause serious environmental pollution, sometimes to levels that can threaten human health, livestock, wildlife, aquatic lives and indeed the entire ecosystem. Every production process goes with wastes generation. Various treatment options are available for treatment of textile wastes before disposal. Traditional disposal method such as ocean dumping is now out of place following numerous incidents of severe negative impacts on the environment after years of disposal. Typical examples are the Love Canal episode of the Niagara Falls in the United States of America and the Mina Mata Bay experience in Japan where several tons of mercury was discharged through effluent into the bay and the inhabitants suffered the effect after over thirty years. There are physical and chemical methods, which, in spite of costs, do not always ensure that the contaminants are completely removed (Hardman et al., 1993). In recent times there has been a tremendous upsurge in the search for cost- effective and environmentally friendly alternatives to traditional methods for dealing with wastes.

Of all the technologies investigated in waste cleaning, bioremediation has emerged the most desirable approach for cleaning up many environmental pollutants. Bioremediation is a pollution control technology that uses biological systems to catalyze the degradation of or transformation of various toxic chemicals to less harmful forms. The general approaches to bioremediation are to enhance natural biodegradation by native organisms (intrinsic bioremediation), to carry out environmental modification by applying nutrients or aeration (bio-stimulation), or through addition of microorganisms (bioaugmentation) (Ashoka et al., 2002). Bioremediation is similar to the use of plants to restore contaminated sites (Phytoremediation). The ability of microorganisms to transform a variety of chemicals has led to their use in bioremediation processes. A number of microorganisms have since been studied to unfold their degradative abilities in remediation of pollutants.

Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bioremediation (Glazer, 1997). Bacteria generally are easier to culture and they grow more quickly than fungi. They are more amenable to molecular genetic manipulations. They are able to metabolize chlorinated and other organic contaminants such as oil and mineralize chemicals using them as carbon or energy source (Glazer, 1997). Diverse fungal cultures have been investigated recently for bioremediation processes (Aust, 1990; Tien and Kirk, 1983; Bumpus and Aust 1993; Akamatsu et al., 1990). By virtue of their aggressive growth, greater biomass production and extensive hyphal reach in the environment, fungi have been seen to perform better than bacteria. The high surface-to-cell ratio of filamentous fungi makes them better degraders under certain niches (Ashoka et al., 2002).

BIOREMEDIATION USING *PHANEROCHAETE CHRYSOSPORIUM*

The basidiomycete, *P. chrysosporium*, belongs to the white rot class of wood-rotting fungi. It produces different extracellular enzymes involved in lignin degradation. The first extracelular enzyme discovered to depolymerize lignin and lignin-substructured compounds *in vitro* was produced by this organism. The enzyme has been described variously as 'ligninase', 'diary/propane oxygenase' and 'lignin peroxidase' (Aitken and Irvine, 1989). A second class of enzyme also produced by *P. chrysosporium* is manganese peroxidase. Because of the requirement of this fungus for divalent manganese in carrying out peroxidase reactions, this enzyme is known as manganese- dependent peroxidase or manganese peroxidases (MnP) (Aitken and Irvine, 1989). Manganese is known to catalyze several oxidation reactions important in lignin degradation, including decarboxylation and demeth(ox)ylation of aromatic substrates. It plays an important role in the degradation of phenol units and non-phenol units, acting together with lipids. In contrast, lignin peroxidase degrades only the non-phenol units and acts with the hydrogen peroxide (Hatakka, 2001). Lignase actually comprises a series of isoenzymic peroxidases.

The need for manganese in the MnP enzyme results from the enzyme's ability to catalyze the oxidation of Mn(II) to Mn(III) in the presence of Mn(III) stabilizing ligands. The resulting Mn(III) complexes can then oxidize the organic substrate (Hatakka, 2001). These enzyme systems are responsible for the aggressive decomposition of lignin by *P. chrysosporium*. Mn-peroxidase can catalyze the oxidation of several aromatic dyes (Barr and Aust, 1994) and monoaromatic phenols, (Barr and Aust, 1994) but these reactions depend on the existence of specific reaction conditions which includes the presence of both divalent Manganese and certain types of buffers (pH and redox potentials) (Atlas and Bartha, 1998).

P. chrysoporium has also been shown to mineralize a variety of recalcitrant aromatic pollutants. Liginase has been shown to catalyze limited oxidation of benzo-α-pyrene and other polycyclic aromatics, as well as a number of phenolic pollutants (Aitken and Irvine, 1987). The fungus can degrade various other xenobiotics such as polyaromatic hydrocarbons and chlorinated aromatic compounds, and also pollutants, which are covalently bound to humic substances (Pointing, 2001). Humic substances consist of aromatic rings connected by flexible and rather long aliphatic chains (Tuomela, 2002). This structure is formed by oxidative ring opening lignin, loss of phenolic and methoxyl groups and an increase in carboxyl and carbonyl group. Humic substances are thus less aromatic and have fewer methoxyl and more carboxyl groups than lignin. During the degradation of xenobiotics, the white rot fungi often polymerize or convert substantial amounts of compounds to humic bound products.

The white rot fungi technology is very different from other well-established methods of bioremediation (e.g. bacterial systems). The differences are primarily due to the unusual mechanisms which nature has provided them with and several advantages for pollutant degradation. One distinct advantage these fungi have over bacterial systems is that they do not require preconditioning to the particular pollutant. Bacteria usually must be pre-exposed to a pollutant to allow the enzymes that degrade the pollutant to be induced. The pollutant also must be in a significant concentration; otherwise, induction of enzyme synthesis cannot occur. Thus, there is a finite level to which bacteria can degrade pollutants. Also because the induction of the degrading enzyme is not dependent on the pollutant in the fungi, the pollutant can be degraded to a near non-detectable level. In contrast to the bacterial system, the degradative enzymes of white rot fungi are induced by nutrient limitation. Thus, cultivation of the white rot fungi on a nutrient-limited substrate, will initiate the process (Tuomela, 2002; Aust, 1995).

The basidiomycete produce different other extracellular enzymes involved in pollutant degradation. They use a variety of mechanisms to accomplish the complete degradation of lignin and a wide variety of other environmental pollutants. The fungi secrete a family of peroxidases to catalyse both direct an indirect oxidation of chemicals. The peroxidases can also catalyze reductions using electron donors to generate reductive radicals. The general, biodegradative ability of this fungus is related to the ability of the fungus to degrade lignin. This ability, which is unique to the group of fungi, is thought to be dependent on the family of peroxidases they secrete (Tien and Kirk, 1984). Through this, they can solubilize highly polymeric substances more complex than lignin (Barr and Aust, 1987). The fungus that degrades lignin is also able to degrade xenobiotics because their enzyme system is unspecific due to the heterogeneous nature of the lignin polymer and because xenobiotics are often aromatic and thus resemble lignin or its degradation products (Orth et al., 1994; Paszozynski and Crawford, 1995; Pointing, 2001). In general, white rot fungi have been found to degrade or oxidize polycyclic aromatic hydrocarbons, chlorinated organic compounds, polychlorinated biphenyls, nitro-substituted compounds, fluorinated aromatic compounds munitions waste, such as trinitrotoluene (TNT), synthetic dyes, synthetic polymers (plastics) and are humic bound synthetic compounds (Fernando and Aust, 1994; Orth et al., 1994; Paszozynski and Crawford, 1995; Pointing, 2001). The lignin peroxidases are somewhat unique in that they have higher oxidation potentials than do most peroxidases (Aust, 1995; Mills et al., 1989). In this way these enzymes have somewhat greater range of chemicals that they can oxidize.

In a somewhat analogous way, some chemicals are first reduced before they can be oxidized by the lignin peroxidases. There seem to be several mechanisms by which this can occur but most significantly, this must be the manner in which the fungi mineralize a number of highly oxidized chemicals such as TNT (2,4,6-trinitrotoluene) and DDT [1,1,1-trichloro-2,2-bis (4-chlorophenyl) ethane]. One method is the indirect oxidation via the cation radical of veratryl alcohol. For compounds that may have sufficiently low oxidation potential but are apparently without access to the heme of lignin peroxidases, indirect oxidation occurs upon inclusion of veratryl alcohol. This mechanism involves oxidation, but many chemicals that are oxidized by white rot fungi are already highly oxidized. For instance, TNT is oxidized by white rot fungi (Stahl and Aust, 1993) but TNT is an oxidant not a reductant. Its metabolism by all other organisms involves reduction, primarily by nitro-reductases (Stahl and Aust, 1993; Parrish, 1977). It is however presently not clear whether *P. chrysosporium* produces nitro-reductases but the mycelium of *P. chrysosporium* is found to reduce TNT (Stahl and Aust, 1993). The fact that TNT is reduced externally by membrane potential of this fungus has significance for the detoxicification of TNT and for the design of TNT bioremediation systems based on the fungus. TNT can be oxidized by Mn-dependent peroxidases subsequent to reduction, and then be mineralized when lignin peroxidases are produced (Stahl and Aust, 1993).

An awareness of environmental problems and potential hazards caused by industrial wastewater has prompted many countries to limit the discharge of polluting effluents into receiving waters (Ezeronye and Okerentugba, 1999; Okerentugba and Ezeronye, 2003; Ezeronye and Ugbogu, 2004; Ezeronye and Ubalua, 2005). Textile manufacturing yields a large quantity of black and highly toxic wastewater that contains high concentration of chromium, phenolics, suspended solids, and high biochemical oxygen and chemical oxygen demands, sulphide azo and diazo compounds (FEPA, 1991). The biodegradative capacities of *P. chryosporium* are remarkable. Of all the white rot fungi, this organism is the most studied and it has emerged a model in bioremediation of toxic, recalcitrant and colored effluent. The organism is unique both in terms of the number of different chemicals involved and its degredative ability. *P. chrysosporium* has been shown to be effective in removing color from textile-dye effluents of wastewater (Ashoka et al., 2000). Unfortunately, due to the complex mechanisms involved in its biodegradative mechanism, the technology has been slow to emerge. Apart from the mechanisms involved in the degradation, the physicochemical parameters of the effluents are very important. Stability testing of lignase and Mn-peroxidase reveals at least three components of inactivation:

1. Thermal inactivation.
2. Rapid, partial inactivation attributable to hydrogen peroxide.
3. And a slower inactivation caused by hydrogen peroxidase.

Thermal inactivation is common to all enzymes. In most cases, thermal inactivation is a direct result of protein denaturation, a disruption of forces responsible for maintaining the tertiary structure of the enzyme. Experiments show that a number of variables besides temperature influence the rate of thermal inactivation of lignase in *P. chrysosporium.* These variables include pH, enzyme concentration, and the presence of veratryl alcohol, manganese and hydrogen peroxide (Aitken and Irvine, 1989). Results of some experiments suggest that hydrogen peroxidase is only responsible for lignase inactivation in the absence of an oxidizable organic substrate (Aitken, 1988). Haemmerli et al. (1987) noted that residual activity of lignase could increase if the enzyme is incubated in the presence of veratryl alcohol (3,4-dimenthoxybenzyl alcohol) a secondary metabolite of *P. chrysosporium* and a substrate for the ligninase. Ligninase inactivation is also known to occur in the course of oxidation reactions, which has been shown to limit the extent of target compound removal in phenol oxidation studies. In the laboratory this can be overcome by manipulating reaction condition specifically by controlling the rate of addition of hydrogen peroxide. Ligninase activity is known to increase with decreasing pH, while rapid enzyme inactivation occurs at a pH near optimum. Thus, stability increases as both pH and enzyme concentration increase.

Unlike ligninase, Mn-peroxidase inactivation increases at a higher enzyme concentration and the effect of hydrogen peroxide on enzyme inactivation is more significant for ligninase and addition of protein can greatly enhance Mn-peroxidase stability. This may be due to a slower rate of reversible denudation at higher concentration of protein (Aitken and Irvine, 1989).

It is clear that the biodegradative activity of *P. chrysosporium* is a complex one. Understanding the mechanisms of the biodegradation role of this fungus is very important if one must explore the unique enzyme system in it for remediation of colored and complex, toxic effluents. The stability of the enzymes in relation to the physicochemical nature of the effluents is an important factor in evaluating both technical and economic feasibility of using this organism commercially in bioremediation projects. Thus, the rate of enzyme inactivation is an important component of the overall kinetics of any proposed enzymatic process. The understanding of these mechanisms has actually been a drawback in the technology and the deployment of this fungus widely in bioremediation. However, continuous research will eventually close the present gap in

knowledge about the use of this organism. This paper is written in the hope that it would stimulate interest and investigations into the development and adoption of biotreatment of colored and toxic effluents in developing countries using the model system of *P. chrysosporium.*

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