

“Degradation of Lignin through Carbon Utilization by the Microbial Ligninolytic Enzymes for Environmental Management”

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Abstract: Lignin is a class of complex organic polymers. Lignins are one of the main classes of structural materials in the support tissues of vascular plants. The lignin degradation indicated that the presence of ligninolytic enzymes could enhance the activity of microorganisms. Lignin is degraded by microorganisms including fungi and bacteria. Lignin peroxidase is a hemoprotein firstly isolated from the white-rot fungus *Phanerochaete chrysosporium* with a variety of lignin degrading reactions, all utilizing hydrogen peroxide as an oxygen source. Lignolytic enzymes have a capacity to degrade the pesticide and it have a potential applications in a large number of fields, including bioremediation, biofuels, food agriculture, paper and pulp textile finishing, denim store washing, cosmetics biosensors and many others.

Keywords: Lignin, Lignolytic Enzymes, Hemoprotein, Biofuels, Biosensors.

I. INTRODUCTION

Lignin is a complex polymer and its degradation takes a number of years. It constitutes the second largest sink next to cellulose for fixed carbon therefore lignin biodegradation occupies a significant portion in the global carbon cycle (Eriksson *et al.*, 1990). Preventative measures as part of a good management program is the first step in reducing pesticide contamination. The sources of this contamination can be broadly referred to as point sources and diffuse sources. Point sources are instances whereby a leak or spillage of a pesticide has resulted in contamination of the environment at a specific point, usually at a high concentration. Diffuse sources are losses of the pesticide into the environment after application through such routes as volatilization, surface runoff and leaching. Ligninolytic fungi or lignin degrading fungi (such as white rot fungi) access their main carbon source ligninocellulose by breaking down the lignin that protects it. They do this by producing enzymes designed to co-metabolically degrade the complex recalcitrant structure of the lignin (Leonowicz *et al.* 2001). The enzymes are extracellular and nonspecific thus they have the ability to degrade a wide variety of organic substances that share features with lignin. Lignocellulose refers to plant dry matter (biomass), so called lignocellulosic biomass. It is the most abundantly available raw material on the earth. It is composed of carbohydrate polymers (cellulose, hemicellulose), and an aromatic polymer (lignin). These carbohydrate polymers contain different sugar monomers (six and five carbon sugars) and they are tightly bound to lignin. Lignocellulosic biomass can be broadly classified into virgin biomass, waste biomass and energy crops. Virgin biomass includes all naturally occurring terrestrial plants such as trees, bushes and grass. Waste biomass is produced as a low value byproduct of various industrial sectors such as agricultural (corn stover, sugarcane bagasse, straw etc.) forestry (saw mill and paper mill discards). Energy crops are crops with high yield of lignocellulosic biomass produced to serve as a raw material for production of second generation biofuel examples include switch grass (*Panicum virgatum*) and Elephant grass. The biological treatment of the effluent from paper and pulp industry where large number of microorganisms including bacteria, fungi and actinomycetes have been implicated in the biodegradation of lignin involving an oxidation process (Kirby, 2005; Gao *et al.*, 2011; Amret *et al.*, 2009). Various enzymes present in fungi like lignin peroxidases, manganese peroxidases, can effectively degrade lignin.

II. MATERIALS AND METHOD

Lignin is a complex organic polymers. Lignins are one of the main classes of structural materials in the support tissues of vascular plants and some algae. Lignins are particularly important in the formation of cell walls, especially in wood and bark, because they lend rigidity and do not rot easily. Chemically lignins are cross-linked phenol polymers. Lignin fills the spaces in the cell wall between cellulose, hemicelluloses, and pectin component, especially in xylem tracheids, vessel elements and sclereid cells. It is covalently linked to hemi-cellulose and, therefore, cross-links different plant polysaccharides, conferring mechanical strength to the

cell wall and by extension the plant as a whole. It is particularly abundant in compression wood but scarce in tension wood. Anaerobic degradation of lignin by micro flora was found to be very limited (Benner and Hodson, 1985).

2.1. Bacterial Degradation – Bacterial degradation of lignin is because both lignin and cellulose together does not support the bacterial growth. Recently reports show delignification by certain bacteria like *Pseudomonas*, *Arthrobacterium*, *Xanthomonas*, *Aeromonas*, *Flavobacterium*, and *Streptomyces* (Crawford and Crawford, 1980; Amer and Drew, 1980). Some *Pseudomonas* spp. has been reported to be the most efficient bio-degraders of lignin as reported by Zimmerman, 1990, and Vicuna, 1988. However non filamentous bacteria have very less capacity to demineralise lignin and are restricted to small portion of lignin which has less molecular weight (Vicuna *et al.*, 1993; Ruttimann *et al.*, 1991).

2.2. Fungal Degradation - Fungi are the nature scavengers whose only known microorganisms found to degrade lignin (Hatakka, 2001; Evans and Hedger, 2001). The wood decaying fungi are classified as:

2.2.1. Soft Rot Fungi– They include imperfect fungi (Deuteromycetes) and molds of Ascomycetes which are known for degradation of lignin (Blanchette, 1995; Daniel and Nilsson, 1998). Soft rot fungi include species of *Monodictys*, *Allescheria*, *Monodictys*, *Graphium*, *Papulospora*, *Paecilomyces* and *Thielavia*. Lignin degradation by fungi is better in hardwood than in softwood. According to reports by Rodriguez *et al.*, 1996, soil fungi *Fusarium oxysporum*, *Penicillium chrysogenum*, and *Fusarium solani* degraded 23.5%, 27.4%, and 22.6% of lignin, from wheat straw, respectively. Another soil fungus *Fusarium proliferatum* had been reported to secrete aryl alcohol and laccase in liquid cultures (Regaldo *et al.*, 1999).

2.2.2. Brown Rot Fungi -They include several species of basidiomycetes. These fungi have ability to easily remove cellulose and hemicellulose from the wood and form brown lignin. The brown colour of lignin is actually the modified lignin. Brown-rot fungi can be classified into two important groups based on the difference in the mechanism: one belonging to *Gloeophyllum trabeum*, which accumulates oxalic acid required for the hydrolysis of polysaccharides and also as a chelator for a Fe (II) H₂O₂ system generating hydroxyl radicals (Shimada *et al.*, 1997) and, the second includes *Poria (Postia) placenta* and *Coniophora puteana*. *Poria placenta* was found to demethoxylate lignin but no evidence of ring opening was found by Davis *et al.*, 1994.

2.2.3. White Rot Fungi– They are most effective degraders of lignin which comprises under of Basidiomycetes and a few species of Ascomycetes. They have capacity to complete degradation of lignin component to CO₂ and water. They cause cell wall erosion and with the progression of decay process. Some white rot fungi degrade lignin without loss of cellulose and cause white-pocket rot and are commonly seen in *Phellinus nigrolimitatus* (Singh, 2006).

Excellent example of both types of wood rot occurs in *Ganoderma applanatum* and *Heterobasidion annosum* (Eriksson *et al.*, 1990). A report by Arora *et al.*, 2002 showed seven species i.e. *Phlebia fascicularia*, *Daedalea flavida*, *Dichomitus squalens*, *T. versicolor*, *P. radiata*, *P. floridensis* and *Phanerochaete chrysosporium* of white rot fungi degrading up to 25% of lignin from wheat straw after 32 days. As per the report of Gilbertson, 1980, white rot fungi are known to occur less predominantly on wood species of gymnosperms than compared to angiosperms.

2.3. Microbial Enzymes in Lignin Degradation

Among these three fungi, the white-rot fungi are more effective for lignin degradation. Important classes of enzymes involved in degradation of lignin are lignin peroxidase (LiP), manganese peroxidase (MnP), and laccase, enzymes.

a) **Lignin Peroxidases (LiP; EC 1.11.1.14)** – LiP is an extracellular glycosylated protein with an isoelectric point of 3.2-4.0 and a molecular weight (MW) of 38-43 KDa. A heme group that is deeply buried in the protein constitutes its activity center. LiP enzymes are produced by the fungi during secondary metabolism in nutrient starved cultures and are glycosylated heme proteins. LiP was first discovered in *Phanerochaete chrysosporium* and since then has become one of the most studied peroxidases (Glenn *et al.*, 1983).

b) **Manganese Peroxidases (MnP; EC 1.11.1.13)** – MnP is found in white rot fungi as a series of glycosylated isoenzymes. Its isoelectric point ranges from 4.2 to 4.5. The MW of MnP ranges from 38 to 62.5 KDa, with the MW of most purified enzymes around 45 KDa. The catalysis by MnP is initiated by binding organic peroxidase or H₂O₂ to the native ferric enzyme which in turn forms an iron peroxide complex (Hofrichter, 2002).

c) **Laccases (Lac; EC 1.10.3.2)** – Lac, a glycoprotein member of the blue multi copper oxidase family, is a copper containing polyphenol oxidase (Hakulinen *et al.*, 2002). Fungal lac is also glycoprotein and is composed of three parts: a peptide chain, a sugar chain, and Cu²⁺ ion. Its isoelectric point is 2.8-4.3 (Gao *et al.*

al., 2011), and its molecular weight ranges from 60 to 390 KDa (Wang and Li, 2003). They are most largely found enzymes for lignin degradation present in many white rot basidiomycetes.

- d) **LMEs** - *Ganoderma* produce lignin-modifying enzymes (LME) and also include LiP, MnP and Lac. *Ganoderma* spp. can secrete a variety of enzymes that can hydrolyze lignin into monosaccharides, such as arabinose, xylose, galactose, fructose and glucose, as well as disaccharides of small molecules such as arabinose, xylose, galactose, fructose and glucose, which serve as carbon and energy sources. However, not all *Ganoderma* strains can produce these enzymes at the same time, and some *Ganoderma* strains can only secrete one or two of them. Further, the types of enzymes produced are influenced by the type of *Ganoderma* strains, medium composition and culture conditions (D'Souza *et al.*, 1996; D'Souza *et al.*, 1999; De Souza Silva *et al.*, 2005; Varela *et al.*, 2000).

III. RESULT AND DISCUSSION

Lignin is an aromatic polymer found in the lignocellulose component of plant cell walls. The lignocellulose content of plant biomass represents a major source of renewable carbon that could be used; to produce bio-ethanol via conversion to glucose and fermentation, to produce chemical feedstock for commercial use. However, a major obstacle to exploiting this renewable carbon is the durability of lignin, a heterogeneous aromatic polymer that encases the cellulose fibers in lignocellulose. Lignin, which comprises 10-30% of lignocelluloses by dry weight, is composed of aryl-C3 units linked via a variety of C-C and C-O ether linkages that are extremely resistant to degradation. White rot fungi and soil litter-decomposing fungi are known to produce manganese peroxidase in multiple forms. They cause lignin breakdown and are involved in several functions like fungal pathogenicity, pigmentation, sporulation, fructification and detoxification. These fungi cannot carry out photosynthesis; rather, they can only absorb nutrients such as lignin, cellulose, hemicellulose, and organic nitrogen from degraded wood and other substrates. Laccase enzyme can catalyze oxidation of aromatic amines, phenolic compounds and other compounds via reduction of molecular oxygen to H₂O₂. Although the enzyme activities of LiP have been studied to a considerable extent what still remains elusive is the location of binding sites (Du *et al.*, 1992). Though former two enzymes have been studied significantly the potential of laccase has not been well characterized (Cameron *et al.*, 2000). At the level of the above study the three enzymes did not present a holistic support for its biodegradation ability as there might be other enzymes which were not covered under the purview of their study (Emtiaziet *al.*, 2001). Thus the available data about the presence of three ligninolytic enzyme makes the fungus MVI.2011 as suitable alternative to the existing remediation system as it widens the scope for its potential to be used in degradation of persistent environmental pollutant from various industries and paper mills in particular. Enzymes from different species of *Ganoderma* were purified and their physicochemical and biochemical properties were studied (Kim and Nho, 2004; Ko *et al.*, 2001; Huie and Di, 2004; Kumaran *et al.*, 2011a; Tian and Zhang, 2005).

IV. CONCLUSION

Lignin is a complex polymer and its degradation takes a number of years. Lignins are particularly important in the formation of cell walls, especially in wood and bark, because they lend rigidity and do not rot easily. Ligninolytic fungi or lignin degrading fungi (such as white rot fungi) access their main carbon source lignocellulose by breaking down the lignin that protects it. Bacteria and fungi are the nature scavengers who have only known micro organisms found to degrade lignin; they are soft rot fungi brown rot fungi white rot fungi. The microbial enzymes that involve in lignin biodegradation are Lignin Peroxidases, Manganese Peroxidases, Laccases, Lignin Modifying Enzymes (LMEs). The carbon degradation is major properties of ligninolytic enzymes, Ligninolytic enzymes were added into the initial composting materials as treatment, and the inactive ligninolytic enzymes were added into the composting materials as control.

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