

# REVIEW ARTICLE ON LIGNOCELLULOSE BIOMASS AS SUBSTRATE FOR PLEUROTUS (OYSTER MUSHROOM) CULTIVATION

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**Abstract-**The biotechnological potential of oyster mushrooms with lignocellulosic biomass has a great interest in research. Lignocellulolytic fungi, especially *Pleurotus* species have attracted a great deal of interest as potential biomass degraders for large-scale biotechnological applications due to their ability to produce vast amounts of valuable products and extracellular lignocellulolytic enzymes. Agro-waste lignocellulosic biomass basically consists of 40 to 50% cellulose, 25 to 30% hemicellulose and 15 to 20% lignin materials which serve as major source of carbon and energy for *Pleurotus* species cultivation. Bioprocessing of plant byproducts using *Pleurotus* species provides numerous value-added products such as basidiocarps, animal feed, enzymes and other useful materials. The bioprocessing of lignin depends on the potent lignocellulolytic enzymes such as phenol oxidases (laccase), heme peroxidases, lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase produced by the organism. The cellulose-hydrolysing enzymes divided into endo- $\beta$ -1,4-glucanase, exo- $\beta$ -1,4-glucanase I and II, and  $\beta$ -glucosidase, they attack cellulose to release glucose. Several of these enzymes have reported having the ability to degrade and mineralize toxic chemicals, such as polycyclic aromatic hydrocarbons (PAHs), atrazine, organophosphorus and waste waters. The biodegradation and bioconversion of agro wastes (lignin, cellulose and hemicellulose) could have vital implication in cleaning our environment. Lignocellulosic biomass is also used in biopopulating that is another industrial application which offers a particular acceptable alternative to search for virgin fiber in paper manufacturing.

Key Words:- Hemicellulose, Basidiocarps, Organophosphorous, *pleurotus*.

## 1. INTRODUCTION

Recently, lignocellulosic biomasses have gained increasing research interests and special importance because of their renewable nature (Asgher et al., 2013; Ofori-Boateng and Lee, 2013). This has attracted the interest of many researchers in the utilization of lignocellulosic wastes. *Pleurotus* are characterized by a white spore print, attached to the gills, often with an essentric stip, or no stip at all, and they are commonly known as Oyster mushrooms (Miles and Chang, 1997). Growing oyster mushroom is becoming more popular throughout the world because of their abilities to grow at a wide range of climate conditions and utilizing various lignocelluloses. In nature, *Pleurotus* species live on parts of plants which are generally poor in nutrients and vitamins. Large amounts of lignocellulosic waste generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro-industries, and they are posing serious environmental pollution problems (Howard et al., 2003). Accidentally, much of the lignocellulosic biomass is often disposed of by burning or just lying values without any important used attached to them. Agro-residues contain three major structural polymers, cellulose, hemicellulose and lignin, which can be easily utilized or broken down by the lignocellulotic enzymes. *Pleurotus* species are the most efficient lignin-degrading organisms, with the ability to produce mainly laccases, lignin peroxidase and manganese peroxidase (Adebayo et al., 2012a). These enzymes present a non-specific biocatalyst mechanism and have been used for bioremediation process due to their ability to degrade azo, heterocyclic, reactive and polymeric dyes (Baldrian and Snajdr, 2006; Forgacs et al., 2004). White-rot basidiomycetes are among the most potent organisms to biodegrade and detoxify a wide range of wastes and pollutants. These fungi selectively attack lignin and related compounds by producing one or more of phenol-targeting redox enzymes, namely the peroxidases and laccases/phenol-oxidases (Ntougias et al., 2012). Prospection for fungi is the ability to secret high levels of lignin-degrading enzymes and novel enzyme variants, with desirable properties for biotechnological applications (Adebayo et al., 2012a). Therefore, the huge amounts of lignocellulosic biomass can be potentially bioconverted into different high value raw materials and products such as bio-ethanol, enriched animal feed, cheap energy sources for microbial cultivation (mushrooms included) and enzyme production, biodegradation and bioremediation of toxic organic compounds (Koutrotsios et al., 2014; Anwar et al., 2014; Asgher et al., 2013; Irshad et al., 2013; Ntougias et al., 2012). The objectives of this review are the compilation of the achievements in the technologies developed between oyster mushrooms and lignocellulosic materials.

Oyster mushrooms are cosmopolitan, and belong to the genus *Pleurotus* (Fungi: Basidiomycetes). Their cap is normally shell-like (about 5 to 20 cm in diameter; 1.9 to 7.8 inches), fleshy, with eccentric or lateral stipe; and their color can be white, cream, yellow, pink, brownish, or dark gray (Martínez-Carrera, 1999). Oyster mushrooms are commercially important in the world mushroom market, and several species are grown commercially on a large and small scale in many countries (Adebayo et al., 2012). *Pleurotus* species have been recognized as mushroom with dual functions to humans; both as food and medicine (Chang and Buswell, 2003). They are nutritive with good quantity of proteins, vitamins and minerals. Medicinally, they are been recommended for obese persons and diabetes patients because of low calorie value (Chang and Buswell, 2003) and very low sugar without starch. Oyster mushroom are generally classified as follow Scientific: *Pleurotus* spp.; Phylum: Basidiomycotina; Class: Basidiomycetes; Subclass: Holobasidiomycetidae, Family: Polyporaceae; Genus: *Pleurotus* Species: sajorcaju, sapidus, ostreatuseous, membranaceous, florida, citrinopileatus, flabellatus, pulmonarius etc.

## 2. BIODEGRADATION OF LIGNOCELLULOSIC BIOMASS

Generally, *Pleurotus* species follow the mechanism employed by white-rot fungi in degrading lignocellulose waste. White-rot fungi degrade lignin by secreting enzymes collectively termed “ligninases”. Ligninases can be classified as either phenol oxidases (laccase) or heme peroxidases [lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase (VP) (Martinez et al., 2005). Production of lignin peroxidases (LiP), manganese peroxidases (MnP) and laccases from *P. ostreatus* and *P. pulmonarius* have been studied (Okamoto et al., 2002; Carabajal et al., 2012; Luz et al., 2012), while VPs have been reported to be produced by *P. eryngii* (Camarero et al., 1999; Ruiz-Duenas et al., 1999) and *P. ostreatus* (Cohen et al., 2002).

## 3. LIGNIN BIODEGRADATION

### 3.1 Laccase

Laccases are glycosylated blue multi-copper oxidoreductases (BMCO) that use molecular oxygen to oxidize various aromatic and nonaromatic compounds through a radical catalyzed reaction mechanism (Claus, 2004; Baldrian, 2006). Laccases couple the electron reduction of dioxygen into two molecules of water with the oxidation of a vast variety of substrates, such as phenols, arylamines, anilines, thiols and lignins (Thurston, 1994). Four copper ions in their catalytic center mediate the redox process. The oxidation reactions catalyzed by laccases lead to the formation of free radicals which act as intermediate substrates for the enzymes (Ferraroni et al., 2007). These mediators can leave the enzyme site and react with a broad range of high-redox potential substrates and thus create non-enzymatic routes of oxidative polymerizing or depolymerizing reactions (Dashtban et al., 2010). Ultimately, laccase-mediator system (LMS) becomes involved in a range of physiological functions such as lignolysis, lignin synthesis, morphogenesis, pathogenesis and detoxification (Mayer and Staples, 2002).

## 4. HEME PEROXIDASE

### 4.1 Lignin peroxidases

Lignin peroxidase is one of the heme-containing glycoproteins and plays a major role in the biodegradation of lignin cell wall constituent (Piontek, 2001). LiP catalyze the H<sub>2</sub>O<sub>2</sub>-dependent oxidative depolymerization of a variety of non-phenolic lignin compounds (diarylpropane),  $\beta$ -O-4 non-phenolic lignin model compounds and a wide range of phenolic compounds (e.g. guaiacol, vanillyl alcohol, catechol, syringic acid, acetosyringone) (Wong, 2009). LiPs oxidize the substrates in multi-step electron transfers and form intermediate radicals, such as phenoxy radicals and veratryl alcohol radical cations. These intermediate radicals undergo non-enzymatic reactions such as radical coupling and polymerization, side-chain cleavage, demethylation and intramolecular addition and rearrangement (Wong, 2009). Unlike the other peroxidases, LiP is able to oxidize non-phenolic aromatic substrates and does not require the participation of mediators due to its unusually high redox potential (Wong, 2009; Wang et al., 2008). The crystal structure of the first LiP has shown that the heme group is buried in the interior of the protein and has access to the outer medium through a channel (Dashtban et al., 2010). Although, the size of the channel is not sufficient to allow the large polymer lignin to access the heme group, small molecule substrates can find a suitable binding site (Piontek, 2001).

## 5. MANGANESE PEROXIDASES (MNP)

Manganese Peroxidases are extracellular glycoproteins and are secreted in multiple isoforms which contain one molecule of heme as iron protoporphyrin IX (Asgher et al., 2008). MnP catalyzes the peroxide dependent oxidation

of Mn (II) to Mn (III), which is then released from the enzyme surface in complex with oxalate or with other chelators. Chelated Mn (III) complex acts as a reactive low molecular weight, diffusible redox-mediators of phenolic substrates including simple phenols, amines, dyes, phenolic lignin substructures and dimers (Wong, 2009; Wesenberg et al., 2003; Asgher et al., 2008).

## 6. VERSATILE PEROXIDASES (VP)

Versatile Peroxidases are glycoproteins with combine properties capable of oxidizing typical substrates of other basidiomycetes peroxidases including Mn (II) and also veratryl alcohol (VA), MnP and the typical LiP substrate, respectively (Wesenberg et al., 2003; Asgher et al., 2008; Ruiz-Duenas et al., 1999). VPs form an attractive ligninolytic enzyme group due to their dual oxidative ability to oxidize Mn (II) and also phenolic and non-phenolic aromatic compounds (Wesenberg et al., 2003). It has been found that VPs can also efficiently oxidize high redox-potential compounds (Gomez-Toribio et al., 2001). It has been suggested that VPs can oxidize substrates spanning a wide range of potentials, including low- and high-redox potentials.

## 7. CELLULOSE BIODEGRADATION

Cellulose is a homopolysaccharide composed of  $\beta$ -D-glucopyranose units, linked by  $\beta$ -(1 $\rightarrow$ 4)-glycosidic bonds. Cellulose contains both nonreducing (NR) and reducing (R) ends. The smallest repetitive unit of cellulose is cellobiose, which can be converted into glucose residues. The cellulose-hydrolysing enzymes (that is, cellulases) have been reported to be produced by species of *Pleurotus* from lignocellulose (Kuforiji and Fasidi, 2008; Carabajal et al., 2012; Luz et al., 2012). The enzyme (cellulases) divided into three major groups: endo- $\beta$ -1,4-glucanase, exo- $\beta$ -1,4-glucanase I and II, and  $\beta$ -glucosidase. The endo- $\beta$ -1,4-glucanase catalyze random cleavage of internal bonds of the cellulose chain, while exo- $\beta$ -1,4-glucanase I attack the chain at reducing ends to release cellobiose, and exo- $\beta$ -1,4-glucanase II attack cellulose at non-reducing end chain.  $\beta$ -glucosidases are only active on cellobiose, and release glucose monomers units from the cellobiose .

## 8. HEMICELLULOSE BIODEGRADATION

The second most abundant renewable biomass which accounts for 25 to 35% of lignocellulosic biomass is hemicellulose (Saha, 2000). They are heterogeneous polymers built up by pentoses (D-xylose, L-arabinose), hexoses (D-glucose, D-galactose), Sugaracids (Ferulic acid and 4-O-methyl-d-glucuronic acid and acetyl group. Many enzymes are responsible for the degradation of hemicellulose. The  $\alpha$ -D-glucuronidase attack 4-O-methyl-d-glucuronic acid, while endo- $\beta$ -1,4- xylanase break the xylan chains and  $\alpha$ -L- arabinofuranosidase attack end chain of L-arabinose. The further reactions are cutting off ferulic acid and removing of the acetyl groups by feruloyl esterase and acetylxylan esterase, respectively. Reduction of xylan to xylobiose is done by  $\alpha$ -D-galactosidase. The  $\beta$ -D-xylosidase release D-xylose, a monomers unit from xylobiose . Like cellulose, hemicellulose is also an important source of fermentable sugars for biorefining applications. Xylanases are being produced and used as additives in feed for poultry and as additives to wheat flour for improving the quality of baked products at the industrial.

## CONCLUSION

Oyster mushroom and their enzymes serve as an efficient alternative for the biodegradation and bioconversion of lignocelluloses and other resistant pollutants. Lignocellulose biotechnology by oyster mushroom could produce numerous value-added products such as fruit body, extracellular enzymes, Animal feed and other products. The bioprocessing of lignin depends on the potent lignocellulolytic enzymes such as phenol oxidases (laccase) or heme peroxidases (lignin peroxidase (LiP), manganese peroxidase (MnP) and versatile peroxidase) produced by the organism. The cellulose-hydrolysing enzymes (that is, cellulases) basically divided into endo- $\beta$ -1,4-glucanase, exo- $\beta$ -1,4-glucanase I and II, and  $\beta$ -glucosidase, they attack cellulose to release glucose, a monomers units from the cellobiose, while several enzymes ( $\alpha$ -D-glucuronidase, endo- $\beta$ -1,4- xylanase,  $\alpha$ -L- arabinofuranosidase, feruloyl esterase, acetylxylan esterase,  $\alpha$ -D-galactosidase and  $\beta$ -D-xylosidase) acted on hemicellulose to give D-xylose from xylobiose. The bioconversion of lignocelluloses majorly involves transformation of lignin, hydrolysis of cellulose and hemicellulose to simple sugar, which can then play vital role in fermentation process. Beside, that the enzymes produced serves in biode-gradation or bioconversion of agro-waste, they can also be used in several biotechnological applications, including detoxification, bioconversion and bioremediation of resistant pollutants. Several of these enzymes have previously reported of having the ability to degrade and mineralize toxic chemicals, such as polycyclic aromatic hydrocarbons (PAHs), atrazine, organophosphorus and wastewater.

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