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Synthesis and Emerging strategies of lignin degradation: A Concise Review

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ABSTRACT

Lignin is a major component of wood, which is recalcitrant in nature and is not easily degraded. Its recycling is essential for the carbon cycle. Waste products from paper, pulp and wood industries impair with soil and water bodies leading to accumulation of lignin. In this review various effective physical, chemical, physico- chemical, thermo- chemicals and electrical method are discussed for lignin removal along with biological deterioration of lignin with the help of organisms (insects, fungus and bacteria). Use of microbes and insect for lignin deterioration is termed as lignin biodegradation. Lignin degradation with the help of fungi is well studied but much less studied in bacteria and insects. The present review deals with the biodegradation of lignin with the help of microbes.

Introduction

The lignocellulosic material of plants consists of mainly three components cellulose, hemicellulose and lignin. After cellulose, lignin is the second most abundant renewable aromatic biopolymer (600- 1000 kd) (1). It is the most abundant aromatic polymer in the biosphere. It is a crucial component of the plant cell wall, imparting firmness and protecting the easily degradable cellulose from attack of pathogens (2, 3). Lignin is thought to act as a kind of glue in the plant cell walls and provides plants an effective protection against parasite attack and represents an obstacle to microbial digestion of structural carbohydrates due to phenolic compound (4). Phenylalanine, provide compressive strength and additional rigidity to the walls and make it water impermeable (5). Lignin makes up about one-quarter to one-third of the mass of dry wood (2). Lignin is an integral part of the cell wall of plant cells e.g. tracheids, xylary fibres and sclereids of plants etc. and is composed of phenylpropanoid units including coumaryl, guaiacyl and syringyl moieties, linked to each other through different kinds of bonds (6). In the chemical pulping process, lignin is removed from wood pulp before it is turned into paper and the extracted lignin is used as a binder in particle board, adhesive for linoleum and raw material for processing into chemicals (such as dimethyl sulfoxide and vanillin) (6). Lignin termed as nature's plastic is one of the key pollutants from paper mill because of its recalcitrant nature. Lignin surrounds cellulose in the plant cell wall forming a matrix, which itself is resistant to degradation. Due to its complicated structure and nonhydrolysable bonds, lignin degradation is more difficult than cellulose or hemicelluloses (7). As a consequence of the paper industry, lignin is most often used by paper mills as a fuel for the recovery of its energy content (8).

Structural organization of lignin:

Lignin is the generic term for a large group of aromatic polymers resulting from the oxidative combinatorial coupling of 4-hydroxyphenylpropanoids (9). These polymers are deposited predominantly in the walls of secondarily thickened cells, making them rigid and impervious. It is commonly accepted that lignin evolved together with the adaptation of plants to a terrestrial life to provide them with the structural support needed for an erect growth habit (10). Accumulation of lignin during development of sclerenchyma and xylem elements accomplish them strength (5). Lignin has a highly branched three-dimensional phenolic structure including three main phenylpropane units, namely *p*-coumaril, coniferyl and sinapyl (Fig. 1). Softwood lignin contains relatively fewer sinapyl units and consists mainly of guaiacyl structures, while hardwood lignin contains guaiacylsyringyl structures.

Lignin Biosynthesis

Several aspects regarding to lignin biosynthesis were given, according to one of them lignin is derived from the polymerization of coniferyl alcohol (11).

Phenylalanine give to coniferyl alcohol and monolignols through the phenylpropanoid pathway which involve several multiple intermediary steps where other important plant products like flavonoids, coumarins and benzoic acid derivatives were also synthesized. The monolignols are toxic and unstable overcome with glycosylation, present in very low levels in the living plant cells. The free radical mechanism is believed to be involved in polymerization of monolignols into lignin (5). Polymerization of monolignols into lignin differs in their substitution pattern and the relative abundance of the different monolignols residues in lignin varies in between and also within the species.

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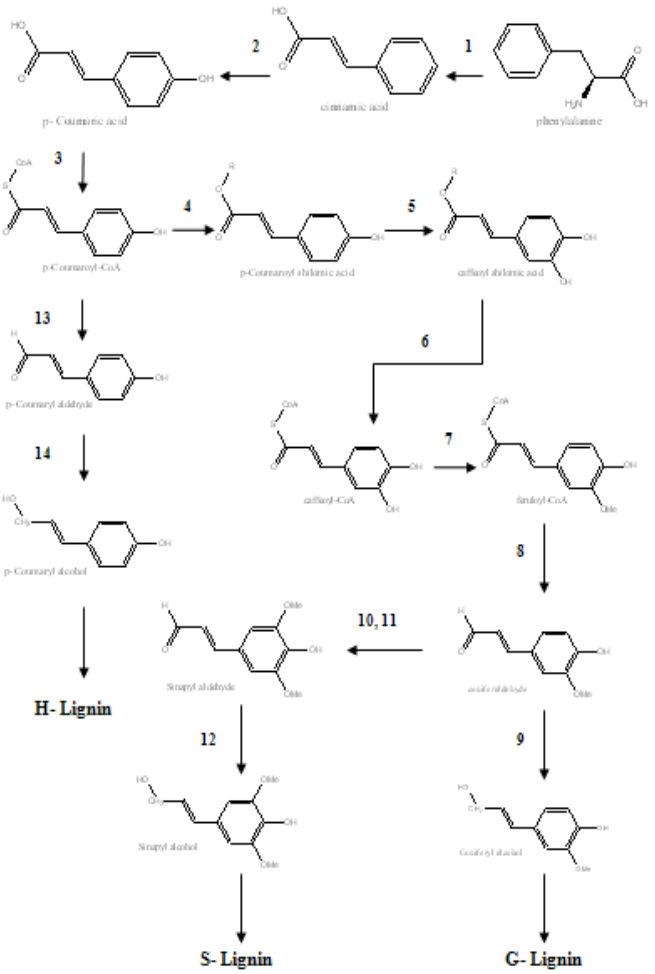

Figure 1

The overview of biosynthetic route toward the monolignols p-coumaryl, coniferyl, and sinapyl alcohol **1;** Phenylalanine Ammonia-lyase (PAL) **2;** Cinnamate 4-hydroxylase (C4H) **3;** 4-Coumarate: CoA ligase (4CL) **4, 6;** p-hydroxycinnamoyl CoA: Quinate/Shikimate p-hydroxycinnamoyltransferase (HCT) **5;** p-Coumarate 3-hydroxylase (C3H) **7;** Caffeoyl- CoA O-methyltransferase (CCoAOMT) **8, 13;** Cinnamoyl- CoA reductase (CCR) **9, 12, 14;** Cinnamyl alcohol dehydrogenase (CAD) **10;** Ferulate 5-hydroxylase (F5H) **11;** Caffeic acid O-methyltransferase (COMT).

In addition to developmentally programmed deposition of lignin, its biosynthesis can also be induced upon various biotic and abiotic stress conditions, such as wounding, pathogen infection, metabolic stress, and perturbations in cell wall structure (12), which suggest that developmental and environmental signals both respond to lignin biosynthesis (10).

Lignin synthesizing enzymes

Phenylalanine Ammonia-Lyase

The first step in the biosynthesis of phenolic compound is taking place by the deamination of L-phenylalanine to trans-cinnamic acid and ammonia, catalyzed by phenylalanine ammonia lyase (PAL) (PAL; EC 4.3.1.5) (13). This enzyme belongs to family lyases, which cleave carbon nitrogen bonds. Induction of PAL by fluorescent pseudomonads was reported in cucumber against *Pythium aphanidermatum* (14). This enzyme is found in tetrameric form in vascular plants and is one of the most intensively studied in plant secondary metabolism because of its key role in phenylpropanoid biosynthesis (15).

PAL involved in catalyzing the phenylpropanoid metabolism plays an important regulatory role in controlling biosynthesis of all phenylpropanoid compounds, including lignin (16). Phenylpropanoids functions as effective antioxidants by donating hydrogen from hydroxyl groups placed with an aromatic ring to terminate free radical oxidation of lipids and other biomolecules (17). Analysis of phenylpropanoid metabolites in transgenic tobacco plants indicates that lignin content is greatly affected by reducing PAL activity (18). PAL subunits are typically encoded by multigene families in angiosperms and show tissue-specific patterns of expression (19).

Cinnamate 4-Hydroxylase

Hydroxylation of cinnamic acid to p-coumaric acid is catalyzed by cinnamate 4-hydroxylase (C4H; EC 1.14.13.11), which is a member of cytochrome P-450-linked monooxygenase superfamily and plays a central role in phenylpropanoid metabolism and lignin biosynthesis. Molecular oxygen is cleaved during this reaction, with one oxygen atom added to the aromatic ring and the other reduced to water (5, 20).

cDNA encoding C4H was isolated from a hybrid poplar (*Populus trichocarpa x P. deltoides*), RNA-blot analysis detected C4H transcripts which were highly expressed in developing xylem. Subcellular localization was confirmed with the help of chimeric C4H-green fluorescent protein (GFP) gene in Arabidopsis (20). C4H activity reduced by antisense expression or sense suppression results in reduction of lignin has been determined by pyrolysis gas chromatography/mass spectrometry (21).

Coumarate 3-Hydroxylase

Coumarate 3-hydroxylase catalyzes the hydroxylation of p-coumarate to form the caffeate (5). The effect of C3H down regulation on lignin structure was analyzed with the help of NMR (22).

Coumaroyl-Coenzyme A 3-Hydroxylase

The enzyme showed a molecular mass of 42,400 ± 1700 Da in gel chromatography and required ascorbate, NADH, or NADPH as cofactors having hydroxylase activity and the diphenol oxidase activity. The enzyme was identified as an alternative in hydroxylation of free p-coumarate in *Silene dioica* (23).

Lignin degradation

Industrial discharge and effluents of paper and pulp mills, alcohol distilleries, textiles and leather industries creates unacceptable problems in soil and lead to anaerobic conditions in water bodies due to blockage of light to lower depths and cessation of photosynthesis (24). Lignin is the structural components of plant cell wall and as it provides stiffness to the wood creates the main problem in waste from the pulp and paper industry. The pulp and paper industry uses cheaper and more effective way to get lignin extraction as they can get to the cellulose fibers which have an adversarial effect on the carbon cycle. Lignin degradation is in a central position in the earth's carbon cycle, because most renewable carbon is either in lignin or in compounds protected by lignin from enzymatic degradation (cellulose and hemicelluloses) (1). Because the lignin protects cell wall polysaccharides from microbial degradation, thus imparting decay resistance, it is also one of the most important limiting factors in the conversion of plant biomass to pulp or biofuels. The removal of lignin from plant biomass is a costly process; hence, research efforts are now aimed at designing plants that either deposit less lignin or produce lignins that are more amenable to chemical degradation (25). Lignin size is one of the major hurdles in degradation of lignin. It may be dimeric compounds or even tetrameric compounds (26, 27). So, an eco- friendly way is needed for removal of lignin from paper and pulp. Here, we are discussing

the synthetic method and biological method of lignin degradation.

Synthetic method of lignin pretreatment

Different technologies have been developed in the last decades for the pretreatment of lignin biomass. The basic aim of the pretreatment process is to remove recalcitrant lignin and increase the porosity of the lignin containing materials and hence improve hydrolysis. It can be generally divided into: physical parameters, chemical parameters, physic-chemical parameters, Thermo- chemical parameters, electrical or a combination of these.

Physical Parameters

Mechanical Comminution: It involves chipping, grinding and milling for reduction in particle size to increase the surface area and the reduction of polymerization for proper hydrolysis of ligno-material (28).

Extrusion: The material is subjected to heat, mixing and shearing which modifies the physical and chemical structures and increases the accessibility for enzyme attack (29, 30).

Chemical parameters

Ozonolysis: Ozone treatment reduces the lignin content of the ligno-wastes. At room temperature and normal pressure, ozone de-lignifies materials by using its oxidative nature. This resulted in an increase of the in-vitro digestibility of the treated material, and unlike other chemical treatments, it does not produce toxic residues (31).

Acid Hydrolysis: Concentrated acids such as sulphuric acid and hydrochloric acid have also been used to treat ligno-materials. Pretreatment with acid can result in improvement of enzymatic hydrolysis of lignin biomasses to release fermentable sugars (28). The highest rate of hydrolysis is obtained with dilute acids because concentrated acids form inhibiting compounds and leads to corrosion (30).

Alkaline Hydrolysis: Some bases can also be used for the pretreatment of ligno-materials, and the effect of alkaline pretreatment depends on the lignin content of the materials. Sodium hydroxide increases the internal surface of materials decreasing the degree of polymerization and crystallinity which disrupts lignin and increase their solubilization. Sodium, calcium and ammonium hydroxides are also useful (32).

Oxidative Delignification: Lignin degradation could be catalyzed by the peroxidase enzyme with the presence of H_2O_2 (33).

Organosolv Process: In the organosolvation process, an organic solvent along with inorganic acid catalysts (HCl/ H_2SO_4) is used to break the internal lignin and hemicellulose bonds. The organic solvents widely used are methanol, ethanol, acetone, ethylene glycol, triethylene glycol, and tetrahydrofurfuryl alcohol (34).

Physico-Chemical Parameters

Steam Explosion: Materials are subjected to sudden high and low pressure under saturated steam. Lignin material undergoes an explosive decompression and redistributed (35). Steam explosion is typically initiated at a temperature of 160-260 °C with corresponding pressure of 0.69-4.83 MPa for several seconds to a few minutes before the material is exposed to atmospheric pressure (31).

Carbon Dioxide Explosion: Under high pressure Carbon-dioxide acts as supercritical fluid thereby increases material digestibility and extract lignin. Supercritical CO_2 would have a lower temperature than steam explosion and more economic compared to ammonia explosion. Supercritical fluid refers to a fluid that is in a gaseous form but is compressed at temperatures above its critical point to a liquid like density (36).

Ammonia Fiber Explosion (AFEX): Lignin biomasses are exposed to liquid ammonia at high temperature and pressure for a certain period of time, and then the pressure is suddenly reduced. Liquid ammonia (anhydrous) at 60-100°C and high pressure results in swelling and physical disruption of materials having low lignin content (37).

Liquid hot water pretreatment: Under pressurized hot water it solubilize materials and reduces lignin content (30).

Thermo- chemical Parameters

Pyrolysis: Heating/treating of materials at temperatures greater than 300 °C has also been used for the pretreatment of lignin biomass (30), (28).

Gasification: Material is converted into syngas which is a mixture of hydrogen and carbon monoxide (30).

Electrical Parameters

Pulsed-Electric-Field Pretreatment

Application of short burst of high voltage when the sample was placed between two electrodes. A critical electric potential is induced across the cell membrane of sample on application of electric field. Developed short burst destroys and changes the structural components of plant sample's cell membrane (38).

Biological Method

All the above mentioned process is harsh and cost intensive for lignin removal. Besides this, biological treatments are environmental friendly and employs micro-organisms (fungi, actinomycetes and bacteria) and insects for lignin degradation. Lignin degrading microbial enzymes have been fascinating as they offer environmental friendly technologies for the paper, pulp and other industries for lignin degradation (39).

Numerous studies were ongoing to explore the microbial degradation of lignin in which very few achievements were reported and role of specific microbes except little fungus for lignin biodegradation is still unclear. Probably this is due to experimental, environmental difficulties and availability of insufficient data of related microbes. Microbial lignin degradation is expertise in aerobic and anaerobic conditions. Various forms of lignin i.e. synthetic, dimeric and Douglas fir wood labeled with C-14 are not degraded anaerobically (40), while beech wood buried in an anaerobic condition showed slight degradation (41).

Fungal lignin degradation

Lignin degradation by fungi is best exemplified by the white rot fungus. *Phanerochaete chrysosporium, Streptomyces viridosporus, Pleurotus eryngii, Trametes trogii, Fusarium proliferatem, Agaricus, Erwenia, Copricus, Mycema, Sterium* are reported to degrade lignin (42).

Lignin of wheat straw get converted into reducing sugars by white-rot fungi *Pleurotus ostreatus* (43), *Phanerochaete sordid* (44), *Pycnoporus cinnabarinus* (45). *Stereum hirsutum* was found to degrade the lignin of Japanese red pine *Pinus densiflora* (46).

A White-rot fungus produces a range of extracellular lignolytic enzymes, including heme-dependent lignin peroxidases, manganese peroxidases, versatile peroxidases, and copper-dependent laccases (47).

More than 600 species of Basidiomycetes including *Clavaria, Clitocybecollybia, Flammula, Hypholoma, Lepiota, Mycena, Pleurotus, Agaricus, Polyporus, Fusarium, Arthrobotrys, Poria, Pholiota, Cephalosporium, Collybi,* and *Humicola* have been found to degrade lignin by secreting extracellular lignin peroxidase and manganese –dependent peroxidase isozyme (48).

Lignin degradation abilities and laccase, manganese-dependent peroxidase (MnP), lignin peroxidase (LiP) and total

cellulase enzymatic profiles of basidiomycetes fungus *Pleurotus ostreatus, Coriolus versicolor, Tyromyces albidus* and *Trametes gallica* were studied in submerged media containing peat with the help of Scanning electron microscopy (SEM) (49).

In the case of fungal lignin degradation, structural hindrance of filaments, their specific requirement such as; humidity, oxygen demand, appropriate temperature, proper pH, long lag period are some limitations which limit industrial application of fungi when lignin concentration is extremely high (50).

Lignin degrading bacteria:

Several bacteria with lignin degradation potential has been identified however the data is very scarce. Hence, isolation and identification of ecofriendly lignin degrading bacteria is an essential task for the future as bacteria simply grow faster and multiply faster and have a wider tolerance of temperature, pH and oxygen limitation than other microbes. Hence, it is anticipated to be better in the production of lignin degrading enzymes due to their vast environmental adaptability and biochemical versatility. The biological degradation of lignin with the help of microbe has been well studied in white-rot and brown-rot fungi, but is much less well studied in Bacteria. The recent published work suggests that bacteria are able to break down lignin but the process of enzymatic degradation is poorly understood. It may seem that extracellular peroxidase and laccase enzymes to be involved in lignin degradation and would be of considerable importance and confers a new perceptive that may be of great industrial significance (51).

Streptomyces viridosporus T7A Gram-positive soil bacteria is well known for lignin degradation by producing the enzyme lignin peroxidase at optimum pH 7.5- 8.5 (52). *Bacillus subtilis* a soil bacterium can produce an endospore that is resistant to various environmental factors such as temperature, acid and other undesirable factor reported to produce laccase which degrade lignin at an optimum temperature of 30°C - 37°C (53). *Pseudomonas Putida,* a flagellated rod-shaped bacterium, produces enzyme β-etherase, Protein ligF and Vanillate O-demethylase oxidoreductase to degrade lignin at 30°C of optimum temperature (54). *Pseudomonas paucimobilis*, a Gram-negative bacterium isolated from human clinical specimens has the ability to degrade various dimeric lignin compounds by producing C alpha-dehydrogenase (55). *Xanthomonas* strain reported to mineralized synthetic (14C) lignin in 20 days (56). Bacteria like *Pseudomonas aeruginosa, Serretia marcescens, Nocardia, Arthrobacter, Flavobacterium, Micrococcus, Xanthomonas* and *Rhodococcus* have also been reported to possess lignolytic activity (57), (58). *Sphingobium sp.* SYK-6 degrades Lignin-derived fragments, convert the biphenyl compound 5, 50-dehydrodivanillate (DDVA) into vanillate by using a set of catabolic genes: ligX, ligZ, ligY, ligW and ligW1 (59). Actinomycetes degraded water soluble grass residue described as acid-precipitable polymeric lignin (60). *Streptomyces* spp. of actinomycetes is the most widely studied bacterial groups for lignin degradation in spruce and maple (61). A kraft lignin-degrading bacterium *Aneurinibacillus aneurinilyticus* isolated from sludge of paper industries degrades lignin and their extract in ethyl acetate was analyzed by GC-MS. The result indicates presence of low molecular weight aromatic compounds such as Guaiacol, Acetoguaiacone, Gallic acid and Ferulic acid signifies that the bacterium can oxidize sinapylic (G units) and coniferylic (S units) alcohol units which are the fundamental architect of the hardwood lignin structure (62). Analysis of woods after three months exposure to a bacterial consortium of Myxobacteriales or Cytophagales indicated extensive wood decay and lignin loss from birch and pine woods (63). Eleven bacterial isolates from various environments were screened for lignin degradation activity in which *Pseudomonas aeruginosa* and *Serratia marcescens* were able to degrade lignin by extracellular lignin peroxidase enzyme (3).

With genomic data available bacterial laccases reported to be widespread and they are thought to play a role in metal tolerance and sporulation (64). More than 100 bacterial laccase sequences were identified and have advantages over fungal laccases (64).

Pseudomonas putida mt-2, *Rhodococcus jostii* RHA1, *Streptomyces viridosporus* T7A were found to possess lignin-degrading activity (65). Dependency of *Streptomyces viridosporus* T7A activity upon hydrogen peroxide indicate use of extracellular lignin peroxidase whereas *Pseudomonas putida* mt-2, *Rhodococcus jostii* RHA1 has their activity in the absence of hydrogen peroxide which indicates use of laccase enzyme (65).

Interestingly a number of *Burkholderia* and *Citrobacter* group isolated from termite guts have aromatic compound degradation capabilities which were used in lignin degradation (66). Gut microflora from *Anoplophora glabripennis* and *Zootermopsis angusticollis* showed depolymerisation, demethylation and ring hydroxylation of lignin (67).

Lignin Degradation by Insects

Lignin degradation by Asian longhorned beetle (*Anoplophora glabripennis*) and the Pacific dampwood termite (*Zootermopsis angusticollis*) was studied using tetramethylammonium hydroxide thermochemolysis and found they were involved in propyl side-chain oxidation (depolymerization), ring hydroxylation and demethylation of ring methoxyl groups (67). The Formosan subterranean termites (*Coptotermes formosanus Shiraki*) have the ability to degrade lignin structure by demethylation, demethoxylation and propyl side chain modification (68). The wood-boring Clearwing borer *Paranthrene robiniae* reported to degrade lignin. *P. robiniae* modify the hardwood structures during the larval stage. The results were validated with solid state ^{13}C cross polarization magic angle spinning (CP/MAS) nuclear magnetic resonance (NMR) spectroscopy, attenuated total reflectance Fourier transform infrared (ATR-FTIR), pyrolysis-gas chromatography/ mass spectrometry (Py-GC/MS), and thermogravimetric (TG) analysis which strongly suggest that structural alteration of lignin is due to degradation of syringyl units (69).

Lignin degrading enzyme:

Complex structure of lignin suggests more than one enzyme is required in combinations for lignin degradation to cleave different parts of the lignin structure efficiently. Lignin-degrading enzymes are the group of oxidoreductive enzymes which have their potential role in bioremediation (70).

Lignin Peroxidase (LiP) and Manganese-dependent Lignin Peroxidase (MnP) are extracellular in nature. For their peroxidase activity presence of extracellular hydrogen peroxide is essential. LiP attacks both phenolic and nonphenolic aromatic residues, has bioremediation potential to decolorize the effluents and hence are used to treat colored industrial effluents and other xenobiotics. MnP catalyzes the oxidation of Mn(II) to Mn(III) which in turn can oxidize phenolic substrates (71).

Lignin peroxidase (Lip)

Lignin peroxidase (EC 1.11.1.14) is a glycoprotein (approximately 37,000 to 43,000 Daltons in size, pI from 3.3 to 4.7), possesing heme proteins at active center, have structural similarity with the peroxidases and utilizes hydrogen peroxide and organic peroxides to oxidize a variety of substrates (72). The substrates for lignin peroxidase include both phenolic and

nonphenolic aromatic compounds, amines, aromatic ethers and polycyclic aromatics which are oxidized to give radicals (72, 73). Lignin peroxidase can catalyze the oxidation of substrates with a reduction potential greater than 1.3 volts. The enzyme is capable to oxidize lignin monomers, dimers and trimers as well as polycyclic aromatic compounds. The oxidation of these substrates results in C_a-$C_ß$ cleavage of lignin model compounds (74).

Manganese peroxidase (MnP)

Manganese peroxidase (EC 1.11.1.13), (molecular masses 45,000 to 60,000 Daltons) belongs to the family of oxidoreductases (73). They oxidize Mn(II) to Mn(III), have a conventional peroxidase catalytic cycle with Mn(II) as a substrate and chelated by organic acid chelators, which stabilize the product Mn(III). Although Mn(III) oxidizes phenolic compounds, but it cannot attack non-phenolic units of lignin. It employs heme as a cofactor and needs Ca^{2+} for activity. White rot fungi *Ceriporiopsis subvermispora* secrete this enzyme and their several isoenzymes to aid lignin degradation (75).

Laccase

Laccases (EC 1.10.3.2), (molecular masses about 60,000 to 65,000 Daltons) are blue-copper phenoloxidases that catalyze the one-electron oxidation mainly of phenolic compounds and non-phenolics in the presence of mediators (76). Laccase can degrade lignin via generating Reactive radicals which cleave covalent bonds and produce monomers. Laccase can also cause radical degradation and depolymerization of larger polymers by oxidizing small organic compounds (77). They are produced by the fungus *Pleurotus ostreatus* play a role in the degradation of lignin, and can therefore be included in the broad category of ligninases (78).

Glyoxal oxidase (GLOX)

Glyoxal oxidase (EC 1.1.3.-), (molecular mass approx 68,000 Daltons) is an extracellular H_2O_2-generating enzyme produced by ligninolytic cultures of *Phanerochaete chrysosporium* have a crucial role in the physiology of lignin biodegradation and also control the regulatory mechanism of oxidase and peroxidase. GLOX production to act as lignin peroxidase required high level of oxygen (79), which mediates through glyoxal and methyl glyoxal in which Glyoxal oxidized to glyoxylic acid and H_2O_2 are releases (73).

Aryl-alcohol oxidase

Aryl alcohol oxidase (AAO) (EC 1.1.3.7) or veratryl alcohol oxidase or aromatic alcohol oxidase are FAD-containing enzyme belonging the family of oxidoreductases, (molecular masses are about 71,000 to 73,000 Daltons) (pI of 3.9) acts on the CH-OH group of donor with oxygen as acceptor and catalyzes the oxidation of aromatic alcohols to aldehydes (73). The systematic name of this enzyme class is aryl-alcohol: oxygen oxidoreductase. It helps in fungal degradation of lignin by providing the hydrogen peroxide required by ligninolytic peroxidases (80).

Versatile peroxidase (VP)

Versatile peroxidase (EC 1.11.1.16) (molecular mass 43 kDa) has both manganese peroxidase and lignin peroxidase activities involved in natural degradation of lignin. It oxidizes hydroquinone in the absence of exogenous H_2O_2 when Mn(II) is present. Chemical oxidation of hydroquinones promoted by Mn(II) (81).

Conclusion

Demand of paper and other wood materials increases as the population of the world is increasing and hence, more waste products are generated through them. The basic aim of the present review is to give a brief outline of lignin degrading various parameters and to provide tools for discovering additional methods of biological delignification and enzymes. There is need of to discover new microbes and insect for their rapid and eco-friendly delignification.

In nature microorganisms seems to be in a close interaction with plants, penetrates cell wall barrier and enters into the host and inhabit them as symbiont which makes them available as a potential candidate for delignification. Researches and discovery of delignifying microorganisms can result in the recycling of carbon from the lignin biomass and can play a significant role in paper, pulp and wood industries. An understanding of the mechanisms of delignifying microorganism will be helpful in their potential use. Degradation of lignin is a complex process because of its recalcitrant nature and complexity of its compounds. Biological degradation is cost effective, low energy requirement, less production of toxic compounds, environment friendly. Although the hydrolysis rate is very slow in biological process hence, it is time consuming as compared with chemical, physical and other processes. But due to their environment friendly nature biological degradation is favored on to other synthetic like chemical and physical method. There is a need to search and test cost effective and eco-friendly micro-organisms and insect for removal of lignin quickly and efficiently. Researches on a wide variety of microorganisms are concern for lignin degradation which includes bacteria and fungi. But unfortunately to date, only a few groups of organisms are reported to capable of degrading complex lignin polymers. Most of the research work has been focused mostly on fungi. Among microbes as compare with fungus, bacteria simply grow in unfavorable harsh conditions such as in variable temperature and pH, and interestingly have a minimum nutritional requirement, have short generation times so multiply at a faster rate but several research conducted previously clearly stated that there is only countable number of bacterial strains were able to degrade lignin as compared to fungus.

In the future, despite the progress achieved, more effort is needed to develop and explore environmental friendly lignolytic enzymes and/or microorganisms having a significant industrial impact. These developments led to the discovery of the lignin-degrading enzyme, and provide the tools for discovering additional enzymes and for evaluating the possible role of non-enzymatic oxidations. Rapid further advances are assured.

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