Review

Recent advances in applications of acidophilic fungal microbes for bio-chemicals

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Abstract: Lignocellulosic feedstock (cellulose, hemicellulose and lignin) has been used for a variety of purposes. Among them, lignin can produce value-added chemicals having phenyl-propanoid subunits known as core lignin, possessing either C-C bonds or ether linkages. It can be depolymerized by microbial activity together with certain enzymes (laccases and peroxidases). Both acetic acid and formic acid production by certain fungi contribute significantly to lignin depolymerization. Natural organic acids production by fungi has many key roles in nature that are strictly dependent upon organic acid producing fungus type. Enzymatic conversion of lignocellulosic is beneficial over other physiochemical processes. Laccases, the copper containing proteins oxidize a broad spectrum of inorganic as well as organic compounds but most specifically phenolic compounds by radical catalyzed mechanism. Similarly, lignin peroxidases (LiP), the heme containing proteins perform a vital part in oxidizing a wide variety of aromatic compounds with H_2O_2. Lignin depolymerization yields polyaromatics, the important ones are BTX (Benzene, Xylene and Toluene), found in several different configurations. However, most modern aromatics complexes enhance the production of p-xylene, benzene and sometimes o-xylene respectively. Thus, this review will provide a concept that chemical and biological modifications of lignin yield certain value added and environment friendly chemicals.

Keywords: Lignocellulosic biomass; Laccases; Peroxidases; Green biochemical; Acidophilic microbes.

Introduction

The processing as well as the withdrawal of fossil fuels are the major prevailing environmental issues these days. Therefore, it is the utmost need of the time to lessen fossil fuels consumption as much as possible. The only reliable solution to this major issue is to exchange the petroleum products with lesser costly environmental friendly (green) chemicals. Approximately, over 10 million tons of petrochemical materials (phenol and its derivatives) are generated annually. Thus, advancement is required to utilize new and natural raw substances for polyphenolic compounds biosynthesis [1].

Lignocellulosic feedstock (the fundamental renewable source of carbon) has been utilized for the broad-scale fuels and chemicals synthesis to lessen the usage of non-renewable fossil fuels [2,3]. A large percentage of carbohydrate polymers such as pectin, hemicellulose, cellulose together with heterogeneous, alkyl-aromatic polymer, lignin constitutes plant cell walls [4].

Lignin is primarily composed of three phenyl-propanoid monomers namely sinapyl alcohol (S), 4-hydroxycinnamyl alcohol (H) and coniferyl alcohol (G) bonded by C–O or C–C linkages and are produced during cell wall biosynthesis by radical coupling reactions [5,6]. Plants utilize this high molecular weight, branched polymers for both water transport and defense. Around 50% of the

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inter-monomer linkages of lignin in most plants are the aryl ether β-O-4 bonds [5]. The internal "nonphenolic" β-O-4 linkages in lignin are bonded to additional monomeric units while lignin polymers being blocked by a *p*-hydroxyl group also called as "phenolic" group (Fig. 1) [7].

Various processes like homogeneous or heterogeneous catalysts availability as well as chemical and physical environments have been taken into consideration to understand the chemistry of predominant β-O-4 linkages cleavage in lignin polymers [8,9]. Remarkable and sophisticated NMR methods for finger printing aryl-ether linkages (other linkages) in lignin have been developed to establish their fate for being having a treatment function [10-12]. Lignin removal coupled with redistribution within biomass is the principal cause of using biomass for both important fuels and products synthesis using carbohydrates [4,13].

Lignin can also be depolymerized easily by concentrated acid hydrolysis. A broad range of genetically modified plants are used for less recalcitrant lignin or lower lignin contents as to improve carbohydrate production from biomass [14-16]. Other technologies by means of novel solvents like ionic liquids [17-19] coupled with organic solvents (Organosolv processes) have the capability to depolymerize the plant cell wall into its respective components [20-22].

Organic Acid Treatment

A broad scale laboratory investigation using a varied majority of organic solvents (ethanol, acetic acid, esters etc.) has been established to obtain remarkable results of both woody as well as non-woody pulping procedures [23-25].

Acetic Acid

Acetic acid, the first organic acid used in laboratory studies for lignocellulosic raw material delignification, proved to be helpful as a pulping solvent [26,27]. The wood pulping properties of acetic acid is far better compared with conventional chemical processes; it also possesses major benefits in contrast to other organosolv processes used at laboratory scales as reported by many researchers [28].

Formic Acid

Delignification can be performed via organosolv extraction. Formic acid, a chemical agent for biomass fractionation, is readily available and cheap organic solvent [29]. During formic acid pulping, β-O-4 bonds of lignin are cleaved by its dissolution into black liquor, whereas solid cellulose remains in the residue after degradation hemicellulose into both monosaccharides as well as oligosaccharides. Lignin precipitates out from the liquor by adding water while formic acid can be reused easily after recovery. Various techniques for pulping biomass fractions via formic acid have been described using peroxy-formic acid mixtures, aqueous formic acid and with acid-catalyzed aqueous formic acid [29,30-32].

Below 70% formic acid strength, pulping of lignocelluloses for delignification is not efficient but it rises as formic acid concentration exceeds 80% [29,33]. The structural together with molecular characteristics of lignin by products and solid residues formed as consequences of formic acid pulping should be established for various applications of biopolymers [34].

Fungal Acid Production

The filamentous fungi have gained remarkable interest due to their characteristics industrial applications as well as significant role in natural ecology as they play a pivotal role for low molecular weight organic acids production [35,36]. Natural organic acids production by fungi has many key roles in nature primarily either due to direct environmental interaction of organic acid or

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decrease in pH prior to their secretion. These roles are strictly dependent upon organic acid producing fungus type [37,38].

Acid-tolerant filamentous fungi have great potential because of consecutive decrease in pH upon organic acid secretion. For ecto-mycorrhizal fungi, this decline in pH proved to dissolve soil minerals and release nutrient ions for plants and microorganism's uptake, thereby enhancing mineral weathering [36]. The formation of oxalic acid by saprophytic and wood rotting fungi results in acid-catalyzed hydrolysis of holocellulose [39-41]. For this reason, Basidiomycota is studied in detail being capable to manufacture oxalic acid [42-45]. Studies on both fungus and plant symbiosis are considered to recognize their ecosystem roles [42,46].

The focus of the synthetic biology is based on working and utilization of biological systems for the benefit of society by fulfilling the demands for sustainable alternatives to fossil fuel as energy plus chemicals sources. Pharmaceutical, cosmetic excipients and food additives are some of the major industrial outcomes of organic acids as they are highly degradable molecules and can potentially replace petroleum-based or synthetic chemicals [47].

A variety of useful organic acids are produced by fungi, such as citric, gluconic, malic and itaconic acids are synthesized by *Aspergillus* and lactic and fumaric acids are formed by *Rhizopus* genera. Large scale bioprocesses can be used for certain organic acids like citric acid having the potential of fungi as organic acid production platforms [35,48].

Lignin inhibits both enzymatic and microbial attack as it is the chief constituent of plant tissue's mechanical support. By forming stable lignin-carbohydrate complexes (LCCs) with polysaccharides, lignin restricts the ruminal degradation and digestion of both cellulose and hemicellulose [49]. Actinomycetes together with white-rot fungi are LCCs degraders [50,51], although anaerobic lignin breakdown, occurring that in the rumen appears to be questionable as oxygen is the main moiety for lignin depolymerization [50,52,53].

The existence of soluble compounds in the rumen exhibiting the likewise spectra for both ultraviolet and infrared compared to lignin were previously reported [54]. All such easily dissolvable substances would not be liberated by disruption of LCCs, rather they might invent from the microbial hydrolysis of encompassing basic structural polysaccharides on LCCs surfaces. Conversely, conventional degradation of lignin model compounds by ruminal microbes coupled with the anticipated ways for their depolymerization utilizing HPLC investigation of the end products were additionally described [55-57]. Synthetic model compounds proved to be highly important to express breakdown of definite lignin attached structures on cell walls of plants. 4-Methylumbelliferone (4-MUF), a lignin analogue, is usually incorporated to lignin structure that can be helpful for detection purposes of lignin degradation as 4-MUF usually fluoresces in free state [58].

Both bacteria and fungi can primarily degrade lignocellulose biomass. However, augmenting the microbial activities is a collection of soil macro-invertebrates, ranging their properties from modest combination and spreading of plant material to a definite dissimilation of the structural polymers of lignocellulose feedstock [59-60]. Termites, being the most abundant and important of these invertebrates that coupled with associated microbial symbionts have the capability to disintegrate a proportion of both cellulose (74-99%) and hemicellulose (65-87%) constituents of the ingested lignocellulosic plant material [61,62].

Bonds in Lignin

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Lignin molecule possesses a variety of structurally correlated phenylpropanoid subunits having either C-C bonds or ether linkages known as core lignin. The major linkage is a phenylglycerol-β-aryl ether (e.g. ring 1→14), trailed by phenylcoumaran (ring→2), diary propane (ring→11), and biphenyl (ring→5) linkages. But diphenyl ethers (ring 12→13) and pinoresinol linkages (ring 5→6) are characteristically less common [63,64]. The breakdown of all these linkages by hydrolysis is almost tough or not possible. The structural and non-repetitive intricacy of lignin is due to its biogenesis [65].

In 1951, Freudenberg with his co-workers reported high molecular weight dehydrogenation polymerizates (DHP) having a proximity to spruce lignin synthesized by coniferyl alcohol dehydrogenative polymerization [66,67]. Further studies revealed the explanation of a complex reaction sequence that usually take place in plant cell walls lignifications [63,64,68,69]. The production of clear majority of phenoxy radicals using extracellular peroxidases starts a new cycle of non-enzymatic polymerization reactions to form oligolignols being condensed further in parallel reactions by initiating from basic identical monomers (coniferyl, sinapyl, and p-coumaryl alcohol). A three-dimensional complex network of non-identical oligolignols constitutes the major final product, lignin. The lignin from dissimilar phylogeny has remarkable structural differences [64].

Softwood lignin possess guaiacyl propane subunits (e.g. ring 13), being polymerizates of coniferyl alcohol monomers. Conversely, a mixture of sinapyl and coniferyl alcohol starts hardwood lignification that yields a characteristic mixture of syringyl and guaiacyl propane subunits (ring 4). Comparable to both types described, the grass lignin showed the greatest complication having 4-hydroxyphenylpropane subunits (ring 14). However, most grass lignin, coupled with hardwoods, have considerable percentage of chemically less recalcitrant linkages (5-10%) as aromatic acids being esterified to core lignin (ring 1→2), normally residing the primary hydroxyl groups at propyl side chains. Being covalently bonded with hemicellulose and possess carbohydrate polymer linkage, it is impossible to depolymerize lignin from lignocelluloses prior to partial denaturation. During the polymerization process in plant cell wall, many ethers and esters are formed by covalent linkages when several intermediates not only react other oligolignols but also with glucuronic acids in hemicelluloses possessing both hydroxyl and carboxyl groups (Fig.2 ring 10) [64,65,68].

Symbiotic Fungi

A subfamily Macrotermitinae, having higher termites, plays a remarkable role by forming a fascinating symbiotic association with external basidiomycete fungi belonging to genus *Termitomyces* that are being cultured in greyish-brown convoluted dynamic combs. The fungal mycelium that fills these combs have plant materials being partially digested by fungus and develop mycotetes (round white nodules) consisting of many conidia (asexual spores). Plant material gets heavy impregnation of fresh termite faeces that ultimately becomes permeated with *Termitomyces* spp. to develop new combs. Termites can easily utilize the older or more seasoned parts of the comb together with the fungal nodules. In 1989, researchers reviewed both biology and importance of this remarkable link that proved to be a key question for fungus role in termite nutrition [70]. Evidence suggests that *Termitomyces*spp. causes incomplete digestion of both plant polysaccharides and lignin within the comb [71,72].

Enzymatic Depolymerization of Lignin

Ligninolytic enzymes that perform the conversion of lignosulphonate considered to be the main lignin degrading enzymes [73]. Enzymatic conversion of lignocellulosic is beneficial over other

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physiochemical processes because of enzymatic specificity in reactions. There has been an expanding literature focusing on the ligninolytic enzymes after their discovery from white rot fungi [74]. This method is a significant alternative to the other methods due to high product yield and lower environmental impact. White rot fungi produce main lignin-degrading enzymes including heme-containing lignin peroxidases (LiP), manganese peroxidase (MnP), versatile peroxidase (VP) and copper containing laccases (benzenediol: oxidoreductase) (Fig. 3) [73].

Laccase (1, 4-benzenediol oxygen oxidoreductase)

Laccases being the core of interest since 19thcentury are one of the oldest enzymes obtained from Japanese lacquer tree as first described by Yoshida in 1883. These are the copper (Cu) containing proteins that contribute to oxidize a broad spectrum of inorganic as well as organic compounds but most specifically phenolic compounds by radical catalyzed mechanism [75].

The production of enzymes has been improved by some specific compounds which act as protein synthesis inducers. The manufacturing of recombinant laccases at industrial level has been increased by the recent success in cellular engineering and fungal molecular technology. Laccases are relatively more stable because they do not use hydrogen peroxidases (H_2O_2) as a cofactor. They can produce water by reducing the molecular oxygen in the presence of substrate (Fig. 4) [76].

Laccases are multi-copper proteins that are characterized by their electron paramagnetic resonance (EPR) spectrum in three distinctive types:

- Type-1 copper: attach to two amino acids (cysteine and methionine) and two histidine ligands, because of these enzymes show blue color.
- Type-2 copper: attach via water and two histidine ligands.
- Type-3 copper: contain two copper ions each of which attach to three histidine ligands.

Catalytic activity of laccases is performed both by type-2 and type-3 which form a trinuclear cluster (Fig. 5) [77,78].

The catalytic activity is generally dependent on three binding sites with these four types of copper ions. Type-1 copper is the main primary electron acceptor and then electron transferred to the tri-nuclear cluster. The oxygen reduction into water also takes place on these binding sites. Laccases remove solely one electron to oxidize its substrate and laccase with its total reduced state contain four electrons consequently electrons gain by oxygen yielding water [79]. Substrate spontaneously forms free radical or a new compound after the removal of proton (Fig. 6) [78].

An extensive amount of literature has examined the source of Laccases from fungi and plants. Its activity was also seen in bacteria viz. *Streptomyces griseus, Azospirillumlipoferum, Marinomonasmediterranea,* and *Bacillus subtilis* [80-82]. There are abundant types of fungi that show Laccases activity including*Neurosporacrassa, Pyriculariabryzae, Pleurotus, Pholiata,Polyporus versicolor* A, B, and *Aspergillus nidulans*. However, researchers show much interest in basidiomycetes like*Agaricusbisporus, Lentimusedodes, Trametes versicolor* and *Pleurotusostreatus*since they produce laccases that are involved in lignin degradation [83]. Laccases from *Trametes versicolor* (LTV) and *Agaricusbisporus*(LAB) are easily commercially available and have various applications in different fields including pulp and paper industry, textiles, environmental aspects, the food processing units, pharmaceutical business and nano-biotechnology [78].

Additionally, voluminous literature covers the LAB and LTV regarding their reactions and production [84].Laccases synthesized specially from white rot fungus (LAB and LTV) can cause lignin degradation due to their ability to further rearrange the phenoxy radical by C_α-C_β cleavage as

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well as the benzyl hydroxyls oxidation. Lignin polymer is too large to penetrate active site of laccase so it could not oxidize directly by laccase. Furthermore, a mediator; an additional compound is required to deal with this limitation [85].

Laccase-Mediator System (LMS)

For the depolymerization of lignin, laccases require a mediating agent known as intermediary substance or mediator. Mostly laccase mediators are low molecular weight and aromatic compounds. The combination of laccases with mediators increases the yield and rates in conversion of laccase-substrate as well as it adds new reactions to substrate without which enzyme shows no or just marginal activity. Consequently, LMS enhances the range of substrate to oxidize compounds with higher redox potential (E°) compared to laccases (LMS E° lies above +1100 mV but laccase allows to oxidize molecule in limited range of +475 to +790 mV) [86].

Numerous artificial mediators remain the subject of wide range of study, from the very first described laccase-mediator; ABTS to the synthetic mediators of -NOH- type (e.g. HBT, N-hydroxyphtalimide (HPI), violuric acid (VIO) and N-hydroxyacetanilide (NHA), the stable one 2,2,6,6-tetramethyl-1-piperidinyloxy free radical or TEMPO. ABTS has been considered the best substrate-mediator laccase. It speeds up the rate of reaction by moving the electron towards electron accepting compounds from the donor substrate. Two stages are involved in the oxidation of ABTS. In the earlier stage, fast oxidation occurs and cation radical ($ABTS^{+}$) is formed, after that di-cation ($ABTS^{2+}$) formed by the slow oxidation of cation radical (Fig. 7) [87].

A large body of literature has explained ABTS application of lignin degradation using laccase. The use of mediators, most probably ABTS is unique for the oxidation of lignin subunits. Many workers examined the Kraft lignin oxidation by *Trametes versicolor* (LTV) laccase and stated that ABTS coupled with laccase enhance the catalytic activity of laccase to generate lignin subunits having an average weight of 5300 g/mol [84]. The mechanism of ABTS oxidation indicates that $ABTS^{2+}$ di-cation only act as an intermediate, for oxidation of non-phenolic structures. Conversely, $ABTS^{+}$-cation radical accounts for phenolic structures [87].

In previous studies, researchers mostly concentrated on the oxidation mechanism of ethers, alcohols and lignin model compounds. Extensive research has described the effects of mediators and laccase enzyme on lignin model compounds to fully recognize the laccase reaction owing to the lignin structure complexity [84] (Fig. 8).

Model Compounds of Lignin

The structural variability and complexity of lignin provoked the use of various lignin model compounds in its place to study the lignin depolymerization [88]. Such model compounds bear a resemblance to lignin polymer and investigation of their reactivity gives understanding about the reactivity of lignin polymer itself. Several factors lead to the use of lignin model compounds:

1. to perceive the interaction between lignin and enzymes by using lignin model compounds in place of lignin due to their simple structure;
2. many model compounds contain lignin-related linkages i.e. β-O-4, α-O-4, β-5, 4-O-5, etc. so their reactivity give the information about lignin-enzyme interaction;
3. the product and analysis of such model compounds are relatively easy as compared to lignin. Many publications give the idea about the interaction of lignin with laccase; though, the lignin degradation mechanism is much more difficult to understand [89].

Lignin Peroxidase

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Lignin peroxidases (LiP) are heme containing proteins, first isolated from *Phanerochaetechrysosporium*. These peroxidases catalyze the oxidation of a broad variety of aromatic compounds in the presence of H_2O_2 [90]. This enzyme had been completely characterized and its catalytic mechanism was studied previously in oxidizing substrate. The enzyme is oxidized using H_2O_2 to LiPI (intermediate of LiP) and water. LiPI then converts to LiPII and substrate radical (VA"") by the oxidation of first molecule of veratryl-alcohol (VA). LiPII use the second veratryl alcohol (VA) by the reduction of the substrate and the enzyme recover in its original form [91].

$$\textbf{Enzyme (LiP)} + H_2O_2 \rightarrow \textbf{LiP I} + H_2O$$

$$\textbf{LiP I} + \textbf{VA} \rightarrow \textbf{LiP II} + \textbf{VA}^+$$

$$\textbf{LiP II} + \textbf{VA} \rightarrow \textbf{Enzyme (LiP)} + \textbf{VA}^+$$

Since 1986, veratryl alcohol (VA) had been a redox mediator for LiP. it did not react with lignin in the absence of veratryl alcohol. Lignin depolymerization via LiP is performed by adding veratryl alcohol [92].

Manganese Peroxidases

For last 25 years, production of heme-peroxidases remained an interesting subject for researchers which include both manganese peroxidase (MnP) and lignin peroxidase (LiP) as discussed earlier [93]. MnP uses H_2O_2 as a co-substrate in the substrate oxidation. Like LiP, MnP also produces the intermediates (MnP-I and MnP-II) in its catalytic cycle [91].

The nature of substrate makes the main difference between MnP and LiP. Unlike LiP, primary substrate of MnP is Mn (II) instead of phenol and produces Mn (III) which is highly reactive and oxidizes a variety of phenolic compounds. Firstly, iron-peroxide complex is formed when native ferric MnP bound to H_2O_2. MnP-compound-I along with a molecule of water produce by the transfer of electrons from MnP. Mn^{2+} oxidized to Mn^{3+} and transfer the electron to the porphyrin intermediate while MnP-compound-I transformed to MnP-compound-II [94]. MnP-II reduces in a similar way and regenerate the native MnP along with a second water molecule. Mn^{3+} chelated with organic acids makes possible Mn^{3+} releasefrom the active site of MnP. This detachment increases the oxidation rate by stimulating the MnP activity. Chelates of Mn^{3+} cause the oxidation of many substrates or the removal of radicals (Fig. 9) [95].

Versatile Peroxidases

A novel peroxidase from *Pleurotuseryngii*was reportedand this peroxidase contains both main peroxidase properties (LiP and MnP). This enzyme, named as versatile peroxidase (VP), indicating that it has properties of both LiP and MnP and can oxidize various substrates including Mn^{2+}, phenolic compounds and non-phenolic aromatic compounds e.g. veratryl alcohol [96]. Versatile peroxidase isolation is performed using white-rot fungi types like *Bjerkandera*spp. strain BOS55, *Pleurotusostreatus*and *Bjerkanderaadusta*[97].

In summary, heme containing peroxidases (LiP, MnP and VP) also have some drawbacks that limit their use in the study. MnP, LiP and VP involve the use of H_2O_2 for their catalytic activity while laccases only require O_2 which they absorb from the atmosphere directly. Peroxidases are extreme expensive and are not commercially available yet, in contrast, laccases are available at low prices. In comparison to peroxidases, laccases offer selection of mediator compounds for the process requirements. Consequently, laccase is a potential enzyme for degradation of lignin with promising applications that might improve efficiency and productivity with low investment cost [98].

Green Chemicals

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By exploring the chemical worth of biomass, green chemical technologies developed to capture the resources and maximize the production of value added plus environmental friendly chemicals. In this integrating approach, high value chemicals co-produce which maximizes the use of all biomass components, waste streams and by-products virtually with keeping environmental footprint low [89].Green chemicals obtained from the lignin are linked to the well-being of the environment with the potential production of renewable fuels, polymer building blocks and aromatic monomers such as phenol, vanillin, benzene, toluene, and xylene (BTX) [99].

Lignin for production of aromatic chemicals

Lignin (the renewable raw material) is probably present in ample amounts for the synthesis of aromatic substances at industrial level. It seems easy to conclude that efficient and direct conversion of lignin into low molecular weight and distinct aromatic compounds is highly remarkable goal. But the synthesis of defined high-volume aromatic chemicals using diverse and physically intricate lignin is feasible and long-term opportunity, although it is a most challenging goal to achieve(Fig.10) [100].

BTX (Benzene, Toluene, Xylene) Chemicals

Lignin can be depolymerized into various aromatic components. As these compounds are obtained from lignin, the first and the foremost duty is to eradicate the oxygen containing functional by decarboxylation, decarbonylation, dehydroxylation, and demethoxylation [101]. Benzene is a resourceful petrochemical building block from which more than 250 products could be formed. Cyclohexane, ethyl benzene and cumene are the chief derivatives. The xylenes product well-known as mixed xylene contains four different isomers: ortho-xylene, para-xylene, meta-xylene and ethyl benzene. Toluene is gaining importance for the xylenes manufacturing through disproportionation of toluene and trans-alkylation with C-9 aromatics [102]. Aromatic complexes are found in several different configurations. However, most modern complexes of aromatics are considered to maximize the yield of para-xylene, benzene and sometimes ortho-xylene [103]. On the other hand, by focusing on phenol and its derivatives, the phenolic hydroxyl and the aromatic ring needs to be remain intact and thus less energy will be required to convert polyphenolic ligneous complex into these compounds [104].

Bonds Cleavage in Lignin

Variety of depolymerization protocols are employed to yield 'green' chemicals from lignin. The production of aromatic chemicals might be achieved through several processing routes using the lignin enriched fractions [105]. The regulated breaking of different linkages in lignin requires detailed information regarding the stability of the bonds under different conditions in addition to understand the lignin decomposition mechanism. In lignin, both ester and ether bonds are easily hydrolysable. Lignin can also be degraded by means of biological methods with micro-organisms, by chemical routes or via sun light (UV) [106].

Monomeric Lignin Molecules

Selective depolymerization involving C-O and C-C bond rupturing could produce a plethora of complex aromatics structures either difficult to generate via conventional petrochemical ways. These compounds are correlated to the fundamental building blocks of lignin and are highly desirable due to their production in reasonable commercial amount. However, two barriers would have to be overcome. The first barrier is the advancement in technology for careful bond-scission to separate out the monomeric lignin structures althoughthis technology would be more difficult to develop

than the other destructive processes that yield phenols or BTX. Secondly, applications and markets for lignin monomers are needed to be developed. For these reasons, this technology has long-term applications and currently market-pull for large scale use is unknown [107].

Conclusions

More recent research work indicates that lignin can be depolymerized into variety of useful chemicals of industrial importance. For the progress of an economical viable lignin valorization path to synthesize aromatic chemicals, advanced methods are required to assess the ideal conditions, appropriate hydrogen donors together with bio-refinery catalysts. There is also much need of the time to further develop this process for the commercial production of high purity lignin.

In nature, lignocellulosic residues obtained via municipal solid wastes, agricultural, grass, wood and forestry substances are available in bulk quantities and have an enormous bio-conversion potential. As a renewable resource, they are an important source of both biologically and chemically useful products. Lignin, when accumulated in sufficient amounts at places where agricultural residues reveal a discarding nuisance result in environmental decline coupled with valuable materials loss that can be helpful in paper and pulp industry as well as biomass fuel production and composting.

Varieties of innovative markets for lignocellulosic residues especially of lignin like Benzene, Toluene and Xylene (BTX) have been identified in recent times. Low cost bioremediation projects by utilizing fungi seem to be promising as they are the source of well-organized lignocellulose depolymerization enzyme machinery. However, additional consideration of the innumerable other enzymes coupled with organic acids for depolymerization reactions and its molecular features will be desired. The most remarkable task is to assimilate various enzymes role and organic acids together with natural lignin degradation using a variety of microbes. Thus, organic acids utilization is much more effective and safe to increase the product quantities and as well as to decrease costs compared to certain other costly manufacturing protocols.

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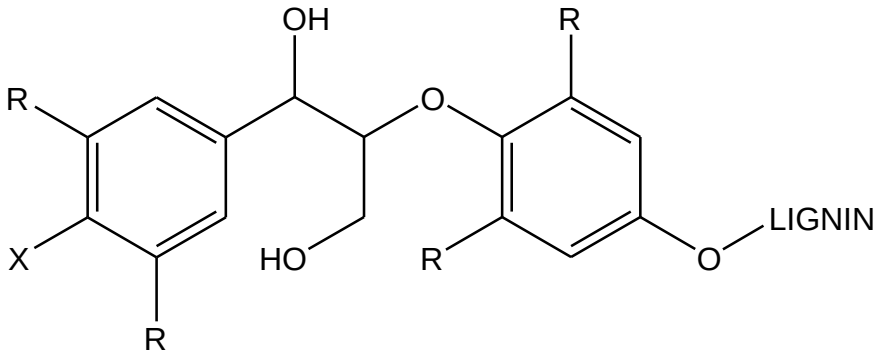
Figures and Figure Legends



R = H or OMe

X = OH (phenolic) or O-Lignin (non-phenolic)

Fig. 1 Lignin polymers can be terminated by a p-hydroxyl group or connected to additional lignin species, referred to as "phenolic" (X = OH) and "non-phenolic" (X = H or O-lignin) groups, respectively.

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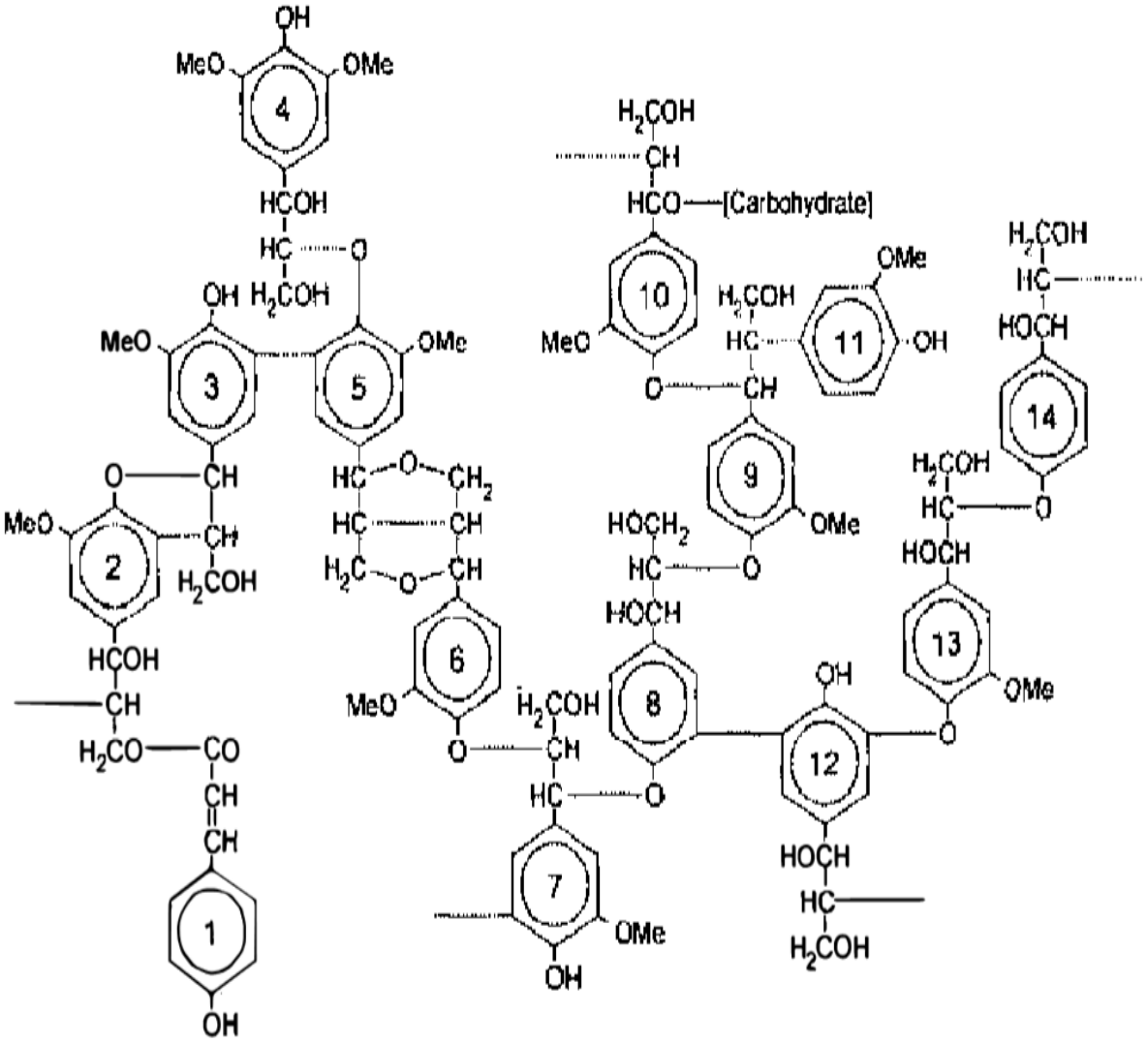

Fig. 2 Ideal lignin molecule showing bonds (adapted from Breznak and Brune 1994 with copyright permission).

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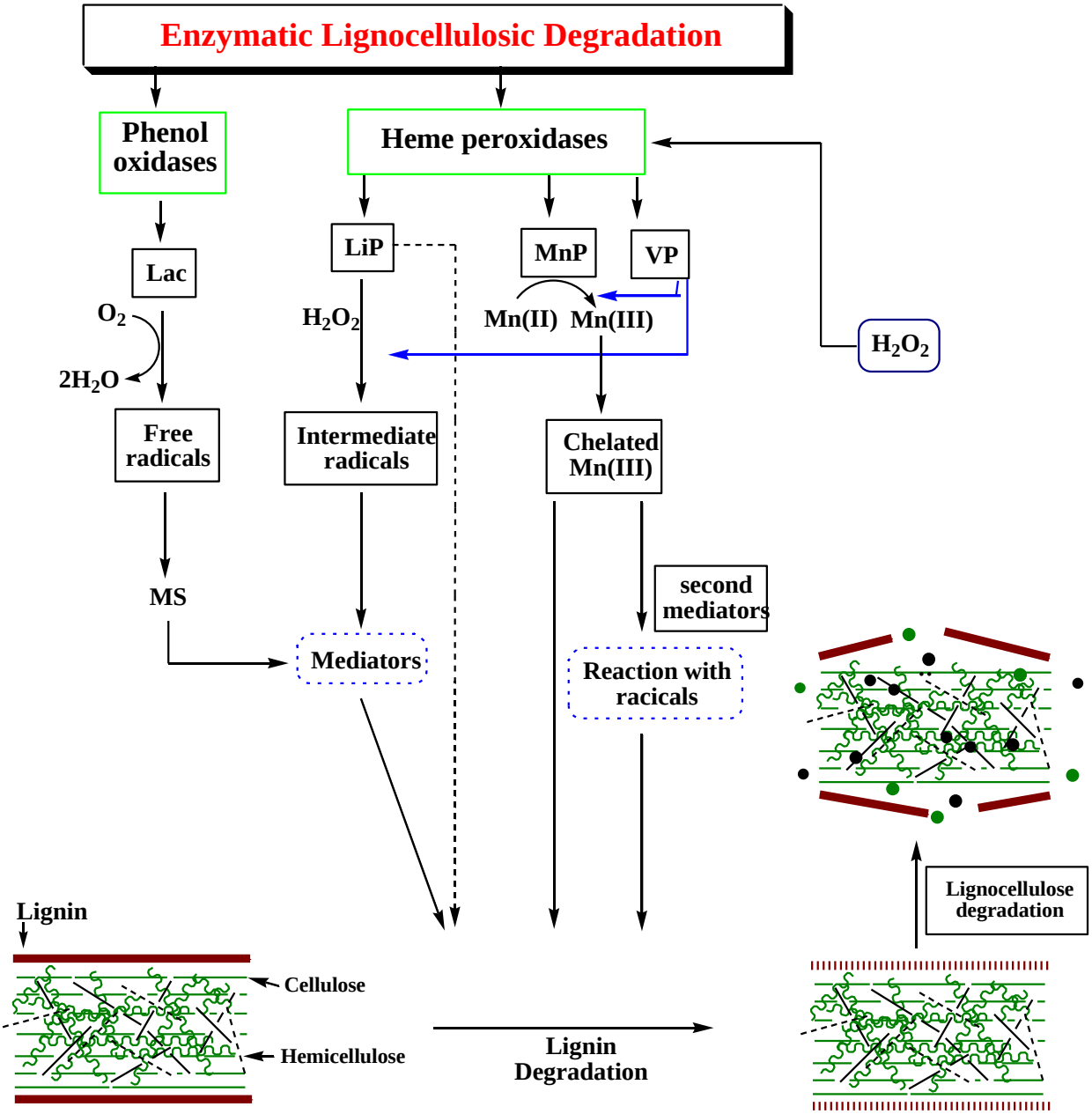

Fig. 3 Schematic representation of the lignin degradation steps and enzymes involved.

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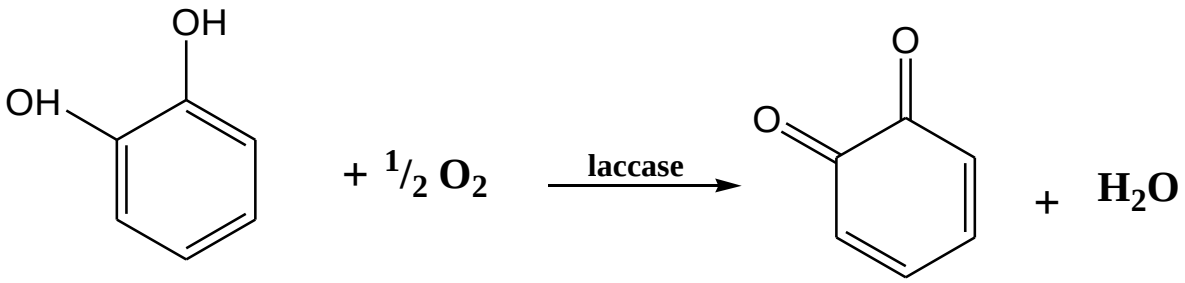

Fig. 4 Oxygen (O_2) reduction into water (H_2O) by laccase.

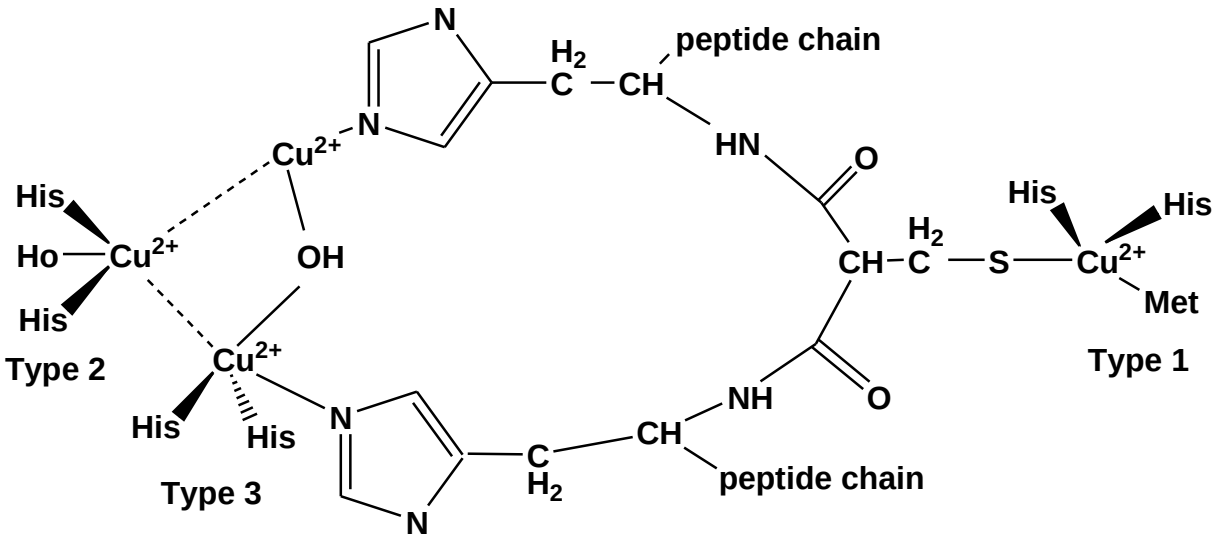

Fig. 5 Schematic diagram of laccase active site; containing four copper which belong to type-1, type-2 and type-3 binuclear copper site on the basis of their electron paramagnetic resonance (EPR).

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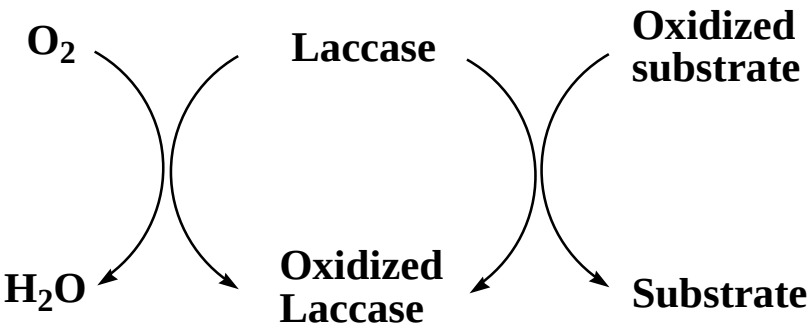

Fig. 6 Schematic representation of redox cycles for oxidation ofsubstrates catalyzed by laccase.

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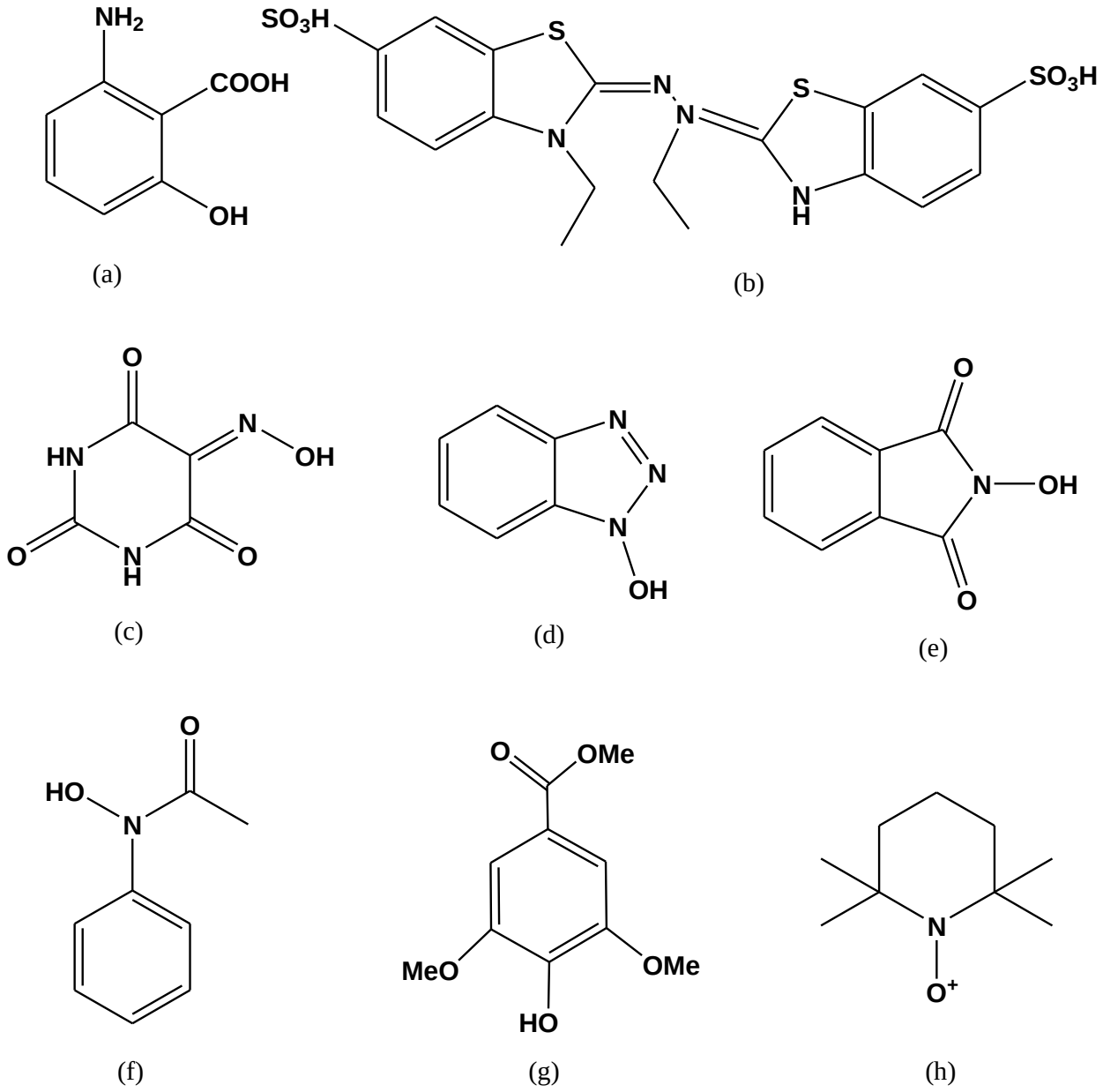

Fig. 7 Examples of laccases mediators. (a) 3-Hydroxyanthranilic acid (HAA); (b) 2,20-azino-bis-(3 ethylbenzothiazoline-6-sulphonic acid) (ABTS); (c) N-hydroxybenzotriazole (HBT);(d) N hydroxyphtaimide (HPI); (e) violuric acid (VLA); (f) N-hydroxyacetanilide (NHA); (g) methyl ester of 4 hydroxy-3,5-dimethoxy-benzoic acid (syringic acid); (h) 2,2,6,6-tetramethylpiperidine-1-yloxy (TEMPO).

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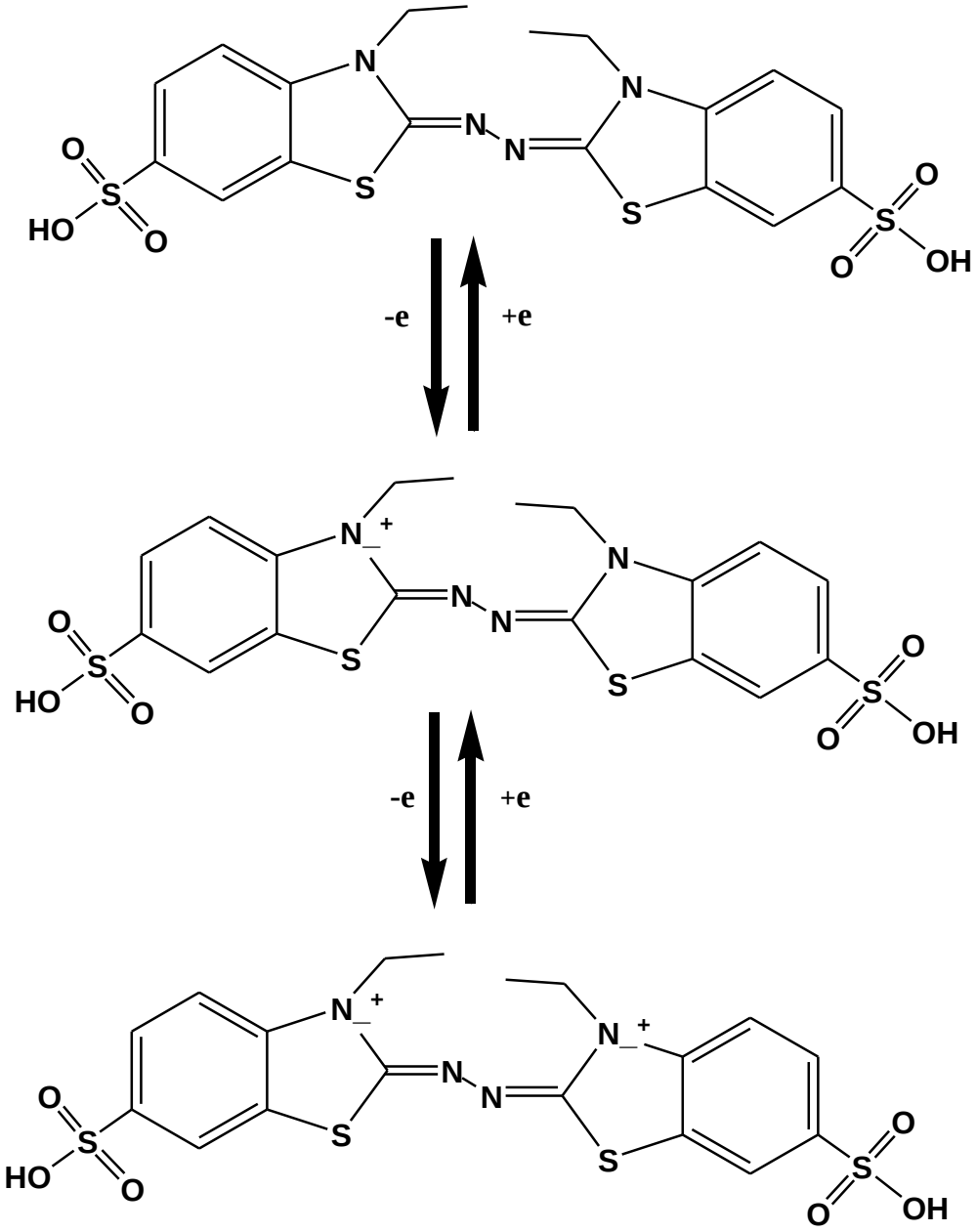

Fig. 8 Oxidation of ABTS catalyzed by laccase.

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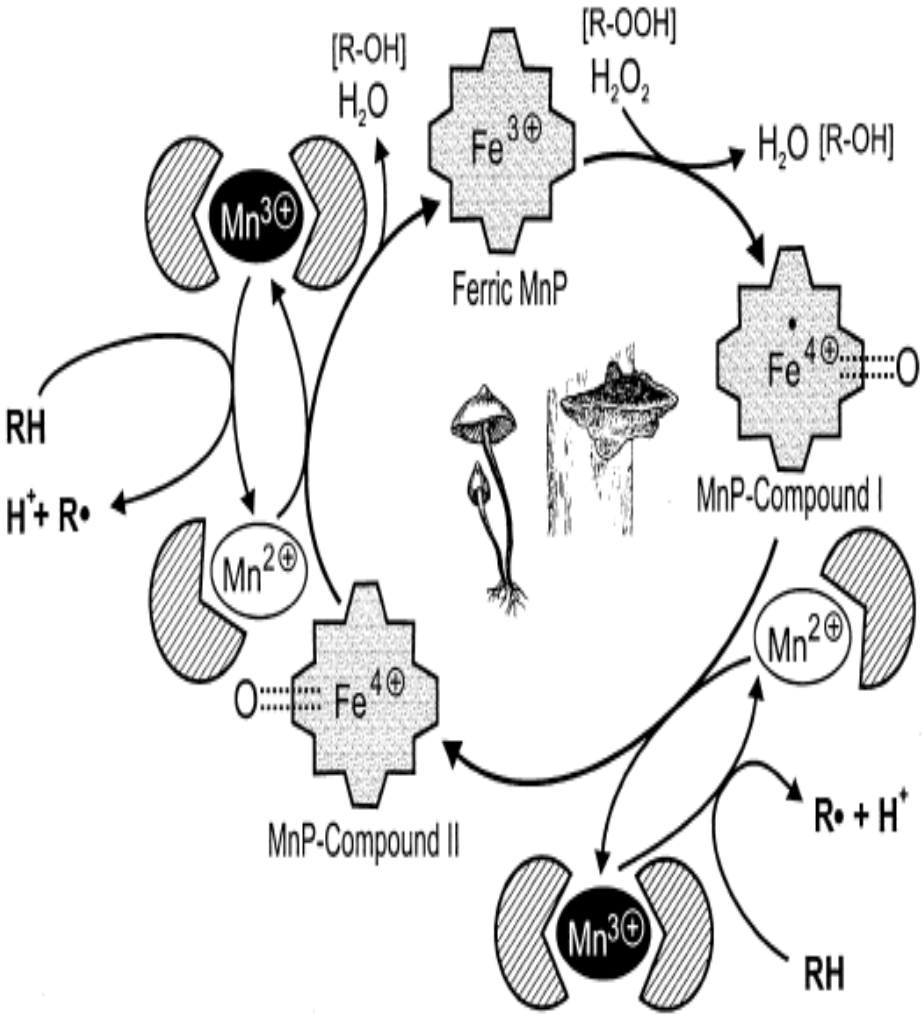

Fig. 9 Schematic representation of manganese peroxidase (MnP) catalyzed redox cycles for Mn^{2+} (adapted from Hofrichter 2002 with copyright permission).

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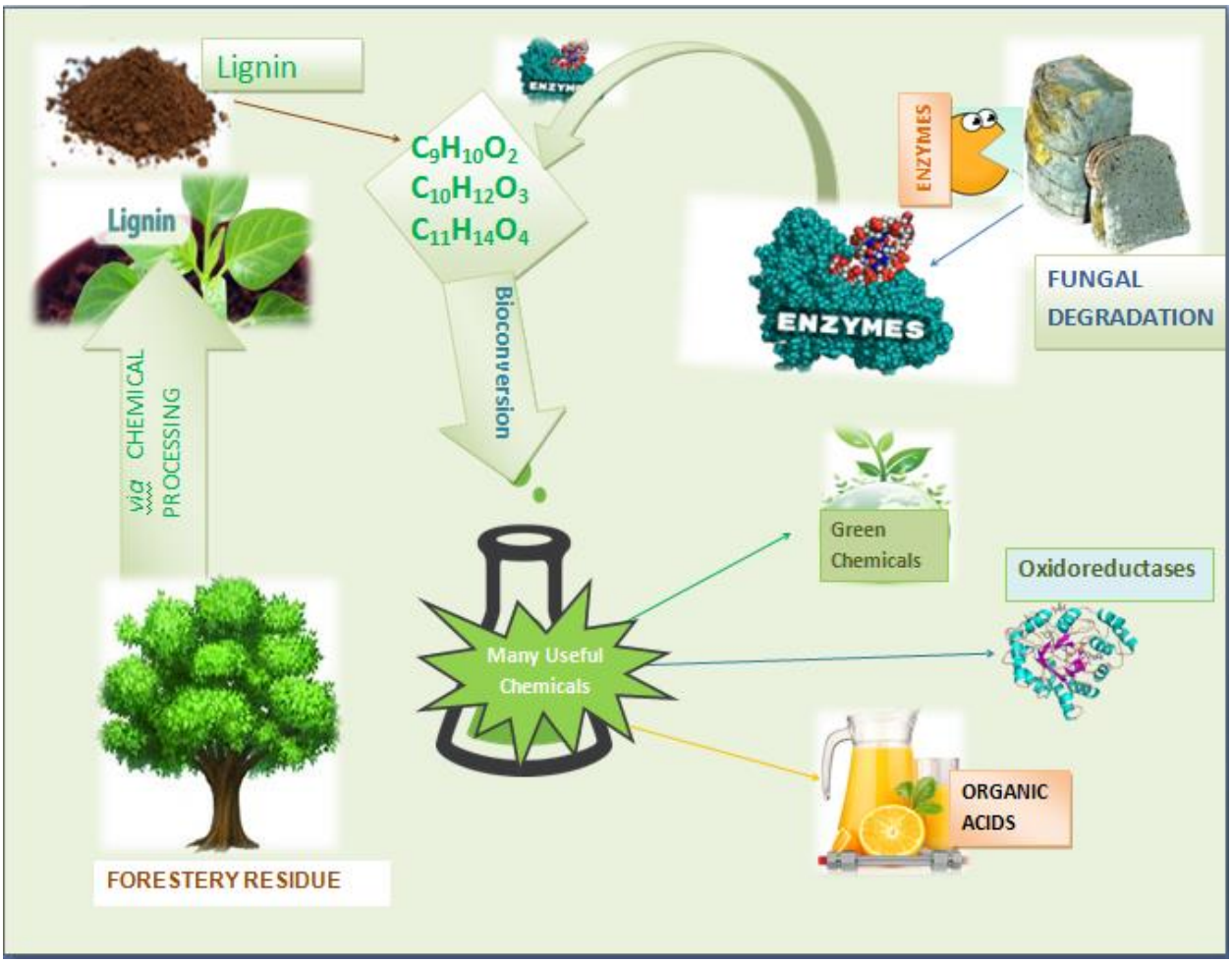

Fig. 10 Commercial Lignin Transformations.