Review

Microbial degradation of lignin: Role of lignin peroxidase, manganese peroxidase, and laccase

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Abstract: Lignin peroxidase (LiP), laccase (LA) and manganese peroxidase (MnP) of white-rot basidiomycetes such as Phanerochaete chrysosporium, Coriolus versicolor, Phlebia radiata and Pleurotus eryngii catalyze oxidative degradation of lignin substructure model compounds and synthetic lignins (DHPs). Side chain- and aromatic ring cleavage products of both phenolic and non-phenolic substrates oxidized by LiP were isolated and characterized by NMR and MS. The cleavage mechanism was elucidated by using $^{18}$O, $^2$H, and $^{13}$C labeled lignin substructure dimers with $^{18}$O$_2$ and H$_2$^{18}O. Recent studies suggested that LiP is capable of oxidizing lignin directly at the protein surface via a long-range electron transfer process. LA and MnP, which oxidize phenolic but not non-phenolic moieties, generally degrade lignin stepwise from phenolic moieties. However, recent studies indicated that MnP and LA can degrade both phenolic and non-phenolic aromatic moieties of lignin with some special mediators.

Key words: Microbial degradation of lignin; lignin peroxidase (LiP); manganese peroxidase (MnP); laccase (LA); aromatic ring cleavage; side chain cleavage.

Introduction. Lignins are three dimensional phenylpropanoid polymers linked by several different carbon-to-carbon and ether linkages between phenylpropane units most of which are not readily hydrolysable. (Fig. 1) Lignin is considerably resistant to microbial degradation in comparison with polysaccharides and most other biopolymers.

Nevertheless, white-rot basidiomycetes such as Coriolus versicolor, Phanerochaete chrysosporium, Phlebia radiata, Pleurotus eryngii etc. are known as typical lignin-degrading microorganisms. During the past 20 years, knowledge of the chemistry and biochemistry of lignin biodegradation by white rot basidiomycetes progressed substantially mainly through two complementary approaches. 1) Chemical and spectroscopic analyses of proto- vs. degraded lignins, and 2) biochemical elucidation of the degradation mechanism of lignin substructure model compounds and synthetic lignins (DHPs).

Higuchi and Nakatsubo synthesized several oligolignols containing major lignin substructures such as $\beta$-O-4, the most frequent interphenylpropane linkage (40-60% in lignin), $\beta$-5 (10%), $\beta$-1 (5%), and $\beta$-$\beta$ (<5%) linkages. The lignin substructure oligomers were used to elucidate lignin degradation mechanisms by P. chrysosporium and C. versicolor, and their enzymes, LiP and laccase (LA).

Mechanism of side chain cleavage of lignin substructure model compounds by lignin peroxidase (LiP). 1. $\beta$-1 Compounds. Kirk and Nakatsubo found for the first time that a deuterated non-phenolic 1,2-diarylpropane-1,3-diol model oligomer is degraded via $\alpha$-$\alpha$ cleavage by a ligninolytic culture of P. chrysosporium to give phenylglycol, $\alpha$-hydroxyacetophenone and benzaldehyde products, with retention of hydrogen atoms at $\alpha$ and $\beta$. They further found using ligninolytic culture experiments with $^{18}$O that the benzyl hydroxyl oxygen atom of the phenylglycol was derived from molecular oxygen.

Subsequently Tien and Kirk and Glenn et al. discovered the enzyme lignin peroxidase (LiP) which cat-
Fig. 1. A structural model of softwood lignin (guaiacyl lignin).

Fig. 2. Degradation pathways of deuterated β-1 lignin models (1,1') by lignin peroxidase (LiP) of P. chrysosporium. (1), deuterium; (2), 4-methoxyphenylglycol; (3), 4-methoxybenzaldehyde; (4), 1-(4-methoxyphenyl)-2-hydroxyethanone; (5), 1-(4-methoxyphenyl)-2-(4-methoxyphenyl) propane-1-one-3-ol.
Fig. 3. Degradation of deuterated arylglycerol-β-aryl ether lignin substructure models by LiP of *P. chrysosporium*. (1), α,β-dideuterated 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether; (2), 4-ethoxy-3-methoxyphenylglycerol; (3), 4-ethoxy-3-methoxybenzaldehyde; (4), guaiacol.

Fig. 4. Aromatic ring cleavage products of arylglycerol-β-aryl-U-13C, OCD ethers (1) by LiP of *P. chrysosporium*. (2), 4-ethoxy-3-methoxyphenylglycerol-β,γ-cyclic carbonate; (3), 4-ethoxy-3-methoxyphenylglycerol-β-methyl oxalate; (4), 4-ethoxy-3-methoxyphenylglycerol-γ-formate.

Fig. 5. Mechanism of aromatic ring cleavage of β-O-4 lignin substructure models by LiP of *P. chrysosporium*. (1), 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether; (2), 4-ethoxy-3-methoxyphenylglycerol-β, γ-cyclic carbonate; (3), 4-ethoxy-3-methoxyphenylglycerol-β-methyl oxalate; (4), 4-ethoxy-3-methoxyphenylglycerol-β-mucorate.
alyzes Ca-Cβ cleavage in the propyl side chains of β-1 compounds, in agreement with in vivo experiments.

Habe et al. synthesized deuterated non-phenolic 1,2-diarylpropane-1,3-diols as substrates for experiments with LiP of P. chrysosporium, and found the formation of phenylglycol, α-hydroxycetophenone and benzaldehyde products with retention of the deuterium at Ca and Cβ of the side chain. The result confirmed that hydrogen abstraction is not involved in the Ca-Cβ bond cleavage (Fig. 2).

Kersten et al. and Hammel et al. showed that LiP acts by catalyzing the 1-electron oxidation of aromatic rings, forming cation radicals, which undergo a variety of nonenzymatic reactions.

1. β-O-4 Compounds. Umezawa et al. found that α,β-dideuterated 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl ether (Fig. 3, (3) was converted by LiP to 4-ethoxy-3-methoxybenzaldehyde (3), and guaiacoylacetaldehyde (4) and guaiacol (5) by Ca-Cβ cleavage, and subsequent O-Cβ cleavage, and 4-ethoxy-3-methoxyphenylglycerol (2) by O-C4 cleavage. Mass spectrometric analysis showed that deuterium at Ca and Cβ of the 4-ethoxy-3-methoxyphenylglycerol, of 4-ethoxy-3-methoxybenzaldehyde and of guaiacoylacetaldehyde were almost quantitatively retained after the Ca-Cβ and O-C4 bond cleavages. (Fig. 3) The results clearly showed that Ca-Cβ cleavage and O-C4 cleavage occurred via the cation radical intermediates by one electron oxidation of the aromatic ring of the substrate by LiP.

In further investigation we identified an alternative Ca-Cβ cleavage reaction of a β-O-4 model compound, 4-ethoxy-3-methoxyphenylglycerol-β,18O-guaiacyl ether, to give 2-guaiacyloxethanol and benzyl alcohol probably via benzaldehyde, in lignolytic cultures of P. chrysosporium. GC-MS analyses of the isolated products showed that 17O of the ether oxygen of the substrate was not retained in the 2-guaiacyloxethanol product. When 4-ethoxy-3-methoxyphenylglycerol(γ-13C)-β-guaiacyl ether used as substrate, the 2-guaiacyloxethanol product was labeled with 13C at the 2-position but not the 1-position.

Mechanism of aromatic ring cleavage of lignin substructure model compounds by LiP. Kirk and Chang compared white-rotted lignin polymer (isolated and purified from white-rotted wood) with non-degraded lignin, using a variety of chemical and physical methods. Among their conclusions was that aromatic rings had been cleaved while still in the polymer.

The mechanism of aromatic ring cleavage of lignin by fungi, however, remained unsolved until 1985. Umezawa and Higuchi synthesized 4-ethoxy-3-methoxyphenylglycerol-β-guaiacyl[U-ring18O,OCDC3]-ether, and 4-ethoxy-3-methoxyphenylglycerol-β-syringyl[U-ring18C,OCDC3]-ether as substrate to elucidate the mechanism of aromatic ring cleavage of the model compounds. The compounds were incubated with ligninolytic cultures of P. chrysosporium in the presence of H3O18. We isolated and identified for the first time βγ and αβ-cyclic carbonates, formate and oxalate esters of arylglycerol from the reaction mixtures as aromatic ring cleavage products (Fig. 4). We finally identified a muconate ester of arylglycerol as an initial ring cleavage product of the dimers by LiP.

The cleavage mechanism of the aromatic ring was further elucidated by experiments using [H,13C] and 18O labeled dimers with 15O2 and H218O. The results showed that the mechanism of aromatic ring cleavage of lignin is completely different from the aromatic ring cleavage reaction for catechol derivatives by dioxygenases: LiP catalyzes the one electron oxidation of the aromatic ring (B) of arylglycerol-β-aryl ether to give aromatic cation radicals which are attacked by H2O, and that the resulting radicals couple with dioxygen to afford the muconate ester of arylglycerol (Fig. 5).

Cleavage of side chains and aromatic rings of a synthetic lignin (DHP) by LiP. Umezawa and Higuchi found that most of the initial stage of degradation reaction of β-O-4 lignin substructure model dimers was catalyzed by LiP. A synthetic lignin (DHP: dehydrogenation polymer of coniferyl alcohol prepared using horseradish peroxidase, M. W. >2200) was prepared and subjected to degradation with LiP to elucidate the mechanism of lignin degradation by this enzyme.

As the case of the degradation of β-O-4 lignin substructure model dimers by LiP, the cyclic carbonates and formate ester of arylglycerols, and arylglycerol were isolated from degradation products of the DHP by LiP; the chemical structures of the products were identified by GC-MS. These results indicated that the lignin polymer is really degraded by the LiP of white-rot fungi.

Active sites of LiP to substrates. Doyle and his group recently found that Trp 171 of LiP protein is hydroxylated at the Cβ position. They found that the hydroxylation process in both wild type and recombinant LiP isozyme H4 is autocatalytic and that Trp 171 may be implicated in catalysis. Site directed mutagenesis of recombinant enzymes with Trp 171 substituted by Phe (W171F) or Ser (W171S) lost all activity for veratryl
alcohol (VA; a LiP substrate) but not for two dye substrates. The result suggested two distinct substrate interaction sites in LiP, a heme-edge site, and a novel site centered around Trp 171 which is required for the oxidation of VA. Stop-flow kinetic studies strongly suggested that an electron-transfer pathway exists within the enzyme protein leading from the heme to a surface site in close proximity to Trp 171.

Johjima et al. confirmed that the binding site of LiP for VA is Trp 171 by using three different chemically modified LiPs against VA acting as a reducing substrate, a reducing reagent for the rapid conversion of LiP back to native LiP, and as an enzyme-bound redox mediator. They further studied the binding properties of LiP for synthetic lignin (DHP) by resonant mirror biosensor techniques, and found that among several lignolytic enzymes only LiP specifically binds to DHP. Kinetic analysis showed that the binding is reversible, and LiP is capable of oxidizing lignin directly at the protein surface by a long-range electron transfer process. A close look at the crystal structure suggested that LiP possesses His-239 as a possible lignin-binding site on the surface, which is linked to Asp-238. This Asp residue is hydrogen-bonded to the proximal His-176. The His-Asp proximal-His motif would be a possible electron transfer route to oxidize polymeric lignin.

Tien’s group studied on the active site of LiP with respect to substrate size using either fungal or recombinant wild type, as well as mutated, recombinant LiPs. A nonphenolic tetrameric lignin model that contains β-O-4 linkages was used as substrate. Both natural and recombinant LiPs oxidized the tetrameric model forming four products, tetrameric, trimeric, dimeric, and monomeric carbonyl compounds. The result indicated that LiP is able to attack any of Ca-Cβ linkages in the tetrameric compound and that the substrate-binding sites is thus well exposed. Mutation of a Trp residue (W171S) completely inhibited the oxidation of the tetramer model. These results are consistent with LiP having an exposed active site capable of directly interacting with the lignin polymer without the need for low molecular weight mediators, such as VA.

**Manganese peroxidase (MnP).** Following the discovery of LiP in *P. chrysosporium*, manganese peroxidase (MnP) secreted from the same fungus was found as another lignin degrading enzyme by Gold’s group, and Crawford’s group, respectively, and subsequent investigations have shown that MnP is distributed in almost all white-rot fungi.

Ten extracellular peroxidase isozymes were purified from the culture of *P. chrysosporium*. These enzymes were designated H₁ to H₁₀, according to their order of elution from an anion exchange column. Isoenzymes H₁, H₃, H₅, H₆, and H₁₀ were identified as LiP isoenzymes, and H₂, H₄, H₇, and H₉ as MnP isoenzymes, respectively.

MnP oxidizes phenolic compounds as well as Mn⁷⁺ to Mn³⁺. Mn³⁺ is stabilized by fungal chelators such as oxalic acid, and the chelated Mn³⁺ oxidizes phenolic compounds. MnP, in the presence of sodium malonate, Mn⁷⁺ and H₂O₂, was found to catalyze Ca-Cβ cleavage, Ca-oxidation and alkyl-aryl cleavages of phenolic β-1 and β-O-4 lignin substructures.

It has been proposed that chelated Mn³⁺ acts as low-molecular weight, diffusible redox-mediator that attacks the phenolic lignin structure. Further investigations showed that the chelated Mn³⁺ system generates reactive intermediates (peroxy radicals) from unsaturated fatty acids such as linoleic acid and their derivatives (lipids).

The MnP-lipid system is strong enough to degrade Ca-Cβ and β-aryl ether bonds in not only phenolic but also nonphenolic lignin model dimmers.

Hammel and his group found that wood block cultures and defined liquid medium cultures of *Ceriporiopsis subvermispora* rapidly depolymerized and mineralized a ¹⁴C-labeled, polyethylene glycol-linked high molecular weight β-O-4 lignin model compound that represents major nonphenolic structure of lignin. The fungus cleaved the model between Ca and Cβ to release benzylic fragments. The fungal degradation on the model and methylated lignin was significantly faster in the presence of Tween 80, a source of unsaturated fatty acids.

Wariishi et al. also found that MnP catalyzes substantial depolymerization of DHP by purified MnP of *P. chrysosporium* in the presence of malonic acid as the chelator. Both guaiacyl- and guaiacyl-syringyl lignin models were degraded substantially.

However, identification of cleavage products of side chain and aromatic ring of lignin substructure models and DHP by MnP, and the chemical degradation mechanism have scarcely been investigated.

**Versatile peroxidase (VP).** Versatile peroxidases (VP) that can oxidize Mn⁷⁺ as well as phenolic and non-phenolic aromatic compounds have been isolated from *Pleurotus* and *Bjerkandera*. VP oxidizes Mn⁷⁺ to Mn³⁺, degrades the nonphenolic lignin model veratrylglycerol-β-guaiacyl ether yielding veratryl aldehyde, and oxidizes veratryl alcohol and p-dimethoxybenzene to
veratryl aldehyde and p-benzoquinone respectively as LiP does. A review on lignin conversion by MnP has been published recently.\(^{35}\)

**Laccase (LA).** In 1928 Bavendamm\(^{36}\) discovered by cultivating wood-rotting fungi in an agar medium containing several phenolic compounds, such as gallic acid, tannic acid, and hydroquinone, that white-rot fungi produced a large darkened zone around the mycerial mat, but no zone of darkening was associated with the growth of brown-rot fungi. Davidson et al.\(^{37}\) subsequently investigated the reaction using 210 species of wood-rotting fungi, and concluded that the white-rotting type coincides with Bavendamm’s reaction in general, and that the reaction is helpful in identifying fungi. The enzyme responsible for Bavendamm’s reaction was extensively studied in the next 10 years, and characterized to be laccase (LA).\(^{38}\) LA, p-diphenol oxidase (EC 1.10.3.2) has been isolated and characterized as a blue, copper containing oxidase from a lac tree (Rhus spp) and several fungi. White rot fungi constitutively produce laccase during primary metabolism.\(^{39}\)

1. **Degradation of β-1 model compounds.** Kawai et al.\(^{40}\) found that phenolic β-1 model compounds are degraded by LiP of *P. chrysosporium* and LA of *C. versicolor* via similar pathways. 1-(3,5-Dimethoxy-4-hydroxyphenyl)-2-(3,5-dimethoxy-4-ethoxyphenyl)propane-1,3-diol (1, Fig. 6) was converted by LA of *C. versicolor* to 1-(3,5-dimethoxy-4-hydroxyphenyl)-2-(3,5-dimethoxy-4-ethoxyphenyl)-3-hydroxypropanone (2) by Ce oxidation, 2-(3,5-dimethoxy-4-ethoxyphenyl)-3-hydroxypropanal (5), 2,6-dimethoxy-p-hydroquinone (4) and its benzoquinone (3) by alkyl-phenyl cleavage (Fig. 6). Their experiment further showed that \(^{18}\)O of \(^{18}\)O\(_2\) was incorporated into etanone, and \(^{18}\)O of H\(_2^{18}\)O into hydroquinone and benzoquinone, respectively.

Based on the structures of the degradation products and the isotopic experiments they concluded that three types of reactions proceeded via phenoxy radicals of the substrates generated by LA; 1) Ce-Cβ cleavage between C\(_1\) and C\(_2\), 2) alkyl-phenyl cleavage between C\(_1\)
and aryl group, and 3) Cα oxidation.

Recently yellow laccase as well as blue laccase have been isolated from solid-state and submerged culture of Panus tigrinus. The yellow laccase had no blue maxima in the absorption spectrum, but catalyzed oxidation of VA and a non-phenolic β-1 dimer. The yellow laccase was suggested to be formed as a result of blue laccase modification by products of lignin degradation, which might play a role as natural electron-transfer mediators for the oxidation of nonphenolic substances.41)

2. Degradation of β-O-4 model compounds. Kirk et al.42) worked on degradation of the lignin model compound syringylglycerol-β-guaiacyl ether by Polyporus versicolor and Stereum frustulatum. They found that the benzyl alcohol group of the substrate was oxidized to a carbonyl group, giving α-guaiacyacetosyringone by whole culture of S. frustulatum and the culture filtrate of P. versicolor. The alkylphenyl carbon-to-carbon bond in both syringylglycerol-β-guaiacyl ether and α-guaiacyacetosyringone was cleaved by culture filtrate of P. versicolor with formation of guaiacycetaldehyde and guaiacycetacetic acid, respectively. The syringyl moieties of both parent compounds were converted to 2,6-dimethoxy-p-benzoquinone by culture filtrate of P. versicolor. Laccase also effected all the above reactions.

Kawai et al.43) recently investigated the degradation of syringylglycerol-β-guaiacyl ether by LA of C. versicolor. They showed that the substrate is mainly converted to the α-carbonyl dimer, 2,6-dimethoxyhydroquinone, and glyceraldehyde 2-guaiacyl ether by alkyl-phenyl cleavage, and to guaiacol by O-Cβ cleavage. Syringaldehyde and guaiacoxethanol as direct Cα-Cβ cleavage products of the substrate were not found.

Subsequent investigation to identify the pathway to give guaiacol showed that α-carbonyl dimer used as substrate is cleaved between Cα and Cβ to give syringic acid and guaiacol as shown in Fig. 7. The result indicated that phenolic β-O-4 compound is degraded not only by alkyl-phenyl cleavage, which has been proposed as a major LA-mediated degradative reaction, but also by Cα-Cβ-cleavage of the Cα-carbonyl dimer previously formed by Cα oxidation by LA. The side chain cleavage of phenolic β-O-4 lignin substructure model compounds with LiP and LA suggested that the same chemical principle, phenoxy radical as intermediate, is involved in the degradation of phenolic lignin substructure model compounds by both enzymes.

Recently, the degradation of nonphenolic lignin model compounds by LA in the presence of appropriate mediators such as 1-hydroxybenzotriazole (1-HBT) and
2,2'-azobis(3-ethylbenzthiazoline-6-sulfonic acid) has been reported.\textsuperscript{44} Kawai \textit{et al.}\textsuperscript{45} found that LA of \textit{C. versicolor} catalyzed \(\alpha\)-\(\beta\) cleavage, \(\alpha\)-oxidation, \(\beta\)-ether cleavage, and aromatic ring cleavage of the non-phenolic \(\beta\)-O-4 lignin model dimer 1,3-dihydroxy-2-(2,6-dimethoxyphenoxo)-1-(4-ethoxy-3-methoxyphenyl)-propane in the presence of 1-HBT. They also found that the oxidation of the substrate in \(\text{H}_2\text{O}_{18}\) resulted in the incorporation of \(18\text{O}\) into three aromatic ring cleavage products and a \(\beta\)-ether cleavage product which are identical with those obtained by LiP.

3. Syringyl polymer. Syringyl lignin model polymer (MW>2200) was degraded by LA of \textit{C. versicolor}.\textsuperscript{46} The polymer was depolymerized partially to form 2,6-dimethoxy-p-hydroquinone, 2,6-dimethoxy-p-benzoquinone, and syringaldehyde. NMR spectra of the degraded substrate suggested that the LA catalyzed the oxidation of benzylic hydroxyl groups to ketones at the polymer stage.

4. Aromatic ring cleavage. Kawai \textit{et al.}\textsuperscript{47} found that 4,6-di-t-butylguaiacol is converted by LA of \textit{C. versicolor} to a ring cleavage product, the muconolactone derivative, which was previously identified by Gierer and Imsgard\textsuperscript{48} as a product in alkaline-oxygen oxidation of the same substrate. The experiment showed that \(18\text{O}\) from \(\text{H}_2\text{O}_{18}\) but not from \(\text{H}_2\text{O}\) is incorporated into the muconolactone derivative. Thus, the pathway A in Fig. 8 was proposed for ring cleavage of 4,6-di-t-butylguaiacol by LA.

**Conclusion.** The main cleavage mechanisms of side chains and aromatic rings of lignin model compounds and synthetic lignin (DHP) by white-rot fungi and their enzymes LiP, and LA have been elucidated using \(2\text{H}, 13\text{C}\) and \(18\text{O}\)-labeled lignin substructure dimmers with \(18\text{O}_2\) and \(\text{H}_2\text{O}_{18}\). Side chain and aromatic rings of these substrates were cleaved via aryl cation radical and phenoxy radical intermediates in reactions mediated by LiP/\(\text{H}_2\text{O}_{18}\) and laccase/O\(_2\)/mediator.

Hydrogen peroxide is only required for the conversion of native LiP and MnP into two electron-deficient reactive species (compound I). Compound I of LiP abstracts stepwise two electrons from the aromatic ring of lignin substrate to yield aryl cation radicals or aryl cations, which are attacked by \(\text{O}_2\) or nucleophiles such as \(\text{H}_2\text{O}\) and R-OH, respectively. The subsequent reactions of the cation radicals and cations are not controlled by the enzyme just as in the non-enzyme-directed coupling of phenoxy radicals of monolignol in lignin biosynthesis. Thus, the role of LiP, LA, and probably MnP in lignin biodegradation could be explained by the following unifying view.

**Enzymatic reaction**

1. LiP/\(\text{H}_2\text{O}_2\) → Phenoxy radicals of phenolic units, and aryl cation radicals or cation radicals of non-phenolic units
2. La/\(\text{O}_2\) → Phenoxy radicals of phenolic units
3. La/\(\text{O}_2\)+Mediators → Phenoxy radicals of phenolic units, and aryl cation radicals or cation radicals of non-phenolic units
4. MnP/\(\text{H}_2\text{O}_2\)+\(\text{Mn}^{2+}\) → Phenoxy radicals of phenolic units

![Fig. 8. Mechanisms for degradation of 4,6-di-t-butylguaiacol by LA of \textit{C. versicolor}. (1), 4,6-di-t-butylguaiacol; (2), muconolactone derivative.](image-url)
units
5) MnP/H₂O₂+Mn²⁺ → Mediators → Phenoxy radicals of phenolic units and aryl cation radicals or cation radicals of non-phenolic units

Non-enzymatic reaction
1) Homolytic or heterolytic cleavage of side chains (Cα-Cβ, alkyl-phenyl), and aromatic rings
2) O₂ attack on carbon-centered radical intermediates
3) Nucleophilic attack on aryl cations and Cα-Cβ by H₂O and R-OH → Degradation products

Recent molecular investigations on linalool oxidases have shown that *P. chrysosporium* has two gene families including ten LiP-type and three MnP-type genes coding different isoenzymes expressed during secondary metabolism. Many linalool oxidase peroxidase genes from other white-rot fungi, and two VP genes from *Pleurotus eryngii* have been cloned. Biochemical and biotechnological approaches to lignin biodegradation open up a new field in biomass conversion, such as biopulping and biobleaching, and treatment of Kraft bleaching effluents and related pollutants by lignin degrading basidiomyecetes and their enzymes. A review article is referred to for molecular biology and engineering of lignin biodegradation.

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