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- Review -

Effect of Superoxide Dismutase and Low Molecular Mediators on Lignin Degradation[*1]

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ABSTRACT

As the biodegradation of wood constituents has been understood as a multi-basidiomycetes and enzymatic processes, this review will focus on the roles of low molecular compounds and radicals working in harmony with fungal enzymes. Wood rotting basidiomycete fungi penetrate wood, and lead to more easily metabolize carbohydrates of the wood complex. The white-rot fungi, having versatile enzymes, are able to attack directly the "lignin barrier". They also use a multi-enzyme system including so-called "feedback" type enzymes allowing for simultaneous degradation of lignin and carbohydrates. The multi-enzymes including laccase support the proposed route by explaining how the high molecular weight enzymes can function in the wood complex. These enzymes may function separately or cooperate each other. In addition, veratryl alcohol oxidase, cellobiose dehydrogenase, arylalcohol dehydrogenase, and particularly low molecular mediators and radicals have an important role in wood biodegradation. However, the possibility of other mechanism as well as other enzymes, as operating as feedback systems in the process of wood degradation, could not be excluded.

Key words; white-rot fungi, low molecular mediator, biodegradation, superoxide dismutase, multi-enzyme, laccase, feedback type enzyme

INTRODUCTION

The biodegradation of wood constituents is presently understood as an enzymatic process. The transformation of free cellulose or hemicellulose into monosaccharides has long been known to be relatively simple. Many cellulolytic as well as some ligninolytic fungi make use of a full range of hydrolases which are capable of producing monosaccharides in large quantities

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from all polysaccharide components of wood. However, it is mandatory for these components not to occur in a wood complex with lignin. Thus, complex structure of lignin which is very resistant against hydrolytic enzymes causes a problem. This is why the research on the biotransformation of wood complex has been carried out for a number of years.

The wood is a compact, in parts almost crystalline complex. Polysaccharide components in microfibrils are densely packed in lignin layers which protects them against the activity of hydrolytic enzymes and other external factors and serves as a stabilizer of the complex structure (Fengel, 1971). This structure endows plants with the necessary stiffness which in plants performs the function of a kind of concrete block with metal rods inside. Its durability comes from a compact structure of the wood complex (Leonowicz *et al.*, 1997).

In 1964 Freudenberg published his lignin model structure based on dehydrogenative polymerization results as well as on other analytical data available at that time. The model represents 18 prominent C_9-units as a part of a total molecule (in nature over 100). In 1966, Forss *et al.* analyzed lignin fragments obtained by decomposition of lignin and fractionated them according to their sizes. The aromatic components of spruce wood may be divided into two groups, low molecular lignins (about 6% of the wood) and lignin with high molecular phenylpropane. Being widely distributed in nature, lignin is the second (after cellulose) most abundant natural polymer in the biosphere and the most abundant aromatic material accounting for about 40% of the solar energy in plants.

Recently laccase(LAC; EC 1.10.3.2) has received more attention. It has been suggested that two classes of extracellular enzymes, peroxidases and LACs, are involved in ligninolysis owing to their ability to catalyse the cleavage of carbon-carbon or carbon-oxygen bonds in lignin or lignin model compounds. Lignin peroxidase(LiP; EC 1.11.1.14) oxidizes the lignin structures by one electron transfer reactions yielding cation radical intermediates that undergo spontaneous ring fission and other bond cleavages. Manganese dependent peroxidase(MnP; EC 1.11.1.13) providing hydrogen peroxide through oxalate and glycolate as the radical mediators (Leonowicz *et al.*, 1984) cooperates with LiP in lignin degradation or directly degrades the polymer using oxalate as the mediator, although to a smaller extent than LiP. LAC oxidizes phenolic lignin models to phenoxy radicals and quinones. This spontaneous rearrangement can also lead to the fission of carbon-carbon or carbon-oxygen bonds in the lignin phenylpropane subunits resulting either in degradation of both side chains and aromatic rings(Kawai *et al.*, 1988), or in demethylation processes (Ander *et al*, 1983). In this respect, LAC can cooperate with various FAD containing oxidases like glucose oxidase (GOD; EC 1.1.3.4), veratryl alcohol oxidase (VAO; EC 1.1.3.7), cellobiose:quinone oxidoreductase (CBQ; EC 1.1.5.1) and cellobiose dehydrogenase (CDH; EC 1.1.99.18). These reducing quinoids and radicals (through $FADH_2$ oxidation) may prevent polymerization processes.

ENZYMES CONCERNED TO LIGNIN DEGRADATION

All enzymes of white-rot fungi known so far can be divided into three groups. The first one attacks directly the wood constituents; this group includes enzymes acting on carbohydrate components (cellulose, hemicellulose) and lignin. In the cellulase complex, three main groups of cellulolytic enzymes were determined: endo-1,4-β-glucanases (EDG; EC 3.2.1.4), two types of exo-1,4-β-glucanases EXG (cellobiohydrolases CBH; EC 3.2.1.91 and glucohydrolases GCH, EC 3.2.1.29) and β-glucosidases (BGS; EC 3.2.1.21). Hemicellulose from hardwood, i.e. branched acetyl xylan, is converted by several different

enzymes such as endo-1,4-β-xylanase (EDX; EC 3.2.1.8), β-xylosidase (BXS; EC 3.2.1.37), α-glucuronidase (AGU; EC 3.2.1.39), α-L-arabinofuranosidase (AAF; EC 3.2.1.55), and acetylesterase (AEE; EC 3.1.1.6). Galactoglucomannans, principal hemicellulose in softwood, require for complete hydrolysis the following enzymes such as endo-1,4-β-mannanase (EDM; EC 3.2.1.78), β-mannosidase (BMS; EC 3.2.1.25), β-glucosidase (BGS; EC 3.2.1.21), and α-galactosidase (AGaS; EC 3.2.1.22).

In lignin degradation, phenol oxidases are involved, including LiP, MnP, LAC, horse-radish peroxidase (HLP; EC 1.11.1.7), and dioxygenases such as protocatechuate 3,4-dioxygenase (P34D; EC 1.13.11.3), 1,2,4-trihydroxybenzene 1,2-dioxygenase (TBH12D), and catechol 1,2-dioxygenase (C12D; EC 1.13.11.1). The second of the three above mentioned groups includes, superoxide dismutase (SOD; EC 1.15.1.1) and glyoxal oxidase (GLO; EC 1.2.3.5) - these enzymes cooperate with the first group enzymes, but they neither attack wood nor contribute to it. The third, very important group, is constituted to GOD, aryl alcohol oxidases (AAO; EC 1.1.3.7 *e.g.* VAO, pyranose 2-oxidase (P2O; EC 1.1.3.10), CBQ and CDH. The feedback type enzymes play a key role in combining the metabolic chains during biodegradation of the wood polymer. All these enzymes may function separately or cooperate with one another. Their extracellular maturation is probably accomplished with the participation of simultaneously secreted proteolytic enzymes (Staszczak *et al.*, 1996).

Some of these enzymes and their isoenzymes were also investigated in terms of their gene structures. For example, in *Phanerochaete chrysosporium* at least ten LiP gene primary structures (Cullen & Kersten, 1996; Gold & Alic, 1993), a multi-gene family encoding MnP (Pease *et al.*, 1989; Godfrey *et al.*, 1990; Gold & Alic, 1993; Orth *et al.*, 1994), and one GLO gene (Kersten and Cullen, 1993) have been cloned and characterized. In the case of LAC, approximately 15 gene structures have been recognized, not only in ligninolytic fungi, but also in *Neurospora crassa* (Germann *et al.*, 1988), *Cryphonectria parasitica* (Choi *et al.*, 1992), and *Aspergillus nidulans* (Aramayo & Timberlake, 1990). Four LAC genes have been recognized in plant pathogenic fungus, *Rhizoctonia solani* (Wahleithner *et al.*, 1996). Among ligninolytic fungi, the LAC genes are known in *Coriolus hirsutus* (Kojima *et al.*, 1990), *Phlebia radita* (Saloheimo *et al.*, 1991), *Coriolus versicolor* (Iimura *et al.*, 1992), *Agaricus bisporus* (Perry *et al.*, 1993), ligninolytic basidiomycete PM1 (Coll *et al.*, 1993), *Pleurotus ostreatus* (Giardina *et al.*, 1995), *Trametes versicolor* (Jonsson *et al.*, 1995), *Trametes villosa* (three genes lcc 3, lcc 4 and lcc 5; Yaver & Golightly, 1996), and *Pycnoporus cinnabarinus* (*lcc3-1* gene) growing in xylidine-induced medium (Eggert *et al.*, 1998).

The majority of the wood degrading enzymes are well known in the literature, *e.g.* in the reviews of Eriksson *et al.*, 1990; Gold and Alic, 1993; Broda *et al.*, 1994; Reid, 1995; Thurston, 1994; Cullen, 1997; Sarikaya *et al.*, 1997; Ander & Marzullo, 1997, or Leonowicz *et al.*, 1997. Therefore, only SODs rarely cited in the context of wood degradation are shortly described below.

SUPEROXIDE DISMUTASES (SODs; EC 1.15.1.1)

These almost universally distributed enzymes containing iron (FeSOD), manganese (MnSOD) or copper and zinc (CuZnSOD) in the active site, are one of the key enzymes that protect cells against oxidative stress by catalyzing the dismutation of superoxide anion radicals ($O_2^{\cdot-}$; SORs) to oxygen and hydrogen peroxide (McCord & Fridovich, 1969; Lehman *et al.*, 1996). In white-rot fungi, SOD was first found by Malarczyk *et al.* in 1995 (in *Pleurotus* genera), and purified from the mycelium of medicinal fungus, *Ganoderma microsporium* by

Pan *et al.* in 1997. The toxic SORs commonly appear during the quinone redox cycling, also in LAC producing ligninolytic fungus, *Pleurotus eryngii* (Guillen *et al.*, 1997). During the cycle in *P. eryngii* (similarly to the data in our earlier report by Leonowicz and Trojanowski, 1965), the cell-bound divalent reduction of quinones (Q) to hydroquinones(Q^{2-}) is followed by extracellular LAC-mediated oxidation of hydroquinones into semiquinones ($Q^{\cdot-}$) which are then autooxidized to quinones. In both quinone oxidation phases, there occurs the production of SOR:

$$Q^{2-} + O_2 = Q^{\cdot-} + O_2^{\cdot-}$$
$$Q^{\cdot-} + O_2 = Q + O_2^{\cdot-}$$

Subsequently SOR reacts as a reducing or oxidizing agent with other radicals produced by ligninolytic enzymes, contributing to various lignin degradation processes, *e.g.* aromatic ring fission (Kawai *et al.*, 1988) or demethoxylation (Potthast *et al.*, 1995).

As an oxidant, SOR produces Mn^{+3} from Mn^{+2}

$$O_2^{\cdot-} + Mn^{+2} + 2H^{+} = Mn^{+3} + H_2O_2$$

and generates hydrogen peroxide and oxygen:

$$2O_2^{\cdot-} + 2H^{+} = H_2O_2 + O_2$$

as a reducing agent, SOR reacts with Fe^{3+} reducing it to Fe^{+2} and yielding oxygen:

$$O_2^{\cdot-} + Fe^{3+} = O_2 + Fe^{+2}$$

the reduced iron and hydrogen peroxide react with each other to form OH-radicals via the Fenton reaction (Wailing, 1975; Goldstein *et al.*, 1993; Pratap & Lemley, 1998):

$$Fe^{+2} + H_2O_2 = Fe^{3+} + OH^{\cdot} + OH^{-}$$

OH-radicals are also produced from SOR and hydrogen peroxide in the Haber-Weiss reaction (Haber & Weiss, 1934):

$$O_2^{\cdot-} + H_2O_2 = OH^{\cdot} + OH^{-} + O_2$$

As the one-electron reduction product of oxygen, SOR is not strong enough for ligninolysis, but oxygen, hydrogen peroxide and hydroxyl radicals produced with its participation are thought to be important in the lignin degradation (Forney *et al.*, 1982; Guillen *et al.*, 1997; Park *et al.*, 1997).

However, the highly active hydroxyl radical can react with DNA, proteins, lipids, or other biomolecules which are important for cells, usually killing them. In this reaction there also appears SOD which catalyzes the dismutation of the superoxide radical $O_2^{\cdot-}$ (SOR) to O_2 and H_2O_2 (Guillen *et al.*, 1997; McCormick *et al.*, 1998) as follows:

$$2O_2^{\cdot-} + 2H^{\cdot} = H_2O_2 + O_2$$

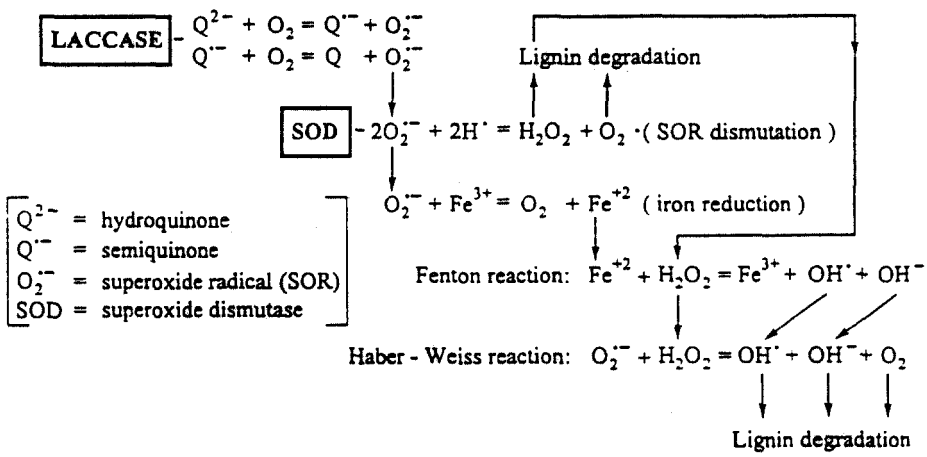

Fig. 1. The possible role of SOD in production of ligninolytic factors and radicals (Malarczyk *et al.*, 1995).

Hydrogen peroxide is subsequently either destroyed to O_2 and H_2O by catalase or it takes part in peroxidative reactions. Consequently, the living cells (also basidiomycetous fungi) commonly possess SOD, catalase and peroxidases which are able to eliminate both $O_2^{\cdot-}$ and H_2O_2.

According to our results, the maximum of SOD activity in lignin-degrading, LAC-, and ligninases-producing fungi appeared when the activities of the ligninolytic enzymes decreased (Malarczyk *et al.*, 1995). This may be caused by the fact that when the ligninolytic enzymes are not active, SOD protects the organisms from the toxic, and reactive radicals. On the other hand, during the high activity of the enzymes, the radicals might be fully involved in biodeterioration processes. SOD is most likely involved in the production of these factors from the intermediates of quinone redox cycling caused by LAC (see Fig. 1). This hypothesis, however, requires further experimental confirmation.

LOW-MOLECULAR MEDIATORS

According to the recent assumptions, the molecular size of wood rotting enzymes does not permit penetration of the wood cell wall (Evans *et al.*, 1994). In the case of fungal cells possessing high enough redox potential the low molecular mediators of internal or external origin migrate from the enzymes and oxidize lignin or wood pulp. The internal mediating precursors are produced as a result of fungal metabolism, matured with the participation of lignin-degrading enzymes, and exported instead of high molecular enzymes into the cell wall environment, where they work in degradative processes as the enzyme "messengers" oxidizing lignin in wood. Many possible low-molecular mass compounds and radicals have been suggested as likely mobile factors to permeate wood cell walls and initiate decay. Some of these, such as veratryl alcohol, oxalate, malate, fumarate, and 3-hydroxyanthranilic acid are produced as a result of fungal metabolism and their secretion enables the fungi to colonize and degrade the wood cell wall structure more effectively than other organisms. Among the above mentioned mediators, the synthesis of veratryl alcohol by *Phanerochaete chrysosporium* was first performed (Lundquist & Kirk, 1978), oxalate was found in *Leucostoma cincta* and *L. personii* (Traquair, 1987), malate and fumarate were recently detected in *Nematoloma frowardii* by Hofrichter *et al.* (1998a), and 3-hydroxyanthranilic acid was identified in *Pycnoporus cinnabarinus* (Eggert *et al.*, 1996).

Veratryl alcohol and oxalate are secondary metabolites of *Phanerochaete chrysosporium* and other white-rot fungi: the former is involved in LiP catalysis as a substrate, and the latter in the action of MnP as a chelator of Mn^{2+} and Mn^{3+}. The reaction of LiP with veratryl alcohol results in the production of a cation-centered radical which is transformed into either veratraldehyde or a number of quinones (de Jong *et al.*, 1994). In the presence of lignin derivatives, the cation radical is complexed to the enzyme and probably acts as a redox mediator in transforming reactions. However, as its half life is extremely short, its action as an independent diffusible mediator is highly dubious. In the MnP system, mediation occurs via a Mn^{3+}-oxalate or other dicarboxylic radical complexes which are capable of oxidizing a variety of lignin-related substrates (Schick Zapanta & Tien, 1997). Apart from oxalate, malate, and fumarate, another dicarboxylic derivative, malonate, has been recently proved to mediate oxidation of Mn^{2+} to Mn^{3+} by means of MnP isolated from white rot fungus *Nematoloma frowardii* (Hofrichter *et al.*, 1998b). Oxalate chelates cations such as Ca^{2+}, Fe^{2+} and NH_4^+(Dutton *et al.*, 1993). As the result of the removal of calcium ions, the cell wall pore size is enlarged, which may permit access by enzyme molecules (Green *et al.*, 1991). On the other hand, oxalate regulates the

concentration of ferric ions for the Fenton's reaction (Wailing, 1975; Goldstein *et al.*, 1993; Pratap & Lemley, 1996). This reaction supplies the degradation environment with highly reactive hydronium ions (H_3O^+) and hydroxyl radicals (HO· and HO^-) which initiate depolymerization of wood.

Apart from lignin degradation supporting 3-hydroxyanthranilate (3-HAA) produced by *Pycnoporus cinnabarinus*, which is a well-known mediator of LAC, it was found that delignification of kraft pulp by LAC can be supported by some external (*i.e.* non-produced by fungi and absent in pulp) low molecular dyes or other aromatic hydrogen donors acting as mediators, such as 2,2'-azino-bis- (3-ethylbenzenthiazoline-6-sulphonic acid) (ABTS) (Bourbonnais *et al.*, 1997) or 1-hydroxy benzotriazole (HBT) (Call, 1994). The discovery of HBT introduced a new class of mediators with the N-OH functional group yielding NO· radical which is stabilized by the mediator structure and selected for lignin oxidation. Other efficient mediators include violuric acid and N-hydroxyacetanilide (Amann 1977; Pfaller *et al.* 1998). In comparison to HBT and ABTS, such mediators as 4-hydroxy-3-nitroso- 1-naphtalene sulfonic acid (HNNS), 1-nitroso-2-naphthol-3,6-disulfonic acid (NNDS), and Remazol brilliant blue R (RBBR) promote delignification to small extent. Promazine (PZ) and chlorpromazine (CPZ) do not act in such a system at all (Bourbonnais *et al.*, 1997). Application of LAC and compounds with -NO, -NOH or HRNOH- groups in delignification was discussed with particular attention paid to effects on pulps by Call and Mucke (1997) in Lignozyme process. Recently, Leontievsky *et al.*(1996 and 1997a) isolated a yellow form of LAC from *Panus tigrinus*, which was probably formed as a result of binding lignin-derived molecules by enzyme protein. This enzyme catalysed the oxidation of veratryl alcohol and of a nonphenolic dimeric compound without the electron-transfer mediator; classical blue LAC requires the presence of ABTS in this reaction(Leontievsky *et al.*, 1997b).

COOPERATIVE ROLES OF ENZYMES IN WOOD BIODEGRADATION

It has been unequivocally stated that lignin degradation is accelerated in the presence of cellulose or its oligomers (Ander & Eriksson, 1975; Hatakka & Uusi-Rauva., 1983). The idea of a feedback-type interdependence of delignification and cellulose degradation processes was postulated for the first time by Westermark and Eriksson (Westermark & Eriksson, 1974a, 1974b). This hypothesis is still valid as the report by Gottlieb *et al.* (1950) concerning the possibility of mycelial growth on lignin as the sole source of carbon has not been confirmed. According to Westermark and Eriksson, depolymerizations of cellulose and lignin are interrelated in certain points and accelerate each other (Westermark & Eriksson 1974a, 1974b, 1975). They discovered the enzyme cellobiose:quinone oxidoreductase (CBQ) which cooperates in a feedback type with LAC and cellulose in the process of depolymerization of both components of the wood complex. Accelerating the degradation of both elements of the wood complex in a feedback system, CBQ thus removes decomposition products of cellulose. The authors suggested that in the system LAC might function as a link in an extracellular "electron transport chain." Lately, Westermark's and Eriksson's schemes have been modified to include the recently discovered enzymes and radicals which are active in degradation of the wood complex (Ander & Marzullo, 1997). The enzyme cellobiose dehydrogenase (CDH) containing both FAD and a heme group may act instead of CBQ (Ander, 1994).

The ligninolytic enzymes - in particular LAC - often carry out their ligninolytic activities by cooperating with low molecular weight compounds acting as redox mediators. The ideal

mediator should "form a high redox potential oxidation product in a highly reversible reaction" (Bourbonnais *et al.*, 1997). Cheaper delignification of wood needs optimization of both factors. The hypothetical relationship among veratryl alcohol oxidase, LAC and other enzymes and radicals in the process of wood degradation is illustrated in Fig. 2.

The function of fungal hyphae in wood and the hypothetical relationship between enzymes and radicals in the process of wood degradation. In the proposed system LAC, LiP and MnP are secreted from fungal hyphae close to the hyphae environment where they cooperate each other and with VAO producing mediating factors. The yielded chelators and mediating radicals are exported further to the wood tissue where they work as enzyme "messengers" in wood degradation. Therefore it is the consecutive proposition of the feedback type enzymatic system, that works in wood degra- dation with a meaningful participation of different ligninolytic enzymes. Ligninolytic radicals are produced by SOD from quinones and semi- quinones yielded by LAC. During fungal growth on medium reach in lignin derivatives the maximum of SOD activity in lignin-degrading environment appeared when the activities of the ligninolytic enzymes decreased (Malarczyk, 1995). It seems that in the system LAC oxidizes lignin-derived radicals to quinones which serve as the oxygen source for GOD (Szklarz & Leonowicz, 1986; Rogalski 1986; Leonowicz *et al.*, 1998) and/or VAO (Marzullo *et al.*, 1995). The latter enzyme produces H_2O_2 and prevents the polymerizing activity of LAC, with formed H_2O_2 serving as a co-substrate for the ligninolytic activity of LiP and MnP.

Thus, GOD and VAO partner enzymes of LAC prevent polymerization and accelerate

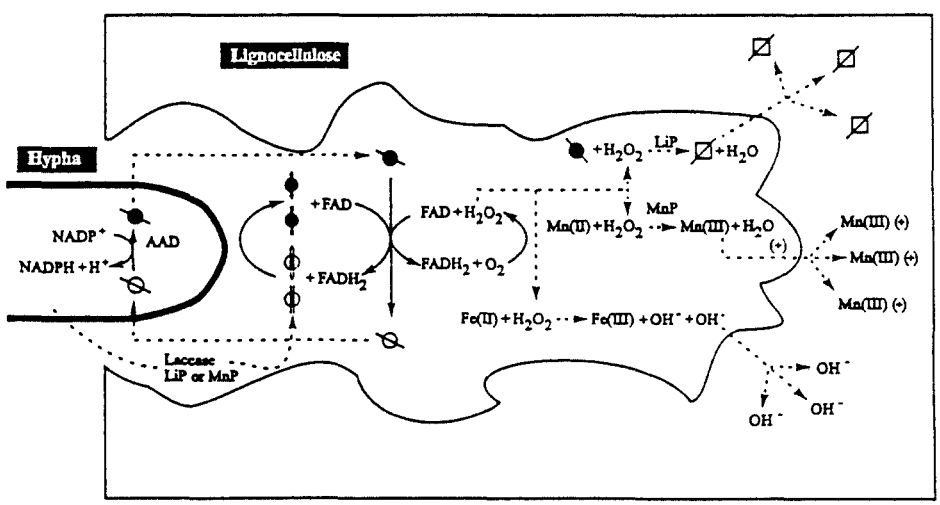

Fig. 2. Hypothetical relationship among fungal hyphae enzymes and veratryl alcohol dehydrogenase with mediators and mediating radicals during degradation of wood. From left to right: AAD - aryl alcohol dehydrogenase with NADP as the prosthetic group; aryl alcohol; aryl aldehyde; LiP; MnP; lignin derived radicals or quinones and their reduced forms, VAO with FAD as the prosthetic group; LiP with Fe; MnP with Mn; metal chelating agents, e.g. oxalic acid (Ander & Marzullo, 1997).

depolymerization (Szklarz & Leonowicz, 1966; Marzullo *et al.*, 1995). Among these, only GOD possesses two functions necessary for acceleration of wood breakdown: by means of oxidation of glucose, it produces hydrogen peroxide necessary for LiP activity, and it reduces quinoids and phenoxy radicals yielded by LAC during oxidation of lignin. However, the fact that VAO reduces quinones and produces hydrogen peroxide cannot be fully taken into consideration because hydrogen peroxide production does not originate in the oxidation of glucose (Marzullo *et al.*, 1995) which is a key metabolite in the degradation of carbohydrates. This probably means that only GOD cooperates with the system of cellulases oxidizing glucose, generated by these enzymes during hydrolysis of cellulose (Leonowicz *et al.*, 1997).

Thus, GOD seems to be a regulating enzyme that might be important in degradation of wood. When oxygen is consumed in the second stageof the GOD glucose reaction, it can be supplied by radicals or quinones generated when phenolic or methoxyphenolic compounds are exposed to the LAC activity (Szklarz & Leonowicz, 1986; Rogalski, 1986). On the other hand, since excess quinones produced by LAC inhibit the enzyme (Szklarz & Leonowicz, 1986; Rogalski, 1986; Rogalski *et al.*, 1995), it can be concluded that GOD counteracting the poisonous level of quinones in the medium enables LAC to continue its function. Taking all these reasons into consideration, over ten years ago we postulated a hypothetical mechanism of degradation of the wood complex (Leonowicz *et al.*, 1986) (see Fig. 3).

According to this mechanism, GOD cooperates with LiP and MnP providing hydrogen peroxide with LAC, and reducing quinones yielded by this enzyme to adequate phenols (Szklarz & Leonowicz, 1986; Rogalski, 1986; Leonowicz *et al.*, 1998). For this reason GOD operates as a feedback system where LIP functions as the first lignin decomposing agent (Hammel *et al.*, 1993; Thompson *et al.*, 1998) and LAC as the demethylating factor (Leonowicz & Trojanowski, 1965; Ishihara, 1983; Leonowicz *et al.*, 1984;

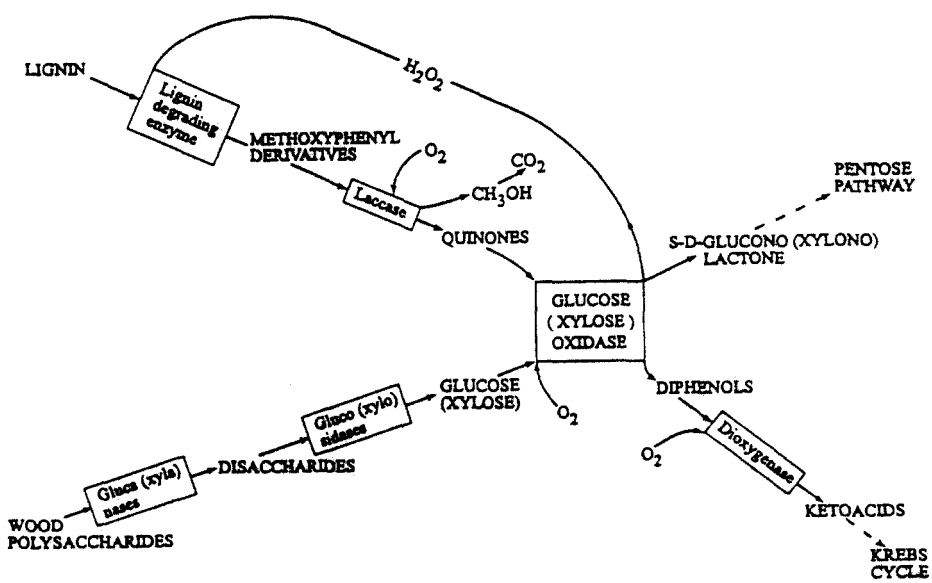

Fig. 3. The hypothetical mechanism of wood degradation by white-rot fungi enzymes (Leonowicz *et al.*, 1986).

Potthast *et al.*, 1995). Glucose which is produced as a result of cellulose hydrolysis by the cellulase complex becomes the substrate for GOD. Oxygen needed by GOD can be replaced by quinones produced by LAC from lignin oligomers. As a result of glucose oxidation, γ-D-gluconolactone is formed, which after certain degradations reinforces the metabolism of the fungus in the pentose phosphate cycle or in glycolysis.

Hydrogen peroxide produced in the reaction catalyzed by GOD activates, in turn, LiP and MnP. Lignin exposed to peroxidases undergoes decomposition into lower-molecular weight fragments containing methoxyl groups. LAC demethylates oligomers yielded by peroxidases and degrades them to even lower fragments (Leonowicz & Trojanowski, 1965; Ishihara, 1983; Leonowicz *et al.*, 1984; Potthast *et al.*, 1995). The generation of excess quinones and possible secondary polymerization is counter-balanced by GOD which reduces them to the respective phenols. The phenols, in turn, become the substrate for P34D (protocatechuate 3,4-dioxygenase) or other dioxygenases such as TBH12D (1,2,4-trihydroxybenzene 1,2-dioxygenase) or C12D (catechol 1,2-dioxygenase) present in fungal cultures (M. Wojtas-Wasilewska *et al.*, 1987; Rieble *et al.*, 1994; Rogalski *et al.*, 1996) which catalyze cleavage reactions of the aromatic rings. The products obtained in the form of keto acids easily find their ways to the Krebs cycle.

The mechanism can occur in those fungi which possess all the above mentioned enzymes, *e.g. P. chrysosporium*, *Phlebia radiata*, and *Trametes versicolor*. In the case of those species in which the presence of LiPs has not been confirmed, the mechanism suggested by Westermark and Eriksson (1974b), where cellobiose:quinone oxidoreductase is the crucial feedback-type enzyme, seems to function very well despite the fact that they transform wood. It is obviously believed that both systems function jointly as it was proposed earlier

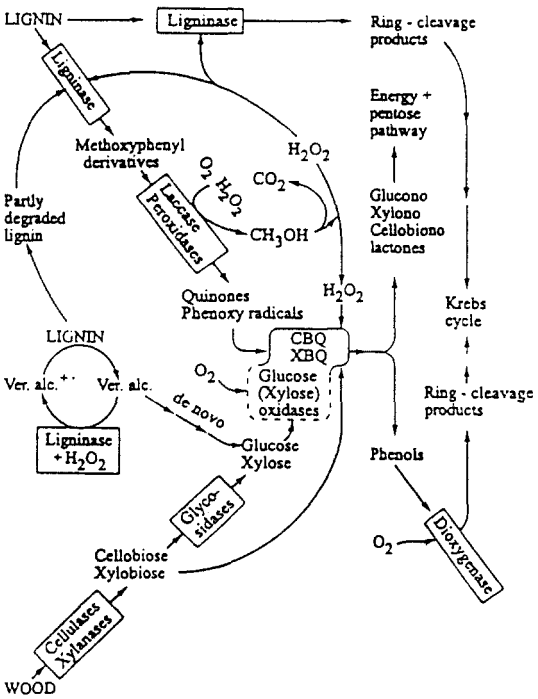

Fig. 4. The hypothetical course for degradation of lignin, cellulose and xylan in wood (Eriksson *et al.*, 1990; Eriksson, 1993).

(Eriksson *et al.*, 1990; Eriksson, 1993).

The current discoveries concerning the role of VAO, CDH, AAD, and particularly low molecular mediators and radicals in wood biodegradation (shown in Fig. 4) support the proposed route by explaining how the high molecular weight enzymes can function in the wood environment that is normally resistant to the microbial attack. However, the existence of other mechanisms as well as other enzymes, also operating as feedback systems in the process of wood degradation, is not out of question.

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