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MINI-REVIEW

Multi-catalysis reactions: new prospects and challenges of biotechnology to valorize lignin

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Abstract Considerable effort has been dedicated to the chemical depolymerization of lignin, a biopolymer constituting a possible renewable source for aromatic value-added chemicals. However, these efforts yielded limited success up until now. Efficient lignin conversion might necessitate novel catalysts enabling new types of reactions. The use of multiple catalysts, including a combination of biocatalysts, might be necessary. New perspectives for the combination of bio- and inorganic catalysts in one-pot reactions are emerging, thanks to green chemistry-driven advances in enzyme engineering and immobilization and new chemical catalyst design. Such combinations could offer several advantages, especially by reducing time and yield losses associated with the isolation and purification of the reaction products, but also represent a big challenge since the optimal reaction conditions of bio- and chemical catalysis reactions are often different. This mini-review gives an overview of bio- and inorganic catalysts having the potential to be used in combination for lignin depolymerization. We also discuss key aspects to consider when combining these catalysts in one-pot reactions.

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Introduction

The depletion of fossil carbon reserves and concern about global warming calls for substitutes of fossil fuels and petrochemicals (Marquardt et al. 2010). For energy production, multiple alternatives are available, e.g., wind, sun, biomass, hydroelectricity, nuclear fission. However, biomass is the only known renewable resource that can be converted to both fuels and chemicals (Zhang 2008). Therefore, biorefineries using lignocellulosic feedstock have been proposed as alternatives to petroleum-based refineries (e.g., Wyman and Goodman 1993; Holladay et al. 2007; Kamm et al. 2007; Zhang 2008; Michels and Wagemann 2010). Recalcitrance to degradation of lignin is a major obstacle to harness lignocellulosic biomass efficiently (Mansfield et al. 1999; Achyuthan et al. 2010; Zakzeski et al. 2010a; Sanderson 2011).

Lignin, a complex three-dimensional amorphous polymer consisting of methoxylated phenylpropanoid units of various types (Achyuthan et al. 2010; Isroi et al. 2011), is the second most abundant constituent of plant biomass next to cellulose, accounting for approximately 20 % of carbon fixed in land ecosystems (Ruiz-Dueñas and Martínez 2009). Despite extensive research, lignin is mostly used in commercial applications of low value, for example, as concrete additive (Doherty et al. 2011) or as low-grade fuel. However, due to its structure, it constitutes a potential renewable source for aromatic added-value chemicals (Holladay et al. 2007).

Novel approaches using new catalytic systems seem necessary in order to exploit lignin as a resource for the production of fine chemicals (Vennestrøm et al. 2010a). The use of multiple catalysts (Marr and Liu 2011; Santacoloma et al.

2011) and the combination of bio- and inorganic catalysts in either sequential or one-pot cascade catalysis (Vennestrøm et al. 2010a) has not been investigated extensively and might be essential to achieve economically sustainable valorization of lignin. Furthermore, combining multiple catalysts in one-pot cascade catalysis would provide advantages over conventional processes focusing on single reactions, mainly by reducing time and yield losses associated with the isolation and purification of reaction products (Murzin and Leino 2008; Vennestrøm et al. 2010a). However, the combination especially of bio- and inorganic catalysts can be complex since enzymes and inorganic catalysts possess various optimal conditions regarding pH, temperature, pressure, and solvent (Marr and Liu 2011).

In this mini-review, we give an overview of various bio- and inorganic catalysts, having the potential to be used in combination for lignin depolymerization, a biopolymer constituting a possible renewable source for aromatic added-value chemicals. The first part focuses on the inorganic catalysts applied for the depolymerization of lignin, the second part deals with lignin degradation in nature and the enzymatic catalysts involved in this process, and the third part discusses aspects to consider when combining both types of catalysts. While several publications on the general combination of enzymatic with inorganic catalysts (e.g., Murzin and Leino 2008; Vennestrøm et al. 2010a; Marr and Liu 2011), lignin-degrading enzymes (e.g., Kersten and Cullen 2007; Hammel and Cullen 2008; Ruiz-Dueñas and Martínez 2009; Wong 2009), and inorganic catalysis for lignin conversion (Zakzeski et al. 2010a) exist, this mini-review combines all these aspects with a specific focus on the depolymerization of lignin for the production of added-value chemicals.

Inorganic catalysis

Depolymerization of lignin and lignin model compounds through inorganic catalysts has been attempted in a plethora of studies. Inorganic catalysts were usually used under harsh reaction conditions. Conversion of lignin depends not only on reaction conditions and catalysts but also on the pretreatment/extraction methods used to isolate the lignin fraction from lignocellulose. So far, the lignin polymer could not be isolated exhaustively due to its molecular association and potential covalent bonds with polysaccharides (Holtman et al. 2007). Depending on the pretreatment method used, undesired chemical transformations like the incorporation of sulfur can occur. Furthermore, the molecular weight of lignin can be reduced and the chemical bond linkages can be altered (Gluckstein et al. 2010). Pretreatment methods resulting in uniform types of lignin of high purity are most desirable. Several publications covering lignin pretreatment exist (e.g., da Costa Sousa et al. 2009; Gluckstein et al. 2010; Zakzeski et al. 2010a). The variation between the lignin feedstocks used in the various studies on lignin transformation impedes the comparison of their performances because a benchmark substrate is lacking. Furthermore, comparability is complicated by the fact that different methods are used for product recovery and also because many studies solely dealt with lignin model compounds.

Chemical lignin transformation reactions can be divided into lignin cracking, hydrolysis, catalytic reduction, and catalytic oxidation reactions. Lignin catalytic cracking processes are adapted from petroleum refineries and work at harsh reaction conditions, especially high temperatures, usually above 300 °C. They can cleave lignin β-O-4 ether bonds as well as relatively unstable carbon–carbon bonds (Thring and Breau 1996) such as Cα–Cβ bonds. Lignin fragmentation by hydrolysis is also achieved at high temperatures, usually above 270 °C. Pandey and Kim (2011) recently reviewed the thermochemical depolymerization and conversion of lignin, including catalytic cracking and hydrolysis processes. These high-temperature processes lead to tar or char formation, volatile components, and complex mixtures of compounds (van Haveren et al. 2008; Zakzeski et al. 2010a; Pandey and Kim 2011).

Paralleling the emerging concept of green chemistry, more and more new chemical catalysts are designed to work in aqueous environments at milder reaction conditions (Hailes et al. 2007; Sheldon 2008). Since an extensive review on inorganic catalysis of lignin was published recently (Zakzeski et al. 2010a), the following section is restricted to a few selected studies where mild reaction conditions were used or that were published just recently.

Lignin reduction

Reductive lignin depolymerization usually removes alcohol, aldehyde, ether, and acid moieties. Therefore, it has the potential to produce simpler bulk aromatic compounds like benzene, toluene, and xylene (B, T, X) as well as phenol (van Haveren et al. 2008), which could be directly used by conventional petrochemical processes (Holladay et al. 2007). Furthermore, the removal of oxygen from lignin makes it a better working fuel (Sanderson 2011). Hydrogenation or hydrodeoxygenation of lignin and lignin model compounds with heterogeneous catalysts, performed at high temperatures and pressures, not only cleaved ether bonds but also hydrogenated aromatic rings, thereby producing an inefficient mixture of compounds (Sergeev and Hartwig 2011). Selective cleavage of lignin model compounds through electrocatalytic hydrogenation at relatively low temperatures (50 °C) was reported (Mahdavi et al. 1997; Dabo et al. 1999; Cyr et al. 2000). Few studies used homogenous catalysts to reduce lignin or lignin model compounds (e.g., Hu et al. 1997;

Plasseraud and Süss-Fink 1997; Schulz et al. 2000). A nickel catalyst was employed for hydrogenolysis of dimeric 4-O-5, α-O-4, and β-O-4 lignin model compounds and several alkyl–aryl and diaryl ethers at temperatures between 80 and 120 °C and pressure of 1 bar of hydrogen (Sergeev and Hartwig 2011). The aromatic C–O bonds of the ethers were selectively cleaved and produced exclusively alcohols and arenes. Furthermore, the ether linkages of the lignin model compounds were broken. Degradation of the α-O-4 model compound yielded 99 % 3,4-dimethoxytoluene and 2-methoxyphenol. Hydrogenation of the β-O-4 model yielded 89 % guaiacol. Thereby, it was demonstrated that the aromatic C–O bonds could be selectively cleaved in the presence of other C–O bonds, while leaving the arene units intact (Sergeev and Hartwig 2011). Whereas these results look promising, the effectiveness of the catalyst on different lignin feedstocks, not just lignin model compounds, has still to be demonstrated. A further issue is the soluble form of the catalyst, which makes separation from products difficult (Sanderson 2011).

Lignin oxidation

Oxidative lignin depolymerization requires catalysts that selectively disrupt the linkages in lignin in order to form specific aromatic alcohols, aldehydes, acids, or other specially functionalized aromatics like vanillin, syringaldehyde, or vanillic acid (Holladay et al. 2007; Zakzeski et al. 2010a). Many of the resulting chemicals might be used as platform chemicals for subsequent organic synthesis or are target fine chemicals (Zakzeski et al. 2010a). The oxidation of lignin and lignin model compounds was also briefly discussed in a recent review on biomass oxidation (Collinson and Thielemans 2010).

Heterogeneous catalysts, more specifically, TiO_2 and Pt/TiO_2, have been used for photocatalytic oxidation to process lignin from wastewater streams of the paper industry (Portjanskaja and Preis 2007; Ma et al. 2008; Portjanskaja et al. 2009). These processes were applied at relatively mild temperatures (~20 °C), but products and percentages of conversion were not specified.

Methyltrioxorhenium (MTO) has been used as a catalyst in the presence of H_2O_2 at 60 °C to cleave the CC double bond of isoeugenol and *trans*-ferulic acid, thereby producing vanillin (Herrmann et al. 2000). Further authors used the catalytic MTO/H_2O_2 system (homogeneous as well as heterogeneous) to oxidize technical lignins and lignin model compounds at 25 °C (Crestini et al. 2005, 2006). Phenolic, non-phenolic, and dimeric lignin model compounds were oxidized to a large extent. The oxidation of 18.2 gL^{-1} sugar cane, red spruce kraft, and hardwood organosolv lignin at room temperature led to high degrees of degradation and produced lignin fragments of increased solubility.

Various studies investigated the electrochemical oxidation of lignin using different electrodes (e.g., Pardini et al. 1992; Parpot et al. 2000; El-Ashtoukhy et al. 2009). Dominguez-Ramos et al. (2008, 2010a, b) used boron-doped diamond electrodes for the oxidation of lignosulfonates. They were able to oxidize approximately 90 % of the total organic carbon in a lignosulfonate solution. However, the structure of the degradation products was not elucidated. For the electrochemical oxidation of kraft lignin, different IrO_2-based electrodes were used (Tolba et al. 2010). Vanillin and vanillic acid were formed as primary intermediates. Apparent degradation rate constants were determined for temperatures ranging from 2 to 60 °C and were highest at 60 °C.

One commercial process for the oxidation of lignosulfonates exists (Faith et al. 1965). Lignosulfonates are produced through sulfite pulping in the pulp and paper industries. Their oxidation produces vanillin in a yield of 10–20 % (*w*/*w* based on the dry weight of lignosulfonates). The spent acid sulfite pulping liquors are oxidized with air at 160–170 °C at alkaline pH. After oxidation, the products are extracted using organic solvents and further purified (Hocking 1997; Ramachandra Rao and Ravishankar 2000).

Due to economic and environmental reasons, production of vanillin from lignosulfonates is a declining process (Sixta 2006). Therefore, Rudolf von Rohr and co-workers investigated an alternative process using kraft lignin as feedstock (Voitl et al. 2009; Voitl and Rudolf von Rohr 2010; Werhan et al. 2011). Kraft lignin is a by-product of the kraft process, which is presently the most prominent pulping process (Auhorn and Niemela 2007).

$H_3PMo_{12}O_{40}$ was used as homogeneous catalyst in an 80 % (*v*/*v*) methanol/water solvent at acidic pH at 170 °C under an atmosphere of 10.8 bar of oxygen containing 8.9 g L^{-1} dry kraft lignin at the start of the oxidation process (Voitl and Rudolf von Rohr 2010). Monomeric products were extracted with chloroform. The process yielded 3.5 % (*w*/*w*) vanillin and 3.5 % (*w*/*w*) methyl vanillate. A total of 10 % (*w*/*w*) of monomers could be extracted, including further products like acetovanillone and methylparaben. Additionally, approximately 60 % (*w*/*w*) of oligomeric products with significantly lower molecular weight than kraft lignin were found in the extract (Voitl and Rudolf von Rohr 2010).

In a more recent study, different metal salt catalysts were tested as replacement for $H_3PMo_{12}O_{40}$ (Werhan et al. 2011). For all studied salts ($CuSO_4$, $CoCl_2$, $FeCl_3$, and $CuCl_2$), higher maximal vanillin concentrations were found than in the presence of $H_3PMo_{12}O_{40}$ or without catalyst. However, only experiments using $CoCl_2$ and $CuSO_4$ as catalyst reached similar concentrations of the valuable methyl vanillate as experiments using $H_3PMo_{12}O_{40}$. The average molecular weight of the product mixture was considerably lower in experiments using metal salts as catalysts compared to experiments using $H_3PMo_{12}O_{40}$. The treatment with $FeCl_3$

or $CuCl_2$ as catalyst led to the lowest average molecular weight (Werhan et al. 2011).

The oxidation of Alcell lignin, soda lignin, and several lignin model compounds was investigated using oxygen with $CoCl_2 \cdot 6H_2O$ as homogenous catalyst dissolved in an ionic liquid (1-ethyl-3-methylimidazolium diethyl phosphate, [EMIM] [DEP]) at 80 °C (Zakzeski et al. 2010b). Ionic liquids (ILs) are mixtures of cations and anions melting below 100 °C. Ethyl acetate extraction after lignin oxidation yielded no monomeric products. However, the difference between the attenuated total reflectance infrared spectra after various reaction times suggested a selective oxidation of the dissolved lignin (Zakzeski et al. 2010b). Treatment of lignin model compounds with the catalyst did not disrupt β-O-4, 5-5, and other linkages. Phenolic functional groups remained unchanged as well. However, alcohol moieties were oxidized to form aldehydes or, at high oxygen pressures or substrate loadings, to form acids (Zakzeski et al. 2010b). In a more recent study, the cobalt-catalyzed oxidation of lignin model compounds in [EMIM] [DEP] was further investigated (Zakzeski et al. 2011).

Further studies for the oxidation of lignin and lignin model compounds conducted at mild reaction conditions with homogeneous catalysts used metalloporphyrin, metallosalen, metallo-TAML, -DTNE, -TACN, polyoxometalate-based, simple metal salt-based, and miscellaneous catalyst systems and have been reviewed recently (Zakzeski et al. 2010a).

Many lignin oxidation studies have been carried out and several used mild reaction conditions to depolymerize lignin. These great efforts lead to processes where about 10 to 20 % (*w*/*w* based on lignin dry weight) of lignin could be converted to value-added monomers (Hocking 1997; Ramachandra Rao and Ravishankar 2000). Although this constitutes a considerable accomplishment, given the complexity of the task, higher product yields are necessary for the processes to become economically attractive. Therefore, new approaches seem indispensable to achieve efficient lignin depolymerization.

Enzymatic catalysis

While lignin depolymerization with inorganic catalysts has still limited success, lignin is efficiently degraded in nature by basidiomyceteous white-rot fungi through multiple enzymes (Levasseur et al. 2008; Ruiz-Dueñas and Martínez 2009; Sánchez 2009; Wong 2009; Collinson and Thielemans 2010; Hatakka and Hammel 2010). This illustrates the potential of biocatalysts for lignin depolymerization. In contrast to inorganic catalysts, biocatalysts can be much more selective and able to distinguish between functional groups (Vennestrøm et al. 2010a) but require mild reaction conditions and are often inactivated in non-aqueous solutions (Doukyu and Ogino 2010). The first part of this section covers the different enzymes involved in natural degradation processes of lignin.

Application of enzymes in industrial processes is challenging since they are usually less stable than inorganic catalysts, not easily recycled, and only work in a relatively narrow temperature window. The second part of this section discusses these challenges and presents ways to improve the biocatalyst properties.

Lignin degradation in nature

After extensive research, the mechanisms of fungal lignin degradation become gradually clearer (reviewed by, e.g., Kersten and Cullen 2007; Hammel and Cullen 2008; Ruiz-Dueñas and Martínez 2009; Wong 2009). Enzymes potentially involved in degrading lignin are divided into lignin oxidases and lignin-degrading auxiliary enzymes, corresponding to their direct or indirect participation to lignin degradation, respectively (Table 1). They have been described at length in previous studies and reviews (e.g., Henriksson et al. 2000; Ozimek et al. 2005; Whittaker 2005; Mayer 2006; Joosten and van Berkel 2007; Hammel and Cullen 2008; Ruiz-Dueñas and Martínez 2009; Wong 2009; Faulds 2010; Giardina et al. 2010; Lundell et al. 2010; Singh Arora and Kumar Sharma 2010). In addition, relevant information on these enzymes is collected in general databases (e.g., http://www.brenda-enzymes.org).

Lignin oxidases

Efficient lignin mineralization in nature is only achieved by basidiomycetous white-rot fungi, which commonly inhabit forest litter and fallen trees (Kersten and Cullen 2007). Degradation of lignin is hindered by its non-phenolic aromatic nature, which prevents direct oxidation by low-redox-potential oxidoreductases (Ruiz-Dueñas and Martínez 2009). Moreover, bulky lignin polymers are often hardly accessible to enzymes. To overcome these limitations, white-rot fungi have developed highly specialized heme-containing ligninolytic peroxidases, i.e., lignin (LiP), manganese (MnP), and versatile (VP) peroxidases, which use H_2O_2 as oxidant (Ruiz-Dueñas and Martínez 2009).

It is assumed that LiPs are able to oxidize the aromatic rings of lignin via long-range electron transfer (Ruiz-Dueñas and Martínez 2009). The unspecific oxidation of the aromatic rings of lignin produces unstable cation radicals, which react further and undergo different non-enzymatic reactions like scission of Cα–Cβ and C4–ether linkages (Martínez et al. 2005). The latter finally leads to the release of aromatic aldehydes, demethoxylation with subsequent methanol release, or aromatic ring cleavage prior to the formation of muconate-type structures (Martínez et al. 2005; Ruiz-Dueñas and Martínez 2009).

Table 1 Enzyme groups potentially involved in the degradation of lignin. The values given refer to the corresponding enzyme group as a whole and depict the temperature and pH ranges in which active enzymes of the associated group have been described. References were obtained from http://www.brenda-enzymes.org

Family	Enzyme group	pH optimum range	pH working Range[a]	Temperature optimum range, °C	Temperature working range, °C[b]	Reaction	References
Lignin oxidases family LO1	Laccase (EC 1.10.3.2)	2–9	0.5–11.5	20–85	0–100	4 benzenediol + O_2= 4 benzosemiquinone + 2 H_2O	Baldrian 2004; Dubé et al. 2008; Haibo et al. 2009; Pakhadnia et al. 2009; Shao et al. 2009; Wang et al. 2010; Wu et al. 2010
	Catechol oxidase (EC 1.10.3.1)	3.5–9	3–9.5	4–70	4–80	2 catechol + O_2=2 1,2-benzoquinone + 2 H_2O	Doğan et al. 2005; Gülçİn et al. 2005; Selinheimo et al. 2006; Sellés-Marchart et al. 2006; Kim et al. 2011
Lignin oxidases family LO2	Versatile peroxidase (EC 1.11.1.16)	3–5	No entries	No entries	No entries	Donor + H_2O_2 = oxidized donor + 2 H_2O	Camarero et al. 1999
	Lignin peroxidase (EC 1.11.1.14)	1–6	1–7	23–70	10–80	(3,4-Dimethoxyphenyl)methanol + H_2O_2=3,4-dimethoxybenzaldehyde + 2 H_2O	Aitken and Irvine 1989; Alam et al. 2009; Ghodake et al. 2009; Gomes et al. 2009
	Manganese peroxidase (EC 1.11.1.13)	2.5–6.8	2.5–11	22–60	10–75	2 Mn(II) + 2 H^+ + H_2O_2=2 Mn(III) + 2 H_2O	Paszczyński et al. 1988; Giardina et al. 2000; Wang et al. 2002; Cheng et al. 2007; Gomes et al. 2009
	Generic peroxidases (EC 1.11.1.7)	3–11.7	2–11.7	10–84	0–90	2 phenolic donor + H_2O_2=2 phenoxyl radical of the donor + 2 H_2O	Shin et al. 1997; Senczuk et al. 2003; Suzuki et al. 2006; Vernwal et al. 2006; Jeoung et al. 2007
	Chloroperoxidase (EC 1.11.1.10)	2.5–6	2–7	30–35	20–50	RH + chloride + H_2O_2 = RCl + 2 H_2O	Petri et al. 2004; Bayramoğlu et al. 2008; Yadav et al. 2010
Lignin oxidases family LO3	Cellobiose dehydrogenase (EC 1.1.99.18)	2.7–9	2.5–10	20–75	10–80	Cellobiose + acceptor = cellobiono-1,5-lactone + reduced acceptor	Schou et al. 1998; Baminger et al. 2001; Sigoillot et al. 2002; Zamocky et al. 2006
Lignin-degrading auxiliary enzymes family LDA1-8	Aryl-alcohol oxidase (EC 1.1.3.7)	5–8.5	2–10	30–55	20–60	An aromatic primary alcohol + O_2 = an aromatic aldehyde + H_2O_2	Guillén et al. 1992; Okamoto and Yanase 2002; Ferreira et al. 2005; Kumar and Goswami 2006
	Vanillyl-alcohol oxidase (EC 1.1.3.38)	7–10.5	6–12	38	No entries	Vanillyl alcohol + O_2 = vanillin + H_2O_2	de Jong et al. 1992; van den Heuvel et al. 2001
	Glyoxylate oxidase (EC 1.2.3.5)	8	6.5 -8.5	40	No entries	Glyoxylate + H_2O + O_2 = oxalate + H_2O_2	Akamatsu and Shimada 1994
	Pyranose oxidase (EC 1.1.3.10)	4–8.5	2.5–11	35–65	25–70	D-Glucose + O_2 = 2-dehydro-D-glucose + H_2O_2	Schäfer et al. 1996; Artolozaga et al. 1997; Leitner et al. 2001; Takakura and Kuwata 2003; Odaci et al. 2010
	Galactose oxidase (EC 1.1.3.9)	5.5–8	5–9	30–50	20–60	D-Galactose + O_2 = D-galacto-hexodialdose + H_2O_2	Alberton et al. 2007; Isobe et al. 2007; Sasaki et al. 2010;
	Glucose oxidase (EC 1.1.3.4)	4–7	3–11	25–70	10–70	β-D-Glucose + O_2 = D-glucono-1,5-lactone + H_2O_2	Crognale et al. 2006; Simpson et al. 2007; Altikatoglu et al. 2010; Hashemifard et al. 2010; Courjean and Mano 2011

Table 1 (continued)

Family	Enzyme group	pH optimum range	pH working Range[a]	Temperature optimum range, °C	Temperature working range, °C[b]	Reaction	References
	Benzoquinone reductase (EC 1.6.5.6)	6–7	5–9	40–45	15–50	NADPH + H^+ + *p*-benzoquinone = $NADP^+$ + hydroquinone	Shimizu et al. 1988
	Alcohol oxidase (EC 1.1.3.13)	3–9	3–10	30–65	18–65	A primary alcohol + O_2 = an aldehyde + H_2O_2	Couderc and Baratti 1980; Patel et al. 1981; Kumar and Goswami 2006; Jadhav et al. 2009; Gvozdev et al. 2010
Lignin-degrading auxiliary esterases family EST	Feruloyl esterase (EC 3.1.1.73)	3–9	2.6–11	20–65	5–75	Feruloyl-polysaccharide + H_2O = ferulate + polysaccharide	Garcia-Conesa et al. 2004; Wong 2006; Aurilia et al. 2007; Moukouli et al. 2008; Hegde and Muralikrishna 2009
Others	Aryl-alcohol dehydrogenase (EC 1.1.1.91)	6.1–8.8	5–9.5	30	30–55	An aromatic alcohol + $NADP^+$ = an aromatic aldehyde + NADPH + H^+	Muheim et al. 1991; Gross and Zenk 1969

[a] PH ranges in which enzymes of the corresponding group have been described as expressing at least 20 % of maximal activity

[b] Temperature ranges in which enzymes of the corresponding group have been described as expressing at least 20 % of maximal activity

MnP oxidizes Mn^{2+} to Mn^{3+} (Gold et al. 1984). Mn^{3+} is subsequently stabilized by organic acids like oxalic acid, which results in Mn^{3+} chelates which act as redox mediators oxidizing phenolic lignin structures (Glenn and Gold 1985; Gold et al. 2000). Furthermore, it has been shown that lipid peroxidation by MnP might play an important role in lignin biodegradation. Mineralization of non-phenolic lignin model compounds as well as ^{14}C-labeled synthetic lignin and ^{14}C-labeled wheat straw was instigated by the presence of an unsaturated fatty acid, i.e., linoleic acid and a surfactant, i.e., Tween 20 or Tween 80 emulsifying water-insoluble components leading to a homogenous mixture of unsaturated fatty acids and water (Kapich et al. 1999a). Subsequently, it was shown that peroxidized unsaturated fatty acids are capable to oxidize non-phenolic β-O-4-linked lignin model compounds irrespective of MnP, indicating that direct contact between MnP and lignin is not necessary but, rather, that the formed peroxyl radicals act as agents for lignin degradation (Kapich et al. 1999b). In nature, lipid substances might be produced by the fungus (Enoki et al. 1999) and are also already present in wood (Hofrichter et al. 2001). VP represents a hybrid of LiP and MnP and is able to directly oxidize non-phenolic lignin units as well as Mn^{2+} (Ruiz-Dueñas et al. 2009).

There are several white-rot fungi that exclusively produce MnP as extracellular peroxidase e.g., *Ceriporiopsis subvermispora* (Lobos et al. 1994), *Dichomitus squalens* (Périé et al. 1996), *Lentinula* (*Lentinus*) *edodes* (Leatham 1986), *Phanerochaete sordida* (Rüttimann-Johnson et al. 1994), and *Pleurotus ostreatus* (Giardina et al. 2000), indicating that MnP might be essential for lignin biodegradation. Other white-rot basidiomycetes produce LiP alongside MnP, e.g., *Phanerochaete chryosporium* (Hatakka 1994), *Phlebia radiate* (Vares et al. 1995), and *Trametes versicolor* (Johansson and Nyman 1993), while VPs have been detected in *Pleurotus* (Camarero et al. 1999) and *Bjerkandera* (Mester and Field 1998) species.

Another group of enzymes potentially involved in the oxidation of lignin are laccases, copper-containing oxidases oxidizing phenolic rings to phenoxy radicals while using molecular oxygen as oxidant (Baldrian 2006). They usually have a low redox potential with few exceptions (Uzan et al. 2010). Therefore, they are unable to directly catalyze the oxidation of non-phenolic aromatic rings of lignin (Ruiz-Dueñas and Martínez 2009). However, they are able to degrade high-redox-potential substrates in the presence of small chemical oxidants acting as redox mediators (reviewed by, e.g., Morozova et al. 2007; Cañas and Camarero 2010; Kudanga et al. 2011). These mediators form radicals, which are able to penetrate the lignocellulose matrix (Ruiz-Dueñas and Martínez 2009). Nevertheless, the role of laccase in lignin biodegradation is still debated. While many white-rot fungi produce laccases, there are no laccase-encoding genes present in the whole genome of *P. chryosporium* (Martínez et al.

2004), a white-rot basidiomycete able to completely degrade all components of lignocellulose (Kersten and Cullen 2007). Furthermore, 17 laccase-encoding genes have been found in *Coprinopsis cinerea*, a basidiomycetous fungus unable to degrade lignin (Hatakka and Hammel 2010). Moreover, in the absence of suitable mediators, laccases oxidize the phenolic groups present in lignin, which results in polymerization (Rochefort et al. 2004). Therefore, it remains unclear if laccases play a role in natural lignin degradation processes. However, since laccases can be produced in sufficient amounts for industrial applications (Hou et al. 2004), whereas this could not be achieved for ligninolytic peroxidases so far (Singh et al. 2011), their potential application for lignin depolymerization in industrial processes in combination with suitable mediators should not be disregarded.

Lignin-degrading auxiliary enzymes

The H_2O_2 needed by the ligninolytic peroxidases and possibly laccases is produced in situ by lignin-degrading auxiliary enzymes (LDA1-8) like, e.g., aryl–alcohol oxidase or glyoxal oxidase (Shah and Nerud 2002). It has been shown that aryl–alcohol oxidase is able to use the aromatic aldehydes produced by lignin breakdown or synthesized by fungi as substrate for H_2O_2 generation in cyclic redox reactions, which also involve aryl–alcohol dehydrogenase (Guillén et al. 2000). Furthermore, there is evidence that FAD oxidases like aryl–alcohol oxidase prevent the recondensation of intermediates during lignin degradation by reducing the de novo formed quinonoids and radical compounds (Marzullo et al. 1995). H_2O_2 has also been shown to be produced by oxidation of lignin-derived hydroquinones through laccases and VPs (Guillén et al. 2000; Gómez-Toribio et al. 2001, 2009) or Mn^{2+} oxidation through laccases (Schlosser and Höfer 2002).

Lignin-degrading auxiliary esterases, i.e., feruloyl esterases, have been shown to hydrolyze ferulate bridges between lignin and carbohydrates in monocotyledons and dicotyledons and to disrupt the cell wall structure in combination with xylanases and might, therefore, be involved in the initial stages of lignocellulose degradation (Topakas et al. 2007; Koseki et al. 2009).

Hydroxyl radical production

Hydroxyl radicals, which are the strongest oxidants in white-rot fungi cultures (Forney et al. 1982; Backa et al. 1992), can initiate the attack on lignocellulose and presumably cause hydroxylation, demethoxylation, and depolymerization of lignin (Evans et al. 1994; Joseleau et al. 1994). They can be produced by cellobiose dehydrogenase via Fenton reaction (Henriksson et al. 2000; Ludwig et al. 2010; Harreither et al. 2011). A further path leading to hydroxyl radical production involves *p*-quinones originating from lignin decomposition. They can be used by quinone reductases, laccases, and peroxidases in redox cycle reactions to activate oxygen (Guillén et al. 1997). This in turn reduces the ferric iron present in wood either by the superoxide radicals or semi-quinone radicals to ferrous iron (Guillén et al. 2000). Subsequent re-oxidation of the ferrous iron by H_2O_2 reduction produces hydroxyl radicals (Guillén et al. 2000).

Potential additional lignin-modifying enzymes

There are some reports of additional enzymes that might have the potential to act on lignin. Chloroperoxidases might contribute to lignin degradation since they have been shown to catalyze the cleavage of the β-O-4 bond of a dimeric lignin model compound (Ortiz-Bermúdez et al. 2003). Furthermore, they might chlorinate lignin and be partly responsible for the natural production of chloroaromatics (Ortiz-Bermúdez et al. 2003).

Two dye-decolorizing peroxidases (DyP) secreted by the jelly fungus *Auricularia auricular-judae* have been shown to oxidize non-phenolic lignin model compounds and high-redox-potential dyes, i.e., Reactive Blue 5 and Reactive Black 5 at acidic pH (activity maximum at pH 1.4), indicating the ligninolytic activity of these enzymes (Liers et al. 2010). The redox potential of five different DyPs has been calculated to be at least ~1.2 V and it has been suggested that this might be sufficient for the oxidation of non-phenolic lignin structures at low pH values between 1.5 and 3.0 (Hofrichter et al. 2010). However, not all DyPs are capable to oxidize non-phenolic lignin model compounds and whether certain DyPs can actually depolymerize lignin has still to be investigated (Hofrichter et al. 2010).

A further group of enzymes hypothesized to take part in the degradation of methoxylated compounds originating from lignin is aromatic peroxygenases (Kinne et al. 2011). Extracellular aromatic peroxygenase from the agaric fungus *Agrocybe aegerita* (*Aae*APO) has been shown to cleave non-phenolic β-O-4 lignin model compounds as well as phenolic dimers but seems to be unable to oxidize polymeric lignin (Kinne et al. 2011). Therefore, *Aae*APO seems not to be directly involved in lignin biodegradation but rather in the oxidation of compounds with low molecular masses such as lignin fragments or lignans (Kinne et al. 2011).

Lignin modification by brown-rot fungi

Besides white-rot basidiomycetes, brown-rot fungi are also capable to degrade lignocellulose in nature. While lignin is not significantly mineralized by brown-rot fungi, its structure is altered, i.e., its aromatic methoxyl content is lower and its

content of conjugated carbonyls and carboxyls as well as phenolic hydroxyls is higher (Yelle et al. 2008). While hydroxyl radicals seem to play a major role in lignocellulose degradation by brown-rot fungi (Hammel et al. 2002), there also appear to be hydrolytic enzymes involved in the process (Cohen et al. 2005). It has been argued that consequently some lignin degradation has to occur because otherwise these enzymes would be hindered to act on their target substrates, e.g., cellulose by the small pore sizes of intact wood (Yelle et al. 2008). Analysis of lignocellulose degraded by the brown-rot basidiomycete *Gloeophylum trabeum* via two-dimensional solution-state nuclear magnetic resonance spectroscopy indicated degradation of lignin side chains during brown-rot (Yelle et al. 2008). However, brown-rotted lignin has been shown to be polymeric and to keep most of its aromatic residues (Jin et al. 1990). Therefore, it has been suggested that lignin is initially depolymerized by brown-rot fungi (Yelle et al. 2008). Subsequently, the content of phenolic units on lignin increases due to hydroxyl radicals reacting with the aromatic rings (Yelle et al. 2008). Thereafter, the lignin units get reconnected through radical coupling of the phenolic units (Yelle et al. 2008).

Lignin degradation by bacteria

Many bacteria have lignin-degrading ability (recently reviewed by Bugg et al. (2011)). They are able to solubilize lignin and modify the lignin structure (Buswell and Odier 1987; Ball et al. 1989; Godden et al. 1992). However, they do not mineralize lignin to a large extent (Buswell and Odier 1987). These bacteria produce several extracellular enzymes that are involved in the degradation of lignocellulosic biomass like peroxidases, cellulases, and esterases (Santhanam et al. 2012). However, at present, the enzymology of bacterial lignin degradation is not well understood (Bugg et al. 2011). Nevertheless, the results suggest that similar enzyme types as in fungi might be involved. It has been reported that *Streptomyces viridosporus* T7A, a lignin-degrading actinomycete, secretes several peroxidases able to catalyze the cleavage of lignin model compounds (Ramachandra et al. 1988). Subsequently, several actinomycetes exhibiting extracellular peroxidase activity have been identified (Mercer et al. 1996). More recently, a DyP has been identified and characterized in the actinomycete *Rhodococcus jostii* sp. RHA1 able to catalyze Cα–Cβ bond cleavage of a lignin model compound (Ahmad et al. 2011). Production of laccases by bacteria has been reported as well (Miyazaki 2005; Kellner et al. 2008; Niladevi et al. 2008). This indicates that suitable enzymes produced by bacteria could potentially be used by themselves or in combination with enzymes from fungi in future conversion processes of lignin. Using bacteria instead of fungi for enzyme production would most likely reduce the practical challenges posed when working with fungi concerning protein expression and genetic manipulation.

Enhancement of biocatalyst properties

The performance of one-pot multi-catalytic reactions with lignin-modifying enzymes (LMEs) can be limited by several reaction parameters, e.g., pH, temperature, and solvent. In general, pH is not a major constraint regarding activity as several LMEs active at rather high or low pH have been described (Table 1). Therefore, the right selection of enzymes allows for reaction conditions to be acidic as well as alkaline. However, enzyme stability might become an issue under extreme pH conditions, which are, e.g., associated to chemical oxidation processes. Temperature seems to be a major limitation as few enzymes are active at temperatures above 90 °C. Most LMEs show activity maxima between 20 and 70 °C. However, some overlap between the temperature ranges of LMEs and inorganic catalytic processes exists (Fig. 1). Moreover, enzymes are often inactivated or denatured in the presence of organic solvents (Ogino and Ishikawa 2001), further limiting their usability for the conversion of lignin in combination with chemical catalysis.

Improving enzyme stability and activity under harsh reaction conditions would lead to a broader application range, thereby increasing the possible combinations with other bio- or inorganic catalysts. In principle, there are three ways to obtain modified enzymes with improved properties. Enzymes can be isolated from extremophiles, obtained by classical protein engineering where stabilizing mutations can be predicted on the basis of the enzyme structure, and produced through directed evolution (Eijsink et al. 2005; Turner 2009). "Directed evolution is a widely-used engineering strategy for improving the stabilities or biochemical functions of proteins by repeated rounds of mutation and selection" (Bloom and Arnold 2009). These three strategies are also often used in combination. Additionally, enzyme immobilization often leads to the enhancement of enzyme stability and sometimes even of activity (Mateo et al. 2007). Furthermore, immobilization facilitates the recovery and reuse of enzymes (Sheldon 2007). In this section, we discuss several scientific and technological advances leading to the effective improvement of LME properties.

Thermostability

Increased enzymatic thermostability often goes along with a general stability increase of the enzyme, making it more tolerant to extreme pHs, high salt concentration, the presence of organic co-solvents, etc. (Arnold 1990; Ogino and Ishikawa 2001). High process temperatures confer advantages like low levels of microbial side-contamination, improved reaction yields, increased solubility of reaction substrate, and product

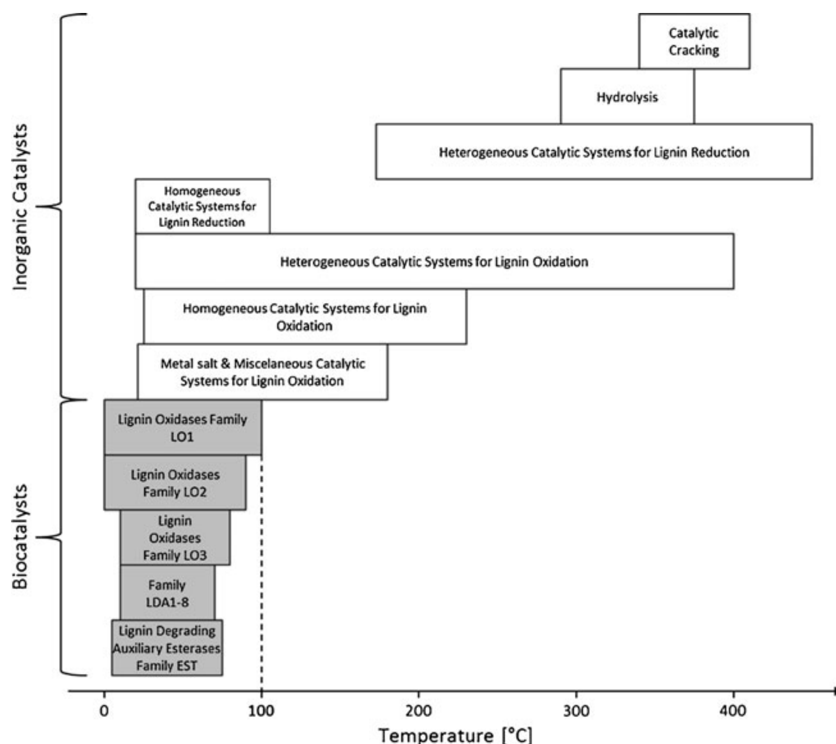

Fig. 1 Temperature ranges of the different enzyme families (*gray background*; data from Table 1) and in which the different types of chemical catalyses of lignin and lignin model compounds (*white background*) were performed (data from Zakzeski et al. (2010a)). *Family LDA1-8* lignin-degrading auxiliary enzymes

and time savings (Arnold 1990; Ogino and Ishikawa 2001; García-Ruiz et al. 2010).

However, increasing stability through point mutations often leads to a trade-off between stability and activity (García-Ruiz et al. 2010). Stability increases are mostly gained through mutations, which establish new interactions and thereby prevent unfolding and denaturation under harsh conditions (Reetz et al. 2006). At the opposite, activity increases are mostly achieved through destabilizing mutations that lead to better access for the substrates to the active sites of the enzymes (Bloom and Arnold 2009). Therefore, to produce enzymes with increased stability and activity, several mutations are often necessary. Several studies reported the increase of enzyme thermostabilities of LMEs through mutations, also often in combination with increased activities (Reading and Aust 2000; Morawski et al. 2001; Festa et al. 2008; García-Ruiz et al. 2010). For instance, a recombinant MnP had a higher activity than the native enzyme produced by *Phanerochaete chrysosporium* but was also more readily inactivated at temperatures of 37 °C and above (Reading and Aust 2000). The recombinant enzyme was modified by the insertion of a disulfide bond, which increased the enzyme's thermostability while retaining its enhanced activity. The newly built enzyme retained most of its activity up to a temperature of 52 °C, where it was inactivated at a similar rate as the native enzyme (Reading and Aust 2000). Other studies applied directed evolution to improve the thermostability of, e.g., a horseradish peroxidase (Morawski et al. 2001), laccase from *P. ostreatus* (Festa et al. 2008), and VP from *Pleurotus eryngii* together with high-redox-potential laccase from basidiomycete PM1 (García-Ruiz et al. 2010). The enzymes were expressed in *Saccharomyces cerevisiae* in all three studies. The half-life of the newly developed horseradish peroxidase was increased by a factor of 3 (Morawski et al. 2001) and that of laccase by a factor of 1.4 (Festa et al. 2008) in comparison to the native enzyme at 60 °C. The study aiming to improve the thermostability of VP and high-redox-potential laccase reported that the VP retained ~30 % of its maximal activity at 65 °C (three times more than the native enzyme) and the high-redox-potential laccase retained 40 % of its maximal activity at 72 °C (ten times more than the native enzyme) (García-Ruiz et al. 2010).

H_2O_2 tolerance of peroxidases

Peroxidases are inactivated by too high substrate, i.e., H_2O_2, concentrations. The oxidative damage of peroxidases has been referred to as suicide inactivation (Valderrama et al. 2002). Since H_2O_2 is needed by peroxidases to degrade lignin, either control of H_2O_2 concentrations or increased tolerance of peroxidases to higher H_2O_2 concentration levels is necessary to run continuous processes. Control of H_2O_2 concentrations could be achieved either by supplying H_2O_2 to fed batches (Mielgo et al. 2003) through electro-chemical methods (Lee and Moon 2003) or through the control of H_2O_2-producing enzymes (Lan et al. 2006). Some studies report on the operation of continuous processes with LiP from *Phanaerochaete chrysosporium* whereby the enzymatic production of H_2O_2 was used as a mean to control the concentration of the latter (Lan et al. 2006; Qiu et al. 2009).

H_2O_2 was supplied by glucose oxidase from *Aspergillus niger*, which was in turn controlled through regulation of glucose concentration and pH. The effectiveness of this combination was demonstrated through the conversion of different dyes. In one study, 90.9 % of rhodamine B was converted over 1 h when H_2O_2 was produced enzymatically compared to only 41.0 % when H_2O_2 was supplied externally every 5 min (Lan et al. 2006). Total supplied H_2O_2 amounts over the course of the whole experiment were 300 μM in both cases (Lan et al. 2006). In another study, LiP and glucose oxidase were co-immobilized on nanoporous gold (Qiu et al. 2009). Some 87.2, 75.5, and 84.6 % of pyragallol red, rhodamine B, and fuchsine, respectively, were converted over 3 h when H_2O_2 was supplied enzymatically compared to only 55.6, 44.3 and 53.5 % when LiP was immobilized as a single enzyme and 1 μM H_2O_2 was supplied externally in one pulse at the beginning of the experiment (Qiu et al. 2009).

Gil-Rodríguez et al. (2008) reported the isolation of a peroxidase from daikon radish (*Raphanus sativus L.* cv. daikon) resistant to H_2O_2, named Zo peroxidase. Future detailed study of the enzyme structure of Zo peroxidase might reveal the properties responsible for its H_2O_2 tolerance and may help in the production of further H_2O_2-resistant peroxidases (Gil-Rodríguez et al. 2008). Several studies reported achieving enhanced H_2O_2 tolerance of ligninolytic peroxidases through mutagenesis. For example, MnP from *P. chrysosporium* with nine times higher H_2O_2 stability (Miyazaki-Imamura et al. 2003) and horseradish peroxidases with 25-, 18-, and 12-fold increased H_2O_2 stability (Ryan and Ó'Fágáin 2007) compared to the wild type were reported. Furthermore, increased H_2O_2 tolerance of a VP from *Bjerkandera adusta* was achieved by producing cross-linked enzyme aggregates of VP in combination with glucose oxidase from *A. niger* (Taboada-Puig et al. 2011).

Stability in non-aqueous solvents

Lignin can be dissolved in some organic solvents (Muurinen 2000; Zhao et al. 2009), making it more available for catalysts. However, enzymes are usually inactivated and denatured in the presence of organic solvents (Doukyu and Ogino 2010) since they generally require water to maintain their native structure by promoting non-covalent interactions like hydrogen bonds, and van der Waals, hydrophobic, and ionic interactions (Singer 1963). Furthermore, polar solvents are able to enter the protein's interior which might induce secondary and tertiary structural changes and are more able than apolar solvents to remove protein-bound water (Serdakowski and Dordick 2008). Additionally, the higher solubility of the substrate in organic solvents constitutes a thermodynamic stabilization of the substrate in its initial state, which leads to a decrease of the observed enzymatic activity (Serdakowski and Dordick 2008). Changing solvent polarity can lead to a more stable transition state and therefore reduce the activation energy of the catalyzed reaction as shown, for example, for subtilisin-catalyzed transesterification reactions in organic solvents (Kim et al. 2000). Therefore, the addition of small amounts of water and the decrease of solvent polarity can lead to more enzymatic activity in organic solvents (Serdakowski and Dordick 2008). Another way to obtain active enzymes in non-aqueous solvents is protein engineering (Serdakowski and Dordick 2008). No LMEs are reported to be organic solvent tolerant by nature in a recent review on the subject (Doukyu and Ogino 2010). However, several studies successfully maintained the enzymatic activity of LMEs in organic solvents (e.g., Dai and Klibanov 1999; Ozawa and Klibanov 2000; van de Velde et al. 2001; Liu et al. 2006; Zumárraga et al. 2007).

Alternatively, biomass can be dissolved in ILs. Several ILs are able to dissolve lignocellulose and allow the separation and at least partial recovery of each fraction. The solubility of carbohydrates and lignin in ILs has been reviewed recently (Zakrzewska et al. 2010). The highest solubility of kraft lignin was achieved using 1,3-dimethylimidazolium methanesulfonate ([Mmim] [CH_3OSO_3]) or 1,3-dimethylimidazolium trifluoromethanesulfonate ([Bmim] [CF_3SO_3]) as solvent (Lee et al. 2009).

Several reviews discuss enzymatic reactions and enzyme stabilization in ILs (e.g., Cantone et al. 2007; van Rantwijk and Sheldon 2007; Gorke et al. 2010; Zhao 2010). Dissolution of lignocellulose in ILs and prospects on possible future uses of ILs in biorefineries are discussed in a recent feature article (Sun et al. 2011). The possibility to directly dissolve lignocellulose and separate lignin from cellulose and hemicellulose might allow an exhaustive utilization thereof and has the potential to facilitate the treatment of lignin with biocatalysts (Sun et al. 2011).

Enzyme immobilization

Enzyme immobilization can improve the properties of biocatalysts by increasing their stability and activity under various conditions (e.g., extreme pHs, organic solvents, elevated temperatures) (Bommarius and Riebel-Bommarius 2004) and allows enzyme reuse and biocatalysts separation from reaction products, thereby permitting the operation of continuous processes (Polizzi et al. 2007). Immobilization techniques have been the subject of several recently published reviews (e.g., Mateo et al. 2007; Brady and Jordaan 2009; Hartmann and Jung 2010; Jochems et al. 2011).

In a recent study, laccase from *T. versicolor* and horseradish peroxidase were co-immobilized on aluminum particles by cross-linking and layer-by-layer coating with alternatively charged polyelectrolytes (Crestini et al. 2011). The resulting multi-enzyme biocatalysts had increased stability compared to

the free enzymes. A total of 2 $g L^{-1}$ milled wheat straw lignin was treated with the biocatalysts in water. Before the start of the experiment, the wheat straw lignin was completely insoluble. After treatment with the biocatalysts, 61.3 and 59.8 % (*w*/*w*) of milled wheat straw lignin were in solution with and without addition of a chemical laccase mediator, respectively. The water-soluble material consisted of polymers with significantly lower average molecular weight than the starting material, demonstrating that lignin depolymerization outweighed lignin solubilization. Control experiments with free enzymes and singly immobilized enzymes (used alone or in mixture, with or without laccase mediator) led to less efficient lignin conversion and depolymerization (Crestini et al. 2011). Furthermore, the immobilized catalysts could be recycled more than ten times. This study gives further indication that a multi-catalyst approach is expedient for lignin depolymerization and demonstrates the advantages of immobilized enzymes over free enzymes due to higher stability and recyclability.

Chemoenzymatic combination

The complex mechanism of lignin degradation in nature and the limited success of lignin depolymerization with single catalysts indicate that efficient lignin conversion to platform and fine chemicals might only be achieved by combining various catalysts. Making use of the unique properties of biocatalysts as well as of chemical catalysts represents a new approach (Vennestrøm et al. 2010a).

One possibility to treat lignin using chemical catalysts as well as biocatalysts would be a sequential treatment in several reactors, allowing optimal reaction conditions for each catalyst. While this might be necessary for certain reactions (especially reactions with chemical catalysts only working effectively at high temperatures), using multiple catalysts in one reactor would offer several advantages, such as process intensification, reduction of product separation and purification steps, material consumption, waste generation, and time and yield losses (Murzin and Leino 2008; Vennestrøm et al. 2010a). However, conducting such one-pot catalytic transformations is challenging. Temperature and reaction media (as already discussed in the previous chapter), pressure, compatibility, and reaction rates are key parameters to consider when combining enzymes and inorganic catalysts (Vennestrøm et al. 2010a). The highest pressure used in chemical catalysis as reported by a recent review (Zakzeski et al. 2010a) was 46 MPa (Kashima et al. 1964). However, these processes were conducted at temperatures between 250 and 450 °C and are therefore unsuitable for enzymatic reactions. The highest pressure for chemical catalysis conducted at 100 °C or below as reported by the same review was 18 MPa (Borthakur 2007). Pressures in this order of magnitude seem to have only limited effects on enzyme stability as pressures of 100–200 MPa are required to dissociate oligomeric proteins (Silva and Weber 1993) and even higher pressures of 400–800 MPa are needed to denature small monomeric proteins (Heremans 1982).

Compatibility between reactants, intermediates, substrates, and bio- and inorganic catalysts that are employed in one-pot processes is more crucial and has to be ensured (Vennestrøm et al. 2010a). Furthermore, reaction rates have to be adjusted in order to avoid the formation of undesired compounds or substrate limitation for one of the catalysts (Murzin and Leino 2008; Vennestrøm et al. 2010a). Therefore, reliable kinetic models describing the transformation processes have to be developed, which is not an easy task because a lot of parameters and variables have to be considered and integrated together in the models, e.g., catalyst–catalyst interactions, mass transfer processes, feedback inhibition, etc. (Murzin and Leino 2008). Recently, processes relying on the chemo-enzymatic conversion of biomass were discussed in a further review, which however did not mention the use of lignin as feedstock (Marr and Liu 2011).

While chemo-enzymatic combinations for lignin transformation have not been extensively explored so far, several interactions between LMEs and chemical transformation processes can be envisioned (Fig. 2). Chemical transformation of lignin could provide compounds that can be used by laccases as mediators (Fig. 2, a). Phenol—a compound that has been shown to act as laccase mediator for the oxidation of, e.g., polycyclic aromatic hydrocarbons like anthracene (Johannes and Majcherczyk 2000)—has been produced by, e.g., hydrocracking of organocell lignin (Meier et al. 1994). Chemical oxidation of lignin has led to the formation of other compounds that have been reported to act as laccase mediators in a recent review (Cañas and Camarero 2010), e.g., vanillin (e.g., Badamali et al. 2009; Partenheimer 2009; Werhan et al. 2011) or aceto vanillone (e.g., Crestini et al. 2005, 2006; Badamali et al. 2009). Therefore, employing laccase in combination with certain chemical catalysts might lead to in situ production of laccase mediators, and consequently the addition of laccase mediators in advance might not be necessary.

Furthermore, the different oxidases can be employed for H_2O_2 production (Fig. 2, b). This has been demonstrated in a recent study (Vennestrøm et al. 2010b). Glucose oxidase from *A. niger* was immobilized on a titanium silicate catalyst. The H_2O_2 produced as a side-product by the glucose oxidase-catalyzed oxidation of one substrate (e.g., β-glucopyranose) was used as oxidizing agent by the chemical catalyst for the oxidation of another substrate (e.g., allyl alcohol, 2-butanol, 2-propanol, crotyl alcohol). A similar combination can be envisaged between FAD-oxidase enzymes and, for example, MTO catalysts that require H_2O_2 as primary oxidant for the oxidation of lignin (e.g.,

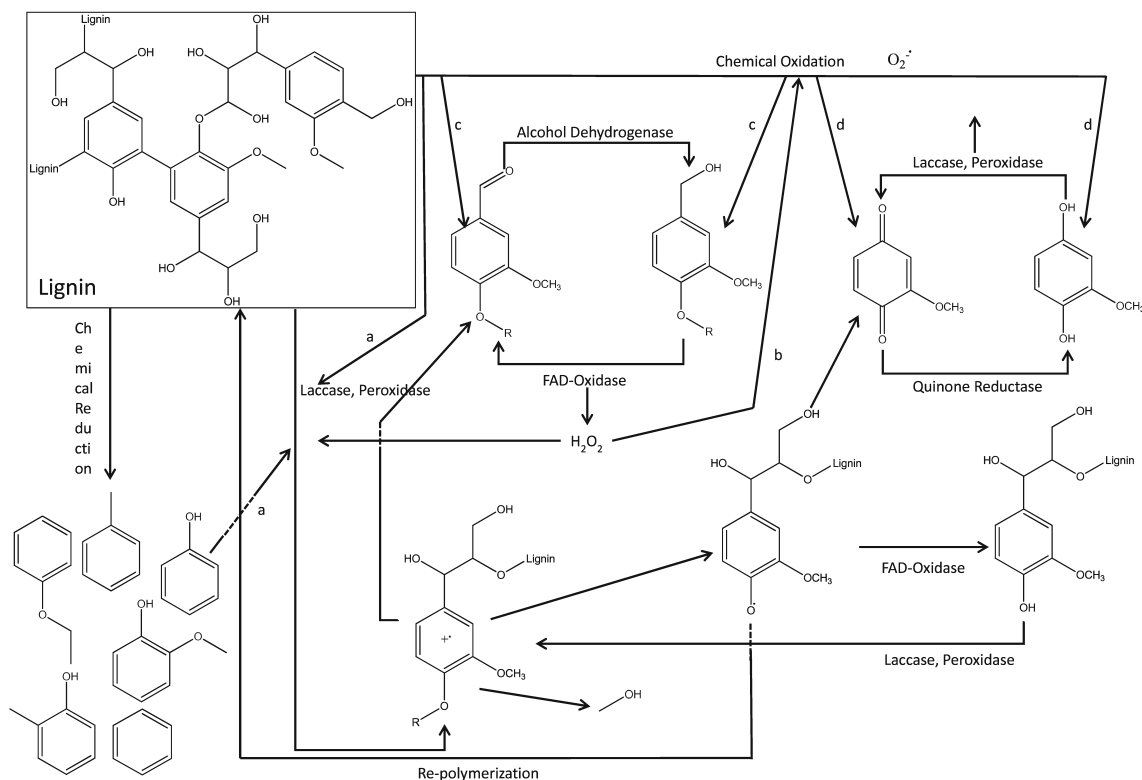

Fig. 2 Scheme depicting possible interactions between biological and chemical lignin degradation (for explanation, see text)

Herrmann et al. 2000; Crestini et al. 2005, 2006). The degradation of lignin or lignin model compounds by the MTO/H_2O_2 oxidation system has led to the formation of aromatic aldehydes (Herrmann et al. 2000; Crestini et al. 2005, 2006) as well as aromatic alcohols (Herrmann et al. 2000) (Fig. 2, c). These products could be directly used by FAD-oxidases and alcohol dehydrogenases in redox cycles for the further production of H_2O_2. Moreover, repolymerization of intermediates like quinonoids and radical compounds during lignin degradation might be prevented by employing FAD-oxidases.

Chemical oxidation of lignin by, e.g., polyoxometalate (POM) (Gaspar et al. 2007) or Co(salen) (Canevali et al. 2002) catalysts can result in quinones and hydroquinones that can be used by quinone reductases, laccases, and peroxidases in redox cycles for the production of superoxide radicals (Fig. 2, d). Furthermore, it has been shown that laccase can re-oxidize reduced POM catalysts. This has been used for the continuous delignification of eucalypt kraft pulp (Gamelas et al. 2007). The POM catalyst ($[SiW_{11}V^{V}O_{40}]^{-5}$) was employed at around 90 °C in a batch reactor for pulp delignification. Laccase from *T. versicolor* was applied in a separate aerated batch reactor for the re-oxidation of the reduced POM catalysts at 45 °C. The re-oxidized chemical catalysts were separated from the enzymes via an ultrafiltration ceramic membrane and returned to the first reactor. By this continuous system, nearly 70 % of pulp delignification was obtained (Gamelas et al. 2007).

Such possible interactions between catalysts show the large potential for new multi-catalyst processes for lignin transformation. Their development might be essential for a more efficient valorization of the biopolymer.

Conclusions

Lignin depolymerization using single inorganic catalysts had limited success. Advances in enzyme engineering and immobilization, emergence of new solvents, and new chemical catalyst design lead to an increasing degree of overlap of reaction conditions in which enzymes and inorganic catalysts can operate. These new possibilities for catalyst combinations open promising perspectives for novel technologies in the field of green chemistry. Sequential or one-pot multi-catalyst processes have the potential to play an important role in the conversion of the renewable feedstock lignin into value-added fine chemicals and should become the focus of future research.

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Conflict of interest The authors declare that they have no conflict of interest.

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