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Oxidative polymerization of lignins by laccase in water-acetone mixture*

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The enzymatic oxidative polymerization of five technical lignins with different molecular properties, i.e. Soda Grass/Wheat straw Lignin, Organosolv Hardwood Lignin, Soda Wheat straw Lignin, Alkali pretreated Wheat straw Lignin, and Kraft Softwood was studied. All lignins were previously fractionated by acetone/water 50:50 (v/v) and the laccase-catalyzed polymerization of the low molecular weight fractions (Mw < 4000 g/mol) was carried out in the same solvent system. Reactivity of lignin substrates in laccase-catalyzed reactions was determined by monitoring the oxygen consumption. The oxidation reactions in 50% acetone in water mixture proceed with high rate for all tested lignins. Polymerization products were analyzed by size exclusion chromatography, FT-IR, and ^{31}P-NMR and evidence of important lignin modifications after incubation with laccase. Lignin polymers with higher molecular weight (Mw up to 17500 g/mol) were obtained. The obtained polymers have potential for applications in bioplastics, adhesives and as polymeric dispersants.

Key words: lignin, organic solvent, laccase, enzymatic polymerization

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INTRODUCTION

Reactions catalyzed by enzymes, besides being environmentally friendly, can be very effective in the transformation of specific substrates due to their high selectivity. As a result, biocatalytic procedures have arisen as feasible alternatives to several traditional chemical processes, even at industrial scale. In the forest based industry, a wide range of enzymatic applications were investigated (Moldes & Vidal, 2011; Kudanga *et al.*, 2008). Particularly, one of the most common enzymes to be applied in relation to the forest industry is laccase (Widsten & Kandelbauer, 2008). Laccases are an integral component of fungal metabolism. Due to their ability to catalyze a broad variety of oxidative reactions, they play an important role in the lignin-degrading pathways in wood-rotting fungi, such as *Trametes versicolor*. Laccases (EC 1.10.3.2) are multi-copper phenoloxidases able to oxidize a wide range of phenolic substrates, including the phenolic moieties typically found in lignin by concomitant reduction of O_2 to H_2O (Baldrian, 2006). Laccases contain 4 copper atoms, one termed Cu T1 and involved in the oxidation of the reducing substrate and electron transfer to the T2/T3 copper cluster, and a trinuclear copper cluster T2/T3, where oxygen binds and is reduced to water. Substrate oxidation by laccase is a one-electron reaction generating a free radical. The initial product of the reaction, i.e. the phenoxy radical, is typically unstable and may undergo a second enzyme-catalyzed oxidation or otherwise a non-enzymatic reaction such as hydration, disproportionation or polymerization leading to new C-O-C and C-C linkages. The bonds of the natural substrate, lignin, that are cleaved by laccase include, Cα-oxidation, Cα-Cβ cleavage and aryl-alkyl cleavage. Laccases can act on non-phenolic compounds by employing mediators, which undergo an oxidation-reduction cycle, thus shuttling electrons between non-phenolic compounds and the enzyme, (Bourbonnais *et al.*, 1995). This makes them attractive catalysts to produce novel lignin polymers with modified physico-chemical and functional properties *via* oxidative polymerization.

Lignin is a complex, three-dimensional aromatic biopolymer that represents 15–35% of the wood (Onnerud *et al.*, 2002) and makes up about 20% of the total mass of the biosphere (Kleinert & Barth, 2008). Lignins are composed of three different types of phenylpropane units, i.e. *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) groups, which are found in various ratios depending on the source (Dashtban *et al.,* 2010). Industrial lignins are by-products of the pulp and paper industry and lignocellulosics bioethanol production processes. The chemical structure and composition of any lignin is affected by (i) the lignocellulosic source and (ii) the pulping process. Most industrial lignins are heterogeneous mixtures with a broad molecular weight distribution, and this has a high impact both on polymer properties like mechanical properties, solubility, or flow behavior and industrial application (Crestini *et al.*, 2010). Fractionation of lignin by solvent extraction has been shown to produce more homogeneous lignin fractions with defined molecular mass distribution and chemical group functionalities (Gouveia *et al.*, 2012; Ropponen *et al.*, 2011), that can be further modified by chemical or enzymatic treatment to obtain novel lignin derivatives with new functionalities. The modification of lignin functionality by oxidative coupling to small molecules and by oxidative polymerization using

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Abbreviations: G, guaiacyl; S, syringyl; H, p-hydroxyphenyl; Mw, weight-average molecular weight; PD, dispersity; KSL, Kraft softwood lignin; SGWL, Soda grass/wheat straw lignin; OHL, Organosolv hardwood lignin; SWL, Soda wheat straw lignin; AWL, Alkali pretreated wheat straw lignin; KSL-Lcc, Laccase treated KSL lignin; SGWL-Lcc, Laccase treated SGWL lignin; OHL-Lcc, Laccase treated OHL lignin; SWL-Lcc, Laccase treated SWL lignin; AWL-Lcc, Laccase treated AWL lignin

laccases in aqueous media has been shown (Van de Pas *et al*., 2011). Nevertheless, like other hydrophobic polymers, lignin is not soluble in water, and this restricts the extent of possible enzymatic modifications (Mattinen *et al*., 2011). Precipitation of the polymerization products may also limit further oxidative polymerization (Buchert *et al*., 2002). Therefore, the use of water-miscible organic solvents to increase the solubility of the substrate and products can be used to enhance enzymatic modification of lignins. In this paper, we have studied the ability of *Trametes versicolor* laccase to modify fractionated lignin by oxidative polymerization in 50% (vol) aqueous acetone. Five different lignins (from softwood, hardwood, straw and grass) obtained by different pulping processes (kraft, soda, organosolv) were fractionated by selective extraction with acetone/water 50:50 (v/v) and the more homogenous soluble fractions were used to determine the influence of the type of lignin in the enzymatic polymerization.

MATERIALS AND METHODS

Lignins. Organosolv lignin from mixed hardwoods (Alcell) was obtained from Repap Technologies Inc. (Valley Forge, PA, USA) and is referred to as OHL. Soda wheat straw lignin (SWL) and soda lignin from mixed Sarkanda grass/ wheat straw (SGWL) were obtained from Granit SA (Lausanne, Switzerland). Indulin AT, a Kraft lignin from softwood (KSL), was obtained from MeadWestvaco (USA). Alkali-pretreated wheat straw lignin (AWL) was obtained from TU Dresden (Germany). Of each lignin tested, the fraction soluble in acetone-water solution 50:50 (v/v) was isolated by selective extraction from 1% (w/v) suspension, at 24°C. The yield of the soluble lignin fractions was 55% (OHL-50), 60% (AWL-50), to 85% (SWGL-50), 96% (SWL-50) and 98% (KSL-50). The soluble lignin fractions were characterized by SEC, FT-IR and ^{31}P-NMR. The isolated lignin fractions soluble in acetone/water 50:50 (v/v) were used as the starting substrates for laccase oxidation.

Enzyme. Laccase from *Trametes versicolor* (30.6 U mg^{-1} of solid) was purchased from Sigma-Aldrich (Taufkirchen, Germany). Enzyme activity was determined spectrophotometrically using syringaldazine as substrate in different acetone:water (v/v) mixtures. The reaction mixture (1 ml) contained 0.027 mM syringaldazine dissolved in acetone:water 50:50 (v/v) and 0.5 μg ml^{-1} laccase. The formation of the syringaldazine radical (ε_{530} = 65 $mM^{-1}cm^{-1}$) was followed in time at 530 nm and 25°C (Sealey & Ragauskas, 1998).

Laccase–lignin reactions. Lignin fractions were solubilized in acetone/air-saturated water 50:50 (v/v) to attain a concentration of 10 mg ml^{-1}. Reaction was started by the addition of laccase solution (0.24 mg ml^{-1}) and the reaction mixture was stirred at 20°C for 24 hours. A control reaction without enzyme was carried out in the same conditions. During polymerization, the formation of a precipitate was observed for all lignins except KSL. The precipitate was separated on a glass filter and characterized (SEC, FT-IR). Reactions were concluded by adding 50 ml deionized water to the soluble fractions and precipitation by lowering the pH to 1.0 with 1M HCl. The reaction products were separated on a glass filter (G4) and dried overnight in a vacuum oven at 60°C. All experiments were carried out at least in duplicate.

Dissolved oxygen consumption measurements. Consumption of dissolved oxygen was followed using a SympHony SB90M5 instrument (Thermo Orion) with a detection limit of 0.1 mg L^{-1}. The experiments were carried out under constant mixing in 15 mL completely filled and sealed glass vessels, in order to avoid entry of oxygen into the reaction mixture during the experiments. The fractionated lignins were dissolved in acetone/water 50:50 (v/v) at a concentration of 1 mg ml^{-1}. The measurements (in duplicates) were done under stirring, using a magnetic stirrer at 250 rpm. The monitoring of the degradation started after addition of 150 ml laccase (0.06 mg ml^{-1}), and the concentration of the dissolved oxygen was monitored continuously for 40 min. The registered response was corrected by subtracting the response obtained for the blank samples (with acetone/water mixture only).

Fourier transform infrared spectroscopy (FT-IR). Fourier Transform Infrared (FT-IR) spectra of the solid lignin samples were obtained in attenuated total reflectance (ATR) mode on a Varian Scimitar 1000 FT-IR spectrometer equipped with a DTSG-detector PIKE MIRacle ATR equipped with a diamond w/ZnSe lens single reflection plate. Spectra were collected in the range 4000–650 cm^{-1} with a resolution of 4 cm^{-1} and with 128 co-added scans. The spectra were baseline corrected and normalized to 1510 cm^{-1}. Shoulders and complex bands were deconvoluted for a good assessment. The assignment of peaks was performed as described by Faix (1991) and Boeriu *et al.* (2004).

Size exclusion chromatography (SEC). The molar mass distribution of lignins was analyzed by alkaline SEC using a TSK gel Toyopearl HW-55F column, 0.5 M NaOH as eluent, UV detection at 280 nm and calibration with sodium-polystyrene sulfonates, according to the procedure described elsewhere (Gosselink *et al*., 2010). Mp (peak molecular weight), Mn (number average molecular weight), and Mw (weight-average molecular weight and dispersity (PD, Mw/Mn) were calculated.

Quantitative ^{31}P-NMR spectrometry. ^{31}P-NMR spectra of lignin samples were recorded on Bruker Avance II 400 MHz spectrometer, after derivatization with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane, using the procedure described by Gosselink (Gosselink et al., 2010). Signal assignment was performed as described by Granata and Agryropoulus (Granata & Argyropoulos, 1995).

RESULTS AND DISSCUSION

In the current work the structural changes that occur during the laccase treatment of low molecular weight lignins from different sources in acetone/water mixture are discussed. Laccase activity in acetone-water mixtures was measured spectrophotometrically using syringaldazine as substrate. Our study reveals a slightly decrease of laccase activity at acetone concentration below 55% (not shown). When more than 55% (vol.) of acetone was added the activity of laccase decreased dramatically (not shown). This agrees with earlier reports showing that an increasing content of organic solvent in the reaction mixture inactivates laccase gradually (Mattinen *et al*., 2011), although the reactivity loss may be partially compensated by the increased reactivity of the substrate.

Oxidative polymerization of lignins by laccase and characterization of products

Five technical lignins of different origin (hardwood, softwood and grasses) and different pulping (organosolv, Kraft and soda) were selected, to cover all structural variation between lignins, i.e. G-type (KSL), SG type

Table 1. Molecular masses of the chemically fractionated lignins before and after laccase polymerization.

Entry	Sample		Mw (g mol^{-1})	Mn (g mol^{-1})	Mw/Mn	Mass increase
1	OHL-50		2010	437	4.6	
2	OHL-Lcc	soluble	5708	714	8.0	2.8
3		insoluble	7219	1063	6.8	3.6
4	SGWL-50		3325	1007	3.3	
5	SGWL-Lcc	soluble	12780	989	12.9	3.8
6		insoluble*	14130	1496	9.4	4.2
7	SWL-50		3777	590	6.4	
8	SWL-Lcc	soluble	15118	1060	14.3	4.0
9		insoluble	18647	1758	10.6	4.9
10	AWL-50		3123	651	4.8	
11	AWL-Lcc	soluble	17359	1303	13.3	5.6
12		insoluble	16017	1930	8.3	5.1
13	KSL-50		4279	1223	3.5	
14	KSL-Lcc	soluble*	3580	534	6.7	0.8

*Partially undissolved in 0.5 M NaOH; Mass increase — represents the ratio between the Mw of the soluble/insoluble product of the laccase-mediated oxidative polymerization and the Mw of the substrate lignin.

(OHL) and SGH type (SGWL, SWL and AWL). The lignins were fractionated with 50:50 acetone/water (v/v) and the low molecular weight (LMW) fractions soluble in the 50:50 acetone/water (v/v) were oxidized in the presence of laccase. For all lignins except KSL, a precipitate was formed during the laccase-catalyzed reactions. SEC data (Table 1) and FT-IR analysis (data not shown) showed that the solid product consisted of an insoluble high molecular weight lignin-polymer, highly condensed, formed after the laccase treatment and represented about 10% (w/w) of the total lignin substrate added in the reaction. The major product of the enzymatic reaction that was soluble in the reaction medium was separated after acid precipitation from the reaction mixture and was characterized to determine the changes in molecular weight and functional group composition upon enzymatic modification. The precipitated "solid" lignins could not be characterized by using ^{31}P-NMR technique because of low solubility in the solvent mixture used for the analysis.

Molecular weight distribution of laccase-modified lignins

The results of gel permeation HPLC analysis confirmed the polymerization of all lignins (Table 1), by the increase in the Mw of the polymerized lignin.

A significant increase of the Mw was observed for all lignins modified by laccase, excepting KSL. KSL represent a particular case, since the product of the polymerization was only partly soluble in 0.5 M NaOH, the solvent used for sample preparation for the SEC analysis and therefore the Mw showed in Table 1 refers only to the NaOH soluble fraction of the product. The highest polymerization degree was achieved in the oxidation of AWL (16017 g mol^{-1} for "solid" lignin and 17359 g mol^{-1} for soluble lignin) when the Mw increased about five fold as compared with the untreated lignin. Polymerization of SWL and SGWL was similar based on the increase of the Mw of the soluble product (about four fold) while a lower increase of the Mw was registered for OHL (about three fold). The highest increase of the Mw was observed in general for the insoluble products while the dispersity was higher for the soluble ones, showing that extensive structure rearrangements are taking place during polymerization process.

Structural modifications of lignin fractions based on FT-IR spectral analysis

FT-IR spectra of all lignin samples, i.e. untreated lignins, the lignin polymers isolated from solution and the minor products precipitated during the reaction, show all typical lignin patterns, with bands at 1605, 1510 and 1425 cm^{-1} (aromatic ring vibrations of the phenylpropane skeleton), 3400 cm^{-1} (aromatic and aliphatic OH groups), 2960, 2925, 2850 and 1460 cm^{-1} (C-H vibration of CH_2 and CH_3 groups) and 1703 cm^{-1} (non-conjugated carboxyl/carboxyl). KSL samples showed typical G bands (1265, 1125, 855 and 815 cm^{-1}), while the hardwood and grass lignins showed also the bands associated to S rings (1330, 1115 and 835 cm^{-1}). Nevertheless, differences have been observed in the intensities of the bands 1330 and 1265 cm^{-1} assigned to S and G units, due to the enzymatic degradation of lignins (as exemplified in Fig. 1 on OHL). A decrease of the intensity at 1460 cm^{-1} may be related to demethylation of S-rings upon laccase treatment. A notable increase of the bands at 1660 cm^{-1} assigned to conjugated C=O is observed in the spectra of all modified lignins, which is probably due to oxidation of lignin side-chains during the enzymatic treatment and the formation of condensed structures by oxidation. Oxidative degradation of lignin side-chains during lignin biocatalytic oxidation has been already reported (Camarero *et al.,* 1997). The formation of more

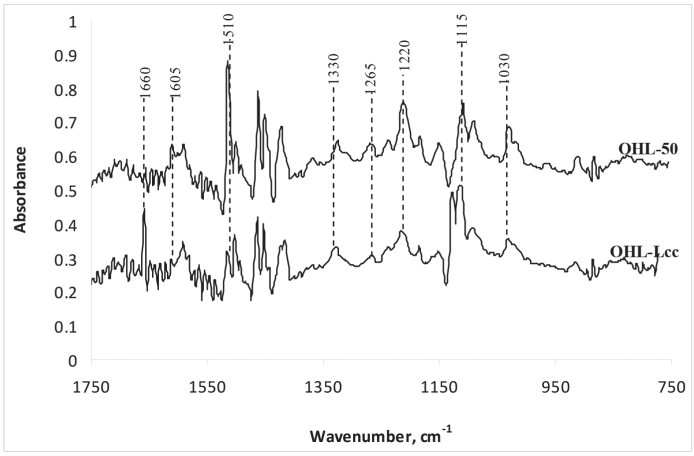

Figure 1. FT-IR spectra of native (OHL-50) and laccase-modified (OHL-Lcc) organosolv hardwood lignin.

condensed structures was also evidenced by the increase of the band at 1115 cm^{-1}, where contribution of C-O deformation in condensed C–O–C is found. Similar results obtained by ^{31}P-NMR analysis will be in detail discussed.

Functional groups composition in native and laccase-modified lignins

The functional group content of the unmodified and laccase-modified lignin was determined by quantitative ^{31}P-NMR analyses. The results are given in Table 2. ^{31}P-NMR spectra of lignins after laccase treatment showed the qualitative and quantitative modifications induced on the lignin structures that can be associated to both side-chain oxidation processes (as shown by the decrease of aliphatic OH groups in lignin side-chains) and oxidative coupling processes. A decrease of the total aromatic OH in all modified lignins was observed, which is consistent with formation of more condensed structures upon oxidative polymerization. This was associated with a decrease of S content (i.e. the percentage of the total phenolic OH) and S/G ratio in treated lignins, which revealed an easier degradation of S units, less condensed than G units, by the laccase treatment, probably because syringyl type substructures were more susceptible to oxidative cleavage of the Cα–Cβ bond than guaiacyl type substructures (Ke & Laskar, 2011). Demethylation of the S units might also occur (Crestini & Argyropoulos, 1998). On the other hand, the content of p-hydroxyphenyl groups (H) increased for all studied lignins, the highest increase being observed for the SGH lignins (SGWL, AWL, SWL), suggesting that the guaiacyl/syringyl units in lignin are susceptible to cleavage by oxygen-active radical species, while the para-hydroxybenzene units are not. Similarly, an increase of the condensed phenolic OH relative to the total phenolic OH was observed, proving as well that oxidative coupling reactions were induced by laccase treatment. The highest increase of condensed phenolic OH was found for OHL and AWL lignins.

The decrease of the aliphatic O–H groups is also observed, which is consistent with recent observations of side chain oxidation and fragmentation of model compounds during laccase mediator system treatments (Liu *et al.,* 2012). Laccase and oxygen oxidize phenols to phenoxy radicals, which subsequently rearranges to various condensation products, semiquinones, and quinones, but oxidation of aliphatic hydroxy groups with laccase has only been reported to take place in the presence of a mediator (Bourbonnais *et al.* 1997). Our results demonstrate that the content of aliphatic hydroxyl decreased for all studied lignins after the laccase treatment process, showing that the side chain oxidation could be achieved without using mediator systems, or small lignin fragments can play the role of mediator in these reactions. Oxidation of aliphatic hydroxyl groups is also supported by the formation of additional conjugated carbonyl groups that was observed in the FT-IR spectra of the polymerized lignins. Although the aliphatic hydroxyl decreased, the relative content of aliphatic hydroxyl groups reported to the total aromatic hydroxyl groups (i.e. ratio Alkyl–OH/Aryl–OH in Table 2) increased for all lignin suggesting that the main reactions during the enzymatic treatment are (i) polycondensation of the phenolic radicals initially formed with formation of new C–C and C-O–C bondings (demonstrated also from the increase of FT–IR band at 1115 cm^{-1}) and (2) oxidation of the side chains. Degradation and depolymerization of lignin is less significant, although it cannot be totally excluded, in view of the high dispersity of the products obtained.

Table 2. 31P-NMR analysis of lignins samples before and after laccase polymerization

Lignin	Aliph OH (mmol g^{-1})	Aromatic OH (mmol g^{-1}/%)				COOH (mmol g^{-1})	Alkyl–OH/ Aryl–OH	S/G
		Cond. OH	S–OH	G–OH	H–OH			
OHL-50	1.12	0.90/27	1.27/39	0.84/26	0.23/7	0.37	0.34	1.51
OHL-Lcc	0.98	0.74/40	0.52/28	0.41/22	0.18/10	0.33	0.52	1.27
SGWL-50	1.56	0.90/28	0.72/23	0.91/29	0.58/19	1.19	0.51	0.79
SGWL-Lcc	0.92	0.37/35	0.17/16	0.24/23	0.26/25	0.66	0.88	0.71
SWL-50	1.60	0.86/32	0.66/24	0.84/30	0.37/14	0.91	0.59	0.79
SWL-Lcc	1.16	0.60/36	0.28/17	0.42/26	0.34/21	0.79	0.7	0.66
AWL-50	1.15	0.89/31	0.73/25	0.93/32	0.35/12	1.07	0.39	0.79
AWL-Lcc	0.98	0.36/41	0.14/16	0.21/24	0.17/19	0.60	1.11	0.66
KSL-50	2.12	1.03/31	0.35/10	1.71/51	0.29/8	0.49	0.63	0.20
KSL-Lcc*	ND	ND	ND	ND	ND	ND	ND	ND

*Slightly soluble in the NMR solvent; ND: not determined; Aryl-OH is the abbreviation of "total phenolic OH"; (%) represents the percentage of total phenolic hydroxyl groups.

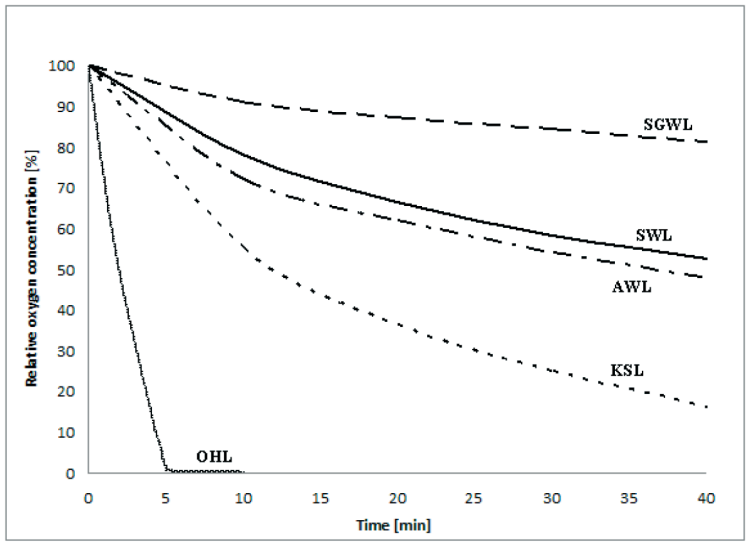

Figure 2. Oxygen consumption vs time in laccase catalyzed reactions of AWL, SGWL, SWL, KSL and OHL lignins

The COOH content was found to decrease for all lignin samples upon laccase treatment, in the order SGWL-Lcc > AWL-Lcc > SWL-Lcc > OHL-Lcc. The lower degree of COOH group concentration decrease could indicate that the OHL and SWL lignins were less degraded by the laccase treatment. These results are consistent with earlier reports showing that condensed structures in lignins are degraded by laccase, mainly via side chain oxidation, demethylation and hydroxylation reactions. These transformations increase the hydrophilic nature of the lignin moieties and introduce activated sites on lignin structures.

Reactivity of lignins in laccase-catalyzed oxidation

Oxygen consumption was used to measure laccase activity against different lignins. Oxygen consumption curves of OHL, SGWL, SWL, AWL and KSL lignins in acetone/water 50:50 (v/v) mixture are shown in Fig. 2. The oxidation degree of lignins decreased in the order of OHL > KSL > AWL > SWL > SGWL.

The laccase treatment on SGH type OHL lignin showed the highest oxygen consumption rate (19.66 mM min^{-1}), with complete oxidation accomplished in about 5 min. The activity of laccase strongly depends on the total amount of phenolic units in lignin structure. OHL lignin showed the highest content of total aromatic hydroxyl among the studied lignins, explaining its higher reactivity. In case of guaiacyl type KSL the initial rate of oxidation was about 60% slower. These results agree with the data obtained by van de Pas *et al.* (2011), who showed that laccase polymerization of unfractionated lignin from hardwood was higher than that from softwood. Among the wheat straw lignins AWL was the most reactive, but the initial oxidation rate was lower compared to the OHL lignin (5.3 mM min^{-1}). Oxidation of SWL by laccase was somewhat slower than that of the AWL, showing that the pretreatment method could slightly affect the rate of oxidation of wheat straw lignin. These results can be related to the molecular properties of the lignins. OHL lignin has the lowest Mw (Table 1, entry 1), is the least condensed (i.e. lowest condensed phenolic and aliphatic-OH content) and the most hydrophobic lignin, having the lowest carboxyl content (Table 3) and this apparently favors the interaction with laccase and results in high initial rates. The content in S units of the OHL lignin was higher than that in G units, as revealed by ^{31}P-NMR analysis. Lignin reactivity depends on the frequency of its structural units, guaiacyl (G) and syringyl (S) monolignols. More syringyl monolignol units, or high S/G lignin monomer ratios induce high lignin reactivity as observed for OHL lignin. The S/G ratios were similar for all wheat straw lignins but the decrease of the ratios was slightly different after the laccase treatment (SWL = AWL > SGWL). Although OHL was the most reactive, the highest increase in the molecular weight as well as the largest changes in functional group content and the formation of more condensed structures was observed for AWL lignin. The order of the extent of polymerization among the studied lignins was AWL > SGWL > SWL > OHL. This suggests that polymer growth in the post-initiation phase is determined by the reactivity and the accessibility of reactive groups in the intermediate polymeric structures obtained in the initiation phase, and this is favored by a low syringyl content and a high p-hydroxyphenyl content, as in all grasses lignins studied. OHL, with the highest S/G ratio and a very low content of H (i.e. lower than 10%) generates the polymers with the lowest molecular weight after laccase-catalyzed polymerization. Grass lignins, however, with the highest content of H-units with free *ortho*-positions in the phenolic ring, are more susceptible for condensation *via* C–C and C–O–C coupling during the radical polymerization step, resulting in high molecular weight polymers.

CONCLUSIONS

Interaction of lignins from different sources and laccase from white rot fungus (*Trametes versicolor*) was investigated in acetone-water system. The good stability at high acetone concentrations (50% vol.) makes laccase a potential candidate for industrial applications. All fractionated lignins used in the study were oxidized by laccase, the extent of the polymerization reactions of different lignins depending on the amount of surface functional groups available for oxidation. ^{31}P-NMR and SEC data revealed that the extent of oxidation of lignins proceeded in water-acetone 50:50 (v/v) mixture increased in the order AWL > SGWL > SWL > OHL. The characteristics of lignins had important influence on laccase reactivity, since laccase from *Trametes versicolor* showed to be more efficient to polymerize wheat straw than hardwood lignin. The polymerization reactions were efficient at quite high concentration of organic solvent (50% acetone), which usually inhibit the activity of oxidases. This finding opens up further possibilities for the utilization of laccases in areas where the solubility of the reactants or products is limited. Solubility of the lignin in the reaction mixture was a key factor of reactivity in the laccase-catalyzed process. The results indicate new pos-

sibilities of enzymatic valorization of lignin. Lignins from various plant materials and pulping processes provide an important source of raw material that may be converted into value-added products using reactions mediated by laccase.

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