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Demethylation and other modifications of industrial softwood kraft lignin by laccase-mediators

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Abstract: Substitution of phenol in phenol-formaldehyde (PF) resin preparations by technical lignins is hindered by the inherently lower reactivity of lignin compared to phenol. Demethylation of an industrial softwood kraft lignin (SKL) to improve its reactivity is the focus of this paper. To this purpose, kraft lignin (KL) was treated with two commercial laccases, NS51002 (L1) and NS51003 (L2), for 24 h in combination with three mediators, 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1-hydroxybenzotriazole (HBT) and 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO). The characterizations of the reaction solution and the resultant KL showed that methanol was released as a result of the methoxy group splitting from the aromatic rings, while such demethylation was dependent on the laccase-mediator system (LMS). The catechol structures formed, which were further oxidized to a quinone structures prone to polymerization, led to molecular mass increment. Also this reaction was LMS dependent. The same is true to the cleavage of β -O-4' linkages, which resulted in depolymerization. The L1-ABTS, L1-TEMPO and L2-HBT combinations are the most efficient and the resulting modified lignin would be suitable to phenol substitution. Challenging is the lignin polymerization following the demethylation, especially in case of L1-ABTS, which might inhibit the reactivity of the treated lignin.

Keywords: ^{13}C -NMR, 2D-NMR, ^{31}P -NMR, demethylation, FTIR, laccase, lignin, mediator, SEC

Introduction

Lignin is, after cellulose, the most abundant macromolecule in nature. Technical lignins are aromatic polymers containing phenolic OH groups, such as kraft lignin (KL) from the kraft pulping process, and are readily available (Rönnols et al. 2017). They are in principle suited for phenol substitution in phenol-formaldehyde (PF) resins, but their lower reactivity leads to deterioration of PF resin quality (Lewis and Lantzy 1989; Dunky and Pizzi 2002; Gandini and Belgacem 2008). A monomeric phenol has three reactive sites against formaldehyde in *para* and *ortho* positions to the OH group, while lignins are variously substituted in these positions. As known, lignin's aromatic nuclei may have *p*-hydroxyphenyl-(H), guaiacyl-(G) and syringyl-(S) structures, while the relative abundance of these units is plant species dependent (Liitiä et al. 2003; Boeriu et al. 2004). In all lignins, the *para* position is occupied by partially hydroxylated propyl chains. The *ortho* positions are either free (H-lignin) or mono-methoxylated (G-lignin) or dimethoxylated (S-lignin). Accordingly, the reactivity towards formaldehyde is in the order of H-lignin > G-lignin > S-lignin. Demethylation has been reported as a way to activate different lignin structures (Hu et al. 2011), in the course of which methoxy groups are converted into phenolic groups, creating new *para* and *ortho* situated OH groups for the reaction with formaldehyde. For example, lignin-based PF resins prepared with demethylated wheat straw soda lignin is able to replace up to 60% of phenol, while the prepared resin still meets the requirements of Chinese national standard for premium type plywood manufacture (Wu and Zhan 2001). An et al. (1995) stated that demethylated KL is suited to a complete phenol substitution and the quality of prepared plywood is acceptable.

A thiol mediated demethylation reaction is found to be effective (Hu et al. 2014). The suitability of an odorless thiol-based reagent (Chae 2008) and iodocyclohexane (Zuo et al. 2008) were investigated for the demethylation of lignin monomers or dimers. The method was also applicable for KL demethylation (Birgersson 2015). On the other hand, more environmentally friendly biochemical methods were also tested successfully (Solomon and

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Lowery 1993; Ibrahim et al. 2011). Laccases (EC 1.10.3.2, *p*-diphenol:dioxygen oxidoreductase) are a group of multi-copper proteins found in many plants, fungi, and microorganisms (Solomon and Lowery 1993). Generally, the laccases need molecular oxygen to oxidize various aromatic and non-aromatic structures. They also play an important role in the biosynthesis of lignin by promoting the oxidative coupling of monolignols (Solomon and Lowery 1993; Solomon et al. 1996). The laccases differ mainly in their structures of the four copper centers, and have different redox potentials. In nature, “electron shuttles” are active between the substrate and laccase, which are called mediators and these combinations are commonly called “laccase-mediator system” (LMS). The mediator can even modulate the redox potential of the LMS system (Crestini and Argyropoulos 1998). LMSs play a versatile role in nature, whereas laccases alone are only able to oxidize phenolic compounds due to their comparatively low redox potentials (Bourbonnais and Paice 1990).

The LMS oxidations occur stepwise, where the initiation is followed by various subsequent reactions including demethylation (Lew 2014; Roth and Spiess 2015). The initiation mechanisms include phenolic hydrogen abstraction for phenolic lignin units and electron transfer (Bourbonnais et al. 1998), hydrogen atom transfer and ionic reaction steps (Fabbrini et al. 2002) for non-phenolic lignin units. The initial oxidative attack is primarily on the phenolic lignin moiety, followed by reactions on the non-phenolic structures (Martínez et al. 1994). Demethylation is limited to the laccases produced by fungi (Leonowicz et al. 1999). Other subsequent reactions include structural degradation (with release of phenolic residues) and oxidation of the side chain hydroxyl groups into aldehydes, ketones and acids (Bourbonnais and Paice 1990). The latter was useful to prepare benzaldehydes from benzyl alcohols (Rosenau et al. 1996) or aromatic methyl groups (Potthast et al. 1995). Additionally, polymerization may occur via the aromatic ring (Lundquist and Kristersson 1985) and ring opening reactions (Higuchi 1990). Compared to monomeric or dimeric models, few studies were dedicated to macromolecular lignins. Studies on lignin degradation by LMS have been reported for the delignification or bleaching of chemical pulps (Du et al. 2013b,c) and in biorefinery applications (Roth and Spiess 2015). Delignification mechanism with LMS was studied by Balakshin et al. (1999) after GPC and NMR analysis. The interaction of LMS and lignosulfonates, leading to polymerization, was also investigated (Areskog et al. 2010). Demethylation of KL by LMS has also been studied, but the chemical analysis was generally restricted to the release of methanol (Ibrahim et al. 2011; Zou et al. 2015).

Clearly, a more comprehensive understanding of the LMS-lignin reactions is needed, which will be in focus of the present paper. Namely, the structural modification of softwood KL by two commercial laccases in combination with three mediators will be studied. The formation of methanol, and the modified lignin structures will be analyzed comprehensively.

Materials and methods

Materials: Softwood kraft lignin (SKL) was separated from industrial black liquor according to the LignoBoost process (Oehman et al. 2006). The SKL has a high purity: 98.45% lignin with low contents of ash (0.35%) and carbohydrates (1.2%) (Wang et al. 2017). The two laccases, N51002 (L1), prepared from a white-rot fungus (*Trametes versicolor*) and N51003 (L2), from *Coprinopsis cinereus*, were obtained from Novozyme (Denmark). All chemicals were purchased from Sigma-Aldrich: 2,2'-azinobis-(3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 1-hydroxybenzotriazole hydrate (HBT), 2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPO), polyoxyethylene sorbitanmonooleate (Tween 80), pre-wetted RC Tubing (Spectra/Por®6 Dialysis Membrane, MWCO 500 Da), 3,5-di-*tert*-butyl-1,2-benzoquinone (98%) and 3-methylcatechol (99%), as well as other chemicals and solvents in AR grades. The PTFE filters were from Milipore (Darmstadt, Germany).

Laccase-mediator treatment of SKL: Two grams (oven-dried weight) of SKL were suspended with one laccase and one mediator in 20 mM pH 5 sodium acetate buffer solution to produce a final volume of 200 ml, with concentrations of 10 mg ml⁻¹ SKL, 0.7 U ml⁻¹ laccase and 1 mM mediator. A small amount (0.5 µl ml⁻¹) of Tween 80 was added to facilitate the suspensions of lignin and laccase. The mixture was incubated under stirring for 24 h at room temperature. After the reaction, a small amount (0.1 ml) of the mixture was sampled for a GC analysis of the MeOH content and the rest was dialyzed (MWCO 500 Da) against running deionized water for 48 h and then lyophilized before further analysis. The recovery yield of modified lignin was around 95–98%.

MeOH detection by GC analysis: The sampled solution was diluted 10 times with deionized water. MeOH was extracted with 1-butanol (1:1, v/v). After standing for 10 min, the top butanol phase was filtered via 0.2 µm PTFE filters into a 2 ml GC vial. The GC instrument (Agilent 6890 GC, injector 250°C and detector 275°C) was equipped with a FID detector for MeOH determination. A total of 1.5 µl of sample was split injected (1/40), using the following: HP INNOWAX column (cross-linked polyethylene glycol, 15 m × 0.25 mm × 0.5 µm), He flow of 50 cm s⁻¹, temperature program: 33°C for 0.5 min → 233°C (50°C min⁻¹), holding time 1 min. Dimethyl sulfoxide (DMSO) was served as an internal standard (IS).

Nuclear magnetic resonance (NMR) spectroscopy: The samples dissolved in DMSO-*d*₆ were analyzed by quantitative ¹³C and 2D ¹H-¹³C heteronuclear single quantum coherence (2D HSQC) NMR spectroscopy on a Bruker Avance 400 MHz instrument using the standard Bruker pulse programs (Zhang and Gellerstedt 2007). From the ¹³C-NMR spectra, the methoxy (OMe) content per aromatic

ring was obtained from the integration ratio of the OMe signal at 55.6 ppm and the aryl peaks at 105–160 ppm; the latter was set as 6 (one aromatic ring). ^{31}P -NMR was performed after adaption and modification of the protocols described in the literature (Granata and Argyropoulos 1995; Pu et al. 2011) to quantify the different hydroxyl groups. In detail, a dried lignin or lignin product sample (30 mg) was dissolved in a mixture composed of 100 μl pyridine/*N,N*-dimethylformamide (1:1), 100 μl *N*-hydroxy-5-norbornene-2,3-dicarboxylic acid imide pyridine solution (20 mg ml^{-1}) as IS and 100 μl chromium acetylacetonate pyridine solution (5 mg ml^{-1}) as a relaxation agent. The reagent 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (100 μl) was then added together with 400 μl chloroform-*d*, and the mixture was stirred for 10 min for completion of the phosphitylation reaction before NMR analysis. For processing, the sharp signal of the IS at 151.9 ppm was used, and the content of hydroxyl groups was obtained by integration of the following shift ranges: aliphatic OH (151.0–145.0 ppm), condensed phenolic OH including syringyl OH (145.0–140.4 ppm), guaiacyl phenolic OH (140.4–138.6 ppm), *p*-hydroxyphenyl phenolic OH (138.6–136.5 ppm) and carboxylic OH (136.5–133.2 ppm). MestReNova (Mnova) 7.1.2 software (Mestrelab Research S.L., Santiago de Compostela, Spain) was applied for data processing.

Fourier transform infrared (FTIR) spectroscopy: A Perkin-Elmer Spectrum 2000 FTIR spectrometer (Waltham, MA, USA) was applied, equipped with an attenuated total reflectance (ATR) system and a Specac MKII Golden Gate (Creekstone Ridge, GA, USA); with 4 cm^{-1} resolution, 16 scans were accumulated at room temperature. A blank scan was performed for background correction. The data processing was conducted by the Spectrum 10™ software as integral part of the equipment.

SEC analysis: The molecular mass distribution (MMD) was determined by means of three columns, Styragel HR1, Styragel HR2 and Styragel HR4, connected in series. Two hundred and fifty microliters of tetrahydrofuran (THF) with the dissolved sample was injected;

THF served as the mobile phase (0.8 ml min^{-1}) by UV_{280nm} detection (AZURA UVD 2.1S, Knauer, Berlin, Germany). The SEC system was calibrated with polystyrene standards with molecular masses from 162 to 3 000 000 Da by refractive index (RI) detection (Waters 2414) using third-degree polynomial fitting. Peak molecular mass (M_p), weight average molecular mass (M_w), number average molecular mass (M_n) and polydispersity (PD) indices (M_w/M_n) were calculated by the Cirrus GPC software version 3.1 from Polymer Laboratories (Agilent).

Results and discussion

Demethylation

As revealed by ^{31}P -NMR analysis (Figure 1), SKL shows features of a typical SKL, containing a noticeable amount of different phenolic rings with a total amount OH_{phen} of 4.57 mmol g^{-1} (Table 1). However, 44% (2.01 mmol g^{-1}) of these are condensed with both *ortho* positions occupied and thus without reactive positions. The rest were mainly G units (2.32 mmol g^{-1}), with one free *ortho* position on each unit, although some H-units were present (0.24 mmol g^{-1}) with two free *ortho* positions.

SKL was treated with the commercial laccases N51002 (L1) and N51003 (L2) in combination with the mediators, ABTS, HBT and TEMPO (Figure 2). After incubation, detectable amount of MeOH was released in all the six reaction solutions. However, the MeOH concentration was too low to be satisfactorily quantified. The more sensitive headspace analysis combined by ion flow tube-mass spectrometry (SIFT-MS) was applied for quantification

Figure 1: ^{31}P -NMR spectra of softwood kraft lignin (SKL) before and after treatment by the laccase-mediator L1 + ABTS.

Table 1: Contents of methoxyl (OMe) groups per aromatic ring (by ^{13}C -NMR) and aliphatic, carboxylic OH groups and phenolic OH groups, such as *p*-hydroxyphenyl (H), guaiacyl (G) and condensed (cond.) units (by ^{31}P -NMR) of softwood kraft lignin (SKL) before and after laccase-mediator treatment.

Sample	OMe per C_6	OH_{aliph} (mmol g^{-1})	OH_{phen} (mmol g^{-1})				Carboxylic. OH (mmol g^{-1})
			G-OH	H-OH	Cond.	Sum	
SKL ^a	0.94	1.91 ± 0.16	2.32 ± 0.04	0.24 ± 0.07	2.01 ± 0.06	4.57	0.46 ± 0.01
L1							
+ABTS	0.55	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b	nd ^b
+HBT	0.79	1.96	1.96	0.27	1.85	4.08	0.45
+TEMPO	0.55	1.92	1.86	0.24	1.71	3.81	0.40
L2							
+ABTS	0.68	2.16	2.22	0.27	2.11	4.60	0.47
+HBT	0.56	1.92	2.09	0.26	1.94	4.29	0.40
+TEMPO	0.86	2.02	2.01	0.26	1.94	4.21	0.44

Laccases, L1=NS51002 and L2=NS51003 and mediators; ABTS, 2,2'-azino-bis-(3-ethyl-benzothiazoline)-6-sulfonate; HBT, 1-hydroxybenzotriazole; TEMPO, 2,2,6,6-tetramethylpiperidin-1-oxyl.

^aDuplicates [mean $\pm$ standard deviation (SD)]. ^bNot determined due to incomplete dissolution of the sample.

Figure 2: The redox reactions of mediators used in the study.

ABTS, 2,2'-Azinobis-(3-ethyl-benzthiazoline)-6-sulfonate; HBT, 1-hydroxybenzotriazole; TEMPO, 2,2,6,6-tetramethylpiperidin-1-oxyl.

in the literature (Zou et al. 2015). The OMe decrement in lignin was further confirmed in this present study by quantitative ^{13}C -NMR (Figure 3). Three combinations, L1-ABTS, L1-TEMPO and L2-HBT, resulted in the highest OMe content reduction, resulting in “OMe/arom. ring” ratios of 0.55–0.56, while the original ratio was 0.94 (Table 1). In the literature, a similar SKL demethylation rate of 40% was reported (Zou et al. 2015).

However, catechol structures were not observed in the LMS-treated SKL by ^{31}P -NMR as no new signal appeared (Figure 1). In a control experiment with 3-methylcatechol, the corresponding catechol signals were detected at 142.23 and 142.17 ppm. This is because the LMS provides an oxidative environment and catechols are strong reductants. In one report, hydroquinone could easily be oxidized into quinone by laccase at room temperature (Cannatelli and

Figure 3: ^{13}C -NMR spectra of softwood kraft lignin (SKL) before and after laccase-mediator treatment (representing by L1+ABTS). *Marked peaks are from contaminated Tween 80 according to (Zhang et al. 2015).

Figure 4: Two-dimensional (2D)-NMR spectra of softwood kraft lignin (SKL) before (bottom) and after laccase-mediator treatment (top) (representing by L1+ABTS).

Ragauskas 2015). However, no quinone structure in the modified lignin was seen by ^{13}C -NMR. The ^{13}C -NMR spectrum of the commercial 3,5-di-*tert*-butyl-1,2-benzoquinone

showed two carbonyls at 180.45 and 179.70 ppm, which were absent in any of the laccase treated SKL samples (Figure 3). The sole carbonyl group signal observed at 172.88 ppm was originated from the residual Tween 80 used in the incubation (Zhang et al. 2015). The charged amount of Tween 80 was low, while its signals at ^{13}C -NMR were sharp and observable due to its shorter relaxing time than that of the polymeric lignin. Obviously these signals did not interfere with the lignin signals (Figure 3). Apparently, any formed quinones must have been reacted further for example via polymerization. A noticeable increase in the molecular mass of SKL was observed (see below), which is a sign for the polymerization. Note that the long laccase reaction time (24 h) used would give the opportunity for the polymerization. Quinone polymerization was in fact reported in the literature (Lundquist and Kristersson 1985).

Other structural modifications

The LMS treatment decreased the total amount of all OH_{phen} groups, from 4.57 mmol g^{-1} to $3.81\text{--}4.29 \text{ mmol g}^{-1}$ (6–16% decrement), except for the sample treated with L2-ABTS, which was apparently unaffected (Table 1). Cleavage of the $\beta\text{-O-}4'$ linkages (see below) could also increase the phenolic content. The OH content of the sample treated with L1-ABTS could not be characterized due to incomplete solubilization, possibly because of its substantial polymerization (see below). OH_{phen} were the preferential targets of the LMS. G units were the most attacked structures, with the extent depending on the LMS combination applied (Table 1). A slight decrease (for L1 laccase) or no decrease (for L2 laccase) of OH_{phen} was observed in condensed units. The content of H units was unchanged after the LMS treatments. However, the quantification of the H units by ^{31}P -NMR is not accurate because the peak intensity is very low and the spectra are noisy by the tail of the G unit signals and there is a baseline shift (Figure 1). Camarero et al. (1994) reported on a significant OH_{phen} decrement during white rot fungi degradation.

In the 2D-NMR analysis, the typical lignin $\beta\text{-O-}4'$ linkages are well visible based on the signals of $\text{C}_{\alpha}\text{-H}$ ($\delta_{\text{H}}/\delta_{\text{C}}$: 4.74/71.3) and $\text{C}_{\beta}\text{-H}$ (4.24/84.2) (Du et al. 2013a). After the LMS treatment, the peak intensities were largely reduced in all of the modified lignins, and for the L1-ABTS treated sample the peaks completely vanished, indicating a comprehensive bond cleavage (Figure 4). This is in accordance with a lignin model study on L1-HBT, where $\beta\text{-O-}4'$ cleavage was reported (Kawai et al. 1999). Another example was

described by Heap et al. (2014). The cleavage will lead to lignin depolymerization. It is also possible that side chain cleavage, including side chain oxidation, would also take place, leading to the disappearance of the β -O-4' signals.

Compared to the starting SKL, some fractions with shorter retention times (lignin fragments with higher molecular mass) were observed, especially for the L1-ABTS system (Figure 5). The peak molar mass (M_p) increased from 2100 to 2300–3900, and reached an extremely high value of 10 000 with L1-ABTS (Table 2). The M_w generally increased three-fold, but reached a 30-fold value of the original SKL in case of the L1-ABTS system (Table 2). A substantial PD increase was seen mainly in the L1-ABTS sample. The influence of the mediator coupling could be essential. Blank experiments were conducted either without laccases or without incubation that exclude the influence of possible contaminations from either the mediators or the laccases. The above mentioned quinone polymerization and cleavage of the β -aryl ether linkages may have been responsible for increases and decreases in

Figure 5: SEC profiles of softwood kraft lignin (SKL) before and after laccase-mediator treatments.

Table 2: The peak (M_p), and number- (M_n), and weight-average (M_w) molecular mass and the polydispersity (PD) of softwood kraft lignin (SKL) before and after laccase-mediator treatment.

Sample	M_p	M_n	M_w	PD (M_w/M_n)
SKL	2100	1300	3800	2.9
L1				
+ ABTS	10 000	4100	115 000	28.1
+ HBT	2400	1700	10 700	6.4
+ TEMPO	2400	1900	24 700	13.1
L2				
+ ABTS	2300	1600	11 000	6.9
+ HBT	2700	1800	15 600	8.9
+ TEMPO	3900	2000	18 400	9.3

the molar mass (MM). Additionally, radical coupling reactions such as 5-5' coupling (Lundquist and Kristersson 1985) may have also contributed to the MM increment.

The FTIR spectra revealed changes between 700 and 800 cm^{-1} (Figure 6). Essentially, no changes occurred after the LMS treatments of SKL in case of L2, regardless of the mediator combinations, whereas absorption decrements around 775 cm^{-1} and increments around 725 cm^{-1} were observed in case of L1 treatment independently of the mediator. These bands are related to hydrogen deformation in substituted aromatic rings (Weigel and Herges 1992). Some alteration of the aromatic structures, for example, via condensation, might have occurred during the L1 treatments.

The initiation reactions by LMS also generate radicals in the side chain, such as benzyl radical. It has been demonstrated in lignin model studies that the benzyl radical is easily oxidized to C_α carbonyl groups leading to benzyl aldehydes, ketones or acids (Camarero et al. 1994). Formation of an acid would be indicative for depolymerization. However, no additional carbonyl groups were observed by ^{13}C -NMR – typical for ring opening reactions (Higuchi 1990) as manifested by signals at 51.6 (MeO) and 166.9 ppm (CO) (Evtuguin et al. 2009) – were not seen either (Figure 3). Thus, the formation of muconic acid does not seem to be a major route in the modification of lignin by LMSs. Alternatively, any formed muconic acid would have reacted further during the targeted 24-h reaction time.

The amount of carboxylic hydroxyl groups, probably located on the side chains, was quantified by ^{31}P -NMR (Table 1). In the literature, decarboxylation has been observed as another common type of laccase reaction

Figure 6: FTIR spectra of softwood kraft lignin (SKL) before and after laccase-mediator treatments.

(Lundquist and Kristersson 1985). Although some COOH reductions were observed after LMS treatments, such a reaction was not observed as a general reaction in the present study.

Influence of LMS combinations

The two laccases originated from different fungi had slightly different redox potentials: 0.7 V for L1 and 0.5 V for L2 (Areskog et al. 2010). The higher oxidation capacity of L1 may explain the generally higher decrement of G-OH groups and of condensed OH_{phen} groups compared to laccase L2 (Table 1), as well as other differences, e.g. the aforementioned changes concerning the aromatic ring substitution patterns observed by FTIR.

Of the tested LMSs, ABTS in combination with L1 caused the most comprehensive modifications, i.e. the most comprehensive demethylation with 41% OMe decrement (Table 1) and the largest MM increment (Table 2 and Figure 5). In principle, laccases with low redox potentials (0.5–0.8 V), like the ones applied in this study, can only oxidize phenolic lignin units (Wong 2009), as oxidation of non-phenolic lignin requires a redox potential higher than 0.9 V (Schmidt 2007). However, the redox potential of L1 is high enough to oxidize the ABTS to both semi-oxidized ABTS[•], with a redox potential of 0.68 V, and fully oxidized ABTS²⁺, with a redox potential of 1.09 (Scott et al. 1993) (Figure 2). Therefore, after ABTS²⁺ formation, the L1-ABTS system was able to oxidize non-phenolic groups, which explains the most comprehensive effects observed. Bourbonnais and Paice (1990) observed the oxidation of non-phenolic model compounds with laccase/ABTS and Bourbonnais et al. (1997) described lignin demethylation by this system.

Conclusion

Demethylation occurred by laccase treatment as indicated by methanol formation and decrease of OMe content. However, the formed catechol intermediates could not be detected on the laccase-mediator modified lignin. Probably, catechols were further oxidized into reactive quinones, which are prone to polymerization leading to elevated MMs. The extent of lignin modification depends largely on the laccase-mediator combinations, among which the most effective was laccase L1 (NS51002) in combination with ABTS, which showed the highest redox potential and which was capable of oxidizing even non-phenolic lignin structures. There are two competing

reactions: demethylation increases the lignin's reactivity, while the elevated MM has the opposite effect. Therefore, the combinations of L1-TEMPO and L2-HBT are preferred over L1-ABTS due to their similar ability to reduce OMe groups, but do not increase the MM too much. The applied 24-h reaction time might have been too long, and thus further work should be done at shorter reaction times.

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