African Journal of Biotechnology Vol. 10(69), pp. 15647-15655, 7 November, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1607
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

The effect of laccase on cellulase-treated lignin in 1-n-butyl-3-methylimidazolium chloride/H_2O homogeneous and H_2O heterogeneous solutions

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Accepted 12 October, 2011

In this study, the effect of laccase earlier obtained by our laboratory on cellulase-treated lignin (CEL) in two different solution systems was further investigated. Results obtained were as follows: After laccase treatment of CEL in the heterogeneous water solution, CEL was then compared with control sample A. Ultraviolet (UV) spectra showed that the total absorbance of sample B increased at 205 and 280 nm; Fourier-transform infrared (FTIR) spectra showed that the carbonyl group obviously appeared; and ^{13}C nuclear magnetic resonance (NMR) spectra showed that the C-γ signals disappeared, the C-α signals significantly increased, and the methoxyl content decreased. After laccase treatment of CEL in the [bmim]Cl/H_2O homogeneous solution, CEL was compared with control sample C. UV spectra showed that the total absorbance of sample D decreased at 280 nm; high-performance liquid chromatography (HPLC) analysis showed that some small absorption peaks disappeared; and ^{13}C NMR spectra showed that the C-γ signals obviously increased, the C-α signals slightly decreased, while the methoxyl content increased. Clearly, for insoluble CEL in the heterogeneous water solution, laccase partly oxidized the hydroxyl group into a carbonyl group, and thereby partly degraded CEL to increase its solubility. In contrast, for soluble CEL in the [bmim]Cl/H_2O homogeneous solution, laccase primarily polymerized small molecule fragments with the CEL macromolecule, increased the methoxyl content of CEL, and thereby played a role in lignin polymerization.

Key words: Cellulase-treated lignin, laccase, [bmim]Cl.

INTRODUCTION

Lignin, the most abundant renewable aromatic biomass on earth, and because of its complex and steady structure, it is hardly decomposed by chemicals and enzymes. Thus, lignin is still a vastly natural polymer that has not been fully utilized, and its existence severely affects the use of cellulose. Owing to different isolation protocols and plant resources, lignin has many different types, such as kraft and sulfite lignins, sulfur-free lignins (including CEL, etc). Conventional kraft and sulfite lignins have been used as dispersants and binders. The new application of sulfur-free lignins is gaining increasing interest, and sulfur-free lignins are expected to be sustainable alternatives to nonrenewable products such as phenolic and epoxy resins (Lora and Glasser, 2002).

In the pulp and paper industry, lignin, often named alkali lignin, is separated from wood for the preparation of fiber material. To dissolve lignin, high temperature, high pressure and strong alkali conditions are often used, which create a series of problems including waste water with toxic aromatic pollutants and human health issues (Huang and Gao, 1999). To develop sustainable alternatives and solve the pollution problems, the application of various environmental benign biological enzymes and

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Abbreviations: **CEL**, Cellulase-treated lignin; **UV**, ultraviloet; **FTIR**, Fourier-transform infrared; **NMR**, nuclear magnetic resonance; **HPLC**, high-performance liquid chromatography; **bmim**, 1-n-butyl-3-methylimidazolium.

green chemical reagents has achieved good future prospects. In nature, ligninolytic enzymes isolated from a group of basidiomycetes known as white-rot fungi can result in the simultaneous decay of lignin. Lignin biodegradation is an oxidative and nonspecific process friendly to the environment (Wang et al., 2008). Among several main extracellular enzymes (lignin peroxidase, manganese peroxidase, laccase and cellobiose dehydrogenase) that participate in lignocellulose biodegradation, fungal laccase is considered to play an important role in lignin degradation (Youn et al., 1995). Thus far, it has been intensively studied in aqueous media such as in the paper and food industries, dye decolorization (Hadzhiyska et al., 2006), bioremediation, organic synthesis, drug synthesis, among others (Mayer and Staples, 2002; Tong et al., 2007).

However, the mechanism of lignin degradation degraded by the laccase is still unclear. The widely accepted view on the degradation mechanism of laccase is that laccase has a biotechnological potential because of its broad substrate specificity and its ability to catalyze the oxidation of a wide range of substrates (typically mono-, di-, and polyphenols, aromatic amines, methoxyphenols, and ascorbate), and may play multiple roles in lignin degradation (Zhang et al., 2009). In all kinds of lignins degraded by enzymes, except for sulfite lignin, others such as conventional kraft lignin and sulfur-free lignins including CEL are often water-insoluble, making the ligninolytic enzymes act only on the surface of lignin particles. Thus, catalytic efficiency of the enzymes is also greatly decreased. Therefore, building a kind of dissoluble solution system in which enzymes can act better is a key solution. Recently, organic solvents have been used for the system where laccase acts (Mustafa et al., 2005). Nowak and Wilkołazka (2007) also found that substrates poorly soluble in water may be used for conversion in regions of organic solvent concentration by laccase from Cerrena unicolor and other laccases. These reviews, however, showed that enzyme activity is heavily inhibited. In addition, traditional organic solvents could result in many environmental problems, including volatility, toxicity, and so on (Andrade and Alves, 2005).

Ionic liquids have been widely accepted for a type of novel “green” solvent. In contrast to conventional organic solvents, ILs exhibit a number of attractive properties, negligible vapor pressure, ease of recycling, thermal stability, etc (Christopher et al., 2004). Earle and Seddon (2000) reported that many reactions are easier in ILs than in conventional organic solvents, even without special apparatus or methodologies. Therefore, these kinds of green chemistry reagents are becoming possible replacements for conventional organic solvents in many reactions (Olivier-Bourbigou and Magna, 2002). Many papers have also reported that some ILs such as 1-butyl-3-methyl- and 1-allyl-3-methylimidazolium chloride ([bmim]Cl and [amim]Cl, respectively) can effectively dissolve biopolymers, as well as different types of lignin samples (Li et al., 2008; Kilpeläinen et al., 2007). Pu and Jiang (2007) have also reported similar results. Additionally, as solvents, ILs have also been widely applied in aspects of biocatalysis, including the activity and stability of all kinds of enzymes in ILs (Kaftzik and Wasserscheid, 2002; Yang and Pan, 2005), and the enzymatic reactions in ILs (Kragl et al., 2002; Rantwijk et al., 2003). Rantwijk and Sheldon (2007) pointed out that enzymatic reactions may be performed in some ILs (either as solvent or co-solvent). Recently, Tavares et al. (2008) reported on laccase activity and stability in co-solvents with ILs. Hinckley et al. (2002) also reported on the capability of laccase to catalyze oxidation reactions in almost total non-aqueous ILs.

All of the aforementioned studies therefore showed that ionic liquids are good solvents for cellulose, and that laccase may catalyze oxidation reactions in a co-solvent system with ILs and water. However, few papers have focused on how does laccase act in such a system and how ionic liquids are used in lignin degradation.

In the current study, we chose CEL (a kind of sulfur-free lignin) and laccase as the research objects. The laccase obtained by our laboratory in a long-term experiment was considered as a new enzyme due to its high optimal temperature compared with the other laccase (Zhang et al., 2009). In this work, the effect of laccase on CEL in a water heterogeneous solution and in the [bmim]Cl/H_2O co-solvents was further investigated. The purpose of this study was to obtain results where laccase catalytic efficiency is far higher in a [bmim]Cl/H_2O homogeneous solution than in a water heterogeneous solution. For this reason, two different systems were designed to dissolve CEL. An aqueous CEL suspension and CEL in the [bmim]Cl/H_2O homogeneous solution were obtained. Unfortunately, we did not get the expected results, but we unexpectedly found that in the absence of the mediator in the water heterogeneous solution, the laccase could degrade CEL partly, whereas in the homogeneous solution, the laccase plays a role in polymerizing small molecule fragments of CEL to the CEL macromolecule. Also, in this study, the change of CEL in two different solvent systems treated by the new laccase from our laboratory was reported for the first time. All results, including UV spectrum, HPLC detection spectrum, FTIR and ^{13}C NMR spectra, indicated that laccase plays different roles in different solution systems.

MATERIALS AND METHODS

Preparation of laccase

Laccase was prepared from *Trametes hirsuta* lg-9 (CGMCC No. 2422) characterized by our laboratory according to the method developed by Zhang et al. (2009). The culture supernatant was first received after *T. hirsuta* lg-9 was incubated at 30°C on a reciprocal shaker (70 rpm) for six days, and then filtered, frozen, centrifuged, concentrated 20-fold, and fractionated by ammonium sulfate precipitation, then dialyzed at 4°C in 0.02 M sodium acetate buffer (pH 4.8). Next, the dialyzed sample was loaded onto a pre-equilibrated DEAE Sepharose FF anionic exchange

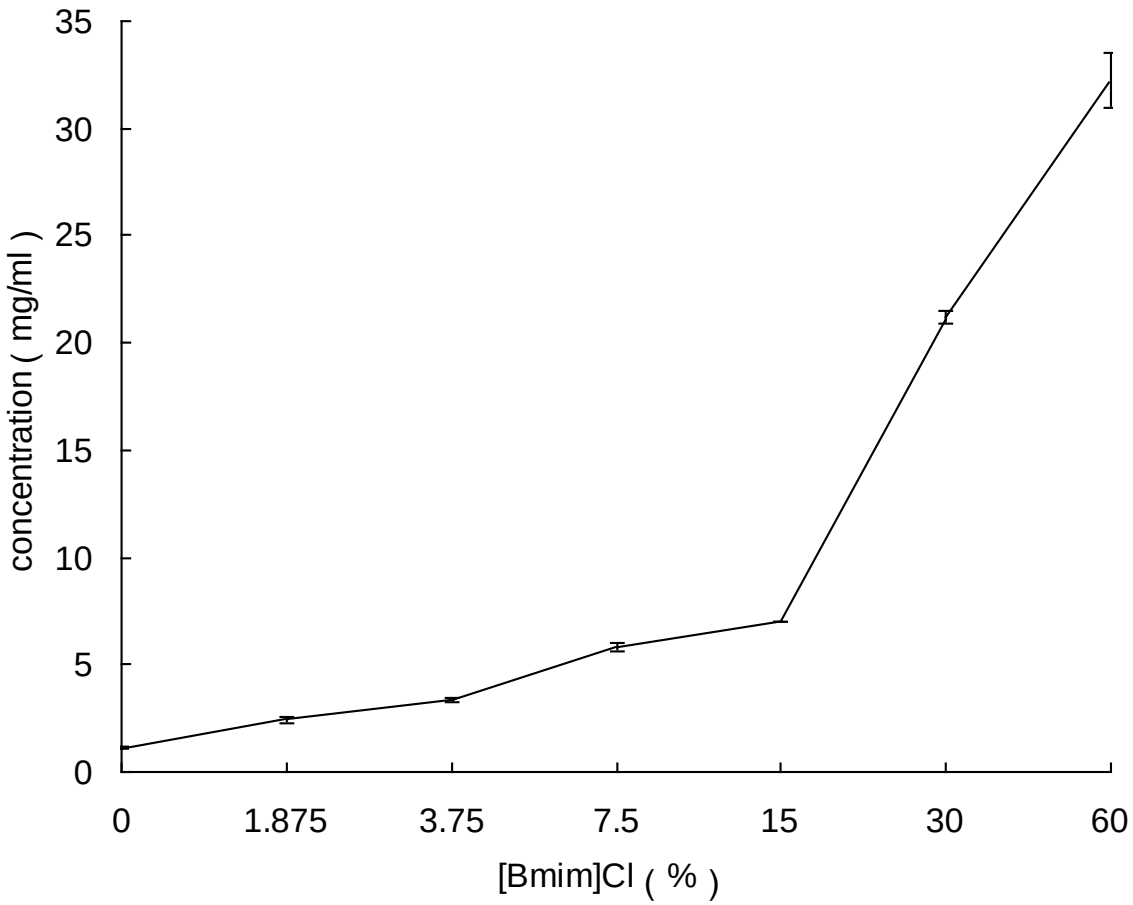

Figure 1. CEL dissolution profiles.

chromatography column. Finally, the received laccase was purified with Sephacryl S-200 size exclusion and detected with SDS-PAGE.

Preparation of CEL

CEL was prepared according to the method developed by Wang et al. (2008). The wood powder of aspens (including duramen and alburnum) was first extracted with a benzene–ethanol (2:1, v/v) solution for 24 h, milled for 48 h in a Lampén mill, and then treated by crude cellulase (1.1 IU/mg), thus producing crude lignin residue. Finally, the crude lignin residue was dissolved in 1 mol/L NaOH. The pH was adjusted to 2.5 with HCl, and the precipitate was formed. The purified residual CEL was obtained by freeze-drying the washed precipitate.

Preparation of [bmim]Cl

[bmim]Cl was prepared according to the method developed by Huddleston et al. (2001). 1-Methylimidazole (Sigma) and 1-Chlorobutane (Sigma) were reacted at 75°C for 48 h. The mixed product was extracted by ethyl acetate, and then dried in a rotary evaporator at 70°C for 8 h. The received yellow liquid was [bmim]Cl. In addition, to remove the remnant water, [bmim]Cl was dried before use. IR spectrum for [bmim]Cl consists of the following peaks (cm^{-1}): 3142.41, 3070.38, 2960.50, 2945.05, 2873.31, 1571.88, 1465.65, 1465.65, 1383.20 and 1170.67. ^{13}C NMR result (ppm) includes the following: δ 136.7 (C–C_1), 123.5 (C_2), 122.2 (C_3), 35.6 (CH_2), 13.3 (CH_3)

Solubility of CEL in the [bmim]Cl/H_2O solution

A total of 320 mg CEL was dissolved adequately in different proportions of [bmim]Cl/H_2O. The mixed solution was centrifuged at 10,000 g for 20 min. The supernatant was determined at 280 nm by UV-3100 after it was diluted 20-fold with various proportions of [bmim]Cl/H_2O. According to the received OD value, the quantity of dissolved CEL was determined by referring to the standard curve (Dence C W., 1992). The standard curve is shown in Figure 1.

Preparation of all kinds of lignin samples

A CEL sample (100 mg) was added to each of the four groups in 20 ml tubes, with serial numbers A, B, C, and D respectively. Afterward, 10 ml H_2O was added to each of the A and B groups, CEL was adequately dissolved, and then the suspension was received. At the same time, 10 ml 45% [bmim]Cl/H_2O was added to each of the C and D groups, CEL was adequately dissolved, and a clear liquid was received. Next, 20 µL laccase without activity was added to each of the A and C groups, and another 20 µL laccase was added to each of the B and D groups. The liquids in all groups were adjusted to pH 5 with HCl and maintained at 30°C for 24 h. Finally, all the groups were kept in a water bath at 80°C for 2 h to

terminate reaction. After determining non-activity, 1 ml centrifuged supernatant was retained for analysis by UV and high-performance liquid. Another centrifuged supernatant in either the C or D group was added to an equal volume of ultra-pure water, which resulted in rapid lignin precipitation. Furthermore, the sediments of each group were washed with ultrapure water and centrifuged, yielding the lignin residue. The lignin residue was also washed with ultrapure water and centrifuged four times. The water used for washing was tested with silver nitrate, and no white precipitate was found. The lignin residue was then freeze dried and the powder was stored in a vacuum desiccator with P_2O_5 for IR and NMR spectroscopy.

Detection of supernatant after treatment

UV detection of supernatant after treatment

The supernatant of the A and B groups was directly detected by UV3100 detector, while the supernatant of the C and D groups required 100-fold dilution with 45% [bmim]Cl/H_2O before being detected by a UV3100 detector.

HPLC detection of supernatant after treatment

The supernatant of each group was detected by reversed-phase c18 column HPLC detection. Chromatographic conditions were as follows: the ratio of methanol and water was 90:10; the injection volume of the A and B groups was 10 µL, respectively; and the injection volume of the C and D groups was 2 µL, respectively.

IR spectral analysis of CEL after treatment

Samples of CEL (5 mg) from each of the A, B, C, and D groups was added to agate mortar, ground with a proper amount of KBr, and then made into tablets which were detected by a NEXUS 470 FT-IR spectrometer, respectively.

^{13}C NMR analysis of CEL after treatment

Four kinds of 20 mg CEL samples from each of the A, B, C, and D groups were completely dissolved using 0.5 ml DMSO-d_6 in NMR tube, respectively. The samples were then assayed with an AVANCE 600 Superconducting UltraShieldTM Fourier Transform NMR Spectrometer (Bruker, Switzerland). The total data acquisition time was 21.5 h, 10240 scans.

RESULTS AND DISCUSSION

The solubility of CEL in different [bmim]Cl/H_2O mass ratio

Taking into account the high viscosity of [bmim]Cl and the low laccase activity in [bmim]Cl alone, [bmim]Cl and H_2O in a certain mass ratio were used as co-solvents to dissolve CEL. The dissolution of CEL is shown in Figure 1. It shows that the higher the mass ratio of [bmim]Cl, the stronger the ability of the solution to dissolve CEL. Through experiments, we selected 45 wt% [bmim]Cl solution as a solvent for two reasons. First, the viscosity of the solvent was reduced and it was easy to dissolve CEL to obtain a homogeneous solution. Second, in this concentration, the laccase activity could be retained for some time.

UV detection of supernatant after treatment

The next step in our study was to form the two different systems to dissolve CEL, and to obtain an aqueous CEL suspension (A, B groups) and a CEL [bmim]Cl/H_2O homogeneous solution (C, D groups). Next, laccase was added to the B and D groups, whose control groups were the A and C groups, respectively. The supernatant of every group was analyzed by UV and HPLC. The sediment was detected by FTIR and nuclear magnetic resonance (NMR) spectroscopy.

UV spectrum of dissolved CEL in supernatant A, supernatant B

The UV spectrum of dissolved CEL in supernatant A and supernatant B is presented in Figure 2a. The total absorbance at 205 and 280 nm increased, indicating that CEL solubility in the aqueous CEL suspension improved after laccase treatment. Using the naked eye, there were clearly visible coloration intensities in supernatant B compared with control A. These proved that laccase can degrade CEL in a water heterogeneous solution; therefore, the concentration of CEL in water increased.

UV spectrum of dissolved CEL in supernatant C, supernatant D

Based on the UV spectrum of dissolved CEL in supernatant C and supernatant D (Figure 2b), the total absorbance in supernatant D at 280 nm was lower than that of supernatant C, indicating that the solubility of CEL in the [bmim]Cl/H_2O homogeneous solution was reduced after laccase treatment. This result basically proved that CEL can be aggregated by laccase in the [bmim]Cl/H_2O homogeneous solution; therefore, the lignin concentration in the solution decreased.

HPLC spectrum of dissolved CEL in supernatant A, supernatant B, in supernatant C, supernatant D

Further analysis of the HPLC spectrum is shown in Figure 3. The total absorbance at 280 nm in supernatant A was lower than that of control B, indicating that CEL solubility in the water heterogeneous solution increased after laccase treatment. Many small absorption peaks in supernatant D decreased at 280 nm compared with those of control C, indicating that small molecular material in the [bmim]Cl/H_2O homogeneous solution reduced after laccase treatment. This evidence confirmed that laccase in the [bmim]Cl/H_2O homogeneous solution can primarily

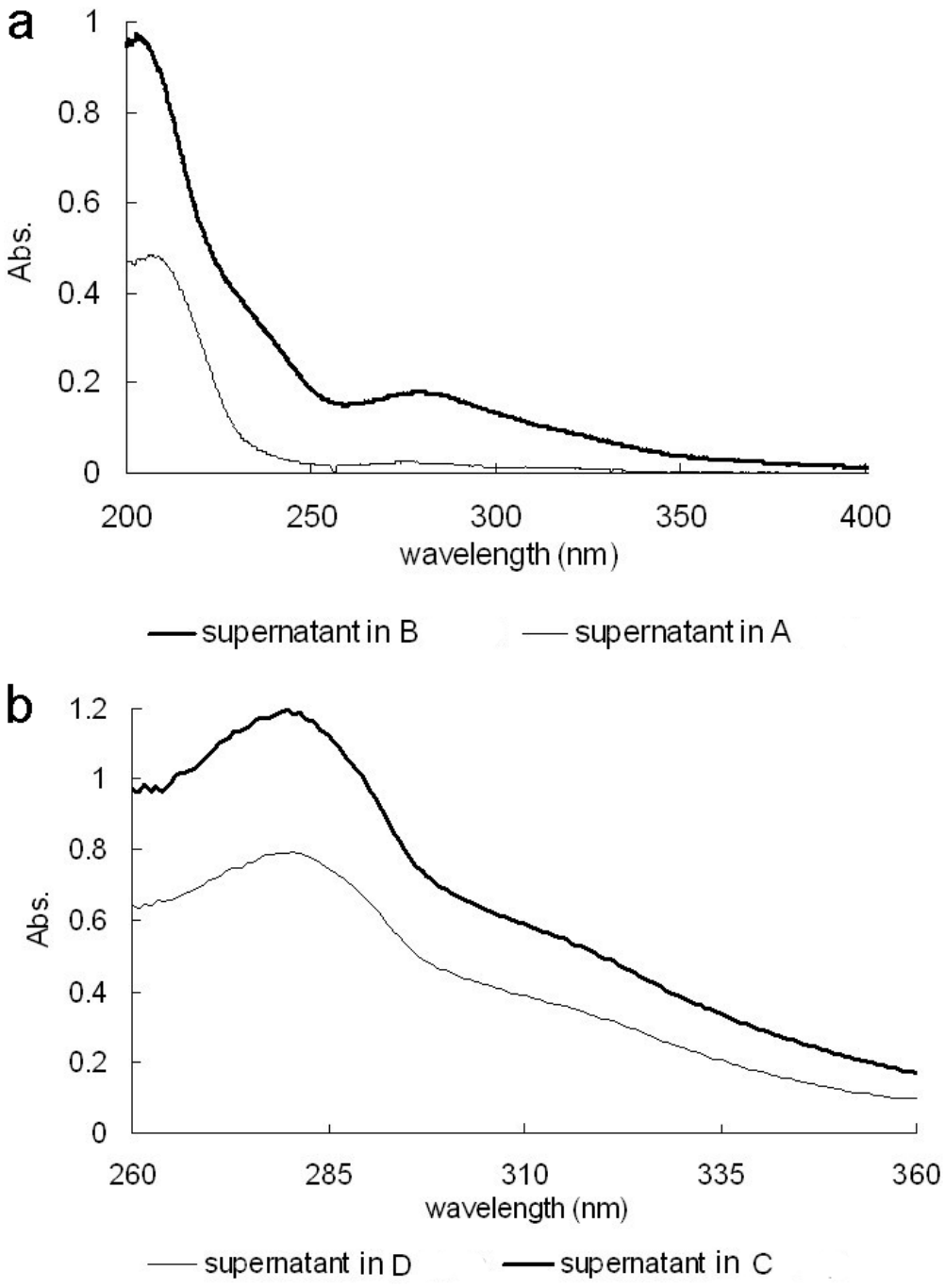

Figure 2. (a) UV spectrum of dissolved CEL in supernatant A and supernatant B; (b) UV spectrum of dissolved CEL in supernatant C and supernatant D.

polymerize small molecule fragments of CEL to the CEL macromolecule. In comparison, in the water heterogeneous solution, there was no apparent change in the small molecule fragments of lignin.

FTIR spectra analysis in all kinds of CEL

FTIR spectra analysis in all kinds of sediment given in Table 1 revealed that the carbonyl group was obviously observed in sample B, whereas it was not observed in other samples because the band at 1712 cm^{-1} was assigned to the carbonyl stretching–unconjugated ketones. This result suggests that the carbonyl group in the lignin (sample A) without laccase treatment was easily reduced to the hydroxyl group, and after laccase treatment, the hydroxyl group was easily oxidized to the carbonyl group. Meanwhile, the carbonyl group was not

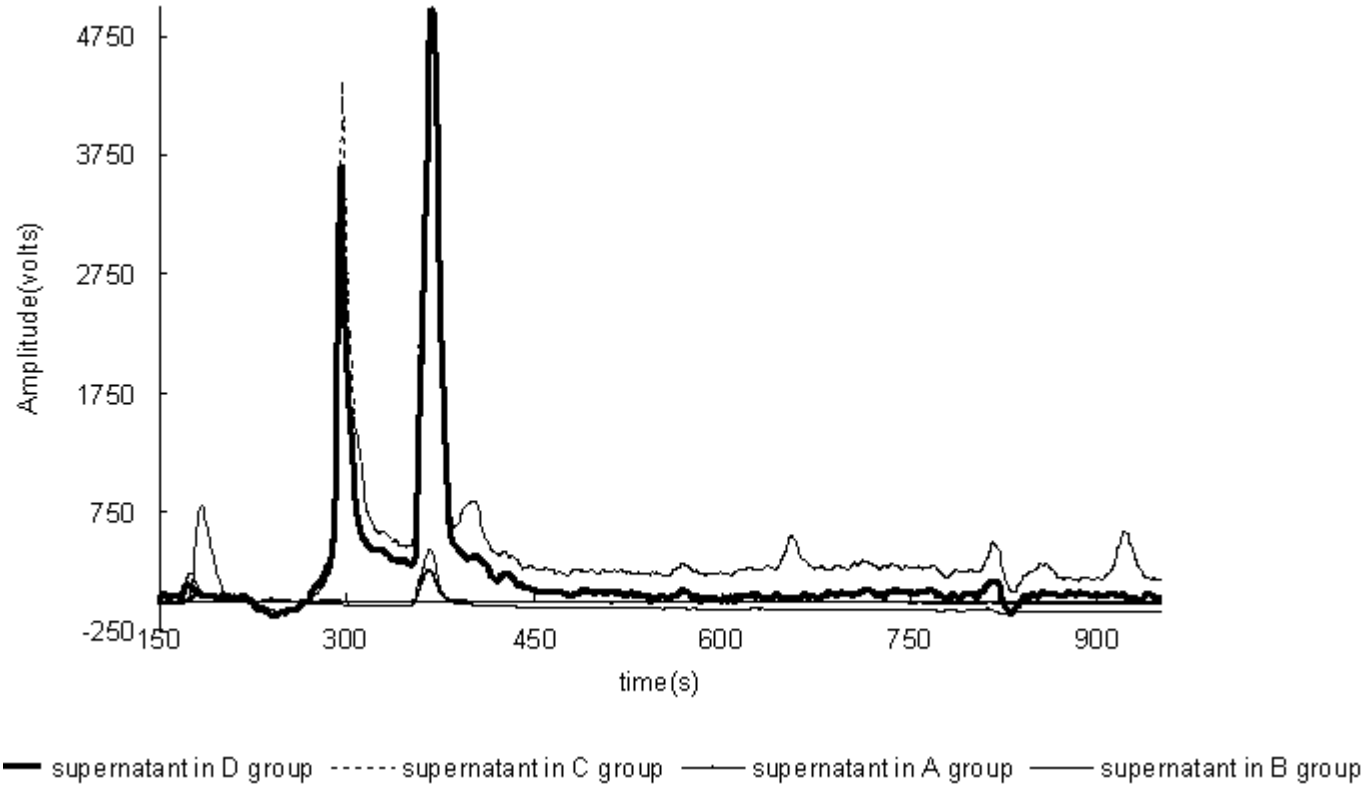

Figure 3. HPLC spectrum of dissolved CEL in supernatant A, supernatant B, supernatant C, and supernatant D.

observed in samples C and D, which showed that due to the presence of ILs, the carbonyl group participated in reactions with ILs. This evidence further confirmed that in the water heterogeneous solution, the laccase directly oxidized the lignin phenolic hydroxyl; therefore, lignin solubility in the water increased. In comparison, in the [bmim]Cl/H_2O homogeneous solution, the content of the functional groups in lignin only underwent slight changes, but these changes were not significant.

^{13}C NMR measurements of the structural changes in all kinds of CEL

The results of the ^{13}C NMR analysis of all kinds of sediment are shown in Table 2. The assignments of ^{13}C signals in the NMR spectral information were derived from studies of model compounds. According to Chen (1998), six aromatic carbons and 0.12 vinylic carbons have an integral region between 162 and 102 ppm; therefore, one aromatic ring (Ar) is equal to the integral value divided by 6.12. The total integral value of the 162 to 128 ppm region divided by 6.12 is thus equivalent to the amount of quaternary carbon atoms per Ar, while the amount of tertiary aromatic carbon atoms per Ar is equal to the difference between 6.12 and the aforementioned amount, and the total integral value of the 58 to 54 ppm region divided by 6.12 is usually attributed to the amount of methoxyl group (OMe) carbon atoms per Ar (Ewellyn et al., 2004). The peaks at 123.5 and 122 ppm were mainly attributed to C-4 and C-2 in imidazole, respectively. The values for C-5 and C-4 in imidazole in sample C were 0.09/Ar and 0.14/Ar, respectively whereas these signals in samples A, B, and D disappeared. Results show that imidazole disappeared after the CEL dissolved in [bmim]Cl (samples C and D) was treated by laccase.

The C-α signals of β-O-4 G and S erythro in sample A disappeared, while their values in samples B, C, and D were 0.96, 1.07, and 0.97, respectively. At the same time, the C-γ signals of β-O-4 S and G threo/erythro in sample B disappeared, and the values in samples A, C, and D were 0.38, 0.91, and 0.98, respectively. These results indicate that C-γ signals disappeared and C-α signals significantly increased after the CEL in water was treated by laccase, whereas C-γ signals obviously increased and C-α signals slightly decreased after the CEL in the [bmim]Cl/H_2O homogeneous solution was treated by laccase. Hence, the former lignin was oxidized by the laccase and β-O-4 bonds were broken, so partial lignin factors were degraded. Meanwhile, after being treated by laccase, the β-O-4 bonds of the latter lignin were not broken; the length of the carbon chain was increased instead. In addition, the amount of OMe in samples A, B,

Table 1. The assignment of FTIR spectra of all kinds of lignin ranges (cm^{-1}).

Peak location range (cm^{-1})	Assignment	Sample A (T×100)	Sample B (T×100)	Sample C (T×100)	Sample D (T×100)
3438	O–H stretching	31.6	26.1	24.3	27.6
2934	C–H stretching	42.5	42.7	42.1	44.1
2855	Methyl/methylene group	59.6	60.1	59.8	61.3
1712	C=O stretch in unconjugated ketone/carbonyl/ester groups	-	74.8	-	-
1593	Aromatic skeleton vibrations plus C=O stretching	31.6	33.1	30.9	34.1
1506	Aromatic skeleton vibrations (G>S)	24.4	25	23.6	26.4
1462	C–H deformations (asym in $-CH_3$ and $-CH_2-$)	25.3	25.8	24.6	27.3
1327	S/G ring	36.2	37.3	36.3	39.2
1268	G ring plus C+O stretching	31.7	31.8	31.2	33.9
1223	Stretching (G' > G)	22.4	21.7	22.9	24.6
1125	Typical of S unit; also secondary alcohol and C=O stretching	11	10.4	11.2	12.5
1033	Aromatic C–H in-plane deformation (G > S) plus C–O deform.	26.5	26.9	28.5	30
920.4	C–H out of plane (aromatic ring)	72.5	74.9	76.1	77.2
832	C–H out of plane in positions 2, 5, and 6 (G units)	69.9	72.6	71.3	73.9

G: Etherified guaiacyl units; G': Non-etherified guaiacyl units; S: Etherified syringyl units; H: p-Hydroxyphenyl.

C, and D were 4.19, 4.07, 4.08, and 4.58, respectively. This evidence further confirmed that the laccase in water degraded the lignin; therefore, the methoxyl content of CEL decreased, whereas in the [bmim]Cl/H_2O homogeneous solution, the laccase polymerized the lignin; therefore, the methoxyl content was increased. These results were consistent with the results of the UV and HPLC spectrum analysis.

Conclusion

In this study, we chose CEL as the substrate and laccase from *Trametes hirsuta* lg-9 as the research object. The purpose of this study was to obtain results where laccase catalytic efficiency is far higher in a [bmim]Cl/H_2O homogeneous solution than in a water heterogeneous solution. For this reason, two different systems to dissolve CEL were designed, wherein an aqueous CEL suspension and a CEL [bmim]Cl/H_2O homogeneous solution were obtained. Unfortunately, we did not obtain the expected results. In this study, however, we reported for the first time the change of CEL in two different solvent systems treated by laccase from our laboratory. Based on all the results of the spectrum analyses, it is clear that the aforementioned results are consistent. Due to the bigger molecular size of laccase in the water heterogeneous solution without any mediator, the laccase alone cannot enter the inner lignin and can only access phenolic hydroxyl groups at the lignin surface (Srebotnik and Messner, 1990). At the same time, the side-chain of lignin is oxidized (Crestini et al., 2003).

In comparison, in the [bmim]Cl/H_2O homogeneous solution, together with the buffered solutions containing the enzymes, all the solution formed a homogeneous phase. Thus, the co-solvents significantly affected the laccase structure, the laccase stability, and the laccase activity of the biocatalysts (Riva, 2006). The specific catalytic mechanism of laccase is, however, still unclear. It is possible that many phenoxy radicals are produced from the inner lignin structures accessed by the laccase, small molecular substances produced in the control C

Table 2. Signal assignment in the ^{13}C NMR spectrum.

Chemical shift ($\times10^{-6}$ppm)	Assignment	Amount (per Ar) A	Amount (per Ar) B	Amount (per Ar) C	Amount (per Ar) D
152.6	C_3/C_5S	1.40	1.37	0.90	1.34
149.6	C_3G	0.13	0.38	0.10	0.38
147.9	C_4G;C_3G'; C_3/C_5S'	0.35	0.70	0.26	0.68
145.2	C_4G'	-	0.11	-	0.09
138.5	C_1S	0.14	0.31	-	0.30
136.5	C_1S; C_2 in Imidazole	0.36	0.12	0.02	0.13
134.5	C_1G; C_4S; C_4S'	-	0.51	0.26	0.50
123.5	C_5G; C_5 in Imidazole	-	-	0.09	-
122.2	C_6G'; C_4 in Imidazole	-	-	0.14	-
119.1	C_6G; C6G'	0.50	0.19	0.51	0.27
115.5	C_5; C_5G'; C_3,C_5H	0.66	0.38	0.95	0.46
111.8	C_2 C_2G'	0.71	0.46	1.15	0.51
104.7	C_2; C_6; C_2,C_6S'	1.87	1.58	1.74	1.46
85.6	C_β and C_α in β-O-4/α-O-4 units	-	0.92	0.47	0.93
72.0	C_α in β-O-4 G and S erythro	-	0.96	1.07	0.97
60.5	C_γ in β-O-4 S and G threo and erythro	0.38	-	0.91	0.98
55.6	Aromatic OMe in G and S units	4.19	4.07	4.08	4.58

G: Etherified guaiacyl units; G': Non-etherified guaiacyl units; S: Etherified syringyl units; S': Non-etherified syringyl units; H: p-Hydroxyphenyl.

group are re-captured (Reale et al., 2004), and the methoxyl content is increased. From the discussion, the laccase from our laboratory could clearly act on CEL whether in the water heterogeneous solution or in the [bmim]Cl/H_2O homogeneous solution. Due to the different dissolution degrees, the results were significantly different. For insoluble CEL in the heterogeneous water solution, laccase partly oxidized the hydroxyl group into a carbonyl group. Consequently, CEL was degraded to increase its solubility. On the other hand, for soluble CEL in the [bmim]Cl/H_2O homogeneous solution, laccase primarily polymerized small molecule fragments of CEL with the CEL macromolecule, increased the methoxyl content of CEL, and accordingly played a role in lignin polymerization. This study will therefore provide a new method for solving the lignin degradation and polymer problem, as well as provide some ideas for industrial processes using laccases.

ACKNOWLEDGEMENTS

This study was supported by the National Science Foundation of China (Grant Nos. 30871985).

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