

## Oxidation of Lignin-Carbohydrate Complex by Laccase/Co(salen) One-Pot Catalysis

(Pengoksidaan Kompleks Lignin-Karbohidrat oleh Lakase/Co(salen) Pemangkinan Satu Periuk)

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### ABSTRACT

Lignin-carbohydrate complex (LCC) is hybrid structures containing covalently linked moieties of lignin and carbohydrates. The nature and amount of LCC affect both industrial processes and practical applications of lignocellulosic biomass. Herein, the LCC was isolated from bamboo by successive solvent extraction and precipitation. The effects and mechanism of LCC oxidation respectively by laccase, Co(salen), and laccase/Co(salen) in the presence of molecular oxygen have been investigated by composition analysis using the standard of National Renewable Energy Laboratory (NREL) and high-performance anion exchange chromatography (HPAEC), GPC, FTIR, and 2D-HSQC NMR. We can conclude that the laccase/Co(salen) one-pot catalysis modified the LCC in such a way that more carbohydrate was removed from the LCC with lower molecular weight of LCC as shown by GPC; the catalytic treatments produced oxidation at lignin side-chains and cleavage of lignin  $\beta$ -O-4',  $\beta$ - $\beta$ ' and  $\beta$ -5' bonds in LCC, and cleavage of benzyl-sugar ether, phenyl glycoside and  $\gamma$ -ester bonds in LCC, as shown by FTIR and 2D-HSQC NMR, especially after the laccase/Co(salen) one-pot treatment. The further insight of LCC degradation was discussed in light of the results obtained in oxidation of the LCC model compound coniferin.

Keywords: Co(salen); lignin-carbohydrate complex (LCC); one-pot catalysis, laccase; oxidation

### ABSTRAK

Kompleks lignin-karbohidrat (LCC) ialah struktur hibrid yang mengandungi gugusan lignin dan karbohidrat yang dikaitkan secara kovalen. Sifat dan jumlah LCC mempengaruhi kedua-dua proses perindustrian dan aplikasi praktikal biojisim lignoselulosa. Di sini, LCC telah diasingkan daripada buluh melalui pengekstrakan dan pemendakan pelarut berturut-turut. Kesan dan mekanisme pengoksidaan LCC masing-masing oleh lakase, Co(salen) dan lakase/Co(salen) dengan kehadiran molekul oksigen telah dikaji melalui analisis komposisi menggunakan piawaian *National Renewable Energy Laboratory* (NREL) dan anion berprestasi tinggi. Kromatografi pertukaran (HPAEC), GPC, FTIR dan 2D-HSQC NMR. Kita boleh membuat kesimpulan bahawa pemangkinan satu pot lakase/Co(salen) mengubah suai LCC dengan cara yang lebih banyak karbohidrat dikeluarkan daripada LCC dengan berat molekul LCC yang lebih rendah seperti yang ditunjukkan oleh GPC; rawatan pemangkin menghasilkan pengoksidaan pada rangkaian sisi lignin dan pembelahan ikatan lignin  $\beta$ -O-4',  $\beta$ - $\beta$ ' dan  $\beta$ -5' dalam LCC dan pembelahan ikatan benzil-gula eter, fenil glikosida dan  $\gamma$ -ester dalam LCC, seperti yang ditunjukkan oleh FTIR dan 2D-HSQC NMR, terutamanya selepas rawatan satu pot lakase/Co(salen). Kajian lanjut tentang degradasi LCC telah dibincangkan berdasarkan keputusan yang diperoleh dalam pengoksidaan koniferin sebatian model LCC.

Kata kunci: Co(salen); kompleks lignin-karbohidrat (LCC); pemangkinan satu periuk, lakase; pengoksidaan

### INTRODUCTION

Biomass is the fourth largest energy source following coal, oil, and natural gas. Biomass refinery as a renewable complement to the petroleum refinery becomes imperative

for clean and sustainable production. The conversion of lignocellulosic biomass plays important role for the utilization of biomass for the production of fuels and chemicals (Ahmad et al. 2020; Nakagawa et al. 2020).

Lignin is the second-most abundant component of plant material and has been proven to be a promising natural aromatic polymer (Huang et al. 2019). However, lignin is difficult to transform due to its complicated structure. On the other hand, lignin can be obtained in large amounts by wood pulping as a by-product, but only < 2% is used efficiently for producing high-value products (Abdelaziz et al. 2020). Consequently, it is far necessary to enlarge understanding of lignin behaviour in structure and mechanism and possibly the use of lignin for producing high-value chemicals.

Lignin is composed mainly of three basic building blocks, *p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, corresponding to *p*-hydroxyphenyl (H), guaiacyl (G), and syringyl (S) structures in lignin, respectively. In addition, lignin is a three-dimensional amorphous polymer with both aliphatic and aromatic constituents via both C-O and C-C bonds (Katahira et al. 2018). Among

these interunit linkages, the  $\beta$ -O-4,  $\beta$ -5,  $\beta$ - $\beta$  and 5-5' units are the main structures. The relative abundance of other linkages such as  $\alpha$ -O-4,  $\beta$ -1, and 4-O-5 diphenyl is relatively low. For further information,  $\beta$ -O-4 interunit linkage (arylglycerol- $\beta$ -aryl ether) is the most frequent in lignin. This linkage occurs at levels of 40%-60% in softwood and hardwood (Azadi et al. 2013; Calvo-Flores & Dobado 2010). Studies confirmed that all lignin units in coniferous species and 47-66% of lignin moieties in deciduous species are bound to hemicelluloses or cellulose molecules in lignin-carbohydrate complex (LCC, Figure 1). The nature and amount of LCC linkages and lignin substructures affect the efficiency of lignin oxidation. Moreover, the yield of aromatic monomers depends on degradation of LCC interunit linkages during lignin conversion. So far, oxidation is commonly used to break the recalcitrant lignin structure into aromatic monomers (Tarasov et al. 2018).

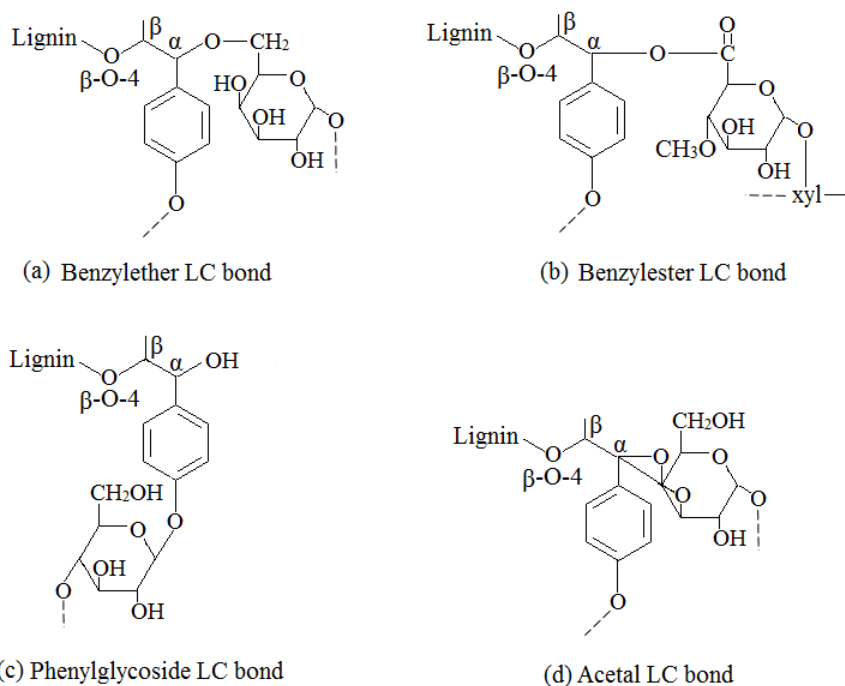


FIGURE 1. Typical lignin carbohydrate (LC) linkages (Lawoko 2013)

Catalysis is often employed to increase the yield of target products (Fernandes et al. 2019). Enzymatic process has the advantage that it can provide the desired product with high selectivity under milder conditions (Sudarsanam et al. 2020). Zhu et al. (2020) explored the use of laccase from recombinant *Escherichia coli* BL21 as a biocatalyst and proposed a mechanism in which

laccase promoted bond cleavage of interunit linkages such as  $\beta$ -O-4,  $\beta$ -5,  $\beta$ - $\beta$ , 4-O-5, and 5-5. Fernandes et al. (2019) combined tert-butylhydroperoxide and titanium catalyst which promotes the oxidation of the lignin structure and other pronounced LCC structural changes. The MoPO/CeO<sub>2</sub> catalyst showed excellent activity and afforded 68 wt% lignin; Highest 12.5 wt%

total monomers including 9 wt% vanillin with 72% GC selectivity were obtained (Rawat et al. 2020). In Huang et al. (2014) study, it was found that the binary catalyst combining Pd complex and polyoxometalate was more active than the one-component catalyst in the selective aerobic oxidation of aromatic alcohol at ambient pressure. Transition metal complexes were also used as catalysts mimicking the biological structure, function, mechanism and process of enzymes. Further, biomimetic complexes were the active catalysts in promoting LCC degradation. Rajagopalan et al. (2008) have reported the axial ligand dependence and substrate selectivity in Co(salen) catalysed oxidation reactions. Similarly, the analysis of lignin-derived chemicals led Díaz-Urrutia et al. (2016) to conclude that ionic liquid-tagged salen-oxovanadium catalyst significantly increased lignin oxidation and LCC oxidation selectivity. Recently, Penin et al. (2020) reported LCC was transformed into monomeric aromatic compounds via oxidative exo- and endo-depolymerisation processes using a vanadate-based biomimetic system.

However, due to the complexity and refractoriness of LCC, efficient conversion of lignin into aromatic chemicals remains a huge challenge (Sivagurunathan et al. 2021). Therefore, one-pot catalysis may be a promising approach affecting the conversion efficiency of lignin. Carrozza et al. (2019) investigated the effects of the use of cholinium lysinate and NiSO<sub>4</sub> in a one-pot process on the physical and chemical alterations in lignin structure. The combination of cholinium lysinate and NiSO<sub>4</sub> increased the depolymerization efficiency of lignin. However, little research has been done on the enzymatic and chemical one-pot catalysis to allow both efficiency and selectivity to be considered. In this work, laccase and Co(salen) were combined to degrade LCC model compound to address the effect of one-pot catalysis on the oxidation mechanism of bamboo LCC.

## MATERIALS AND METHODS

### MATERIALS AND CHEMICALS

Bamboo chips (*Dendrocalamus hamiltonii* Nees et Arn. ex Munro) was provided by Forestry Bureau of Puer City, Yunnan Province, China. The following chemicals and materials were purchased from Sigma-Aldrich. Laccase (Lac, *Trametes versicolor*, powder, light brown, 23.1 U/mg); coniferin (4-(3-hydroxy-1-propenyl)-2-methoxyphenyl β-D-glucopyranoside, lignin-carbohydrate model compound, Scheme 1). All other materials and chemicals (Sinopharm Chemical Reagent Co. Ltd., China) used in this work were of commercially

available reagent grade and were used as received. Milli-Q-Plus ultrapure water was used in all experiments.

Co(salen) was synthesized by condensation of ethylenediamine with salicylaldehyde in methanol and by reaction of salen and cobalt acetate in refluxing methanol (Ng et al. 2019).

### ISOLATION OF BAMBOO LCC

LCC was isolated from bamboo following the reported method (Figure 2) (Zhang et al. 2016).

The air-dried bamboo chips were milled to particle sizes between 40 meshes - 100 meshes using a micro plant grinding machine. The powders were extracted for 6 h with a mixture of toluene/ethanol (2:1, v/v). The extractive-free powders were then ball-milled. The ball-milled bamboo powders were extracted with dioxane/water (96/4, v/v) for 72 h. The insoluble matter was then extracted with acetic acid/water (1/1, v/v) for 72 h. The solution was dried in vacuum, and was then extracted with dimethyl sulfoxide to obtain the solution. The solution was added into dichloroethane/ethanol (2/1, v/v), and then the precipitate was washed with dichloroethane/ethanol (2/1, v/v) one time, and was washed with ethyl ether three times. The solid matter was dissolved in acetic acid/water (1/1, v/v). The solution obtained after separation was added into a certain amount of acetone, and the precipitate was obtained after standing. The precipitate was washed with acetone/acetic acid (99/1, v/v) one time, was washed with ethyl ether three times, was washed with petroleum ether one time, and then dried in vacuum. Finally, bamboo lignin-carbohydrate complex (LCC) was obtained.

### CATALYTIC OXIDATION AND CHARACTERISATION OF BAMBOO LCC

Catalytic oxidation of bamboo LCC was performed in an autoclave with agitation. Conditions used for catalytic oxidation were as follows:  $T=70\text{ }^{\circ}\text{C}$ ;  $t=60\text{ min}$ ;  $\text{pH}=8.0$ ;  $p(\text{O}_2)=0.40\text{ MPa}$ ; bamboo LCC, 100 mg; catalyst, 0.2 wt% to the dry LCC (laccase/Co(salen)=1:1, wt %); acetonitrile/THF mixture, 100 mL (10:1). Control reactions were done in the absence of catalyst to check for the catalytic effect. At end of reaction, the catalyst was separated, and liquid phase containing LCC was dried in vacuum at  $40\text{ }^{\circ}\text{C}$  for next analysis. LCC samples were then designated as control-LCC, laccase-LCC, Co(salen)-LCC, and laccase/Co(salen)-LCC, corresponding to control-treated LCC, laccase-treated LCC, Co(salen)-treated LCC, and laccase/Co(salen)-treated LCC, respectively.

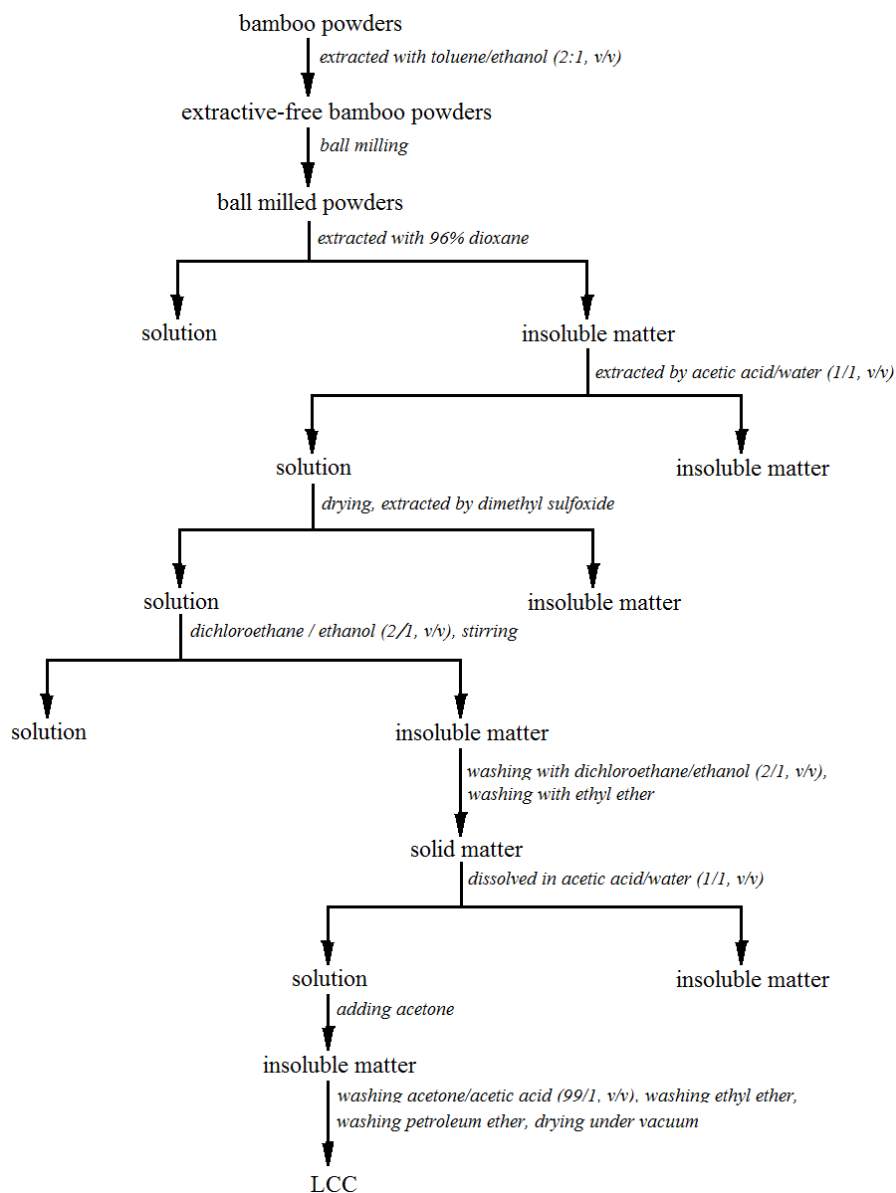


FIGURE 2. Scheme for isolation of bamboo LCC

The chemical composition of LCC samples was analysed according to the standard of National Renewable Energy Laboratory (NREL) (Sluiter et al. 2008). LCC (30 mg) was hydrolysed with in sulfuric acid solution (1.0 mL, 2%) at room temperature for 1 h. After that, water was added to the mixture to achieve a 3%  $\text{H}_2\text{SO}_4$  concentration. Then, the solution was heated in an autoclave at 120 °C for 1.5 h. The resulting suspension was then filtered. The filtrate was diluted to obtain a suitable concentration of monosaccharides (0.1-0.2 mg/mL) for analysis with high-performance anion exchange

chromatography (HPAEC) equipped with iColumn and iDetector. The analysis was carried out using water as the eluent at the flow rate of 1.0 mL/min, where the column temperature was kept at 18 °C. Fucose was used as an internal standard.

Acetylation of LCC was carried out in pyridine via reaction with acetic anhydride prior to GPC analysis. Then, molecular weights and molecular weight distributions of LCC samples were analysed in tetrahydrofuran (1 mL/min) using an Agilent 1100 GPC. Stainless steel column (30 cm×i.d.7.5 mm) and differential refractometer were

used. The column temperature was 35 °C. The calibration curve was obtained using monodisperse polystyrene compounds of known molecular weight as reference substances (Steinmetz et al. 2019).

FTIR tests of LCC samples were conducted on a Bruker Equinox 55 FTIR spectrometer using KBr pellets. The spectra were recorded within a spectral range of 400 to 4000  $\text{cm}^{-1}$  with a resolution of 4  $\text{cm}^{-1}$ . LCC linkages between lignin and carbohydrate (Figure 4(Est), Figure 4 B( $E_1$ ), Figure 4(BE<sub>2</sub>)) and  $\beta$ -O-4 bonds (Figure 4(A)) in LCC samples were quantitatively determined with 2D-HSQC NMR using the central solvent peak as an internal reference ( $\delta_C$  39.6/ $\delta_H$  2.48) (Deshpande et al. 2018). Conditions used for HSQC analysis were as follows: Bruker DRX 500-MHz NMR spectrometer; 140 mg, 0.50 mL DMSO- $d_6$ ; 0.1 s acquisition; 90° pulse; 20,000 Hz  $^{13}\text{C}$  spectral width, 5000 Hz  $^1\text{H}$  spectral width; 1.0 s acquisition delay ( $d_1$ ); 316 K; standard pulse program hsqcetetgspisp2.

#### CATALYTIC OXIDATION OF LCC MODEL COMPOUND AND CHARACTERIZATION OF DEGRADATION PRODUCTS

Conditions for catalytic oxidation of coniferin were used as follows:  $C_0$ , coniferin = 1 mM,  $C_{\text{catalyst}}=0.2$  wt% (laccase/Co(salen)=1:1, wt %),  $T=30$  °C,  $t=60$  min,  $\text{pH}=8.0$ ,  $p(\text{O}_2)=0.40$  MPa. The reaction mixture was extracted with dichloromethane and the reaction products were quantified with gas chromatography/

mass spectrometry (GC/MS; Agilent HP6890-5973 GC-MS; Agilent VF5-ms capillary column, 30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$ ; 80 °C/5 min  $\rightarrow$  10 °C/min, 280 °C  $\rightarrow$  280 °C/20 min), using 1,4-dimethoxybenzene, xylitol, phenyl- $\beta$ -D-galactopyranoside and phenyl- $\beta$ -D-galactopyranoside as internal standards for coniferyl alcohol, glucose, coniferin and glucovanillin, respectively. Control reactions were also done in the absence of catalyst.

## RESULTS AND DISCUSSION

### CATALYTIC OXIDATION OF LCC MODEL COMPOUND

The catalytic oxidation of LCC by the laccase and Co(salen) was explored using coniferin as LCC model compound. The well-established combination of enzyme and biomimetic complex was investigated, laccase with Co(salen) (laccase/Co(salen)). Control experiments were also carried out (Figure 3). The efficiency comparison of various catalysts was first analyzed on the coniferin by conversion determination for evaluation of the coniferin degradation. As shown in Figure 3, the conversion rate produced by the laccase/Co(salen) (11.8%) was higher than that with the Co(salen) (17.3%) and laccase (28.4%). This is in agreement with the difference in their catalytic potential. The conversion difference may also come from the difference in the biomimetic catalysis used (Richard 2016). In addition, because the laccase/Co(salen) one-pot catalysis used in coniferin oxidation was known to be capable of providing synergistic effect (Kim et al. 2020), which will increase the conversion.

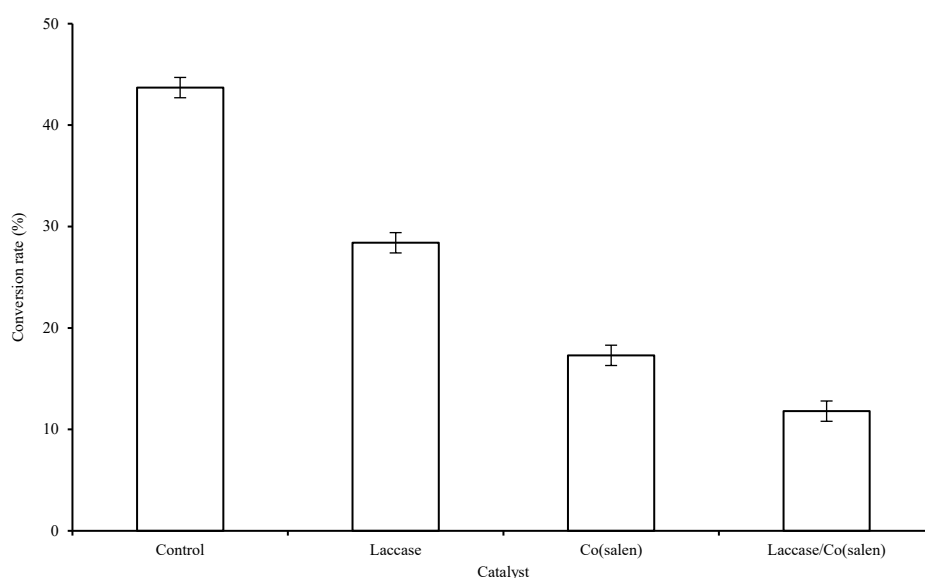


FIGURE 3. Efficiency comparison of various catalysts in coniferin oxidation

## CATALYTIC OXIDATION OF LCC

To obtain structural information of LCC, the LCC samples were analyzed with 2D HSQC NMR. The assignments for the signals of lignin, carbohydrate, and lignin-carbohydrate linkage were made according to previous reports (Del Río et al. 2016; Qin et al. 2018; Sette et al. 2013; Torres et al. 2021). Typical HSQC NMR

spectra of LCC is shown in Figure 4. Glycoside phenyl, benzyl ether, and  $\gamma$ -ester LCC linkages were observed in HSQC spectra. The signals at  $\delta_C/\delta_H$  103-98/4.8-4.3 ppm were attributed to CH-1 of various internal carbohydrates units. The signals at  $\delta_C/\delta_H$  81.2-79.2/4.6-4.5 ppm assigned to  $\alpha$ -CH benzyl ether lignin-carbohydrate (LC) bonds. Meanwhile, LCC samples showed the presence of the

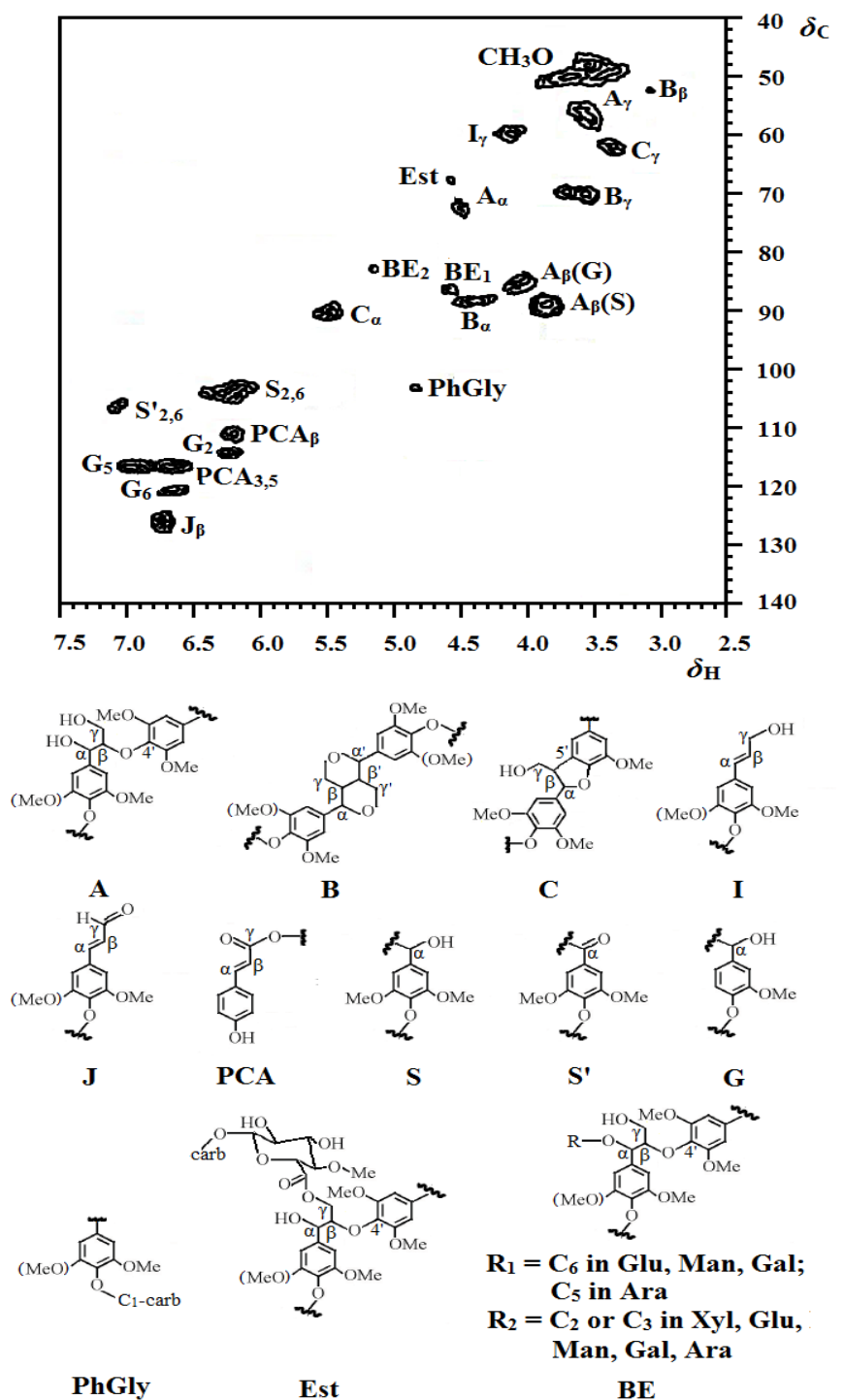


FIGURE 4. Typical HSQC NMR spectra of LCC. (A)  $\beta$ -O-4 aryl ether linkages; (B) resinol substructures; (C) phenylcoumarane substructures; (I) cinnamyl alcohol end groups; (J) cinnamaldehyde end groups; (PCA) p-coumaric acid (ester); (S) syringyl units; (S') oxidized ( $C_\alpha=O$ ) syringyl units; (G) guaiacyl units; (BE) benzyl-sugar ethers; (PhGly) phenyl glycoside; (Est)  $\gamma$ -ester



CH<sub>2</sub>-γ signals of γ-esters in region of δ<sub>C</sub>/δ<sub>H</sub> 64.1-63.0/4.2-4.16 ppm. Phenyl glycoside linkages afforded a variety of signals of carbohydrate C-1 at δ<sub>C</sub>/δ<sub>H</sub> 103.1-100.5/5.3-4.9 ppm. As the aim of this work was focused on LCC structures, we limited the discussion to the main lignin substructures of LCC, such as β-O-4', β-β' and β-5'. Signals from C<sub>γ</sub>-H<sub>γ</sub> correlations in the β-O-4', β-β' and β-5' substructures appeared at δ<sub>C</sub>/δ<sub>H</sub> 60/3.6, 71.4/3.96-4.2 and 66.68/3.69 ppm, respectively. The C<sub>β</sub>-H<sub>β</sub> correlations in β-O-4' substructure linked to G lignin units and S lignin units were attributed at δ<sub>C</sub>/δ<sub>H</sub> 84.3/4.3 and 86.4/4.1 ppm, respectively. Finally, signals for C<sub>α</sub>-H<sub>α</sub> correlations in β-O-4' substructures were observed at δ<sub>C</sub>/δ<sub>H</sub> 71.8/4.8 ppm. The signals of C-H correlation at the α and β position in β-β' substructure appeared at δ<sub>C</sub>/δ<sub>H</sub> 83.7/4.2, and 54.5/3.1 ppm, respectively. In addition, C-H correlation at the α position in β-5' substructure was found at δ<sub>C</sub>/δ<sub>H</sub> 87.5/5.4 ppm.

As shown in Table 1, the content of β-O-4' in the laccase/Co(salen)-LCC was appreciably lower than that in other LCC samples. Therefore, as expected, molecular weight of LCC decreased with improving catalysis (do Pim et al. 2021). With regard to the treatment with the laccase/Co(salen) system, it is worth mentioning that the one-pot catalysis used is among the most successful in removing the condensed lignin units from LCC. While the content of β-β' and β-5' bonds for laccase-LCC was respectively 7.5 per 100Ar and 4.1 per

100Ar, respectively, 4.8 per 100Ar and 3.3 per 100Ar for Co(salen)-LCC, laccase/Co(salen)-LCC had low β-β', β-5' content of 3.2 per 100Ar and 2.4 per 100Ar, respectively. This suggested that the combined catalysis in one-pot with laccase and Co(salen) selectively removed refractory lignin units from LCC, resulting in extended degradation of LCC. The reason may be explained that the laccase/Co(salen) catalysis inhibited condensation reactions via phenoxy radicals (Michniewicz et al. 2007). Meanwhile, it was observed that laccase/Co(salen) one-pot catalysis had the strong and positive effect on cleavage of lignin and carbohydrate bonds such as benzyl-sugar ethers, phenyl glycoside and γ-ester, as shown in Table 1, which was also the reason that the carbohydrate content and molecular weight of laccase/Co(salen)-LCC was low (Du et al. 2013; Steinmetz et al. 2020). In fact, the data of lignin and carbohydrate content in LCC indicated that the carbohydrate content and molecular weight of LCC depended on the catalysis. The carbohydrate contents in LCC, control-LCC, laccase-LCC, Co(salen)-LCC and laccase/Co(salen)-LCC were 42.8%, 39.4%, 37.3%, 36.6% and 35.8%, respectively; the molecular weights of LCC, control-LCC, laccase-LCC, Co(salen)-LCC and laccase/Co(salen)-LCC were 8746, 5217, 4058, 3266 and 2143, respectively (Table 1). This indicated that the laccase/Co(salen) one-pot catalysis promoted the degradation of carbohydrate and lignin of LCC (Ren & Fasan 2020).

TABLE 1. Content of lignin, carbohydrate, and lignin β-O-4' bonds, lignin β-β' bonds, lignin β-5' bonds, benzyl ether, PhGly, Est in LCC sample

Sample	Lignin content (%)	Carbohydrate content (%)	Molecular weight (g/mol)		β-O-4' bonds (per 100Ar)	β-β' bonds (per 100Ar)	β-5' bonds (per 100Ar)	Benzyl ether <sup>b</sup> (per 100Ar)	PhGly (per 100Ar)	Est (per 100Ar)
			M <sub>w</sub>	M <sub>w</sub> /M <sub>n</sub> <sup>a</sup>						
LCC	57.2	42.8	8746	2.5	42.7	12.7	7.2	22.4	8.7	7.6
Control-LCC	60.6	39.4	5217	2.26	38.4	9.2	5.3	18.0	5.2	4.3
Laccase-LCC	62.7	37.3	4058	1.75	32.6	7.5	4.1	13.9	3.5	2.6
Co(salen)-LCC	63.4	36.6	3266	1.41	25.6	4.8	3.3	7.4	2.1	1.3
Laccase/Co(salen)-LCC	65.2	35.8	2143	1.26	18.3	3.2	2.4	3.2	1.4	0.4

<sup>a</sup>M<sub>w</sub>, weight-average molecular weight; M<sub>n</sub>, number-average molecular weight; M<sub>w</sub>/M<sub>n</sub>, polydispersity of LCC preparations. <sup>b</sup>Benzyl ether = BE1 + BE2

FTIR characterization was conducted to further verify the structural differences among the different LCC samples, and the spectra are shown in Figure 5. The intensity of the band at  $1740\text{ cm}^{-1}$  in laccase/Co(salen)-LCC sample, which originated from C=O stretching, was stronger than Co(salen)-LCC and laccase-LCC. This suggests enhanced oxidation in LCC due to the one-pot catalysis with laccase/Co(salen) (Dai et al. 2019),

which was confirmed by the results of HSQC analysis. The bands at  $1603\text{ cm}^{-1}$  and  $1507\text{ cm}^{-1}$  in the spectra of LCC samples were typical of aromatic ring vibrations of lignin. Increasing catalysis promoted degradation of lignin according to the decreasing intensity of the band of aromatic ring. Increasing catalysis also promoted degradation of lignin  $\beta$ -O-4 bonds, which resulted in increase of lignin phenolic OH ( $1370\text{ cm}^{-1}$ ) (Suzuki et

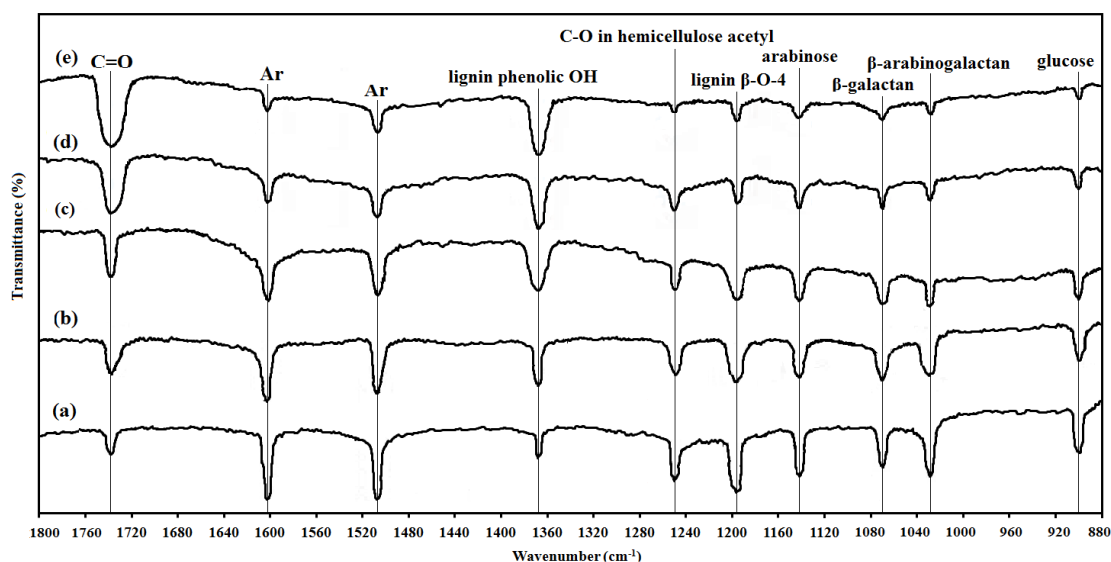


FIGURE 5. FTIR spectra of LCC samples. (a) LCC, (b) Control-LCC, (c) Laccase-LCC, (d) Co(salen)-LCC; (e) Laccase/Co(salen)-LCC

al. 2020). For all LCC samples, typical signals from carbohydrate of LCC were observed at  $1056\text{ cm}^{-1}$  (C-O in hemicellulose acetyl),  $1144\text{ cm}^{-1}$  (arabinose),  $1073\text{ cm}^{-1}$  ( $\beta$ -galactan),  $1030\text{ cm}^{-1}$  ( $\beta$ -arabinogalactan),  $926\text{ cm}^{-1}$  (glucose) (Qin et al. 2018; Singh et al. 2005), but laccase/Co(salen)-LCC had weaker intensity of the absorbance than Co(salen)-LCC and laccase-LCC. This result suggests that laccase/Co(salen)-LCC contained less carbohydrate, which was also proved by the data in Table 1.

## CONCLUSIONS

In light of the results obtained in this study on bamboo LCC oxidation by laccase, Co(salen) and laccase/Co(salen), the LCC degradation reactions include carbohydrate removal, lignin side-chains oxidation, and cleavage of  $\beta$ -O-4',  $\beta$ - $\beta'$ ,  $\beta$ -5', benzyl-sugar ether, phenyl glycoside and  $\gamma$ -ester bonds in LCC. All reactions will eventually lead to LCC finally degradation. Different

combinations of laccase and Co(salen), namely laccase, Co(salen) and laccase/Co(salen), will lead to differences in the actual oxidation routes and eventually differences in the LCC degradation effects. The insight of LCC degradation in catalytic oxidation could be greatly improved by using LCC model compound coniferin as substrate followed by characterisation of conversion rate and degradation products of coniferin so that more comprehensive information about improving LCC degradation by laccase/Co(salen) one-pot catalysis could be obtained.

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