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A one-pot catalysis combining laccase with Cu(salen) for selective removal of refractory lignin units during oxygen delignification of bamboo kraft pulp

Uma catálise em recipiente único combinando laccase com Cu(salen) para a remoção seletiva de unidades de lignina refratárias durante a deslignificação de polpa Kraft de bambu

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Abstract

The catalysis is an alternative to extend the delignification of pulp with molecular oxygen. This work thus focused on the delignification selectivity and selective removal of refractory lignin units by the one-pot catalysis combining laccase with Cu(salen) after oxygen delignification of bamboo kraft pulp. The one-pot catalysis showed a higher efficiency than the single-catalyst catalysis in relation with delignification selectivity and pulp brightness. The determination of the molecular weight showed that the combined catalysis remarkably enhanced the cleavage of connection bonds in pulp lignin. Furthermore, the quantitative analysis of the refractory linkages with 2D-HSQC- and ^{31}P-NMR techniques indicated that the5-5', β-5' and β-β' units were removed by the one-pot catalysis to a large extent, following the corresponding order of cleavage reactivity. This rationalization was further theoretically confirmed by using the 5-5' model compound method.

Keywords: Oxygen delignification; One-pot catalysis; Residual lignin; HSQC; ^{31}P-NMR;β-β'; β-5';5-5'.

Resumo

A catálise é uma alternativa para aumentar a deslignificação de polpa com oxigênio molecular. Este trabalho enfocou a seletividade da deslignificação e a remoção seletiva de unidades de lignina refratárias pela catálise em recipiente único combinando a laccase com Cu(salen) após a deslignificação por oxigênio de polpa Kraft de bambu. A catálise em recipiente único mostrou uma eficiência maior do que a catálise por catalista único em relação à deslignificação seletiva e à brancura da polpa. Determinação do peso molecular mostrou que a catálise combinada melhorou fortemente a clivagem de ligações em polpa de lignina. Ademais, a análise quantitativa das ligações refratárias com técnicas de 2D-HSQC- e 31P-NMR mostraram que as unidades -5', β-5' e β-β' foram removidas em grande parte pela catálise em recipiente único, seguindo a ordem correspondente de reatividade de clivagem. Esta racionalização foi ainda confirmada teoricamente usando o modêlo do método composto 5-5'.

Palavras-chave: Deslignificação com oxigênio; Catálise one-pot; Lignina residual; HSQC; ^{31}P-NMR;β-β'; β-5';5-5'.

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INTRODUCTION

Oxygen delignification is widely used in the pulp and paper industry and, due to its effective pre-delignification prior to the subsequent bleaching sequences, is considered necessary in TCF (total chlorine free)/ECF (elemental chlorine free) bleaching of pulp. In the meanwhile, regulatory pressures have driven the industry to improve oxygen delignification due to its environmental, technical and economic benefits (Bonfatti Júnior & Silva Júnior, 2018). Although double stage oxygen delignification has shown some potential, the delignification selectivity is however susceptible to cellulose degradation. When delignification is beyond the level of 50%, severe cellulose degradation takes place, resulting in severe deterioration of pulp strength. This selectivity issue is thus a drawback of oxygen delignification (Bacarin et al., 2017; Jafari et al., 2015).

In view of minimizing these negative effects on the delignification selectivity, some approaches aimed at extending oxygen delignification have been proposed and implemented in the industry. Among many advantages of the new techniques catalysis could maintain its high efficiency, the ease of implementation and its relatively low cost, especially when compared to other methods. Thus, catalysis applied to pulp delignification becomes an alternative to enhance the degradation of pup lignin and to improve the delignification selectivity (Chaurasia & Bhardwaj, 2019). Enzymatic catalysis presents advantages in relation to other techniques, such as decreased chemical requirements in subsequent bleaching sequences, improved delignification selectivity and increased pulp brightness with equivalent amount of chemicals (Lin et al., 2018). Furthermore, methyltrioxo rhenium (MTO), metal salts, polyoxometalates, metalloporphyrins and cobalt-Schiff base complexes are the important groups of homogeneous catalysts reported to be active in bleaching reactions with oxygen (Crestini et al., 2006; Lu et al., 2019; Pan et al., 2015; Zhao et al., 2013; Zucca et al., 2014). However, enzymatic catalysis usually gives a low efficiency and chemical catalysis a low selectivity (Ma et al., 2018). Therefore, achieving a viable extended delignification is more likely if the two catalyses are combined. This may be a simple and cheap technique that allows the efficiency and selectivity of delignification to be considered simultaneously.

Further developments in this area have to be associated with structural studies of pulp lignin. The present work approached this topic in association with isolated lignin and lignin model compound considering the recalcitrance of refractory lignin. The aim was to focus on the selective removal of refractory lignin units by the one-pot catalysis, which is expected to improve the understanding of factors responsible for limiting the selective delignification of pulp.

MATERIAL AND METHODS

Material

The pulp used in this study was an unbleached bamboo Kraft pulp of kappa number 22.6, viscosity 1328 ml/g and brightness 28.6% ISO. It was chelated with DTPA for 30 min at 50°C and thoroughly washed with water prior to the oxygen delignification experiment.

The following reagents were purchased from Sigma-Aldrich: cyclohexanol (99.0%), tetrahydrofuran (99.0%), 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (99.0%), acetone-d_6 and DMSO-d_6 (99.9%atom D). NaY molecular sieve (surface area, 584 m^2/g; pore size 2.2 nm), and MCM-41 molecular sieve (surface area, 1052 m^2/g; pore size 4.8 nm) were obtained from Sinopharm Chemical Reagent Co. Ltd. (China). Laccase was supplied by Novozymes (recombinant laccase from *Bacillus pumilus*, 22.3 U/mg). All other chemicals (Sinopharm Chemical Reagent Co. Ltd., China) were of p.a. grade (if not otherwise stated) and were used as received.

The immobilized catalysts Cu(salen) NaY (1.88% Cu, S_{BET}270.10m^2/g, D_{BET} 2.17 nm) and laccase MCM (17.3% laccase, S_{BET} 923.6m^2/g, D_{BET} 4.27 nm) were previously synthesized (Zhang & Zhou, 2012; Zhou & Tang, 2016) and used in this work.

In addition, a 5-5' lignin model compound was synthesized starting from the corresponding phenol dihydrocreosol by alkylation with dimethylsulfate under alkaline conditions. Dihydrocreosol (0.2 mol) was dissolved in NaOH solution (30%, 20 mL) with stirring. Dimethylsulfate (0.4 mol) was then added, and the reaction was performed at 60 °C for 2 h. After cooling, the mixture was neutralized with ammonium chloride, and the model compound was extracted with ethyl acetate. The crude compound was obtained by vacuum-evaporation and purified *via* crystallization in hexane-ethyl acetate. ^{1}H-NMR ($CDCl_3$) δ 2.36 (s,6H, CH_3), 3.65 (s, 6H, OCH_3), 3.86 (s, 6H, OCH_3),6.65-6.76 (m, 4H, CH); ^{13}C-NMR ($CDCl_3$) δ 21.27(CH_3), 55.77 (OCH_3), 60.64 (OCH_3), 112.57 (CH),123.54 (CH), 132.57 (C) 132.80 (C), 144.61 (C), 152.28 (C).

Milli-Q-Plus ultrapure water was used in all trials.

Catalytic treatment

The catalytic oxygen delignification of bamboo Kraft pulp was carried out in a semi-batch reactor (250 ml) equipped with a stirrer under laboratory conditions at 90°C for 80 min at the catalyst concentration of 0.2wt% (enzyme catalyst/biomimetic catalyst=1:1, wt%), pulp concentration of 8wt%, pH value of 9, and $p(O_2)$ of 0.80 MPa. The control trials were also carried out under identical conditions in the absence of catalyst. The delignification selectivity was then calculated according to Equation 1-Equation 3.

$$A = \frac{L_1 - L_2}{L_1} \times 100\ \% \qquad \text{Eq. (1)}$$

$$B = \frac{V_1 - V_2}{V_1} \times 100\ \% \qquad \text{Eq. (2)}$$

$$S = \frac{A}{B} \times 100\ \% \qquad \text{Eq. (3)}$$

A, delignification rate (%); L_1, lignin content (Klason lignin and acid-soluble lignin) of pulp before delignification; L_2, lignin content of pulp after delignification; *B*, viscosity reduction rate (%); V_1, viscosity of pulp before delignification; V_2, viscosity of pulp after delignification; *S*, delignification selectivity.

Conditions used for the catalytic treatment of the lignin model compounds were as follows: $C_{0,\text{model compound}}$=1 mM, C_{catalyst}=0.2 wt% (enzyme catalyst/biomimetic catalyst=1:1 (wt %), *T*=90°C, *t*=80 min, pH=9, $p(O_2)$=0.80 MPa.The reaction mixture was extracted with dichloromethane and quantitatively analysed for the reaction products on an Agilent HP6890-5973 GC-MS instrument, using 4-propylbenzoic acid as an internal standard.

Replicates were carried out and good reproducibility was obtained, with a coefficient of variation of less than 3%.

Residual lignin isolation

The residual lignin was isolated from each pulp following a procedure developed by Argyropoulos et al. (2002).

The pulp obtained in various treatments was initially acetone-extracted in a Soxhlet extractor for 24 h, thoroughly washed with water, dewatered and vacuum-dried. It was then hydrolyzed in buffer solution (acetic acid/sodium acetate, pH 4.8) under a mild condition with cellulase (2267.5 U/g, Novozymes). After that, the obtained residue was subjected to a very mild acidolysis at a consistency of 4% with 0.05 M HCl solution in dioxane/water (85:15, v/v) under nitrogen positive pressure. Subsequently the mixture was filtered to remove the hydrolyzed pulp and the solution was acidified with 1 M HCl to pH 2.0, the precipitated lignin was then centrifuged, thoroughly washed with 0.01 M HCl, and freeze-dried. Finally the lignin was further purified by extraction with dichloromethane to remove accumulated extractives. Lignin samples isolated from the various oxygen delignified pulps were designated as BKPL (bamboo Kraft pulp lignin), CL (control pulp lignin), LaL (laccase catalysis plus lignin), LaML (laccase MCM catalysis plus lignin), CuL (Cu(salen) catalysis plus lignin), CuYL (Cu (salen) NaY catalysis plus lignin), La/CuL (laccase/Cu (salen) catalysis plus lignin) and LaM/CuYL (laccase MCM/Cu (salen)NaY catalysis plus lignin).

Yield and chemical composition of the various lignin samples (Table 1) were analysed following to the National Renewable Energy Laboratory standard analytical method (NREL/TP-510-42618). The determination of the sugar content was done with ionic chromatograph (Metrohm ICS-883, Swiss) equipped with iColumn and iDetector. Replicate determinations were carried out with a coefficient of variation less than 3%.

Table 1. Description of the residual lignins.

Lignin Yield (wt%)	Sugar Content (wt %)[a]						Elemental Content (wt %)		
	Glu	Xyl	Gal	Ara	Man	Total	C	H	N
67.2	0.15	0.36	0.04	0.02	0.05	0.62	58.3	5.9	0.72
62.4	0.08	0.27	0.06	0.03	ND[b]	0.44	59.2	5.7	0.68
61.7	0.16	0.16	ND[b]	0.07	0.08	0.47	60.4	6.1	0.47
65.3	0.08	0.13	ND[b]	ND[b]	0.06	0.27	61.7	6.2	0.52
68.3	0.11	0.21	ND[b]	ND[b]	0.05	0.37	59.5	5.7	0.64
67.9	0.06	0.17	ND[b]	ND[b]	ND[b]	0.23	58.3	5.8	0.58
63.7	0.13	0.21	ND[b]	0.02	0.07	0.43	61.6	5.9	0.42
66.2	0.03	0.19	ND[b]	ND[b]	ND[b]	0.22	59.6	6.2	0.71

[a] Glu, glucose; Xyl, xylose; Gal, galactose; Ara, arabinose; Man, mannose. [b] ND, Not detectable.

Analytical methods

The molecular weights of lignin preparations were analyzed by Agilent 1100 GPC (USA), based on the procedures described in a previous study (Liu et al., 2018).

The 2D-HSQC NMR spectra were recorded with the central solvent peak as an internal reference (δ_C 39.6/δ_H 2.48) following the reported procedure with minor modifications in a Bruker DRX 500-MHz NMR spectrometer after 140 mg of each lignin preparation was dissolved in 0.50 mL of DMSO-d_6 (Latif et al., 2019), in which the standard pulse program hsqcedetgpsisp2 was employed in the HSQC test. Conditions used for analysis were as follows: ^{13}C spectral width, 20,000 Hz; ^{1}H spectral width, 5000 Hz; temperature, 318 K; 90° pulse; acquisition time, 0.1 s; and 1.0 s acquisition delay (d_1). Quantitative ^{31}P-NMR spectra of the isolated lignins were acquired operating at an inverse gated decoupling sequence according to the method described by Korntner et al. (2015). Prior to the determination the lignin was derivatized with 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) and cholesterol was used as an internal standard. Conditions used for ^{31}P-NMR analysis were as follows: pulse width, 90°; relaxation delay between pulses, 5 s; and 300 transients.

RESULTS AND DISCUSSION

Response to oxygen delignification

Figure 1 showed the results obtained after oxygen delignification of bamboo Kraft pulp using different catalysts. Delignification selectivity (Figure 1a) and pulp brightness (Figure 1b) were found to significantly evolve with enhancing catalysis. In detail, the oxygen delignified pulps with application of biomimetic catalyst (Cu(salen), Cu(salen) NaY) and immobilized catalyst (LaccaseMCM, Cu(salen) NaY) displayed high values of the two indices compared to laccase and neat catalyst (laccase, Cu(salen)).

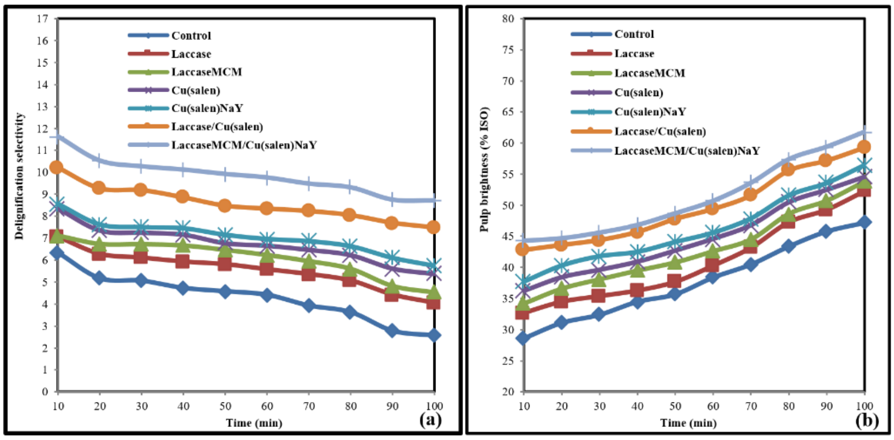

Figure 1. Catalysis *vs* oxygen delignification. (a) delignification selectivity; (b) pulp brightness.

Moreover, further improvement can be obtained by the combined catalysis (laccase/Cu(salen), laccaseMCM/Cu (salen) NaY) in the aspect of delignification and bleaching of the pulp. Following this, a notable gap was found between one-pot catalysis and one-catalyst catalysis in terms of the delignification selectivity and pulp brightness. For example, according to the data obtained in the experiment performed for 80 min, while the value of delignification selectivity/pulp brightness was 5.07/47.3, 5.61/48.6, 6.23/50.5 and 6.64/51.6 respectively for laccase-, laccaseMCM-, Cu(salen)-and Cu(salen)NaY-trials, it was 8.04/55.6 and 9.31/57.4 respectively for laccase/Cu(salen) and laccaseMCM/Cu(salen)NaY-trials. This proves a synergistic effect occurred in the one-pot catalysis (Jiang & Cheng, 2016; Yi et al., 2019).

Effect of catalysis method on depolymerization of pulp lignin and refractory lignin units

In order to clarify the level of lignin depolymerization during oxygen delignification of bamboo Kraft pulp, the molecular weights of various residual lignins were determined with GPC. As expected, the molecular weight of the one-pot catalysis-lignins (La/CuL, LaM/CuYL), especially for the LaM/CuYL lignin sample, was appreciably lower than that of the one-catalyst catalysis samples (Table 2). This resulted in some differences in the PD of the two types of lignin samples (Table 2). The enhanced catalysis made the structure of pulp lignin more homogeneous, decreasing the PD of the residual lignin and indicating the effective degradation of refractory lignin units of the pulp (Giummarella et al., 2019).

Table 2. Molecular weight and substructure content of lignin samples.

Lignin Sample	(g/mol)^{a}/PD^{b} (GPC)	/100Ar (HSQC NMR)		mmol/g (^{31}P-NMR)	
		β-O-4'	β-β'	β-5'	5-5' Condensed Units^{d}
BKPL	11824/1.625	30.3	12.8	6.6	0.48
CL	11355/1.443	29.7	12.4	6.2	0.43
LaL	10309/1.304	27.2	11.3	5.7	0.38
LaML	8903/1.277	26.6	10.1	5.2	0.36
CuL	8256/1.185	22.5	6.7	3.3	0.27
CuYL	5341/1.172	21.2	6.4	2.8	0.24
La/CuL	2537/1.147	12.8	1.5	0.63	0.07
LaM/CuYL	1633/1.136	10.2	0.63	0.27	0.05

^{a}weight-average molecular weight; ^{b}polydispersity (M_w/M_n); ^{c}141.7-140.4 ppm.

In addition, the isolated lignins were studied using HSQC (Figure 2a) and ^{31}P-NMR (Figure 2b) spectroscopic techniques. Figure 2 presented the typical NMR spectra of BKPL; those of other lignin samples were not provided in this paper. Information on the different types of structural features was collected and analysed to follow the changes occurring within the bamboo Kraft pulp lignin during different catalytic processes. Analysis of the lignins by the HSQC technique showed that the major substructures of lignin, such as β-O-4' aryl ether (A; $\delta_{C\alpha}/\delta_{H\alpha}$ 71.5/4.83, G-type $\delta_{C\beta}/\delta_{H\beta}$84.6/4.26, S-type$\delta_{C\beta}/\delta_{H\beta}$ 86.7/4.16, $\delta_{C\alpha}/\delta_{H\alpha}$ 64.5/3.36 in A' α-ethoxylated β-O-4', $\delta_{C\alpha}/\delta_{H\alpha}$ 80.7/4.52 in α-acylated β-O-4', $\delta_{C\gamma}/\delta_{H\gamma}$ 62.4/4.23 in A' α-ethoxylated β-O-4'), resinol (β-β', B; $\delta_{C\alpha}/\delta_{H\alpha}$ 85.7/4.63, $\delta_{C\beta}/\delta_{H\beta}$ 54.5/3.13, $\delta_{C\gamma}/\delta_{H\gamma}$ 71.4/4.25 and 3.83), and phenylcoumaran (β-5', C; $\delta_{C\alpha}/\delta_{H\alpha}$ 87.4/5.41, $\delta_{C\beta}/\delta_{H\beta}$ 54.7/3.77, $\delta_{C\gamma}/\delta_{H\gamma}$ 62.7/3.74) moieties were present in variouslignin preparations (Zhao et al., 2019).

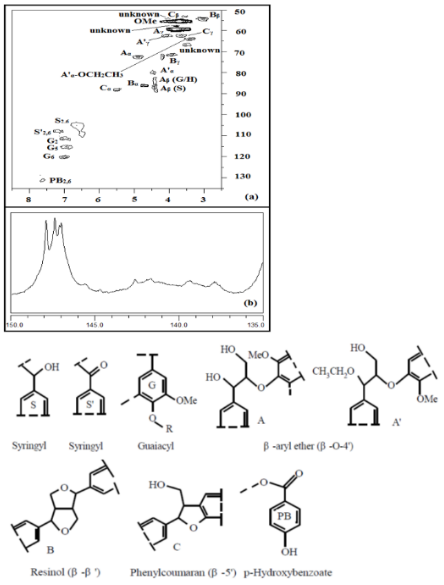

Figure 2-TypicalNMR spectra of BKPL. (a)HSQC NMR; (b)^{31}P-NMR.

As is known to all, β-O-4' bonds are the main interunit linkages and probably the most important substructures in lignin, which greatly affects both the chemical and physical properties of lignin, and the condensed linkages such as β-β', β-5' and 5-5' bonds are found to be critical for selective extended delignification, amongst which 5-5' substructures showed the highest resistance (Akim et al., 2001). Therefore, the β-O-4', β-β' and β-5' units were quantitatively analyzed by the HSQC technique and the content of 5-5' condensed units was determined with ^{31}P-NMR (Wang et al., 2017).

As shown in Table 2, the content of β-O-4' linkages in the BKPL sample was 30.3/100Ar, which decreased to 12.8/100Ar in La/CuL and 10.2/100Ar in LaM/CuYL. This showed that the one-pot catalysis removal rate of β-O-4' units was nearly twice that of the one-catalyst catalysis. In contrast, the condensed lignin units were also removed to a large extent during the one-pot catalysed oxygen delignification of the pulp, in agreement with the evolvement of catalytic power. While the one-catalyst catalysis samples (LaL, LaML, CuL, CuYL) respectively presented 6.4-11.3 β-β' units/100Ar, 2.8-5.7 β-5' units/100Ar and 0.24-0.38 mmol 5-5' units/g, sample LaM/CuYL had a much lowerβ-β', β-5' and 5-5'content, of 0.63/100Ar, 0.27/100Ar and 0.05 mmol/g, respectively. Comparatively, for the condensed units, the cleavage reactivity of the 5-5' linkage was the highest, and the cleavage reactivities decreased in the order of5-5'>β-5'>β-β'. These results theoretically proved that the combination of laccase with Cu(salen) had a positive impact on the selective removal of refractory lignin units in the catalytic oxygen delignification of bamboo Kraft pulp. This is generally quite different from the reported results that lignin condensation often occurred during alkaline oxygen delignification and the condensed structures generated were believed to be less reactive to extended delignification, resulting in a poor delignification selectivity (Dipankar, 2010; Fu & Lucia, 2003; Gomes et al., 2018; Zhao et al., 2018).

In addition, attention should be payed specifically to the development of relatively inert 5-5' substructures within the residual lignins. The substantial decrease observed within the residual lignin LaM/CuYL could be attributed to the fact that the combined catalysis may inhibit secondary condensation reactions. Such reactions are likely to occur between phenoxy radicals generating new 5-5'related units under oxidative conditions (Nanayakkara et al., 2011). Akim et al. (2001) observed in their study that the 5-5' condensed units in residual lignins decreased from 0.44 mmol/g to 0.38 mmol/g during the early phases of oxygen delignification of spruce Kraft pulp, and then started to build up again to a value of 0.49 mmol/g due to the accumulation of new 5-5' units, which potentially demonstrated the superiority of the one-pot catalysis used for pulp oxygen delignification in this work.

Effect of catalysis method on depolymerization of 5-5'lignin model compound

In an effort to further support and confirm this rationalization catalytic trials on a 5-5' lignin model compound (compound 1, Figure 3) in the presence of these catalysts were performed. The main oxidation products were characterized in every case as the compounds 2, 3 and 4. The reaction pathway was hence proposed as follows.The formation of these compounds could be rationalized on the basis of the epoxidation by the superoxo complex L-Cu-O-O•which are active species of Cu(salen) and laccase (Shimazaki And Yamauchi, 2011). The superoxo radicals·attacked C_1 on the aromatic ring, which in turn initiate depoxidation at the side chain position by the superoxo. This step could direct the further oxidative cleavage of the 5-5' linkage to yield the corresponding benzylic alcohol (compounds 2, 4). Simultaneously, the side-chain oxidation yielded the benzylic aldehyde (compound 3). This behavior had been analogously reported by our group in the catalytic oxidation of a β-O-4 lignin model compound by metalsalen complex (Zhou And Lu, 2016). Canevali et al. (2002) also proposed a similar C-C cleavage pathway for the catalytic oxidation of dimeric lignin model compounds using Co(salen) as catalyst, and benzoquinone and aldehyde compounds were also obtained in their studies.

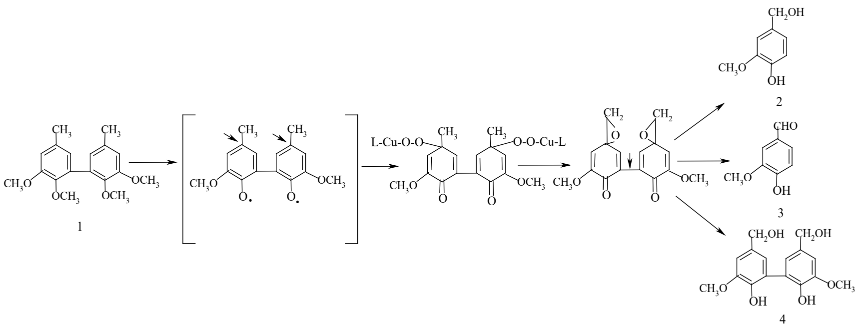

Figure 3. Proposed reaction pathway for the catalytic oxidation of 5-5' lignin model compound.

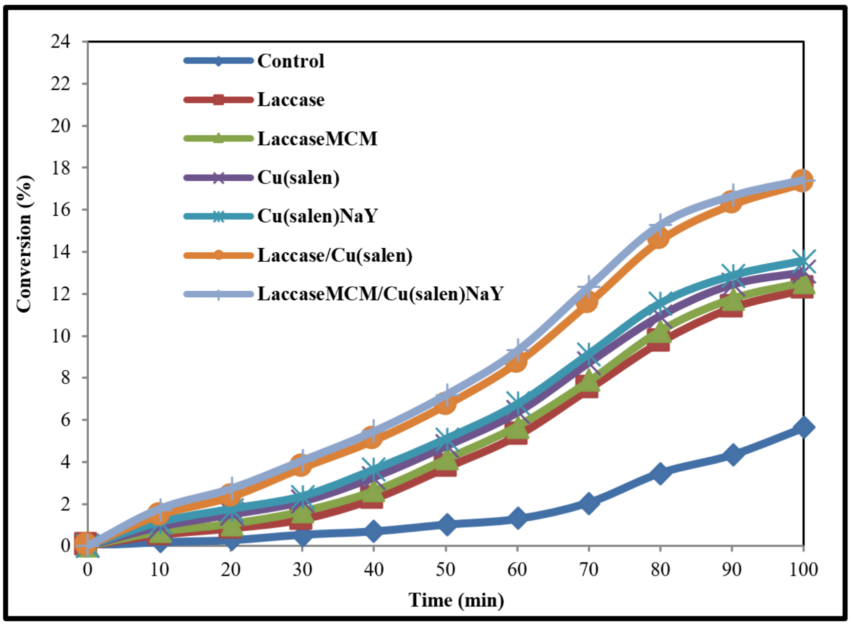

Figure 4. Conversion of the 5-5' model compound.

In Figure 4 the conversion rates of 5-5' lignin model compound with the different catalysts tested are shown, as determined by GC-MS technique, which could be considered as an index of the relative catalytic efficiency of the different catalytic methods toward the selective removal of refractory lignin units. A comparison between single-catalyst catalysis (laccase, laccaseMCM, Cu(salen), Cu(salen)NaY) and one-pot catalysis (laccase/Cu(salen), laccaseMCM/Cu(salen)NaY) indicated that the latter offered a better performance in oxidation of the 5-5' model than the former, and the performance was further enhanced with the use of the immobilized catalyst (laccaseMCM/Cu(salen)NaY). This could be due to the support (MCM, NaY) providing a high stability which is important for superoxo intermediates to retain the activity under oxidative conditions (Kianfar et al., 2018). Under such experimental conditions the homolytic cleavage of the peroxydic bond competes with epoxidation (Bataineh et al.,2016). In fact, the corresponding neat catalysts including laccase, Cu(salen) and laccase/Cu(salen)proved to be the least active ones.

CONCLUSION

Lignin characterization furnished very useful information about the catalytic performance of the combination of laccase and Cu(salen) toward the selective removal of refractory lignin units during oxygen delignification of bamboo Kraft pulp. Fortunately, under reaction conditions typical of industrial oxygen delignification, laccaseMCM/Cu(salen)NaY is an active catalyst in the oxidation of both refractory pulp lignin units and refractory lignin model compounds. The generation of a synergistic effect and the reactions exerted by the enhanced superoxo complex L-Cu-O-O· may play a critical role in the selective removal of refractory lignin units, and thus in the improvement of the delignification selectivity.

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