



Amination of biorefinery technical lignin by Mannich reaction for preparing highly efficient nitrogen fertilizer

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ABSTRACT

To develop a novel lignin-based highly efficient nitrogen fertilizer, the amination of the biorefinery technical lignin was conducted by Mannich reaction synergy with phenolation pretreatment. Subsequently, the structural transformations of lignin samples and the reaction mechanism were investigated in detail. The soil column leaching experiment was also performed to research the nitrogen release behavior of aminated lignin in soil. The results indicated that the amounts of active sites in lignin were significantly increased to 8.26 mmol/g from the original 2.91 mmol/g by phenolation. In addition, the Mannich reaction was highly selective for occurring at *ortho*- and *para*-positions of phenolic hydroxyl groups in the phenolated lignin, in which the latter was favored. Moreover, the nitrogen content in the aminated lignin was highly depended on the types of amination reagent instead of the proportion of reactants in this study. Under an optimal condition, aminated lignin with a high nitrogen content (10.13%) and low C/N ratio (6.08) could be obtained. Besides, it was especially noteworthy that the prepared APL in this study has a favorable nitrogen release behavior in soil. Thus, it is believed that these aminated lignin derivatives could be used for the preparation of various lignin-based highly efficient nitrogen fertilizer.

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1. Introduction

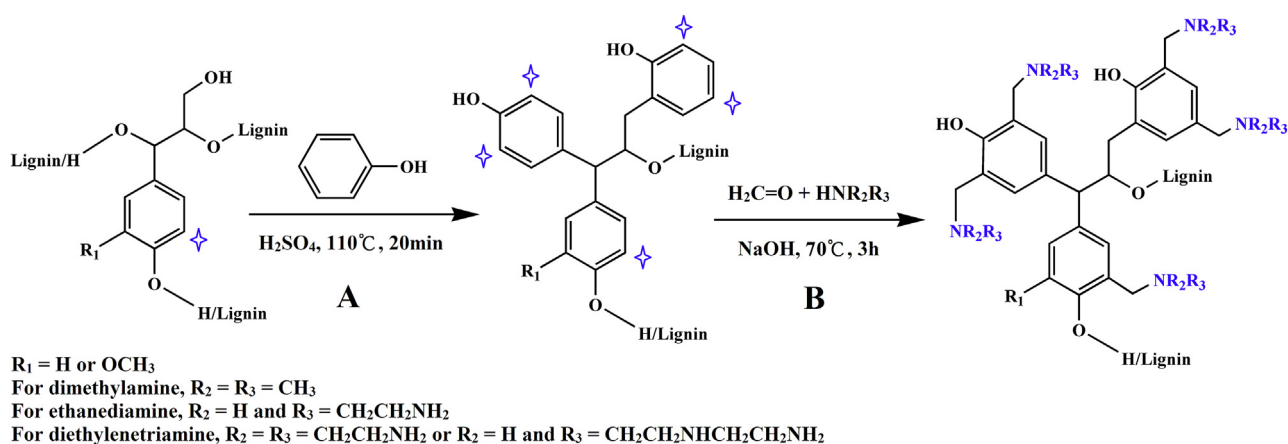
With the industrial development and total population growth, the global depletion of fossil fuels is gradually increasing, which has resulted in not only the scarcity of petrochemical but also serious environmental issues (environmental pollution, global warming etc.). Therefore, there is an immediate need to exploit sustainable and eco-friendly new energy sources [1]. The lignocellulosic biomass, as the natural and renewable resource, exists abundantly in nature and is considered to be a promising candidate to replace fossil fuels [2,3]. Recently, with the extensive application of the concept for converting biomass into biofuels, the modern biorefinery industry is rapidly developed. Meanwhile, large amounts of biorefinery technical lignin as a by-product are generated [4]. Unfortunately, due to the complex structure and low chemical reactivity, the application of lignin was severely

restricted [5,6]. Only less amount of technical lignin was applied to produce value-added products, while the most of them were simply burned as low grade fuels or directly discarded as wastewaters [7]. The low-value use pattern not only caused adverse environmental impact, but also decreased significantly the use efficiency of biomass and economic benefit of biorefinery process, which have beset these biorefinery enterprises for a long term. Thus, it is vital to explore new approach for the high-value utilization of lignin.

Actually, previous researches working on lignin valorization have verified that the biorefinery technical lignin possesses a large potential to be utilized for preparing various lignin-based functional materials [1,2,5]. For example, the lignin could be used as a substitution for the commercial polyol to prepare lignin-based rigid polyurethane foam due to the presence of numerous hydroxyl groups in the structure of lignin [8]. Moreover, the lignin, as a low-cost, eco-friendly and renewable natural polymer materials, is also able to be applied for preparing various adsorbents, which exhibits an excellent adsorption capacity and removal rates for the heavy metals or dyes in the wastewater [9–13]. Furthermore, the lignin possesses a certain degree of antioxidant abilities, which was mainly attributed to its methoxy and phenolic hydroxyl groups [14]. Consequently, lignin could act as an antioxidant addition to

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Scheme 1. Chemosynthesis scheme of the aminated lignin with high nitrogen content (A) The phenolation of lignin; (B) The Mannich reaction of phenolated lignin under alkaline condition. The * mark means reactive sites of the Mannich reaction.

manufacture various antioxidant products, such as antioxidant packing film [15]. Besides, it is especially notable that lignin, the natural urease inhibitor, could effectively inhibit the activity of urease in the soil. Thus, urea is retained in the soil by lignin for a longer period [16]. Meanwhile, as a humic acid precursor substance, lignin could be thoroughly degraded into humus by soil microorganisms, thereby raising soil organic matter content as well as soil fertility [17,18]. These favorable properties enable lignin to be a promising raw material for the preparation of highly efficient nitrogen fertilizer [19,20].

In the past two more decades, lignin has been applied to prepare various slow release fertilizers [19,21–23]. In general, they can be classified into two major categories: the lignin-based coated fertilizer and lignin-based organic-*N*-compounds fertilizer [24,25]. For the first category, urea granules are commonly coated by spraying a mixture of lignin and various types of addition agents, such as rosins and linseed oil [20] or starch [24], in the surface of urea granules. However, due to the poor film forming properties and low water resistance, the slow release capacity of lignin-based coating is limited. Therefore, to obtain a better slow release effect, the double-coated technology and modification of lignin, such as esterification or Mannich reaction, was also administered [19,25]. For the second category, the current approach is mainly to introduce nitrogen into the structure of lignin via ammoxidation [26,27]. Actually, the Mannich reaction, as one of the most important fundamental reaction in organic chemistry, is also an efficient approach to introduce amine groups into lignin, which has been widely applied in the modification of lignin and the chemosynthesis of lignin-based functional materials [28–31]. Nevertheless, due to the low chemical reactivity and less reactive sites in the lignin, the aminated lignin has a relatively low nitrogen content, which severely restricted its

application in fertilizer industry. Therefore, to introduce more amine groups, the activation of lignin must be performed before the Mannich reaction, which mainly includes phenolation [28], depolymerization [32] and demethoxylation. As compared to the other activation methods, the phenolation, which could graft phenol onto the lignin and thereby increase dramatically the content of active sites, is the most efficient.

Thus, in the present study, the aminated lignin with higher nitrogen content was prepared by Mannich reaction synergy with phenolation pretreatment, as an activated method. Subsequently, the structural transformations of all lignin samples and the reaction mechanism were investigated in detail by FTIR, various NMR analysis and elemental analysis. The soil column leaching experiment was also performed to research the nitrogen release behavior of aminated lignin in soil. Besides, to explore the optimum chemosynthesis condition, the influences of proportion of reactants and types of amination reagent on the nitrogen content of aminated lignin were evaluated. It is hoped that this study could facilitate the value-added application of the biorefinery technical lignin.

2. Materials and methods

2.1. Materials

The biorefinery technical lignin (L) was supplied by Shandong Longlive Bio-technology Co., Ltd., China. The general production processes were as follow: the corn cob was firstly subjected to a hydrothermal pretreatment to degrade the hemicelluloses into xylo-oligosaccharides, then the residue was further treated by a diluted

Table 1
The reaction condition for the amination of PL.

	PL ₂ ^a (g)	Formaldehyde (g)	Amination reagent (g)			Temperature (°C)	Time (h)
			Dimethylamine	Ethanediamine	Diethylenetriamine		
APL ₁	5	10	14	–	–	60	3
APL ₂	5	20	28	–	–	60	3
APL ₃	5	30	42	–	–	60	3
APL ₄	5	10	–	7.5	–	60	3
APL ₅	5	20	–	15	–	60	3
APL ₆	5	30	–	22.5	–	60	3
APL ₇	5	10	–	–	13	60	3
APL ₈	5	20	–	–	26	60	3
APL ₉	5	30	–	–	39	60	3
ALP ₁₀	5	20	–	15	–	70	3
ALP ₁₁	5	20	–	15	–	80	3
ALP ₁₂	5	20	–	15	–	60	4
ALP ₁₃	5	20	–	15	–	60	5

^a PL₂ means the phenolated lignin with the weight ratio of reactants, 1:2, lignin/phenol.

alkaline solution to fractionate into lignin and cellulose-rich substrates. Finally, the obtained lignin was directly used as the biorefinery technical lignin without any further purification. Phenol, 37% formaldehyde aqueous solution, 40% dimethylamine aqueous solution, ethanediamine and diethylenetriamine were all analytical grade and purchased from Sigma-Aldrich (Beijing, China).

2.2. Phenolation pretreatment of the biorefinery technical lignin

The phenolation of the biorefinery technical lignin was conducted under the acidic condition according to the previous literature [33]. Briefly, 10 g lignin was dissolved in a certain amount of phenol, and the weight ratio of lignin to phenol were 1:1, 1:2, 1:4, 1:6 for PL₁, PL₂, PL₃, PL₄, respectively. In addition, the sulfuric acid (5 mL) was also added to the mixture as a catalyst. Then, the homogenous mixture was stirred at 110 °C for 20 min. Once the reaction completed, the mixture was slowly added to hydrochloric acid aqueous solution (pH = 2) under continuous stirring to precipitate the phenolated lignin (PL). Subsequently, the precipitates, termed as PL, were filtered, washed with acidified water followed by deionized water until neutral pH, and finally freeze-dried. The scheme of the phenolation reaction is showed in Scheme 1A.

2.3. Amination of the phenolated lignin

The amination of the phenolated lignin was carried out under alkaline condition by Mannich reaction (Scheme 1B). Specifically, 5 g PL was firstly dissolved in 10 mL 0.4 mol/L sodium hydroxide solution for about 10 min under continuous stirring to ensure the sufficient dissolution of PL. Then, a certain amount of amination reagent (40% dimethylamine aqueous solution, ethanediamine or diethylenetriamine) and 37% formaldehyde aqueous solution were added, in turn, into the above solution (Table 1). Subsequently, the mixture was kept at a specified temperature (60 °C, 70 °C, 80 °C) for a specified time (3 h, 4 h, 5 h) with continuous stirring. Afterward, the mixed products were dialyzed using the dialysis tubing with a molecular

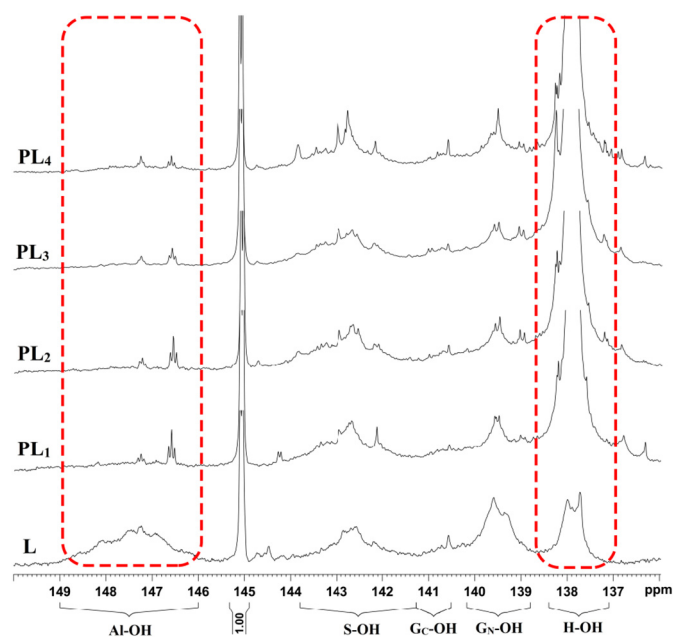


Fig. 2. The ^{31}P NMR spectra of alkali lignin (L) and phenolated lignin (PL₅). PL₁, PL₂, PL₃, and PL₄ refer to the phenolated lignin with different weight ratio of reactants, 1:1, 1:2, 1:4, 1:6, lignin/phenol, respectively. Abbreviations: Al-OH, aliphatic hydroxyls; S-OH, non-condensed syringyl phenolic hydroxyls; Gc-OH, condensed guaiacyl phenolic hydroxyls; Gn-OH, non-condensed guaiacyl phenolic hydroxyls; H-OH, *p*-hydroxyphenyl phenolic hydroxyls.

weight cut-off of 1000 Da. Finally, the aminated lignin (APL) was obtained by freeze-drying.

2.4. Characterizations of L, PLs and APLs

^{31}P , ^{13}C , ^1H , and 2D-HSQC NMR spectra were recorded on a Bruker AVIII 400 MHz spectrometer (Bruker, Karlsruhe, Germany). To quantify the OH groups of L and PLs, the quantitative ^{31}P NMR spectroscopy was performed as described in the published literatures [34,35]. In detail, a dried L or PL sample (20 mg) was dissolved in 0.5 mL of solvent (anhydrous pyridine and deuterated chloroform 1.6:1, v/v) at room temperature. After complete dissolution, 0.1 mL of cyclohexanol as an internal standard (IS) and 0.1 mL of chromium (III) acetylacetonate solution as a relaxation reagent were added successively. Then, the mixture was reacted with 0.1 mL of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) as the phosphorylating reagent. Finally, the mixture was vibrated for 10 min to ensure the completion of phosphorylation reaction and transferred to a 5 mm NMR tube for analysis. The acquisition parameters were pulse angle 30 °C, relaxation delay (d_1) 2 s, data points 64 K, and scan 2048. Besides, the ^1H and 2D-HSQC analyses were performed after dissolving 30 mg samples in 0.5 mL dimethyl sulfoxide- d_6 (L and PLs) or a solution with 0.5 mL D_2O and a few drops

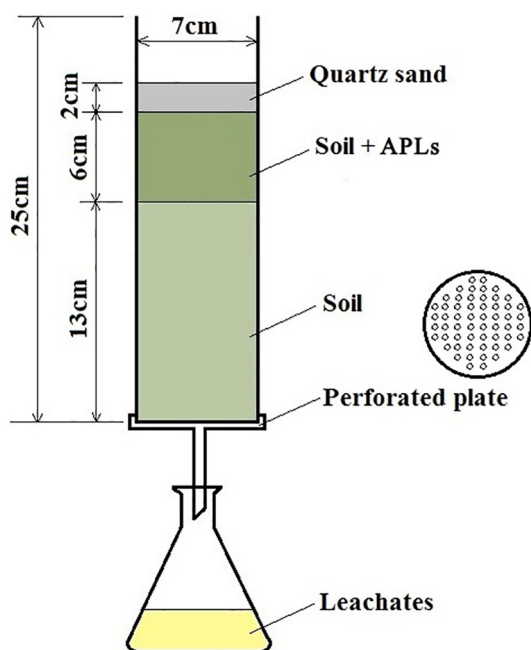


Fig. 1. The schematic drawing of equipment used in the soil leaching experiment.

Table 2
Quantitative analysis of L and PL by 2D-HSQC and ^{31}P NMR spectra.

	2D NMR S/G/H	^{31}P NMR (mmol/g)					Active sites ^a (mmol/g)
		Al-OH	S-OH	Gc-OH	Gn-OH	H-OH	
L	44/38/18	1.31	1.01	0.23	1.17	0.87	2.91
PL ₁	5/7/88	0.24	1.08	0.24	0.78	2.58	5.94
PL ₂	4/7/89	0.13	1.25	0.24	0.91	3.44	7.79
PL ₃	4/7/89	0.1	1.07	0.24	0.85	3.49	7.83
PL ₄	5/6/89	0.08	1.17	0.25	0.88	3.69	8.26

^a Active sites means the amount of available sites on the structure of L or PL₅ for the subsequent Mannich reaction.

of sodium deuteroxide (7.5 M NaOD) (APLs) according to the previous literature [36]. The ^{13}C NMR analysis of L and PL was also conducted after dissolving 140 mg samples in 0.5 mL DMSO- d_6 . Moreover, Fourier transform infrared spectra (FTIR) of the L, PLs and APLs were recorded on a Bruker TENSOR37 FTIR spectrometer (Bruker, Michigan, USA).

2.5. Thermogravimetric analysis of L and APLs

The Thermogravimetric (TG) and differential thermogravimetric (DTG) analysis were conducted by using a thermal gravimetric analyzer (TGA Q50, TA instruments, Delaware, USA). The samples were heated

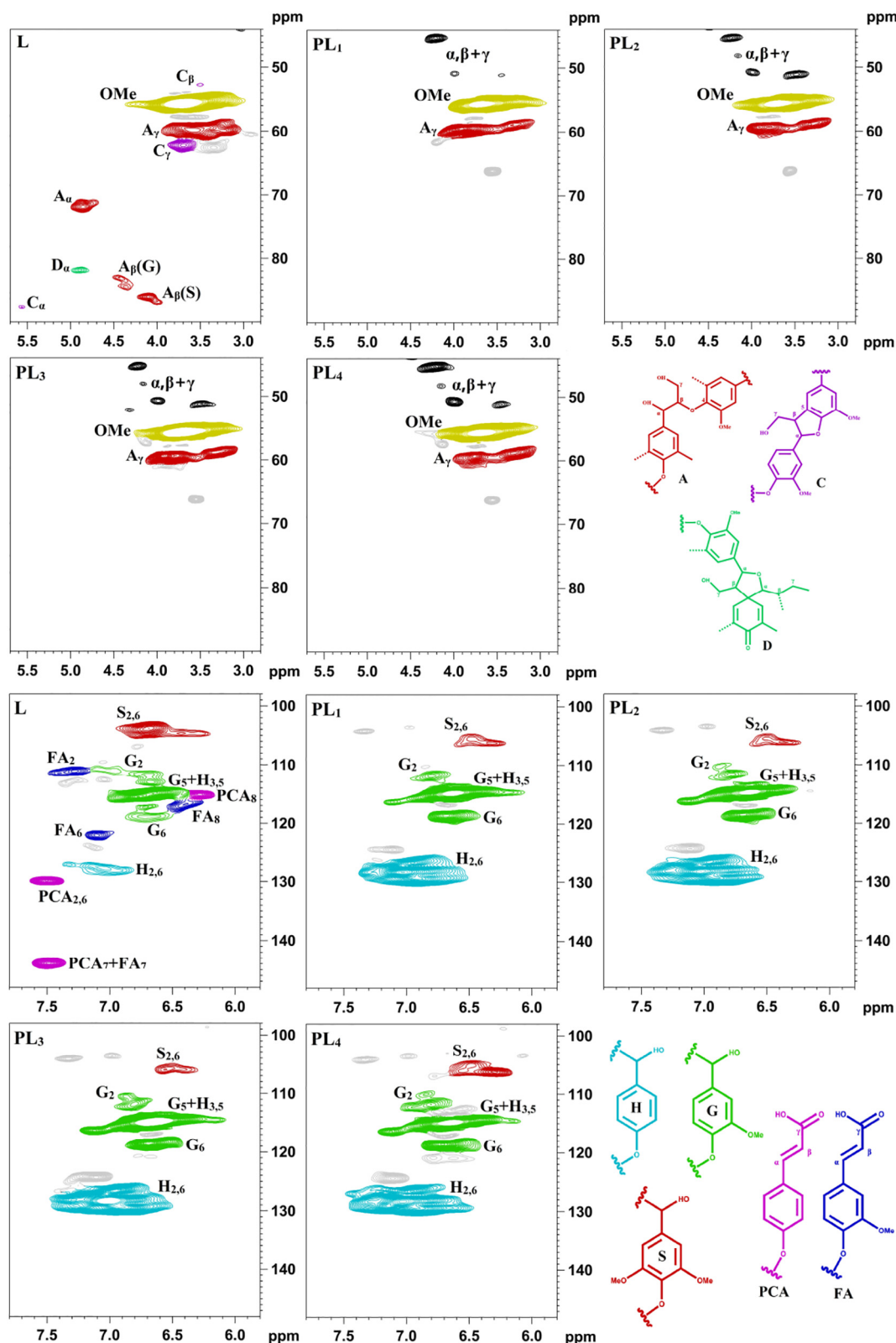


Fig. 3. The 2D-HSQC NMR spectra of L and PL₅.

from 30 °C to 700 °C at a heating rate of 10 °C/min under a highly purified nitrogen atmosphere.

2.6. Elemental analysis of APLs

The C, H and N contents of all APLs were examined in triplicate by a PerkinElmer 2400II elemental analyzer (Perkin Elmer, Massachusetts, USA). The final values are the averages of three measurements.

2.7. Soil column leaching experiments

In order to investigate the nitrogen release behavior of APLs in soil, the soil column leaching experiments were conducted according to previous literatures [37–39]. Simply, the soil was collected from the surface layer of farmland (0 cm to 20 cm depth) and dried at room temperature for 5 days after removing the roots of plants. Then, the soil was sieved by a 2 mm sieve. Subsequently, 700 g dry soil as the lower-layer soil was firstly filled into the bottom of Plexiglas column (inner diameter = 7 cm, height = 25 cm) and the rest dry soil (300 g) mixed thoroughly with APLs (treatment group) or urea (control group) was filled into the top of the Plexiglas column according to the designed soil bulk density (1.4 g/cm³) (Fig. 1). The equivalent dose of 100 mg N kg^{−1} dry soil was applied at different treatments. Moreover, the surface of each soil column was covered with a certain amount of quartz sand to minimize disturbance. Blank experiment (CK) without any fertilizers was also performed under the same conditions. Each treatment was repeated for three times.

Throughout the experiment, the soil water content was maintained at 75% field capacity by weighing and adding water if necessary and all the soil columns were incubated at ambient temperature. After 1, 4, 7, 14, 21, 28, 42, 56 days, 200 mL of deionized water were added slowly into each soil column and the leachates were collected in 250 mL conical flask. Then, the leachates were filtered and stored at −24 °C until

chemical analyses. The ammonium (NH₄⁺) and nitrate (NO₃[−]) content were determined by AutoAnalyzer 3 (Bran Luebbe, Hamburg, Germany) and their cumulative leaching amount were calculated to evaluate the nitrogen release behavior of APLs in soil. The final values are the averages of three repetitions.

3. Results and discussion

3.1. Phenolation of lignin

In order to increase the content of active sites and introduce more amine groups into the skeleton of lignin, the phenolation pretreatment of the biorefinery technical lignin was conducted under acidic conditions. Before the phenolation, the lignin was detailedly characterized by ³¹P, ¹³C and 2D-HSQC NMR analyses. These results showed there was a relatively high content of non-condensed guaiacyl phenolic hydroxyl groups (1.17 mmol/g) and *p*-hydroxyphenyl phenolic hydroxyl groups (0.87 mmol/g) in the L, which contain one and two active sites for Mannich reaction, respectively (Fig. 2 and Table 2). Therefore, the biorefinery technical lignin is an ideal raw material for the amination. After phenolation under different phenol/lignin feed ratios, the signals between 137.1 and 138.4 ppm, which represent for *p*-hydroxyphenyl phenolic hydroxyls, were found to be obviously stronger than that of L (Fig. 2), suggesting that the content of free *p*-hydroxyphenyl units was dramatically increased after phenolation process. On the contrary, as compared to the L, all the PL displayed a relatively weaker signal at 146–149 ppm originating from aliphatic hydroxyl groups, which was mainly ascribed to the substitution of aliphatic hydroxyl groups by phenol during phenolation [28,40], as showed in Scheme 1A. These phenomena demonstrated the phenol had been successfully grafted onto the side chain of lignin.

To obtain more evidences about the successful graft of phenol during the phenolation, the 2D-HSQC NMR analysis was conducted (Fig. 3).

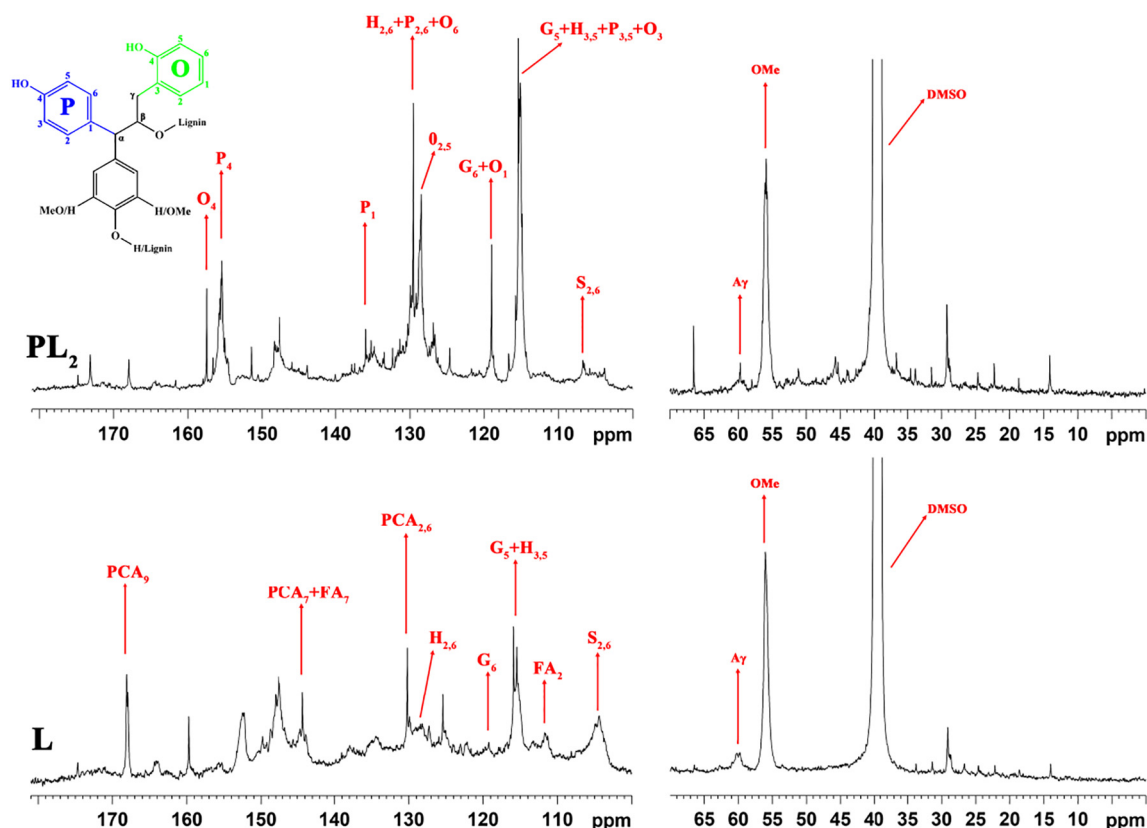


Fig. 4. The ¹³C NMR spectra of L and PL₂. P and O refer to the introduced groups via *para*-substitution and *ortho*-substitution of phenol, respectively.

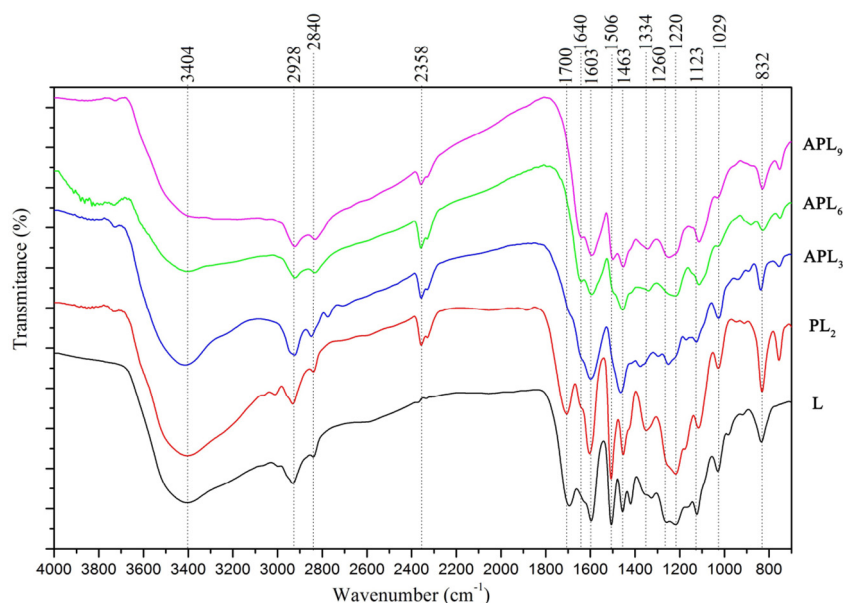


Fig. 5. FT-IR spectra of L, PL₂ and APL_s.

These signals in the spectra were assigned according to the previous publications [36,40]. From the side-chain region of L and PL, it could be clearly found that these signals referring to side-chain region (C_{α} - H_{α} , C_{β} - H_{β} , C_{γ} - H_{γ}) were mostly vanished and some new signal appeared at δ_C/δ_H 45–50/3.5–4.5 ppm in all the PL, which was due to that the phenolation occurred in side-chain region of lignin, then the substitution of functional group (e.g. hydroxyl) in the side-chain region by the phenol, electron-donating groups, enable the original signal (C_{α} - H_{α} , C_{β} - H_{β} , C_{γ} - H_{γ}) in side-chain region to shift to upfield [40]. Moreover, in the aromatic region of L and PL, a new signal corresponding to $C_{2,6}$ - $H_{2,6}$ in oxidized syringyl units located at δ_C/δ_H 104/7.35 ppm appeared in all the PL, which implied that a part of syringyl units would be oxidized under the given conditions. The disappeared signals derived from *p*-coumaric acid and ferulic acid in all the PL suggested that these compounds were removed and cleaved during phenolation process [41]. In addition, the intensity of signals related to *p*-hydroxyphenyl units in all the PL were significantly increased as compared to that of raw lignin (L), implying that the phenol has been successfully introduced to the lignin. Meanwhile, the signal attributed to normal $S_{2,6}$ was also shifted to downfield after phenolation, which may suggest that phenolation reaction occurred in side-chain region of lignin, thereby influenced the chemical environment of $S_{2,6}$ closest to side-chain region. These results were completely consistent with the aforementioned ^{31}P NMR analysis and demonstrated that the phenol had reacted with lignin successfully.

^{13}C NMR spectra were also given in Fig. 4 to provide some detailed information about the structural changes of lignin during phenolation. Firstly, some basic signals referring to S, G and H unit could be detected in the spectra, mainly including these signals at 152.3 ppm ($S_{3,5}$, etherified), 147.6 ppm ($S_{3,5}$, non-etherified), 137.8 ppm (S_4 , etherified), 125.4 ppm (S_4 , non-etherified), 104.4 ppm ($S_{2,6}$), 148 ppm (G_3 , etherified), 147.5 ppm (G_4 , etherified), 135 ppm (G_1), 119.2 ppm (G_6), 128.3 ppm ($H_{2,6}$), 115.5 ppm (G_5 and $H_{3,5}$) [36,41]. In addition, it could be easily found that these signals belonging to the aromatic carbon in the PL became stronger than those of L, especially these signals from $H_{2,6}$, G_6 , G_5 and $H_{3,5}$, which arose mainly from that these new signals assigned to the introduced groups overlapped partially with these original signals of lignin, such as $P_{2,6}$, O_6 and $H_{2,6}$, as well as $P_{3,5}$, O_3 and $G_5 + H_{3,5}$, as showed in Fig. 4. On the contrary, these signals at 168.1 ppm, 144.3 ppm, 130.2 ppm and 111.7 ppm are attributed to PCA_9 , $PCA_7 + FA_7$, $PCA_{2,6}$, and FA_2 , respectively, but completely cannot

be determined in the spectra of PL₂. An obvious shift of $S_{2,6}$ from 104.4 to 106.7 ppm indicated that some condensation occurred during acidic phenolation. Similarly, these signals from 60 ppm to 90 ppm referring to the side-chain carbon (C_{α} , C_{β} and C_{γ}) were also shifted to upfield (42–55 ppm) after phenolation, implying the attachment of phenol occurred in side-chain region of lignin. These results were in good agreement with the aforementioned 2D-HSQC analysis. Furthermore, some new signals at 157.5 ppm, 155.4 ppm, 135.9 ppm and 128.5 ppm appeared in the spectra of PL₂, which were originated from the introduced groups after phenolation. It was especially noted that the signal of P_4 at 155.4 ppm was stronger than that of O_4 at 157.5 ppm, indicating that both the *para*-substitution and *ortho*-substitution of phenol existed in the reaction, while the *para*-substitution was predominant, which was probably due to the smaller steric hindrance during the *para*-substitution [42].

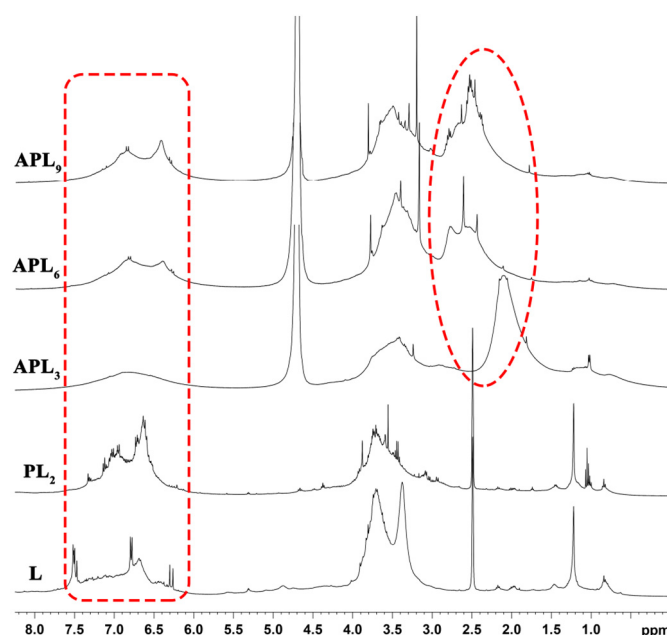


Fig. 6. The ^1H NMR spectra of L, PL₂ and APL_s.

To accurately quantify various hydroxyl groups and the active sites in all samples and explore the optimum phenolation condition, the quantitative data of L and PL from ^{31}P NMR analysis was given in Table 2. As showed in this table, the only effect of phenol/lignin feed ratio on the degree of phenolation was investigated in detail and the 110 °C, 20 min were directly chosen as the optimal reaction temperature and time according to previous literatures [33,40]. From this table, it could be obviously observed that the content of Al-OH was continually decreased in PL (from 1.31 mmol/g in L to 0.08 mmol/g in PL₄) with the increase of phenol/lignin feed ratio. Meanwhile, the content of H-OH exhibited a converse trend from 0.87 mmol/g in L to 3.69 mmol/g in PL₄. This fact implied that the degree of phenolation was gradually improved and more phenols could be introduced onto the structure of lignin by phenolation reaction. In addition, as compared to L, a slight increase in the contents of S-OH and G_C-OH as well as a decrease in the content of G_N-OH could be found in all the PL, implying a slight condensation reaction has occurred during the phenolation under the acidic conditions [43]. Furthermore, the amounts of active sites in L and PL were also calculated. As shown in Table 2, the amount of active sites was gradually increased in the PL since more phenols were used during the phenolation, which was paralleled to the content of H-OH. Nevertheless, it was also noteworthy that the active sites in the phenolated

lignin were only slightly increased after the feed ratio (phenol/lignin) reached to 2:1, suggesting that the reactive sites for phenolation reaction were nearly saturated in given condition. Thus, considering the economic cost of modification, the PL₂ instead of PL₄ was chosen for the subsequent amination.

3.2. Amination of the phenolated lignin by Mannich reaction

The APL was synthesized by Mannich reaction under the alkaline condition (Scheme 1B), and different amination reagents as well as proportions of reactants were used during the Mannich reaction (Table 1). The structural characteristics of the products were analysed by FT-IR and showed in Fig. 5. From the infrared spectra, it could be clearly found that these products exhibited some typical absorption peaks of lignin. For example, the wide peak at 3404 cm^{-1} is assigned to the hydroxyl groups in aliphatic and phenolic structures. The peaks at 2928 cm^{-1} and 2840 cm^{-1} originate from the C—H asymmetrical and symmetrical stretching vibrations in methyl and methylene structures, respectively [44–46]. Moreover, the peaks at 1603 cm^{-1} and 1506 cm^{-1} are attributed to the aromatic skeletal stretching vibrations. The peaks at 1463 cm^{-1} and 832 cm^{-1} derived from C—H bending and out-of-plane deform vibrations, respectively [46]. In the aromatic

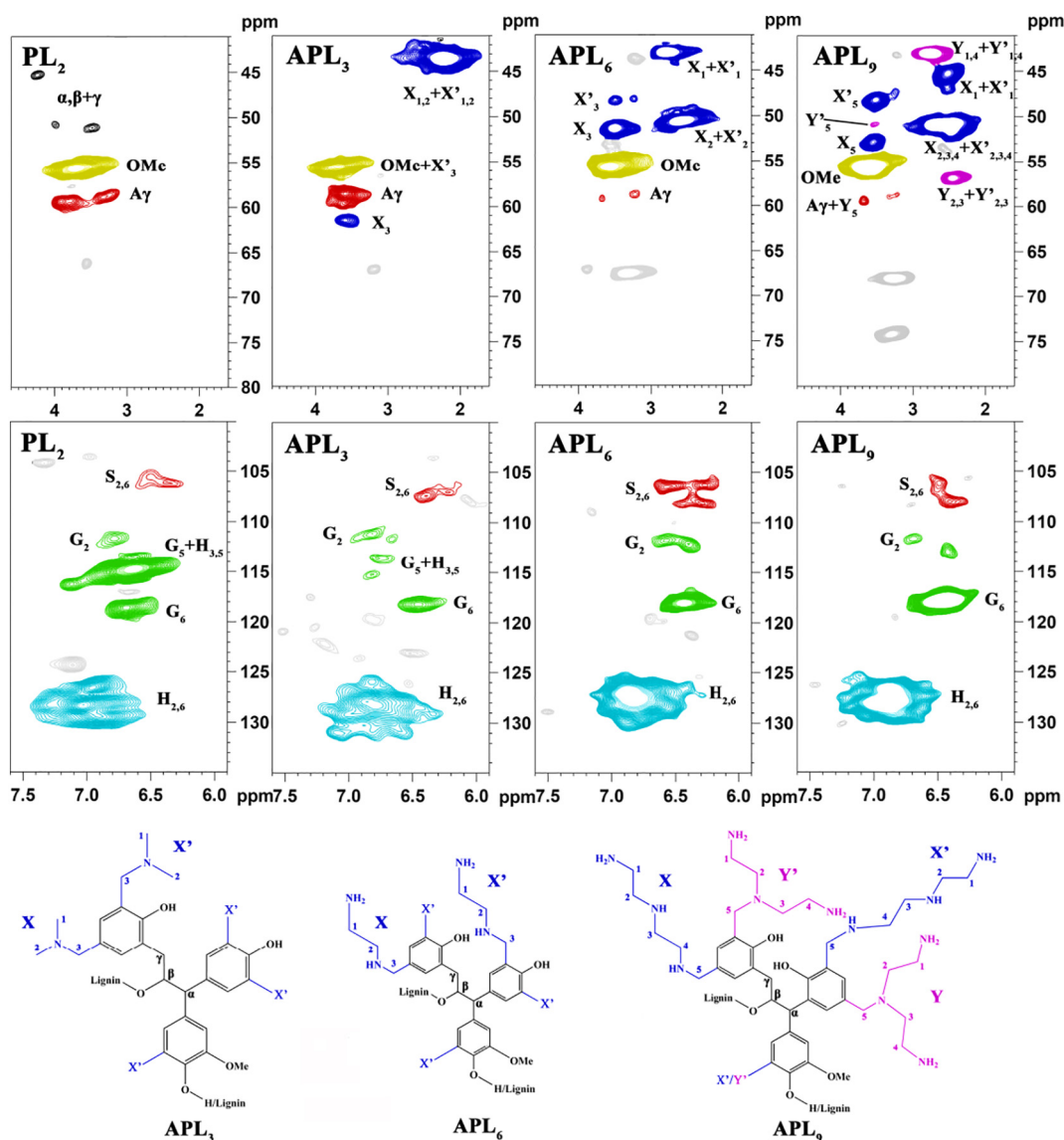


Fig. 7. The 2D-HSQC NMR spectra of PL₂ and APL₅.

structures, the peaks at 1260 cm^{-1} and 1220 cm^{-1} are assigned to the guaiacyl and syringyl structure, respectively, and the peak at 1123 cm^{-1} originates from ether bond in the lignin structure [21,30,41–43], suggesting that the skeleton structure of lignin was not destroyed during Mannich reaction. In addition, some obvious change in the intensity of peaks could be observed in the infrared spectra of these APL. For example, the intensity of peaks at 2928 cm^{-1} and 2840 cm^{-1} originating from the C—H stretching vibrations in methyl and methylene structures was significantly increased after Mannich reaction, especially in the spectra of APL₉. This was due to the successful introduction of amination reagent containing numerous methyl and methylene during Mannich reaction. Besides, since the Mannich reaction occurred at the aromatic region of lignin, the intensity of peaks referring to C—H vibrations from the aromatic skeleton of lignin was markedly decreased in the spectra of APL, such as 1603 cm^{-1} , 1506 cm^{-1} , 1463 cm^{-1} and 832 cm^{-1} . Furthermore, it was noted that a new peak appeared at 1640 cm^{-1} , arising from the N—H bending vibrations in —NH₂ structure [30], in the spectra of APL₆ and APL₉, implying that the amine had been grafted onto the lignin structure. However, this signal was absent in the spectra of APL₃, which was due to the absence of NH₂ structure in the amine group introduced to APL₃, as illustrated in Scheme 1.

¹H NMR analysis was also performed to provide more evidence about the successful introduction of amine groups. As showed in Fig. 6, an obvious increase in the intensity of signal from aromatic proton (6–8 ppm) was observed in the spectra of PL₂. Subsequently, this signal was dramatically reduced after Mannich reaction, implying these amination reagents had successfully reacted with aromatic ring of PL₂. Moreover, it should be noted that some new signals at 2–3 ppm assigned to the introduced amine groups could be found in the spectra of APL, which was consistent with the subsequent 2D-HSQC NMR analysis.

To further investigate the structural transformation of the lignin during Mannich reaction, the 2D-HSQC NMR analysis was carried out. As displayed in Fig. 7, some new signals arose in the side-chain region of these lignin derivatives after amination, which were mainly ascribed to the amine groups introduced during Mannich reaction. The result indicated that these amination reagents had been successfully grafted onto the phenolated lignin by Mannich reaction. Furthermore, based on the NMR spectra, it could be found that the Mannich reaction mainly occurred at the *ortho*- and *para*-positions of the phenolic hydroxyl groups. However, these signals referring to the *para*-substituted groups in all APL, e.g. X₃ in APL₆, were stronger than those of the corresponding *ortho*-substituted groups, e.g. X'₃ in APL₆, implying that the *para*-substitution was favored during the synthesis of APL, which was probably due to the smaller steric hindrance in *para*-positions of phenolic hydroxyl groups. Similarly, it is very noteworthy that there are two different amine groups in APL₉, namely X and Y, as exhibited in Fig. 7. Specially, X group holds distinctly dominant position in APL₉ as compared to Y group due to the smaller steric hindrance, which could be proved by the different intensities of the signals assigned to the X and Y. In addition, in the aromatic region of APL, these signals attributed to G₅ and H_{3,5} almost completely vanished and these signals of G₂, G₆ and H_{2,6} were also slightly shifted to upfield after amination. The result demonstrated that these amine groups were introduced to the G₅ and H_{3,5} position of the phenolated lignin by Mannich reaction, which is in good agreement with the previous report [25].

3.3. The thermogravimetric analysis of aminated lignin

The TG and DTG curves of L, APL₆ and APL₉ were depicted in Fig. 8. From the curves, it could be found that the weight loss trends of aminated lignin were similar to the original technical lignin, which was consistent with the previous report [30]. During the first stage (before $150\text{ }^{\circ}\text{C}$), the weight loss of aminated lignin was attributed to the evaporation of residual moisture. Then, when the temperature was

raised from $150\text{ }^{\circ}\text{C}$ to $300\text{ }^{\circ}\text{C}$, the low molecular weight lignin fragment would decompose and evaporate from these samples [30,47]. As shown in the DTG curves, two peaks of weight loss rate could be observed at $213\text{ }^{\circ}\text{C}$ ($-1.3\%/min$) and $255\text{ }^{\circ}\text{C}$ ($-1.6\%/min$) for L in this stage. However, the thermal decomposition rates of all aminated lignin were lower than that of L, and the maximum weight loss rate were $-1.2\%/min$ at $264\text{ }^{\circ}\text{C}$ for APL₆ and $-1.1\%/min$ at $256\text{ }^{\circ}\text{C}$ for APL₉ in this stage, which may result from the loss of low molecular weight lignin fragment during the dialysis of aminated lignin. The third pyrolysis stage occurred at $300\text{ }^{\circ}\text{C}$ – $500\text{ }^{\circ}\text{C}$ and the maximum weight loss rates of L, APL₆ and APL₉ were attained, respectively, at $351\text{ }^{\circ}\text{C}$ ($-3.3\%/min$), $361\text{ }^{\circ}\text{C}$ ($-2.3\%/min$) and $345\text{ }^{\circ}\text{C}$ ($-2.7\%/min$), which was due to the cleavage of inter-unit linkages of lignin and the evaporation of monomeric phenols [48]. At the last stage (above $500\text{ }^{\circ}\text{C}$), the only slight weight loss could be observed in the TG curves of all sample. The above results implied that the obtained APL contained more macromolecule and less low molecular weight lignin fragment, which contributed to improve the slow release effect of aminated lignin.

3.4. The elemental analysis of aminated lignin

The elemental analysis was also conducted to determine the nitrogen content of all APLs (Table 3). It was found that the nitrogen content of these products (APL₄ ~ APL₉) was significantly higher as compared with that of APL₁, APL₂ and APL₃, which was ascribed to the factor that an ethanediamine or diethylenetriamine molecule contains more nitrogen element than a dimethylamine molecule. Nevertheless, when more ethanediamine or diethylenetriamine were employed during the synthesis process of APL, only slight increase in the nitrogen content of the product could be observed, which was probably attributed to the nearly saturated active sites (G₅ and H_{3,5}) as revealed by the aforementioned 2D-HSQC analysis. In short, these results revealed the type of amination reagent was a more crucial factor than the proportion of reactants to affect the nitrogen content in the products. Furthermore, it was surprised to note that the APL₅ and APL₆ possessed higher nitrogen content than that of APL₉, and the highest nitrogen content (9.65%) appeared in APL₆. Therefore, it could be speculated that ethanediamine is

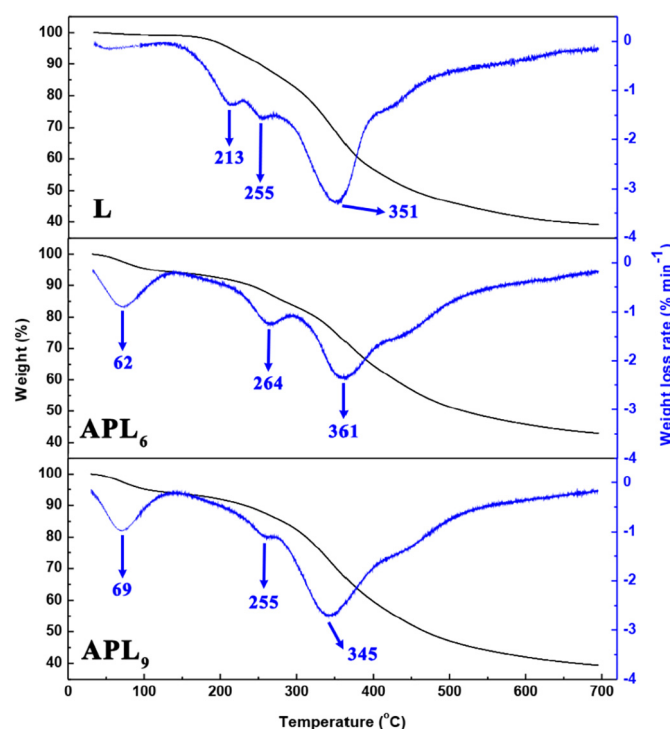


Fig. 8. TG and DTG curves of L, APL₆ and APL₉.

Table 3
The element analysis of APLs.

Samples	Elements contents (%)			C/N
	C	H	N	
APL ₁	64.40	6.93	5.36	12.01
APL ₂	64.34	7.00	5.44	11.83
APL ₃	64.46	7.08	5.42	11.89
APL ₄	61.75	6.81	8.15	7.58
APL ₅	61.46	7.11	9.51	6.46
APL ₆	62.02	6.26	9.65	6.43
APL ₇	60.89	6.53	8.31	7.33
APL ₈	60.41	6.92	8.85	6.83
APL ₉	63.30	6.51	9.25	6.84
APL ₁₀	61.63	6.15	10.13	6.08
APL ₁₁	61.78	6.02	10.18	6.07
APL ₁₂	62.93	6.99	9.53	6.61
APL ₁₃	63.37	6.11	9.62	6.59

probably the most ideal amination reagent for the synthesis of high N-containing aminated lignin as compared to diethylenetriamine and other long-chain aliphatic amines. In addition, a higher reaction temperature and longer reaction time were also employed during Mannich reaction to investigate their effect on nitrogen content of product. As shown in Table 3, the nitrogen content of APL₁₀ had a noteworthy increase as compared with that of APL₅. However, only a slight increase in the nitrogen content of product could be detected with the further increase of reaction temperature from 70 °C (APL₁₀) to 80 °C (APL₁₁). Besides, no significant difference could be observed among the nitrogen contents in the APL₅, APL₁₂ and APL₁₃, suggesting that the longer reaction time had slight effect on the nitrogen content of product. Based on the above analysis, an optimum chemosynthesis condition was

obtained, namely a feed ratio of 1:3:4 (lignin/ethanediamine/formaldehyde) at 70 °C for 3 h during the Mannich reaction.

It was reported that the lignin-based product could be easily degraded by soil microorganism and then release the organic bound-nitrogen when its C/N ratio is lower than 20 [21]. Thus, to evaluate the biodegradability of these APL in soil, the C/N ratios were calculated and listed in the Table 3. The result displayed the C/N ratios of all the products were much lower than 20, ranged from 6.07 to 12.1, indicating that these APLs possessed an excellent biodegradability and could efficiently release nitrogen in the soil to meet the nutrient requirements of plants.

3.5. The nitrogen release behavior of aminated lignin in soil

The nitrogen release behavior of aminated lignin in soil was also investigated by the soil column leaching experiment. As shown in Fig. 9, the cumulative leaching amount of NH_4^+ was far less than that of NO_3^- in all treatments, which was due to the loss of NH_4^+ by volatilization of NH_3 or a fast oxidation from NH_4^+ to NO_3^- by nitrification in soil [38]. Moreover, it could be found from the Fig. 9 that when the urea was used as the nitrogen fertilizer, most of nitrogen was released quickly into the soil environment within 28 days, which was not synchronous with the nutritional demand of the plants, thereby possibly resulting in not only serious loss of nitrogen by leaching and volatilization but also the nitrogen starvation of crops in the later growth stage [19,22,38]. In addition, only slight difference about the cumulative leaching amount of total nitrogen could be observed between the aminated lignin and blank group (CK) in the first 28 days. This phenomenon suggested that the obtained aminated lignin included less low molecular weight fragment with poor slow release effect, which was in good agreement with the TG analysis. Furthermore, it was especially

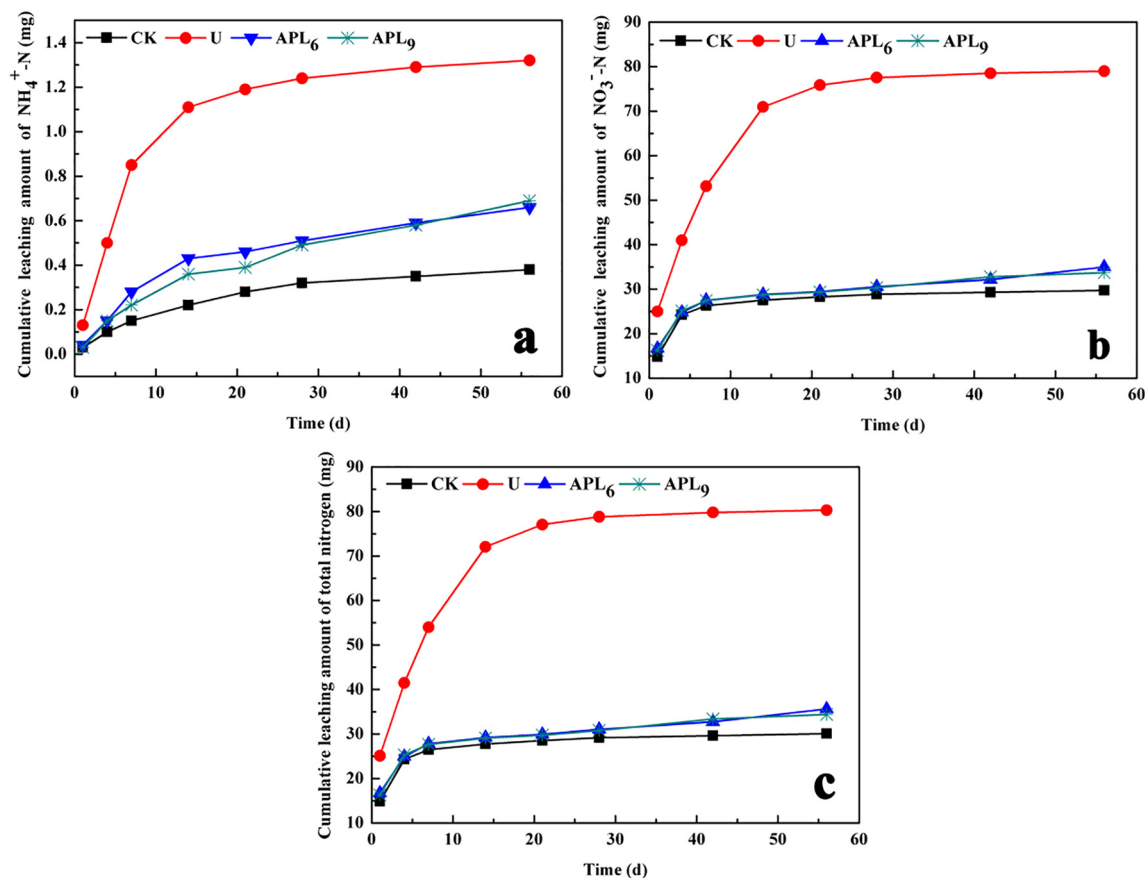


Fig. 9. The nitrogen release behaviors of blank experiment (CK), pure urea particles (U) and aminated lignin (APLs) in soil. (a) Cumulative leaching amount of NH_4^+ -N; (b) Cumulative leaching amount of NO_3^- -N; (c) Cumulative leaching amount of total nitrogen.

noteworthy that the cumulative leaching amounts of NH_4^+ and NO_3^- from treatments groups (APLs) were gradually increased after 28 day, while only a slight change could be found in the control group (U) and black experiment (CK) at the same stage. These results indicated the aminated lignin began to degrade and release slowly the nitrogen in soil environment after 28 days, thus could supply nutrient for the crop in a long period, which is complementary with pure urea. Therefore, it is believed that the obtained aminated lignin has a great potential to be used as the controlled release component in the preparation of lignin-based coated urea fertilizer and matrices fertilizer [19,25–27], which would facilitate the value-added application of biorefinery technical lignin.

4. Conclusions

The present study showed that the chemical reactivity of lignin was significantly improved by phenolation. The type of amination reagent instead of the proportion of reactants was the main factor for influencing the nitrogen content of the product during Mannich reaction. Additionally, ethanediamine is regarded as the most favorable among these selected amination reagents. Furthermore, an optimal product with high nitrogen content (10.13%) and low C/N ratios (6.08) was obtained under a feed ratio of 1:2 (lignin/phenol) at 110 °C for 20 min during the phenolation and a feed ratio of 1:3:4 (lignin/ethanediamine/formaldehyde) at 70 °C for 3 h during the Mannich reaction. In addition, it was especially noteworthy that the prepared APL in this study has a favorable nitrogen release behavior in soil. Therefore, it is believed that this study could facilitate the application of lignin in fertilizer industry, thereby extend the lignin valorization in the biorefinery process.

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Notes

The authors declare no competing financial interest.

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