Green Chemistry

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/greenchem

Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

The Degradation of Lignin in *Phyllostachys Heterocycla* cv. var. *Pubescens* in Ethanol Solvothermal System

Libin Hu[a], Yiping Luo[a], Bin Cai[a], Jianmei Li[a], Dongmei Tong[a], Changwei Hu[a]*

Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

The degradation of lignin to small molecules is significantly important for the use of biomass to produce biofuel as the C/O or H/O ratio in lignin is much higher than those in cellulose and hemicellulose. The present work studied the selective degradation of lignin in *Phyllostachys heterocycla* cv. var. *pubescens* in ethanol solvothermal system. The results revealed that 45.3% of lignin was degraded at 220 °C for 2 h, while significant conversion of hemicellulose and cellulose was avoided. The cleavage of β -O-4 linkage was found to be responsible for the dissolution of lignin from *Phyllostachys heterocycla* cv. var. *pubescens* into the liquid phase. The cracking of phenylpropyl side-chain C-C bonds followed the order: C_α-C_β, C_1-C_α, C_β-C_γ. The maximum yield of 10.6 wt% to 4-ethyl phenols based on the converted lignin in *Phyllostachys heterocycla* cv. var. *pubescens* was obtained under optimized condition.

Introduction

With the decrease of energy resources and worldwide deterioration of environment, the interests to utilize biomass for the production of energy materials and chemicals are increasing recently. Lignocellulosic materials constitute the bulk of the dry weight of woody and grassy plant, and as such are amongst the most abundant biochemicals on the earth [1,2]. Lignocellulosic biomass is mainly composed of three components: cellulose, hemicellulose and lignin. The conversion of all the components could be realized by direct pyrolysis where only low-quality bio-oil mixture that has high oxygen and water content was obtained, which cannot be used in conventional gasoline and diesel fuel engines [3]. To obtain high-quality biofuel, selective conversion of these components could be a promising way. Fermentation is especially suitable for the production of ethanol from the carbohydrates in biomass. The chemical conversion of carbohydrates, which involves mainly the deoxygenation via dehydration and/or decarboxylation followed by condensation to produce biofuel, attracts widely interest [4,5]. In the researches on the conversion of carbohydrates, lignin is needed to be removed generally from the raw biomass materials firstly to facilitate the further processing. That is, by decreasing the content of lignin, the recalcitrance of lignocellulose to chemical or enzymatic digestion to biofuel is highly loose[6-8]. Therefore, the delignification of biomass keeping the carbohydrates unchanged is of significant importance. On the other hand, lignin is a better source for the production of biofuel because more C and H are contained with less O content compared to cellulose and hemicellulose. That is, the H/C_{eff} ratio for lignin as defined in Eq. 1, which represents the effective H/C ratio after deoxygenation in the way of dehydration, is much higher than that for cellulose and hemicellulose [3]. Thus, it could be the most promising material for the production of bio-fuel. The utilization of lignin could take full advantage of raw biomass without residual waste.

$$H/C_{eff} = \frac{moles\ of\ H - (2 \times moles\ of\ O)}{moles\ of\ C} \qquad (1)$$

Lignin is a complex plant-derived biopolymer that accounts for 25~35% of all terrestrial biomass, with a molecular structure largely composed of phenylpropanoid residues derived from three hydroxycinnamyl alcohol-based monomers (*p*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol) [9-12]. Due to the structural complexity, lignin was known as the most recalcitrant to be decomposed. The linkages in lignin can be classified into two broad groups, namely, aryl ether bonds (C-O-C) containing β-O-4, α-O-4, 4-O-5 and condensed bonds (C-C) containing β-β', β-1, β-5, α-1, 5-5', *etc* [13]. A large number of studies on the dissociation of dimeric [14-16], trimeric [17,18], tetrameric [19,20] model compounds and extracted lignin [21,22] have been reported for the upgrading refinery of bio-oil through hydrodeoxygenation [23,24]. Generally, the aryl ether bonds, especially for β-O-4, accounted for the main parts and degraded prior to the condensed bonds [16]. Three types of C-C bonds, that is, C_1-C_α, C_α-C_β and C_β-C_γ bonds, existed in the phenylpropyl group and behaved differently during degradation [19]. Specific phenols containing different carbon numbers could be obtained through controlling the cleavage of the C-C bonds in phenylpropyl group. It was reported that the C_α-C_β bond was the weakest and could be cracked to form aldehyde group [18]. However, the investigation of the cracking of C_1-C_α and C_β-C_γ bonds was rarely reported. Based on the researches on model compounds and pure lignin, the investigation of the dissociation and degradation of lignin in actual biomass could provide more information concerning the dagradative rules and more guiding significance for the ultilization of lignin to obtain

value-added chemicals.

Phyllostachys heterocycla cv. var. *pubescens* (short for *pubescens* afterwards) is a kind of typical lignocellulosic biomass which consisted of hemicellulose, cellulose and lignin. In this work, ethanol solvothermal process was used to study the degradation of lignin in *pubescens*, with objectives to simultaneously extract and decompose the lignin in *pubescens* and make clear the mechanism of the decomposition of lignin in actual biomass.

Experimental

Materials

Pubescens, purchased from Anji country of Zhejiang province in China, was ground to 80 meshes and washed by distilled water for three times. Before use, it was dried in an oven over night at 110°C. The elementary composition was shown in Table S1. The anhydrous ethanol and other reagents used in the experiment were all purchased (AR) and used without further purification.

Ethanol solvothermal process

The selctive degradation of lignin in *pubescens* was carried out in a 250 ml stainless steel autoclave reactor equipped with a temperature controller and a stirrer, which was performed for several aging experiments under similar conditions to reduce the instrumental error before being used. In a typical run, 5 g of *pubescens* and 100 ml ethanol were loaded in the reactor. Then the reactor was sealed and the inner air was replaced by nitrogen. The initial pressure was added to 2.0 MPa with nitrogen. The reactor was heated from room temperature to desired temperature (160-240 °C) in 0.5 hours and hold for 2 hours. The reaction time was optimized in the range of 1-8 hours at 240 °C. Afterwards, the reaction was quenched rapidly by cooling water. After being cooled down to room temperature, the reactor was opened and the mixture was poured out and filtered. The filtrate was rotary-evapored to constant weight. The obtained thick liquid was noted as liquid product (LP). The solid residue (SR) was washed by ethanol for three times and then dried overnight at 110 °C. For the second-step degradation of lignin derivatives, the liquid fraction was loaded into the autoclave for a second run. The procedure of the second run was similar to the first run. Higher temperature was conducted ranging from 220-300 °C for the second run, while the effect of reaction time was investigated at 280 °C.

Chemical Titration.

Classical chemical titration methods were used to analyze the variation of the content of the three main components in the solid residues obtained under different reaction conditions. The details of the titration could be found in the references [2,25]. The average deviation of the titration is less than ±0.5 wt%.

Determination of cellulose: When biomass powder was treated with nitric-acetic acid mixture under heating, intercellular substance was dissolved and cellulose was separated to single fiber. While lignin, hemicellulose and other substance were also removed. Starch and pentosan were hydrolyzed. After the impurities were removed with water, cellulose was oxidized by $K_2Cr_2O_7$ with H_2SO_4 to carbon dioxide and water.

$$C_6H_{10}O_5 + 4K_2Cr_2O_7 + 16H_2SO_4 = 6CO_2 + 4Cr_2(SO_4)_3 + 21H_2O$$

The remainder of $K_2Cr_2O_7$ was titrated by $(NH_4)_2Fe(SO_4)_2$ solution. The same amount of $K_2Cr_2O_7$ never reacted with cellulose was also titrated by $(NH_4)_2Fe(SO_4)_2$ solution. Then, the content of cellulose was determined based on the difference value of $(NH_4)_2Fe(SO_4)_2$ solution.

$$K_2Cr_2O_7 + 6FeSO_4 + 7H_2SO_4 = 3Fe_2(SO_4)_3 + Cr_2(SO_4)_3 + K_2SO_4 + 7H_2O$$

Determination of hemicellulose: Starch and other water-soluble carbohydrates, which would disturb the determination of hemicellulose, was removed by boiling 80% $Ca(NO_3)_2$ solution. After washed with water, the remainder was treated by HCl with high concentration to hydrolyze hemicellulose to a sugar solution. After neutralized with NaOH, total sugar content was determined by copper-iodine method. Sugar from the hydrolysis of hemicellulose reduced Cu（Ⅱ) to Cu_2O. Through determination of Cu_2O content, the content of hemicellulose was determined. Adding sulfic acid to the solution, KIO_3 and KI both contained in the alkaline copper solution would react and release iodine under acid condition.

$$KIO_3 + 5KI + 3H_2SO_4 = 3I_2 + 3K_2SO_4 + 3H_2O$$

After oxalic acid added, iodine reacted with Cu_2O.

$$Cu_2O + I_2 + H_2C_2O_4 = CuC_2O_4 + CuI_2 + H_2O$$

The remainder of iodine was titrated by $Na_2S_2O_3$ solution.

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$

Determination of lignin: After sugar, organic acid and other soluble compounds were separated by 1% acetic acid, chlorophyll, fat and other fat soluble compounds were separated by acetone. After washed by water, lignin was oxidized by $K_2Cr_2O_7$ with H_2SO_4.

$$C_{11}H_{12}O_4 + 8K_2Cr_2O_7 + 32H_2SO_4 = 11CO_2 + 8K_2SO_4 + 8Cr_2(SO_4)_3 + 32H_2O$$

The remainder of $K_2Cr_2O_7$ was titrated by $(NH_4)_2Fe(SO_4)_2$ solution, which was the same with the determination of cellulose.

FT-IR analysis.

The FT-IR spectra were recorded on a Nicolet Nexus 670 fourier transform infrared spectrometer in the rang of 4000-400 cm^{-1} with a resolution of 2 cm^{-1}. 1 mg dried samples were blended with 100 mg KBr and pressed into thin pellets.

^{13}C CPMAS NMR.

The ^{13}C CPMAS solid-state NMR experiments were carried out at room temperature with a BRUKER ADVANCE Ⅲ 500 MHz instrument. Spectra were acquired with a 4 mm Bruker CPMAS probehead, using the combination of cross-polarisation (CP), magic angle sample spinning (MAS) and high-power proton decoupling methods. A total of 800 scans were accumulated for each sample. The spinning rate was 37878 Hz, and the relaxation delay was 5 s. Spinal 128 decoupling was used during acquisition.

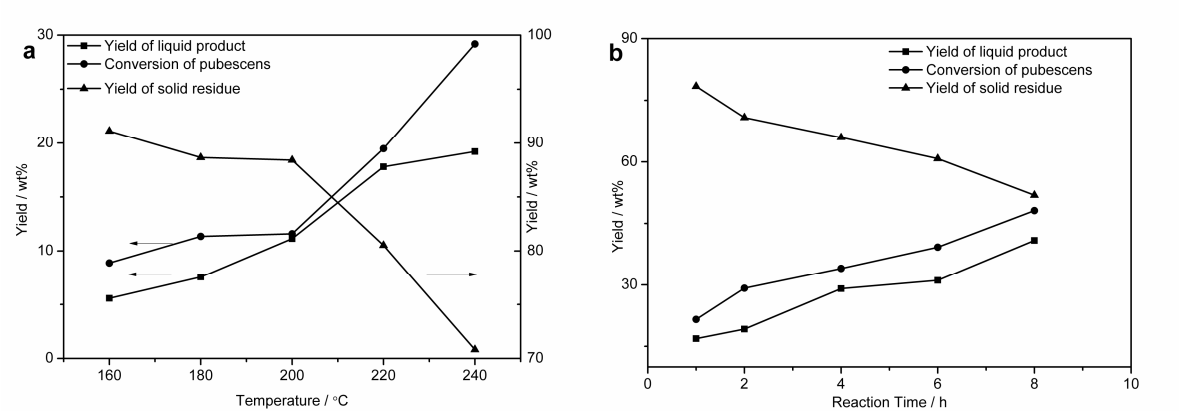


Fig. 1. Effect of reaction temperature and time on the conversion of *pubescens*, the yield of liquid product and solid residue. (a) ethanol solvothermal reactions at different temperature in 2 h, (b) ethanol solvothermal reactions for different time at 240 °C.

Qualitative 2D HSQC NMR analysis.

The 2D HSQC NMR spectra of liquid products were recorded on a BRUKER ADVANCE 400 MHz spectrometer. About 50 mg sample was dissolved in 0.5 ml deuterated dimethyl sulfoxide (DMSO-d_6). Heteronuclear single quantum coherence (HSQC) experiments had the following parameters: aquired 10 to 0 ppm in F2 (^{1}H) dimension using 1024 data points for an acquisition time of 64 ms, 220 to 5 ppm in F1 (^{13}C) dimension using 256 data points, 1.5 s pulse delay, $^1J_{C\text{-}H}$ of 145 Hz, and 16 scans. The solvent peak (δ_C 39.5 ppm and δ_H 2.5 ppm) was used for the chemical shift calibration.

GPC.

The molecular weight and distribution of liquid products were analyzed using the gel permeation chromatography (GPC). A Waters GPC device equipped with a 1515 pump, a Waters model 717 auto sampler, and a 2414 refractive index detector was used, with Waters Strygel columns HT4 and HT3 in series. Empower III GPC software was used for running the GPC instrument and for calculations. Both the columns and the RI detector were maintained at 45 °C in analysis. A rotary-evapored liquid product of 2 mg was dissolved in 1 ml THF and filtrated over a 0.45 μm Teflon filtrate pad. THF was used as the eluent at a flow rate of 1.0 mL/min and an injection volume of 30 μl was used. A calibration curve was obtained using monodisperse polystyrene standards.

GC-FID.

The liquid product was redissolved in acetone and detected by GC-FID quantitatively. The GC-FID (Fuli 9750) was equipped with an HP-innowax column (30m×0.25mm×0.25μm) and the temperature program was set as rising from 50 °C to 200 °C by 10 °C /min, holding for 5min, and then rising to 250 °C by 5 °C /min, holding for 15min. The products was identified qualitatively by GC-MS (with the same column and conditions with GC-FID) firstly, and the retention time was determined by standard substances. Phenylacetonitrile was used as internal standard to quantify the content of products. Every sample was tested for three times.

Result and discussion

The influence of solvothermal reaction temperature and time.

The conversion of *pubescens* at different solvothermal reaction temperature ranging from 160 °C to 240 °C in 2 h was carried out. As shown in the Fig. 1(a), the yield of liquid product increased continuously with the increase of temperature and 19.2 wt% liquid product was achieved at 240 °C. Moreover, the conversion of raw materials increased slowly below 200 °C and then accelerated rapidly at above 200 °C. About 11.6 wt% conversion of *pubescens* was obtained with up to 88.4 wt% residue remained at 200 °C. However, when the temperature raised to 240 °C, the residue decreased to 70.8 wt%. The results were similar to those reported by Hossein Mazaheri *et al* [26], where the conversion of oil palm fruit press fruit fiber (FPF) was 24.5% at 210 °C in 45 min and increased obviously with the increase of temperature. It suggested that it was more effective for the conversion of *pubescens* at higher temperature.

When the reaction time was extended from 1 h to 8 h, the conversion of *pubescens* at 240 °C gradually increased from 21.6 wt% to 48.1 wt %, as illustrated in Fig. 1(b). The yield of liquid product obtained was 40.9 wt% for 8 h reaction, while there was only 51.9 wt% residue. Thus, the extension of reaction time was in favour of the conversion of *pubescens* in ethanol system. Above all, the decomposition of *pubescens* was obviously influenced by the reaction temperature and time. In our previous work [27], *pubescens* conversion of 58.8 % was obtained under hydrothermal liquification process at 300 °C for 4 h. However, only 22.6 % yield of liquid product was achieved, which was similar to that at 240 °C for 2 h in this paper (19.2 wt%). It suggested that ethanol solvothermal process promoted the conversion of *pubescens* to liquid products under mild conditions.

The variation of the content of the three components.

The variation of the content of the three components, that is, hemicellulose, lignin and cellulose, in the raw materials and residues after reactions was summarized in the Table 1. The dried *pubescens* was composed of 17.9wt % hemicellulose, 46.6 wt% cellulose, and 25.4 wt% lignin. After the reactions in 2 h, the content of hemicellulose and lignin in the residues decreased

Table 1. Chemical titration of *pubescens* and solid residues.

Condition	Hemicellulose		Lignin		Cellulose	
	Remained ratio[a](wt%)	Conversion (%)	Remained ratio[a](wt%)	Conversion (%)	Remained ratio[a](wt%)	Conversion (%)
Pubescens	17.9	0	25.4	0	46.6	0
160 °C 2h	17.3	3.4	21.1	16.9	43.8	6.0
180 °C 2h	17.5	2.2	21.0	16.9	42.1	9.7
200 °C 2h	17.1	4.3	18.1	28.7	42.7	8.5
220 °C 2h	13.7	23.2	13.9	45.3	42.8	8.2
240 °C 2h	9.0	49.7	13.5	47.0	42.0	9.9
240 °C 1h	8.8	50.8	15.1	40.8	44.5	4.7
240 °C 4h	7.0	61.2	12.8	49.6	39.6	15.0
240 °C 6h	5.0	72.0	11.6	54.3	38.1	18.4
240 °C 8h	3.3	81.8	9.0	64.5	34.2	26.6

[a] Based on the weight of *pubescens* feedstock.

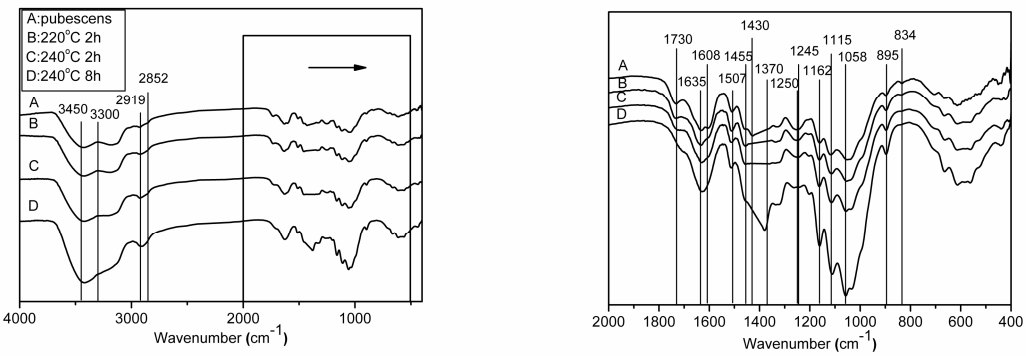


Fig. 2. The FT-IR spectra of solid samples. The left-hand is the whole spectra ranging from 400-4000 cm^{-1}. The right-hand is the partial spectra ranging from 400-2000 cm^{-1}. (A) *Pubescens*, (B) Residue from reaction at 220 °C for 2 h, (C) Residue from reaction at 240 °C for 2 h, (D) Residue from reaction at 240 °C for 8 h.

gradually with temperature. Significantly, the conversion of cellulose maintained at a value below 9.9 wt% for 2 h reactions over 160-240 °C temperature range. In our previous work [28], the conversion of hemicellulose, cellulose and lignin in *pubescens* was 99.0 wt%, 52.6 wt% and 24.6 wt% with hydrothermal decomposition at 220 °C for 30 min. It indicated that ethanol restrained the conversion of hemicellulose and cellulose, and accelerated the conversion of lignin. Due to the high solubility of lignin, the employment of small molecule alcohol as a solvent could increase the conversion of lignin [29].

As shown in Table 1, along with the increase of reaction time, the conversion of both hemicellulose and lignin increased obviously. Especially, the conversion of hemicellulose raised up to 81.8 % after 8 h reaction. It showed that longer reaction time promoted the decomposition of hemicellulose, and it was different from our previous work [28] due to the differnce of solvents, where little effect of reaction time on the conversion of hemicellulose was observed in water/cyclohexane system. Yueyuan Ye *et al.* [30] showed that the highest extractability of ~25% and delignification of ~35% were obtained with 65% ethanol from lignin-rich cornstalk residue obtained from ethanol production process. In this paper, a lignin conversion of 64.5% in *pubescens* was obtained. However, cellulose was also damaged obviously for longer reaction time at 240 °C. The reason for cellulose degradation would be the removal of hemicellulose and lignin which incorporated with cellulose in *pubescens* for its protection. The selective extraction of hemicellulose and lignin was further confirmed by XRD, and SEM (details in Fig. S1-S2). Thorsten vom Stein reported the highly integrated fractionation of soluble hemicellulose sugars, lignin and cellulose-pulp from beech wood in 2-methyltetrahydrofuran and water biphasic solvents with oxalic acid catalysis at mild temperature (80-140 °C) [31]. In this paper, 23.2% of hemicellulsoe and 45.3% of lignin in *pubescens* were converted with only 8.2% of cellulose converted at 220 °C for 2 h. Higher seperation efficiency was obtained under ethanol solvothermal treatment. Lignin derivates was enriched in liquid phase and carbonhydrates-riched solid residue was obtained meanwhile.

FT-IR analysis of *pubescens* and solid residues.

Figure 2 showed the Fourier transfrom infrared spectra (FT-IR) of *pubescens* and the solid residues. The dominant peaks of –OH, $-CH_3$ and $-CH_2$ groups at 3450-3300, 2919 and 2852 cm^{-1} were observed in all spectra. The main difference in the FT-IR spectra of the biomass components appeared at wavenumber below 2000 cm^{-1}. According to the literature [32,33], the peaks at 1370, 1250, 1162, 1115, 1058 and 895 cm^{-1} were assigned to the absorbance of cellulose. The characteristic peaks of hemicellulose appeared at 1730, 1608, 1430 and 1245 cm^{-1}, while those of lignin presented at 1635, 1507, 1455 and 834 cm^{-1}. The characteristic

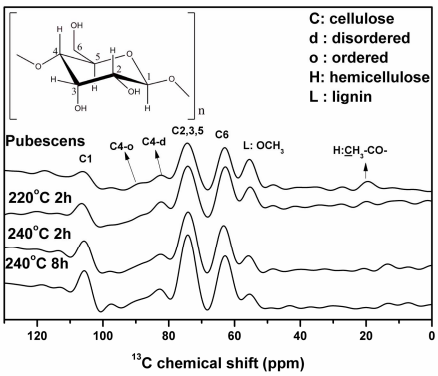


Fig. 3. ^{13}C CPMAS solid-state NMR spectra of solid samples. H represents the hemicellulose and the characteristic signal of hemicellulose is the methyl carbon of the acetyl group. L represents the lignin and the characteristic signal of lignin is the methoxyl carbon. C represents the cellulose and there are seven signals of cellulose carbons, as shown at the top-left corner of the spectra.

Table 2. Data of GPC analysis of liquid products.

Reaction conditions	*Mn* (Da)[a]	*Mw* (Da)[b]	Polydispesity[c]
180 °C 2 h	709	1532	2.16
200 °C 2 h	539	1254	2.33
220 °C 2 h	483	1091	2.26
240 °C 2 h	448	841	1.88
240 °C 1 h	477	957	2.01
240 °C 4 h	408	787	1.93
240 °C 6 h	391	749	1.92
240 °C 8 h	427	794	1.86

[a] *Mn* = number-average molecular weight, 1Da= $1/N_A$ g, N_A was Avogadro's Constant; [b] *Mw* = weight-average molecular weight; [c] Polydispersity = Mw/Mn.

absorbance of hemicellulose and lignin presented in *pubescens* weakened with the increase of reaction temperature and time, indicating that the content of hemicellulose and lignin decreased. The cellulose infrared absorbance of reside B, C and D was similar to that of *pubescens*, while the increasing of absorbance indicated the increase of the relative content of cellulose in residues after reactions. The variation of infrared absorbance between the raw matieral and residues reflected the changes in the contents of hemicellulose, cellulose and lignin, consistent with the chemical titration.

^{13}C CPMAS NMR of solid samples.

^{13}C CPMAS NMR spetra of wood samples presented signals corresponding to all the different carbons of the main wood components. Fig. 3 showed the ^{13}C CPMAS NMR spectra of *pubescens* and solid residues. According to the literature [34,35], the signals are assigned to differrent components as follows: methyl carbon of the acetyl group in hemicellulose at 21 ppm; aryl methoxy carbon in lignin at 56 ppm; cellulose carbon of C-6 at 63 ppm, C-2, C-3 and C-5 at 74 ppm, C-4 disordered at 84 ppm, C-4 ordered at 89 ppm, and C-5 at 105 ppm. Obviously, the signals assigned to hemicellulose decreased after ethanol treatment and vanished when temperature rised up to 240 °C, indicating the decrease of hemicelulose. Meanwhile, the decrease of the signals of methoxyl group showed the reduction of lignin. On the contrary, the resonance signals of C-1, C-2, C-3, C-5 and C-6 in cellulose increased obviously and it showed the increase of the relative amount of cellulose in residues after ethanol treatment. The results were in agreement with those obtained by chemical titration and FT-IR studies. In addition, the C-4 disordered and C-4 ordered signals were related to the amorphous and crystal structure of cellulose respectively. The decrease of C-4 ordered signal demonstrated the depletion of crystal cellulose in the reaction at 240 °C for 8 h.

Qualitative 2D HSQC NMR analysis of liquid fractions.

2D ^{13}C-^{1}H correlation HSQC NMR has been widely used to characterize lignin structures. To obtain a further comprehensive structural characterization of the liquid fraction, the liquid products were subjected to 2D HSQC NMR analysis. The assignment of the main cross-signals according to literature [36,37] was listed in Table S2. The spectra of the liquid products and the main substructures of lignin were presented in Fig. 4. As illustrated in Fig. 4 (a), a large amount of lignin intermediates and some xylose from hemicellulose were present in the liquid fraction. The HSQC spectra demonstrated that **S**-type and **G**-type were the main unit sturctures in aromatic C-H correlation region. In aliphatic C-H correlation region, the signals of **A**, **B** and **C** linkages decreased apparently, indicating the cracking of these linkages during the ethanol solvothermal treatment. The results showed that lignin substructure was further depolymeried at higher temperature and for longer time. Especially, the decrease of signals of **A** linkage was the most prominent, indicating that the cleavage of **A** linkage was the most predominant approach when lignin was dissociated from *pubescens* into liquid fractions. The analysis of HSQC NMR spectra suggested the enrichment of lignin intermediates and some xylose from hemicellulose in liquid product. A comparision of Fig. 4 (a), (b) and (c) showed that the signals of methoxyl group and xylose in liquid products increased clearly with the increase of reaction temperature and time. It revealed the decrease of methoxyl group in lignin and acetyl group in hemicellulose which was in accordance with the ^{13}C CPMAS solid-state NMR spectra of corresponding solid resiues. The results confirmed that more lignin and hemicellulose was cut down into the liquid fraction from the *pubescens*.

The main cleavage routes of lignin were displayed in Fig. 5. The cracking of lignin linkages, especially for the most important **A** linkage of lignin, would lead to the formation of phenolic hydroxyl group and phenylpropane unit, which was benefit to achieve more monomeric phenolic compounds. As shown in Fig. 3 (b) and (c), the decrease of correlation signals of C_α-H_α of **A**, **B** and **C** linkages indicated the cleavage of C_1-C_α bond, and it would lead to the production of phenol and methoxyl phenols. The decrease of C_β-H_β correlation signals reflected the cleavage of C_α-C_β bond, and it would produce the aromatic aldehyde group. The cleavage of C_β-C_γ bond caused the formation of ethyl phenolic compounds, which lead to the decrease of C_γ-H_γ correlation signals.

GPC analysis.

Many reports have proved that extracted lignin or organosolv lignin distributes a large molecular weight range. The liquid products obtained in this work had a lower molecular weight (*Mw* 749-1532 Da) than the reported ethanol organosolv lignin (*Mw*

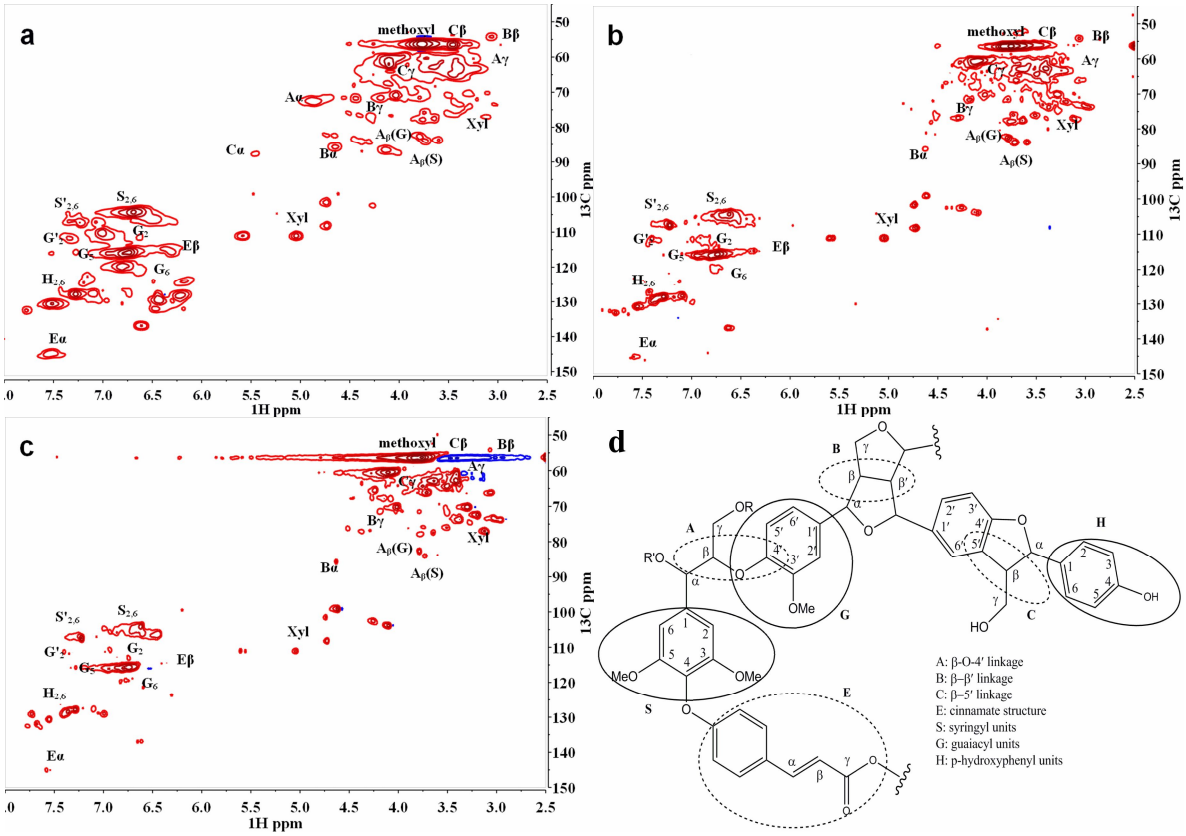


Fig. 4. The 2D HSQC NMR analysis of liquid products. (a) The HSQC spectra of liquid product from reaction at 220 °C for 2 h; (b) The HSQC spectra of liquid product from reaction at 240 °C for 2 h; (c) The HSQC spectra of liquid product from reaction at 240 °C for 8 h; (d) The hypothetical substructure of lignin.

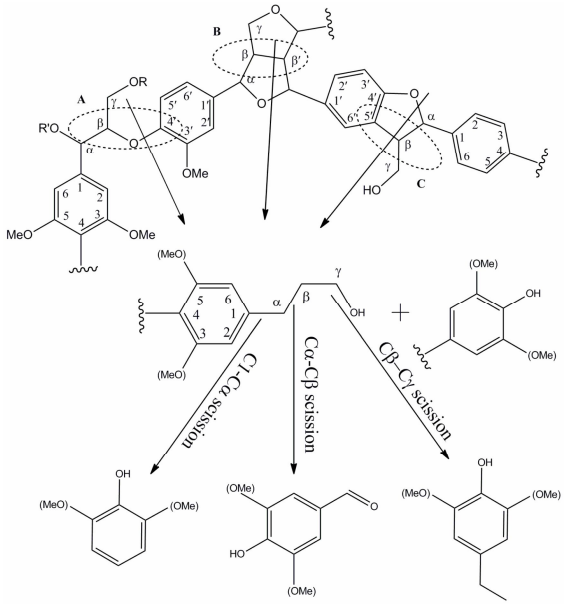


Fig. 5. Proposed degradative routes of lignin in *pubescens*. A, B and C represent the β-O-4, β-β' and β-5' lingkages respectively. Firstly, lignin undergoes the cleavage of the aryl ether bonds to form phenolic hydroxyl group and the phenylpropyl structure. Then, the phenylpropyl structure undergoes the scission of different C-C bonds to generate specific phenolic compounds.

4.2-5.1 kDa, 3.2-6.5 kDa, 5.1-5.7 kDa) [36,38,39], as shown in Table 2. The difference in molecular weight range could be due to the difference in raw matierials and reaction temperature. In this paper, when reaction temperature increased from 180 °C to 240 °C, *Mn* decreased from 709 Da to 448 Da, while *Mw* decreased from 1532 Da to 841 Da. *Mn* decreased to 391 Da and *Mw* decreased to 749 Da in the reaction at 240 °C for 6 h. The polydispersity was ranged from 1.86 to 2.33, suggesting a similar and narrow molecular weight distribution. It suggested that higher temperature and longer time promoted the depolymerization of the oligomer intermediates to small molecules. However, the molecular weight increased after 240 °C reaction for 8 h. This phenomenon was caused by the degradation of cellulose to oligosaccharides, as proved by the analysis of SR. The GPC results confirmed that the ethanol solvent was effective to selective conversion of lignin.

GC-FID analysis of liquid fractions.

Through the GPC results (details in Table 2), the main components in liquid products were oligomers, while the yield of monomers was very low. The phenolic compounds detected by GC-FID were classified into four types on the basis of the cleavage way (as described in Fig. 5), that is, benzofuran, phenols, 4-ethyl phenols and aromatic aldehydes. The influence of temperature on the yield of phenolic compounds was shown in the Fig. 6 (a). The detailed composition of these four types of

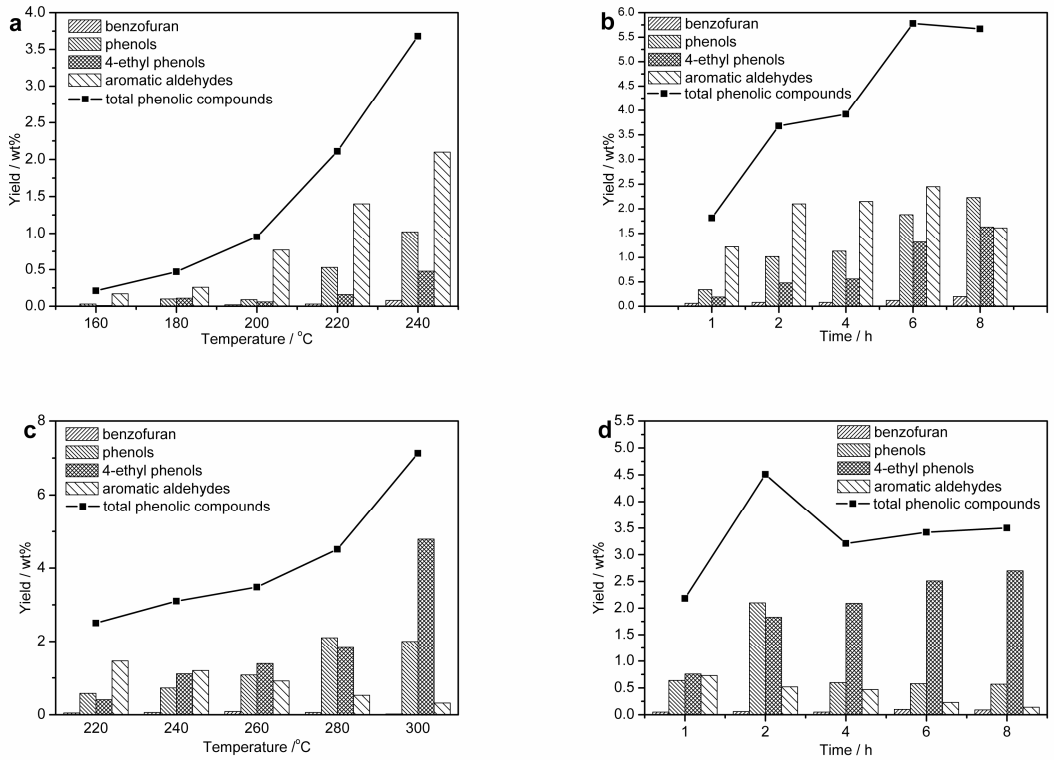


Fig. 6. The yield of phenolic compounds under different conditions detected by GC-FID. (a) One-step reactions of *pubescens* at different temperature in 2 h; (b) One-step reactions of *pubescens* at 240 °C in different time; (c) Second-step degradation at different temperature in 2 h of liquid fraction obtained at 220 °C for 2 h; (d) Second-step degradation at 280 °C in different time of liquid fraction obtained at 220 °C for 2 h.

phenolic compounds was described in supplementary information Table S3. The total yield of phenolic compounds increased with reaction temperature. Especially, The total yield of phenolic compounds increased rapidly when the reaction temperature was above 200 °C and the maximum yield of 3.7 wt% based on lignin in *pubescens* was obtained at 240 °C. The detected total phenolic compounds which were accounted for the majority in the aromatic products were associated with the broken of lignin linkage especially for **A** linkage. In a certain sense, the yield of total phenolic compounds could be taken for the degradative efficiency of **A** linkage. The cleavage of **A** linkage was predominant in ethanol solvothermal treatment of *pubescens*, which mainly occurred at about 200 °C and was promoted with the increase of temperature. The cracking of **A** linkage lead to the rapid increase of conversion of pubscens (as shown in Fig. 1 (a).) and the decrease of molecule weight of liquid products (as shown in Table 3.). Additionally, the aromatic aldehydes were the main products, which reflected the cracking of C_α-C_β bonds. The yield of aromatic aldehydes increased rapidly when the reaction temperature was higher than 200 °C, indicating that the cleavage of C_α-C_β bonds was highly promoted above 200 °C. When the temperature raised up to 220 °C, the yield of phenols increased obviously, which indicated the cleavage of C_1-C_α bonds. It suggested that the cracking of C_1-C_α bonds mainly occurred at above 220 °C. Subsequently, more 4-ethyl phenols were obtained with the increase of reaction temperature, indicating the increase of cleavage of C_β-C_γ bonds. The above data showed that the order of cracking of side-chain carbon bonds during the dissociation of lignin from *pubescens* was as follows: C_α-C_β, C_1-C_α, C_β-C_γ. Besides, because it was difficult to break C_1-C_α and C_β-C_γ bonds simultaneously for the formation of benzofuran, the yield of benzofuran was always very low.

Figure 6 (b) and Table S4 showed the influence of reaction time on the yield of phenolic compounds at 240 °C. The yield of total phenols rised up rapidly for longer time, and it demonstrated that longer reaction time was benefit for the cleavage of A linkage. The results were also coincided with the GPC results as shown in Table 2. Meanwhile, the cleavage of A linkage led to the increase of conversion of *pubescens*, as illustrated in Fig. 1 (b). Then, the slightly decrease of total yield would be due to other side reactions after reaction for 8 h, that is, decomposition or repolymerization. The results showed that higher temperture and longer time were benefit for the cracking of lignin bonds and for the enhancement of the yield of phenolic compounds, but longer time lead to other side reactions. However, the highest total yield of phenolic compounds was only 5.8 wt% based on the lignin in *pubescens* or 12.8 wt% based on the converted lignin. Significantly, the yield of aromatic aldehydes increased at first and then fell down as the reaction time extended. It has been proved that vanillin could be decomposed to guaiscol through decarbonylation [40]. Phenols became the predominant products in liquid phase when reaction time was extended to 8 h. The yield of 4-ethyl phenols and benzofurans also increased gradually with the increase of reaction time. Above all, the results confirmed that longer reaction time could enhance the cracking of lignin

linkages and phenylpropyl C-C bonds during the ethanol solvothermal treatment of *pubescens*.

Further degradation of liquid fraction.

According to the GPC and 2D NMR results, there were many lignin oligomers in the liquid fractions. With an attempt to further decompose the lignin oligomers to obtain more phenolic compounds, the further degradation of liquid fraction was carried out at higher temperature. The further degradation of the liquid fractions from the reaction at 220 ^{o}C for 2h was carried out, and the effect of reaction temperature on the further reactions was shown in Fig. 6 (c). The detailed composition of these four types of phenolic compounds was summarized in Table S5. With increased temperature, the yield of phenolic compounds increased obviously. The total yield of phenolic compounds raised up to 7.1 wt% at 300 ^{o}C for 2 h. The results showed that more phenolic compounds were achieved than the one-step reactions, suggesting the further decomposition of lignin linkages in lignin-riched liquid fraction. The yields of phenols and 4-ethyl phenols increased with rising temperature. Especially, a yield of 4.8 wt% to 4-ethyl phenols based on the lignin in *pubescens* was obtained at 300 ^{o}C for 2 h, that is, a yield of 10.6 wt% to 4-ethyl phenols based on the converted lignin was obtained. It indicated that the cleavage of C_β-C_γ bonds need higher temperature. 4.4% 4-ethyl phenols based on lignin had been achieved from hydrogenolysis of corn stalk lignin under ethanol/water system by Yueyuan Ye [41], and was considered to be approximate to the yield obtained from petrochemical route. In this paper, more 4-ethyl phenols were obtained under mild conditions. It showed that ethanol solvothermal process was an efficient routes to achieve phenolic compounds from lignin. Generally, the cracking of phenylpropyl C-C bonds was still following the order of C_α-C_β, C_1-C_α, C_β-C_γ. However, due to the instability of aromatic aldehyde group, phenols and 4-ethyl phenols were enriched and the yield of aromatic aldehydes decreased in further degradation of liquid fractions under higher temperature.

For the further degradation at 280 ^{o}C, as illustrated in Fig. 6 (d) and Table S6, when the reaction time was extended, the total yield of phenolic compounds increased firstly and then decreased. The reason would be that the side reactions were aggravated under higher temperature. Significantly, 4-ethyl phenols were enriched through further degradation with the reaction time prolonging. Especially, when the reaction was extended to 8 h, the cleavage of C_β-C_γ would be the main route of the degradation of lignin intermediates, and the content of 4-ethyl phenols was accounted for predominance. The results indicated that higher temperature was benefit to produce more phenolic compounds, while longer reaction time would lead to improved selectivity to specific phenols.

Above all, the two-step strategy, that is, firstly the lignin was dissolved at about 220 ^{o}C to the liquid phase, and then the further degradation of the liquid fraction at higher temperature prevented the conversion of carbohydrates (already being separated) and promoted the degradtion of lignin oligomers. The lignin component in *pubescens* was seperated from the carbohydrates and degraded to small molecular derivates mainly through the cracking of **A** linkage and C-C bonds in phenylpropyl. The cleavage of lignin bonds was enhanced and more phenolic compounds were obtained. The C_α-C_β bond was the weakest one and easy to be scissored, followed by the cleavage of C_1-C_α and C_β-C_γ bonds. Through controlling the cracking of lignin bonds, specific phenols could be obtained. Significantly, in the second-step degradation, the cracking of C_β-C_γ bond was highly promoted and the yield of 4-ethyl phenols was highly enhanced to 4.8 wt%, that is, 10.6 wt% based on the converted lignin. The results demonstrated that it was an efficient route to separately degrade the lignin in *pubescens* to specific value-added chemicals.

Conclusions

In this paper, lignin was obviously and selectively degraded with a small quantity of hemicellulose and cellulose converted at 220 ^{o}C for 2 h. The liquid fraction was mainly consisted of lignin oligomers and phenolic compounds. The cleavage of β-O-4' linkage was the predominent approach in the dissociation of lignin from *pubescens*. The cracking of the phenylpropyl C-C bonds was related to the reaction temperature and followed the order of C_α-C_β, C_1-C_α, C_β-C_γ. The results provided a significant guidance to the degradation of lignin in actual biomass. The second-step degradation of liquid fraction promoted the depolymerization of lignin oligomers and enhanced the selectivity to 4-ethyl phenols. Ethanol solvothermal process was an efficient method for degradation of lignin in *pubescens*.

Acknowledgements

This work is financially supported by the National Basic Research Program of China (973 program, No.2013CB228103) and the Special Research Fund for the Doctoral Program of Higher Education of China (No. 20120181130014). The characterization of the residue from Analytical and Testing Center of Sichuan University are greatly appreciated. The supervision of academician Qingshi Zhu is highly acknowledged.

Notes and references

[a] *Key Laboratory of Green Chemistry and Technology, Ministry of Education, College of Chemistry, Sichuan University, Chengdu, Sichuan, 610064, China. Fax: +86-28-85411105; Tel: +86-28-85411105; E-mail: changweihu@scu.edu.cn, gchem@scu.edu.cn*

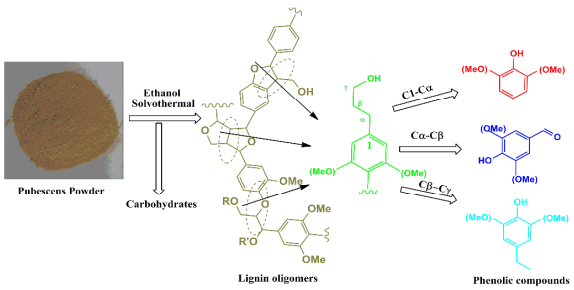
† Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

‡ Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

1 A. A. Peterson, F. Vogel, R. P. Lachance, M. Fröling, M. J. Antal Jr., J. W. Tester, *Energy Environ. Sci.*, 2008, **1**, 32.
2 W. Y. Qi, C. W. Hu, G. Y. Li, L. H. Guo, Y. Yang, J. Luo, X. Miao, Y. Du, *Green Chem.*, 2006, **8**, 183.
3 T. P. Vispute, H. Zhang, A. Sanna, R. Xiao, G. W. Huber, *Science.*, 2010, **330**, 1222.
4 E. L. Kunkes, D. A. Simonetti, R. M. West, J. C. Serrano-Ruiz, C. A. Gärtner, J. A. Dumesic, *Science.*, 2008, **322**, 417.
5 A. D. Sutton, F. D. Waldie, R. Wu, M. Schlaf, L. A. 'Pete' Silks III, J. C. Gordon, *Nature Chem.*, 2013, **5**, 428.
6 S. Y. Ding, Y. S. Liu, Y. Zeng, M. E. Himmel, J. O. Baker, E. A. Bayer, *Science*., 2012, **338**, 1055.
7 C. Chapple, M. Ladisch, R. Meilan, *Nat. Biotechnol*., 2007, **25**, 746.
8 F. Chen, R. A. Dixon, *Nat. Biotechnol*., 2007, **25**, 759.
9 K. David, A. J. Ragauskas, *Energy Environ. Sci.*, 2010, **3**, 1182.
10 J. M. W. Chan, S. Bauer, H. Sorek, S. Sreekumar, K. Wang, F. D. Toste, *ACS Catal*., 2013, **3**, 1369.

11 C. O. Tuck, E. Pérez, I. T. Horváth, R. A. Sheldon, M. Poliakoff, *Science*., 2012, **337**, 695.
12 C. Crestini, F. Melone, M. Sette, R. Saladino, *Biomacromolecules*., 2011, **12**, 3928.
13 F. S. Chakar, A. J. Ragauskas, *Ind. Crop. Prod*., 2004, **20**, 131.
14 A. Beste, A. C. Buchanan, *J. Phys. Chem. A.*, 2012, **116**, 12242.
15 B. Holmelid, M. Kleinert, T. Barth, *J. Anal. Appl. Pyrolysis*., 2012, **98**, 37.
16 R. Parthasarathi, R. A. Romero, A. Redondo, S. Gnanakaran, *J. Phys. Chem. Lett.*, 2011, **2**, 2660.
17 D. S. Argyropoulos, L. Jurasek, L. Krištofová, Z. Xia, Y. Sun, E. Paluš, *et al*. *J. Agric. Food Chem.*, 2002, **50**, 65.
18 C. Gardrat, R. Ruggiero, M. T. Rayez, J. C. Rayez, A. Castellan, *Wood Sci. Technol.*, 2013, **47**, 27.
19 T. Mester, K. Ambert-Balay, S. Ciofi-Baffoni, L. Banci, A. D. Jones, M. Tien, *J. Biol. Chem.*, 2001, **276**, 22985.
20 D. W. Cho, J. A. Latham, H. J. Park, U. C. Yoon, P. Langan, D. Dunaway-Mariano, P. S. Mariano, *J. Org. Chem.*, 2011, **76**, 2840.
21 C. L. Chen, E. A. Capanema, H. S. Gracz, *J. Agric. Food Chem.*, 2003, **51**, 1932.
22 P. R. Patwardhan, R. C. Brown, B. H. Shanks, *ChemSusChem*, 2011, **4**, 1629.
23 S. Crossley, J. Faria, M. Shen, D. E. Resasco, *Science*., 2010, **327**, 68.
24 A. G. Sergeev, J. F. Hartwig, *Science*., 2011, **332**, 439.
25 X. H. Починок, in *МЕТОДЫ БИОХИМИЧЕСКОГО АНАЛИЗА РАСТЕНИЙ*, ed. A. C. Оканенко, Издательство <Науква думка>, 1976, ch. 3, pp. 116-164.
26 H. Mazaheri, K. T. Lee, S. Bhatia, A. R. Mohamed, *Bioresour. Technol.*, 2010, **101**, 7641.
27 J. Luo, Y. Xu, L. J. Zhao, L. L. Dong, D. M. Tong, L. F. Zhu, C. W. Hu, *Bioresour. Technol.*, 2010, **101**, 8873.
28 Y. Xu, L. B. Hu, H. T. Huang, D. M. Tong, C. W. Hu *et al*. *Carbohydr. Polym.*, 2012, **88**, 1342.
29 C. Li, M. Zheng, A. Wang, T. Zhang, *Energy Environ. Sci.*, 2012, **5**, 6383.
30 Y. Ye, Y. Zhang, J. Fan, J. Chang, *Ind. Eng. Chem. Res.*, 2012, **51**, 103.
31 T. Stein, P. M. Grande, H. Kayser, F. Sibilla, W. Leitner, P. D. María, *Green Chem.*, 2011, **13**, 1772.
32 L. V. A. Gurgel, K. Marabezi, L. A. Ramos, A. A. S. Curvelo, *Ind. Crop. Prod.*, 2012, **36**, 560.
33 J. Long, X. Li, B. Guo, F. Wang, Y. Yu, L. Wang, *Green Chem.*, 2012, **14**, 1935.
34 P. Sannigrahi, D. H. Kim, S. Jung, A. Ragauskas, *Energy Environ. Sci.*, 2011, **4**, 1306.
35 T. Melkior, S. Jacob, G. Gerbaud, S. Hediger, L. Le Pape, L. Bonnefois, M. Bardet, *Fuel*., 2012, **92**, 271.
36 G. Hu, C. Cateto, Y. Pu, R. Samuel, A. J. Ragauskas, *Energy Fuels*., 2012, **26**, 740.
37 D. Yang, L. X. Zhong, T. Q. Yuan, X. W. Peng, R. C. Sun, *Ind. Crop. Prod*., 2013, **43**, 141.
38 R. E. Hage, N. Brosse, P. Sannigrahi, A. Ragauskas, *Polym. Degrad. Stab*., 2010, **95**, 997.
39 P. Obama, G. Ricochon, L. Muniglia, N. Brosse, *Bioresour. Technol.*, 2012, **112**, 156.
40 J. Barbier, N. Charon, N. Dupassieux, A. Loppinet-Serani, L. Mahé, J. Ponthus, M. Courtiade, A. Ducrozet, A. A. Quoineaud, F. Cansell, *Biomass Bioenergy*., 2012, **46**, 479.
41 Y. Ye, Y. Zhang, J. Fan, J. Chang, *Bioresour. Technol.*, 2012, **118**, 648.

Graphic Abstract:



The cracking of phenylpropyl side-chain C-C bonds in lignin followed the order of C_α-C_β, C_1-C_α, C_β-C_γ.