Effective biomass fractionation and lignin stabilization using a diol DES system

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Abstract

A sustainable and renewable biorefinery will increase the economic viability of lignocellulose-derived products. In this study, a diol-based deep eutectic solvent (DES) was developed to reduce the recalcitrance of bamboo, facilitate saccharification and valorize the lignin fraction. The DES pretreatment dramatically enhanced glucan digestibility by the effective removal of lignin (as high as 85.45%) and xylan (91.12%). Notably, the recovered lignin from DES pretreatment was protected during the fractionation, showing well-preserved β-O-4 structures (31.82%-59.06%). The

mechanism of lignin protection was analyzed to be accomplished by incorporating the diol hydroxyl functional groups into the α position of the lignin β-O-4 structure *via* etherification. This study highlighted that diol DES is a promising pretreatment solvent to valorize both cellulose and lignin fractions with high enzymatic hydrolysis yield and high-quality lignin co-product.

Keywords: diol DES, delignification, lignin protection, lignin valorization

1. Introduction

The depletion of fossil fuels and the consequent environmental issue have forced society to seek renewable, sustainable, and eco-friendly alternatives [1]. Lignocellulosic biomass is the most abundant renewable resource on earth and has been deemed as an ideal alternative to petroleum [2]. Lignocellulosic biomass is composed primarily of cellulose, hemicellulose, and lignin [3], in which cellulose and hemicellulose are mainly comprised of sugars that can be depolymerized and further converted into chemicals and fuels. As to lignin, it is constituted of guaiacyl (G), syringyl (S), and/or *p*-hydroxyphenyl (H) units, which are bonded through a variety of C-O (*e.g.*, α-O-4, β-O-4 and 4-O-5) and C-C (*e.g.*, β-5, β-β, 5-5, and β-1) linkages [4]. As widely acknowledged that lignin and hemicellulose are covalently crosslinked with each other and interact with cellulose *via* hydrogen bonds and van der Waal forces, resulting in the natural recalcitrance of biomass [5]. Therefore, pretreatment has been investigated as a pivotal processing step for the effective biomass conversion [6].

In a conventional biorefinery or pulping processes, lignin is often treated as waste by either being combusted for heat generation or just discarded [7]. Recently, lignin-first biorefinery concept has been emphasized to make full use

of biomass. These studies are targeted at obtaining reactive lignins that could be used as the ideal candidate for catalytic depolymerization into aromatic monomers, such as phenol, guaiacol, 4-ethyltoluene and others [8]. This objective necessitates the preparation of lignin with a similar structure to the native lignin, *i.e.*, abundant β-O-4 bonds and few condensed structures. However, lignin isolation is still challenging due to its inherent heterogeneity, complexity, and the tendency to suffer from irreversible degradation and condensation reaction [9]. In recent years, several pretreatment solvents such as alkaline solution, organic solvents, and ionic liquids have been applied to fractionate good quality of lignin effectively. However, these methods have not fully addressed the aforementioned issues. Alkaline lignin showed a relatively abundance of impurities, low molecular weight uniformity, and condensed structure [10]. Organosolv and ionic liquid lignins also frequently contain undesired lignin condensation products due to high temperature and acidic conditions [11,12]. In order to protect the intact lignin structure and prevent the condensed reactions, Shuai et al. [13] developed a formaldehyde stabilization strategy preserving lignin structure for near theoretical monomers yield in the subsequent hydrogenolysis. Despite the high conversion yield, using toxic 1,4-dioxane and formaldehyde is unavoidable during the processing. To replace these toxic solvents, alcohol solvent-based pretreatments have been also studied. For instance, Wen et al. [14] found that the ethanol solution pretreatment resulted in α-etherification of lignin side chains. Morteza et al. [15] also reported that glycerol incorporation could significantly prevent lignin condensation and lead to the preservation of β-O-4 linkage-rich lignin. These studies may offer a guide for the lignin-first strategy.

DES pretreatment has recently been considered a promising approach to dissociate and recover lignin (*i.e.* DESL), at the same time enhance the digestibility of the resulting substrates. DESL preparation features mild reaction conditions, high purity and uniformity [16,17]. Additionally, compared to traditional ionic solvents, DES is biocompatible, biodegradable, low-cost, and environmentally-friendly, significantly facilitating its application in biomass pretreatment [18]. DESs comprise at least one hydrogen bond acceptor (HBA) and one hydrogen bond donor (HBD) counterpart, forming a transparent liquid characterized by the low freezing point at mild conditions [19]. Previous studies [20,21] have illustrated that the glycosidic (C-O-C) bonds in both hemicellulose and lignin-carbohydrate complex (LCC) could be cleaved during DES pretreatment. Up to now, a large number of different DES systems such as using organic acid (ChCl/ lactic acid and ChCl/ formic acid), alcohol ((ChCl/ ethylene glycol and ChCl/ glycerin), and lignin-derived compounds (ChCl/*p*-hydroxybenzoic acid and ChCl/guaiacol) have been investigated [18,21]. Typically, they can result in over 70% delignification and over 90% enzymatic saccharification. However, the structural integrity of the recovered lignin in reported articles is frequently significantly altered even under mild conditions [22]. For instance, Shen et al. [23] demonstrated that the β-O-4 linkages had been degraded from 69.52/100Ar (CEL) to 11.84/100Ar (110 °C) in the pretreatment of eucalyptus using ChCl/lactic acid. Wang et al. [24] also illustrated the significant degradation of β-O-4 bonds from 53.24/100Ar (CEL) to 5.35/100Ar (120 °C) in the pretreatment of hybrid Pennisetum using Lewis acid catalyzed ChCl/glycerol. Thus, these DES-induced lignin changes make it difficult to

utilize further. The exploitation of reactive lignin seems to be the key in the application of lignin-first DES pretreatment.

To avoid undesired lignin condensation in traditional DES pretreatment, we herein proposed to use 1,4-butanediol (BDO), a carbocation scavenger, as the HBD, with the aid of trace $AlCl_3$. With this system, a high level of delignification could be achieved at a relatively mild pretreatment condition. Heteronuclear single quantum coherence (HSQC) nuclear magnetic resonance (NMR) results revealed that this strategy significantly preserved the β-O-4 substructure without compromising lignin removal and glucan digestibility. The mechanism of the lignin protection during the pretreatment was systematically analyzed. To the best of our knowledge, this study for the first time reports the protection of the lignin's integrity during the DES pretreatment, which could increase the value of DES lignin and benefit the whole DES based biorefinery processing.

2. Experimental section

2.1 Material preparation

2.1.1 Feedstock and chemicals preparation.

Moso bamboo culms were provided by Xianhe Paper Industry Co. Ltd (Zhejiang Province, China). Bamboo culms were first immersed in tap water for 12 h and then fibrillated by a twin-screw extruder (TSE). After being dewatered and air dried (containing 7.27% water), the crushed bamboo was stored at RT before use. Cellulase (CTec 2, Novozymes) and xylanase (X2753) were obtained from Sigma-Aldrich (Shanghai, China). All other chemicals were purchased from Macklin Biochemical Co., Ltd (Shanghai, China) and used as received.

122 **2.1.2 DES Synthesis, pretreatment and lignin recovery.**

123 We synthesized the DES by mixing ChCl, 1,4-BDO, and $AlCl_3$ into a 500
124 mL three-neck flask at a molar ratio of 25:50:1, and then heated in an oil bath at
125 90 °C with constant agitation until forming a homogeneous and transparent
126 liquid. The DES was sealed and stored in a desiccator for further use. For the
127 DES pretreatment, 10 g dry biomass was mixed with 100 g DES in a three-neck
128 flask which was then transferred into an oil bath at various temperatures of 80-
129 140 °C for 1 h with constant agitation. After pretreatment, the mixture was
130 immediately transferred into a 1 L beaker containing 300 mL of acetone/water
131 (1:1, v/v). After magnetic stirring for 2 h, the mixture was vacuum filtrated and
132 then washed with another 200 mL fresh acetone/water (v/v, 1:1) twice. The solid
133 portion was further washed using DI water until neutral pH, then stored in a
134 refrigerator for further analysis. The filtrate (including the additional 200 mL
135 acetone/water) was collected and rotary-evaporated at 50 °C to remove the
136 acetone. Next, 1 L DI water was introduced to regenerate the dissolved lignin
137 fraction. Finally, the precipitated lignin was separated by centrifugation, and then
138 water-washed three times, and freeze-dried. The dried lignin was sealed and then
139 stored in a desiccator in dark for further study. After the lignin precipitation, the
140 DES was recovered through rotary evaporation to remove the water at 70 °C, and
141 the recovered DES was weighed to determine the recovery yield.

142 **2.2 Substrates characterization**

143 **2.2.1 Enzymatic hydrolysis**

144 Enzymatic hydrolysis was conducted in 150 mL flasks with a solid loading
145 of 2.5% at a 20 mL working volume. In brief, 0.5 g (dry weight) of raw bamboo
146 and pretreated substrates were weighed into the flasks, followed by 1 mL 1 M

147 acetate buffer to regulate the pH around 4.8. Cellulase (25 FPU/g-glucan) and
148 xylanase (150 U/g-xylan) were then introduced. Finally, DI water was
149 supplemented to provide a total volume of 20 mL. The sealed flasks were then
150 transferred into a shaker (at 50 °C and 150 rpm), and incubated for 72 h. After
151 enzymatic hydrolysis, an aliquot of 1 mL was taken for measuring the sugars
152 output through the high-performance liquid chromatography (HPLC).

153 **2.2.2 Compositional analysis of raw and pretreated bamboo**

154 The chemical compositions of the samples in this study were analyzed in
155 accordance with the procedure proposed by the National Renewable Energy
156 Laboratory (NREL) [25]. Briefly, 0.3000 g samples (20-80 mesh) were first
157 hydrolyzed with 3.0 mL of 72 (w/w)% sulfuric acid in a shaker (at 30 °C) for 1
158 h. Water was then added to dilute sulfuric acid to 4 (w/w)%, which was finally
159 autoclaved at 121 °C for another 1 h. All the monomeric sugars in this study were
160 measured by high-performance liquid chromatography (Agilent 1260 series,
161 Agilent Technologies, Santa Clara, CA) equipped with a Bio-Rad Aminex HPX-
162 87H column.

163 **2.2.3 Substrates characterizations**

164 X-ray diffraction (XRD) studies were conducted on a Bruker Advanced D8
165 diffractometer (Bruker, Germany) equipped with a Cu-Kα X-ray generator at a
166 40 kV voltage and a 40 mA current. The test was performed within 2θ range of
167 10°-40° at a 2°/min scanning rate. The crystallinity index (CrI) was calculated
168 based on the flowing equation:

169 $$CrI = \frac{[I_{002} - I_{am}]}{I_{002}} \times 100\%$$

I_{002} is the maximum intensity of the diffraction peaks at ~18°, and I_{am} is the minimum intensity at ~22° of the amorphous portion [26].

Cellulose degree of polymerization was measured according to a viscosity procedure (termed as DP_v). Prior to the test, α-cellulose was acquired according to the method detailed in the supporting information. The limiting viscosity of the samples was measured through the ISO 5351 standard [27]. First, 100 mg of completely dried sample was weighed into the flask, and then adding 10 mL DI water. The suspensions were mixed with magnetic stirring until it was completely dispersed. Then, 10 mL of bi(ethylenediamine) copper (II) hydroxide solution was added to dissolve α-cellulose. Finally, the flasks were placed in awater bath (25 °C) for the viscosity test with a Ubbelohde-viscosity meter. The measurement was performed in triplicate, and the final result represented an average. The DP_v values were calculated based on the limiting viscosity values using the flowing equation:

$$DP_v = \left(\frac{0.75 \times [\eta]}{0.0050246256405154}\right)^{\frac{1}{0.905}}$$

Where [η] was obtained from the published study according to the correction parameter of the viscometer and the time interval between two scale lines.

Fourier transform infrared (FTIR) analysis was conducted to investigate the chemical structure variations of bamboo during the pretreatment. It was accomplished with a Bruker TENSOR27 spectrometer in transmittance mode with 32 scans at 4 cm^{-1} resolution over the wavenumber range of 4000-400 cm^{-1}.

Microstructure morphology of the original and pretreated bamboo was observed with a Hitachi 3400-N scanning electron microscope (Hitachi, Japan)

using a 15 kV accelerating voltage. Samples were first mounted on an aluminum stub through electric tapes and then spray-coated with gold.

2.2.4 Lignin Characterization

The lignin recovery yield was calculated based on the removed lignin after DES pretreatment, and the calculating equation was shown as follows:

$$\text{Lignin recovery yield (\%)} = \frac{\text{Lignin recovered from the lignin - rich liquid (g)}}{\text{Total removed lignin from the initial bamboo (g)}} \times 100\%$$

Raw bamboo's cellulolytic enzyme lignin (CEL) was isolated by sequential enzymatic hydrolysis and dioxane extraction. The details of preparing CEL can be found in our previous publication [6]. Two-dimensional heteronuclear single quantum coherence nuclear magnetic resonance (2D-HSQC NMR) were performed through a Bruker Ascend^{TM} 600 MHz spectrometer. The acquisition parameters were as follows: 166 ppm spectral width in F1 (^{13}C) dimension with 256 data points and 12 ppm spectral width in F2 (^{1}H) dimension with 1024 data points, a J_{C-H} of 145 Hz, a 1.0 s pulse delay, and 128 scans.

According to our previous study, the lignin hydroxyl groups were determined by ^{31}P NMR [6]. It was also performed on the Bruker 600 MHz instrument. In brief, oven-dried lignin samples (~20 mg) were dissolved in a solvent (~0.4 mL), which was composed of anhydrous pyridine and deuterated chloroform (1.6:1, v/v). chromium acetylacetonate and cyclohexanol were used, respectively, as the relaxation and internal standard. An excess amount of 2-chloro-4,4,5,5-tetramethyl-1,3,2-dioxaphospholane (TMDP) (~0.1 mL) was added as a phosphitylation reagent to react with the mixed solution. The mixture was immediately transferred into the NMR tube and then subjected to the test.

The regenerated lignin's microstructural changes and surface characteristics were observed with a field emission scanning electron microscopy (FE-SEM, Regulus 8100, Hitachi, Japan) working at 10 kV acceleration voltages. Prior to the SEM analysis, the samples were first oven-dried overnight at 50 ℃. The dried samples were mounted on an aluminum stub through electric tapes and then sputter coated with gold.

3. Results and discussion

3.1 Substrates characterization

3.1.1 Compositional analysis of the raw and pretreated bamboo.

This study adopted a twin-strew extruder to fibrillate the bamboo culms which is superior to the normal milling process in terms of the low energy consumption and ease to operation. The fibrillated bamboo was then subjected to the DES pretreatment and the solid recovery of the pretreated biomass is summarized in Fig. 1a. As shown, the solid recovery yield of the pretreated bamboo decreased from 88.63% to 51.20% with the pretreatment temperature increasing from 80 °C to 120 °C, indicating that more lignocellulose was dissolved as the pretreatment temperature increased. However, solid recovery increased from 51.20% to 55.95% when further increasing the temperature from 120 °C to 140 °C. This was induced by the formation of pseudo lignin under severe condition [28], and further discussion will address this later. The recovery yields of glucan, xylan and lignin removal were calculated based on the dry weight of original and pretreated bamboo. As shown in Figure 1b, hemicellulose and lignin were readily removed from biomass solids during the DES pretreatment. At 80 °C, 79.39% hemicellulose was retained with 18.65%

delignification. As the pretreatment temperature increased, the recovery yield of hemicellulose was reduced considerably from 79.39% (80 °C) to 55.02% (90 °C), 36.55% (100 °C), 27.74% (110 °C) and 8.88% (120 °C), and no hemicellulose was detected using a temperature of 140 °C. At the same time, the delignification efficiency was substantially increased from 18.65% (80 °C) to 85.45% (120 °C), indicating the highly effective delignification by the proposed DES. However, further increase of temperature beyond 120 °C decreased the lignin removal, with values of 77.25% and 61.69% at 130 °C and 140 °C, respectively. This effect was ascribed to the agglomeration of the dissolved lignin and the formation of pseudo lignin that precipitated on the substrate surface. Nevertheless, the glucan recovery yield was maintained at nearly 95% (94.29%-97.79%) during the pretreatment, confirming that glucan was barely hydrolyzed or degraded throughout the pretreatment. It should be noted that the sole ChCl/BDO without the addition of $AlCl_3$ was incapable of removing xylan and lignin (data not shown), even at high temperatures, indicating $AlCl_3$ as a key promoter in the proposed DES [18].

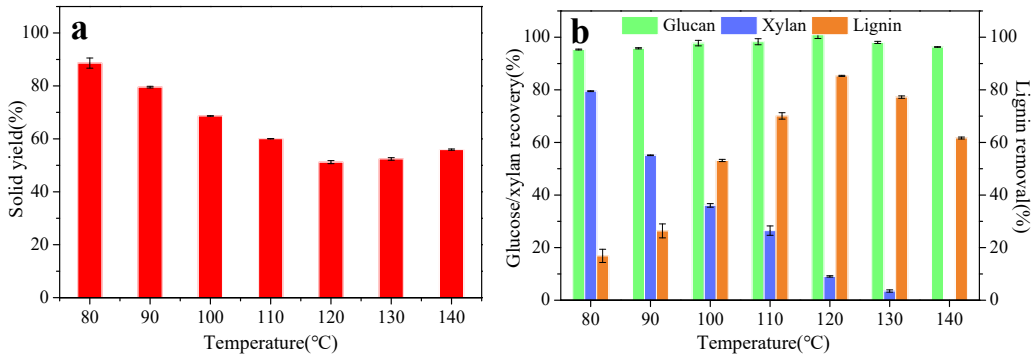


Fig. 1 Solid yield of substrates (a) and detailed components variations (b).

3.1.2. SEM analysis of substrates before and after pretreatment

SEM analysis was applied to investigate the microstructure changes of biomass during the DES pretreatment. As illustrated in Fig. S1, discernible variations of the bamboo fibers were observed during the pretreatment. The size of pretreated substrates decreased with the pretreatment temperature increasing. At 80 °C and 90 °C, the bamboo fiber length was close to that of the raw sample, and their surface was clear and smooth (Fig. S1b and c). When the DES pretreatments were performed at higher temperatures of 100-140 °C, the fiber length clearly decreased, and some cracks were evidently observed on the pretreated samples (Fig. S1e-h). Moreover, spherical-shaped and nub-like lignin debris were observed on the bamboo fibers' surface (see white arrows in Fig. S1). It has been widely reported that the small debris could be ascribed to the lignin migration of inner regions to the external surface, and the generation of pseudo lignin [23].

3.1.3 Impact of the pretreatment on the substrates crystallinity and cellulose degree of polymerization (DP_v)

The CrI (crystalline index) of the original and pretreated bamboo are shown in Fig. 2. The CrI of the untreated bamboo was 59.63%, and it gradually increased to 67.03% with the temperature increasing from 80 to 120 °C. This change was mainly ascribed to the removal of amorphous lignin and hemicellulose throughout the pretreatment. After that, the CrI of pretreated bamboo slightly decreased from 67.03% to 65.17% with the temperature increasing from 120 to 140 °C, which was possibly due to the swelling effect on the crystalline zone induced by the permeation of DES solvent under harsher reaction conditions [26].

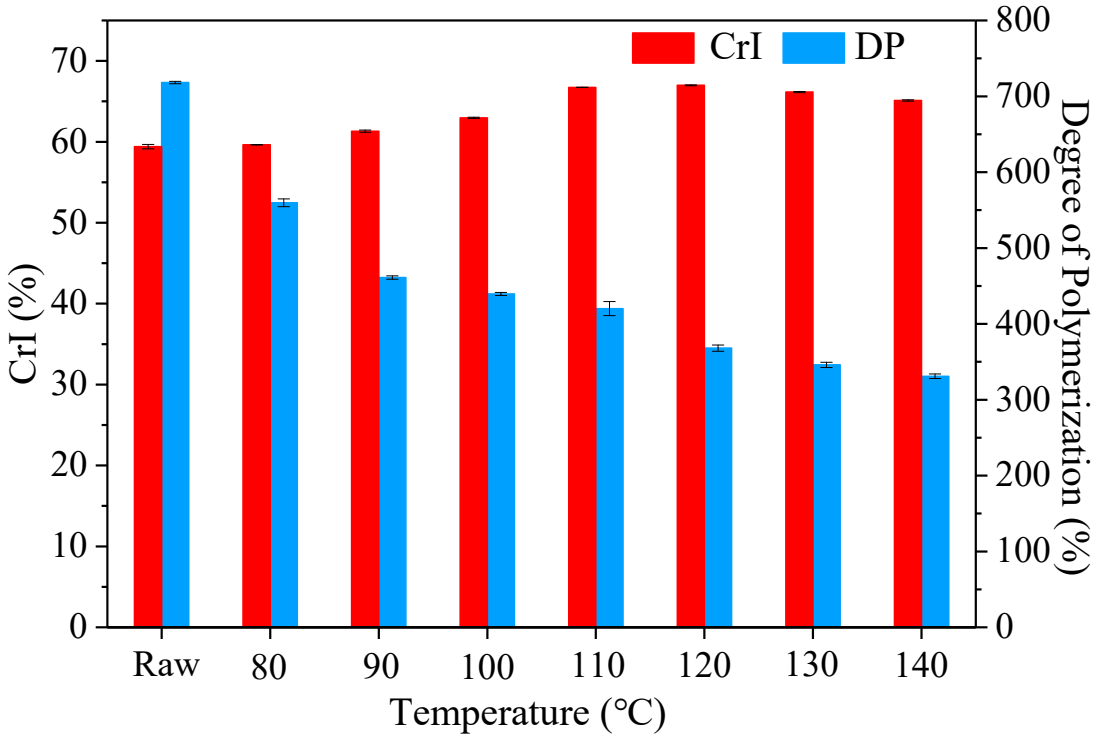


Fig. 2 The CrI of substrates and cellulose DP_v from original and pretreated bamboo

Cellulose DP_v is another important character affecting cellulose saccharification. As shown in Fig. 2, the cellulose DP_v of raw bamboo was 718, clearly lower than woody biomass like poplar and eucalyptus, which is because of the abundance of short parenchyma cells in bamboo [6]. Following the pretreatment, the cellulose DP_v was significantly decreased to 559, 461, 439, 420, 368, 346, and 330 as the temperature increased from 80 °C to 140 °C. The results indicated that the DES could cleave the glycosidic bond in cellulose and expose more reducing ends that facilitate the enzymatic hydrolysis of the substrates.

3.1.4 FTIR spectroscopy analysis of the raw and the pretreated bamboo

FTIR spectroscopy is widely employed in investigating the structure changes of the raw and pretreated substrates. As shown in Fig. S2, peaks at 896, 1101, 1056 and 1426 cm^{-1} are ascribed to C-O-C stretching in amorphous

cellulose, C-O vibration in crystalline cellulose, C-O stretching of cellulose, C-H stretching of cellulose and C-H deformation (asymmetric) of cellulose [29]. Clearly, these signals significantly increased after the pretreatment, indicating cellulose enrichment in the pretreated biomass solid. Additionally, the peak at 1730 cm^{-1}, which is assigned to carbonyl groups in branched hemicellulose, was observed to be weakened with increasing pretreatment temperature and eventually disappeared at 130 and 140 °C, this is caused by hemicellulose removal and the degradation of LCC (lignin-carbohydrate complex) structures [30]. In addition, the peaks at 1602 and 1507 cm^{-1} correspond to aromatic skeletal vibration of lignin, and the peak at 1237 cm^{-1} was assigned to the C-O vibration of the guaiacyl ring of lignin [29]. The intensity of these peaks became inconspicuous, especially under severe DES pretreatment conditions, which was attributed to the lignin removal. However, the peak at 1315 cm^{-1} relating to C-O vibration in syringyl ring of lignin became conspicuous with increasing pretreatment temperature. This result revealed that the G unit was more readily removed from biomass in the pretreatment.

3.1.5 Enzymatic digestibility of the raw and pretreated bamboo

Enzymatic hydrolysis was performed to evaluate the impact of the DES pretreatment on sugar release. As shown in Fig. 3, after 72 h enzymatic hydrolysis, the saccharification yield of glucan and xylan of the raw bamboo was only 9.25% and 1.43%, respectively. After the DES pretreatment, the enzymatic hydrolysis was considerably enhanced. Specifically, the glucan saccharification yield was increased from 9.25% (raw substrate) to 24.24%, 41.40%, 81.92%, 91.16% and 100% at temperatures of 80, 90, 100, 110, and 120 °C, respectively. The same trend was also found in the xylan digestibility which increased from

1.43% to 100%. Remarkably, the glucan hydrolysis yield ranged from 41.40% to 100% as the temperature increased, 4 to 11 times higher than that of the raw bamboo. Generally, hemicellulose and lignin in the cell wall are two factors inhibiting enzymatic digestibility, in which they are crosslinked with each other, forming a networked structure that covers the cellulose. Moreover, non-productive adsorption of lignin to the enzyme generates a lignin-enzyme complex, which also significantly lowers saccharification efficiency [31]. To evaluate the importance of hemicellulose and lignin removal, the relationship between glucan saccharification ratio and the removal of lignin and xylan was characterized. As shown in Fig. S3, a strong linear correlation between the glucan hydrolysis yield and lignin removal (R^2=0.9451) as well as xylan removal (R^2=0.9386) was observed, implying that the removal of both lignin and hemicellulose highly reduced the recalcitrance and thus significantly improved saccharification efficiency.

Furthermore, as described in Fig. S4, a strong negative correlation between the glucan hydrolysis yield and DP_v (R^2=0.8624) was also observed. This result indicated that the enzymatic hydrolysis yield was significantly enhanced with the decrease of cellulose DP_v, which was induced by the depolymerization of cellulose chains, thus increasing the specific surface area of cellulose and exposing more reducing ends [32].

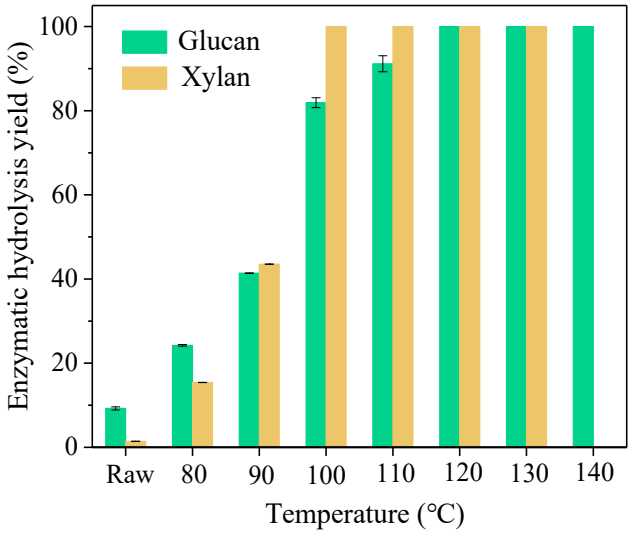


Fig. 3 Glucan and xylan saccharification yields of the raw and the DES pretreated bamboo under different pretreatment temperatures.

3.2 Lignin characterization

3.2.1 Carbohydrates Analysis of Regenerated Lignin.

As shown in Fig. S5, the recovery yield of the DES-induced lignin was over 80% for all the pretreatment runs (except 80 °C), which was significantly higher than conventional pretreatment methods such as alkaline and some organosolv processes [33-35].

Compositional analysis was performed to investigate the purity of these regenerated lignins. As shown in Table S1, only trace carbohydrates were detected (less than 0.3% glucan and 2.01% xylan). In addition, with the pretreatment temperature increasing, the amount of the associated carbohydrates declined, and they were barely detected at temperature higher than 120 °C, indicating the high purity of our regenerated lignin. The nearly pure lignin

prepared in this study is favorable for the lignin valorization, such as catalytic depolymerization and nanocomposites manufacturing.

3.2.2 FTIR spectroscopy analysis of the original CEL and regenerated lignin

The lignin of the original and regenerated lignin exhibited similar FTIR spectra, indicating the structural integrity of the regenerated lignins (Fig. S6). The characteristic signals at ~1700 cm^{-1} attributed to C=O stretching vibrations in carbonyls, non-conjugated ketones, and ester of lignin groups [36] were strikingly enhanced as the pretreatment strengthened. Additionally, the absorption bands at 1602, 1459 and 1513 cm^{-1} corresponded to the lignin aromatic skeleton have no significant variation [37]. This data implied that the aromatic skeleton of regenerated lignin was not significantly disintegrated during the treatment. Further, the peaks at 1148, 1032 and 1357 cm^{-1} attributed to the characteristic signals of guaiacyl unit [38] were weakened in all the runs. The peak at 1255 cm^{-1} corresponded to syringyl unit, and the peak at 832 cm^{-1} related to *p*-hydroxyphenyl (H) unit [36] indicated no significant change with the temperature increasing. These results suggested that the regenerated lignin contains more S units but less than G units, and the 2D-HSQC NMR analysis further confirms this.

3.2.3 Morphology of the regenerated lignins

The morphological features of the CEL and recovered lignin were investigated *via* SEM, as shown in Fig. 4. The CEL featured as irregular and lump-shaped agglomerates, which is composed of interconnected and homogeneous ellipse-like particles. Interestingly, after our DES pretreatment, all the regenerated lignin had a spherical shape. DES lignin in previous article was in flake-like or unregular shapes [39]. Uniquely, herein, the BDO DES generated

lignins with a regular sphere shape. The size of the lignin spheres was observed to be several microns and it seems to be enlarged with increasing the temperature. The possible mechanism of generating the regular spherical lignin was proposed (Fig. S7) [40]. In brief, after adding water to the lignin-abundant DES, lignin nuclei were formed through aggregating the large and homogenous hydrophobic lignin, and then the nuclei gradually grew into micro spherical lignin through absorbing the small lignin particles, which formed a hydrophobic nucleus and a hydrophilic shell. During the nucleation process, lignin started to aggregate through the hydrophobic interaction which formed stable nuclei, and the surface of the nuclei was hydrophilic. After that, the hydrophilic lignin debris adsorbed onto the surface of the nuclei, thus promoted the nuclei to grow into micro spherical lignin. Besides, the BDO grafted onto the α-position of lignin aliphatic side chain during our DES pretreatment could increase the hydrophilicity of the lignin, which further promoted the swelling growth process of the lignin spheres. Further studies concerning the mechanism and application of the lignin microspheres are ongoing and will be reported in our future studies.

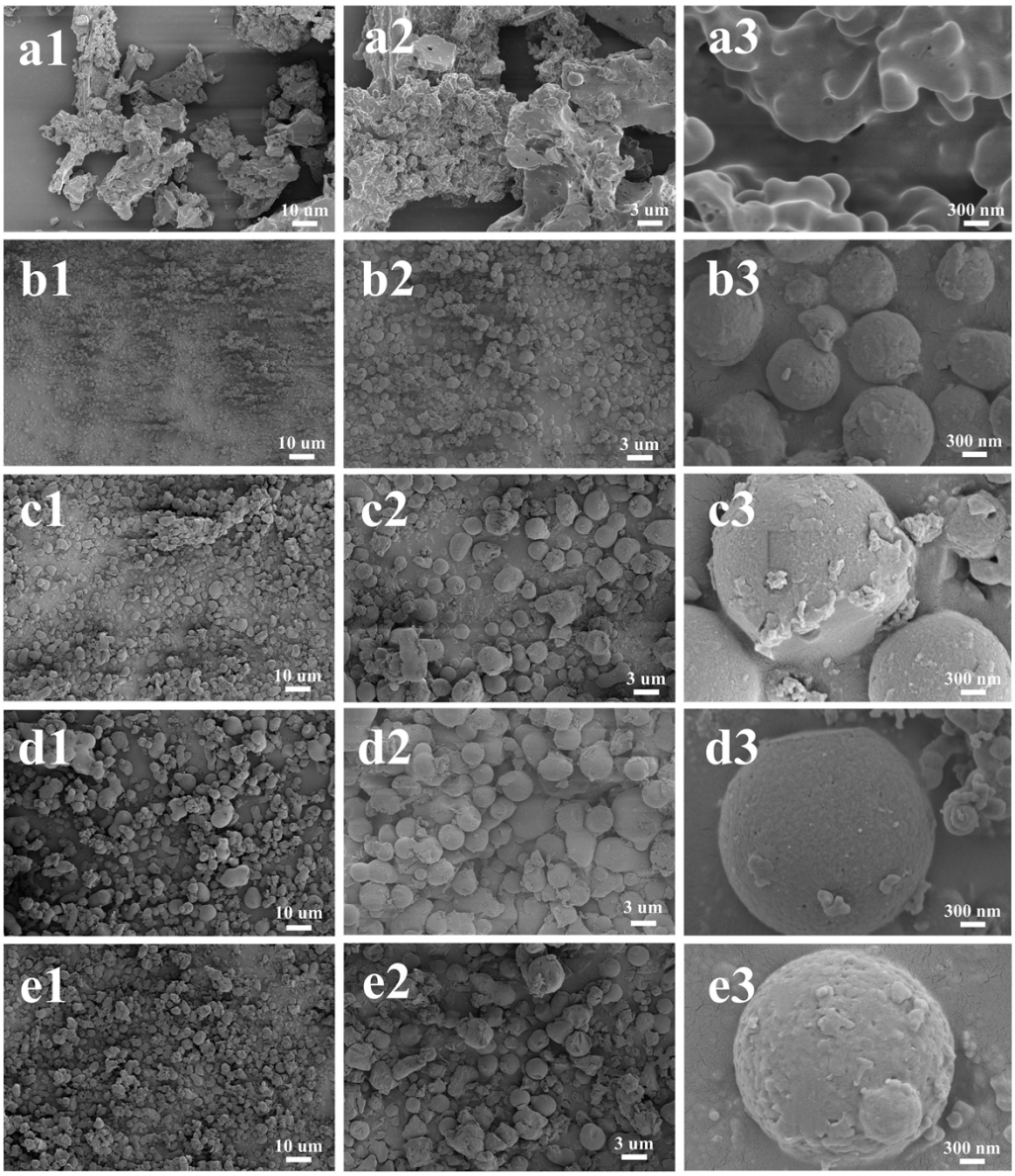


Fig.4 SEM images of CEL (a1-a3), recovered lignins at different temperatures of 90 °C (b1-b3), 100 °C (c1-c3), 120 °C (d1-d3), and 140 °C (e1-e3).

3.2.4 HSQC NMR analysis of CEL and recovered lignin

For the detailed structural information of the recovered lignins in different pretreatment conditions, 2D-HSQC NMR analysis was conducted. CEL was prepared as a control to observe the structural changes during the pretreatment process. The side-chain (δ_C/δ_H 50.0-90.0/2.50-6.0 ppm) and aromatic (δ_C/δ_H 100.0-150.0/9.0-5.5 ppm) regions of the HSQC spectra of the regenerated lignins

are shown in Fig. 5 and Fig. 6. The main lignin cross-signals of the 2D-HSQC spectra, as demonstrated in Table S2, were referred to the previous publications [23,39,41].

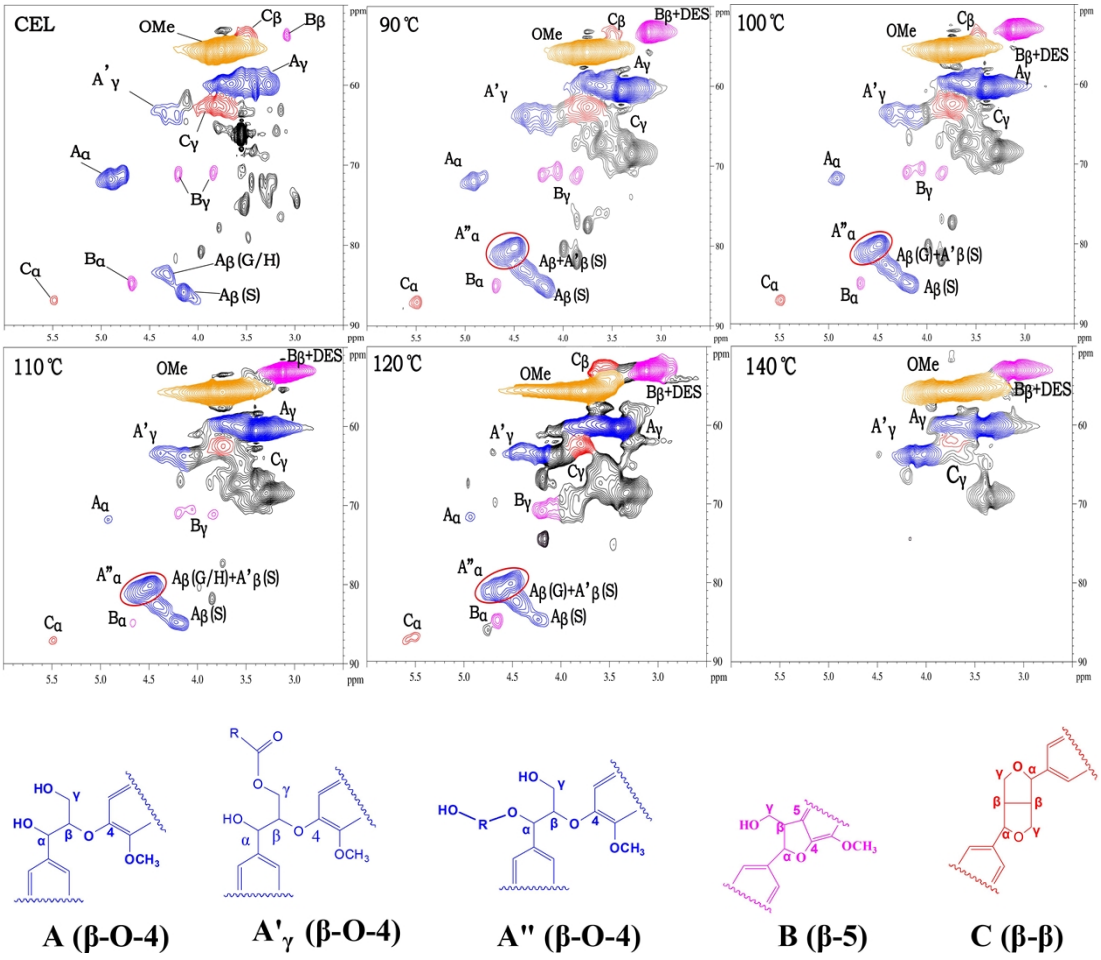
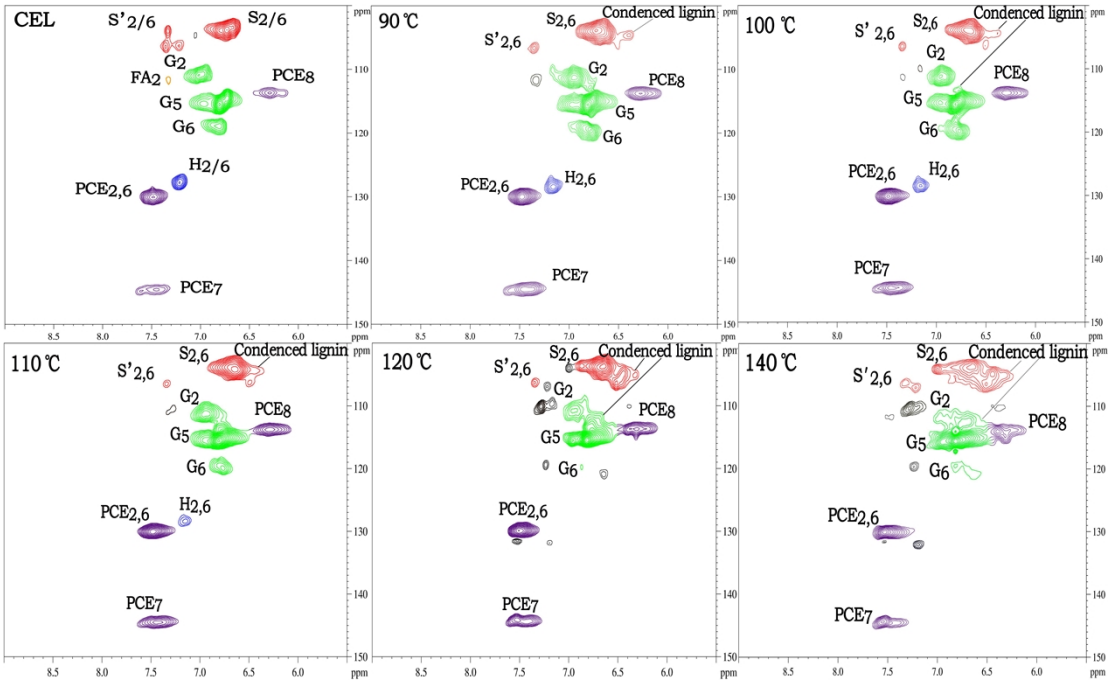


Fig. 5 Side-chain regions of CEL and the regenerated lignin fractions in the 2D-HSQC NMR spectra under different pretreatment temperatures.



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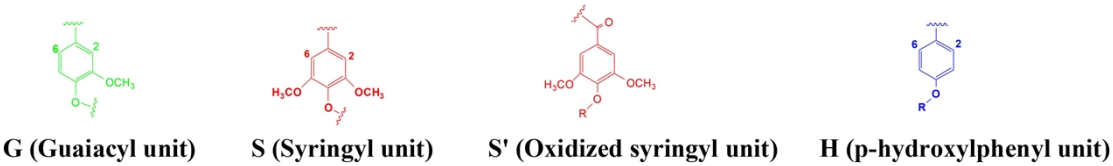

G (Guaiacyl unit) **S (Syringyl unit)** **S' (Oxidized syringyl unit)** **H (p-hydroxylphenyl unit)**

422 **FA (Ferulic acid)** ***p*-CE (*p*-Coumarate)**

423

424 **Fig. 6** Aromatic regions of the CEL and regenerated lignin fractions in the 2D
425 HSQC NMR spectra under different pretreatment temperatures.

426

427 In the side-chain region (Fig. 5), CEL showed pronounced signals of β-O-4
428 (A_α), β-β (B_α), and β-5 (C_α) linkages at 71.62/4.85, 84.73/4.65, and 86.83/5.42
429 ppm. Additionally, the γ-acetylated β-O-4 linkages (A_γ') were also observed,
430 indicating the existence of LCC structure in bamboo [42]. After the DES
431 pretreatment, the A_α signal of regenerated lignin was weakened even at the mild
432 temperature of 90 °C. Meanwhile, a new signal was discovered at 80.05/4.49
433 ppm in the recovered lignin HSQC NMR spectra (see red rings in Fig. 5). This

data was assigned to β-O-4 structure derivatives (A”$_\alpha$) which is caused by the graft of 1,4-BDO onto the α-position of the β-O-4 linkage (Fig. 7, route A) [16,31]. During the pretreatment, the position of the β-O-4 structure was hydrolyzed to form a positively charged carbon by the departure of leaving groups, which normally lead to the condensation in most cases (Fig. 7, route B). However, in this study, 1,4-BDO acted as a nucleophile to attack the carbocation intermediates, forming the α-etherified lignin that suppressed the condensation [16,43]. In addition, as shown in Fig. S8, lignins regenerated at the temperature lower than 110 °C all exhibited a brown color, similar to native CEL, and in evident contrast to lignin recovered from severe conditions of L140 °C, which has a very dark color, suggesting more condensed structures [44]. As expected, the proposed DES system protected the lignin structure and preserved β-O-4 linkage in regenerated lignins (Table 1). The β-O-4 bond content in regenerated lignins was as high as 59.06/100Ar (90 °C), 51.95/100Ar (100 °C), and 46.47/100Ar (110 °C), which is close to the native CEL (59.19%). Previous reports concerning DES pretreatment only focused on the delignification and lignin recovery, while the lignin quality was not considered as much as the quantity [18,45,46]. In this study, we proposed a novel strategy by introducing carbocation scavenger into the DES system, which generated lignins possessing a similar structure to the native lignin. Even at the temperature of 120 °C, β-O-4 bond content can still reach 31.82%, indicating a strong protective effect of our system. Nevertheless, no aryl ether was detected when further increasing temperature to 140 °C. In the case of β-β and β-5, they were barely affected during the DES pretreatment except that at 140 °C where no β-β or β-5 was found.

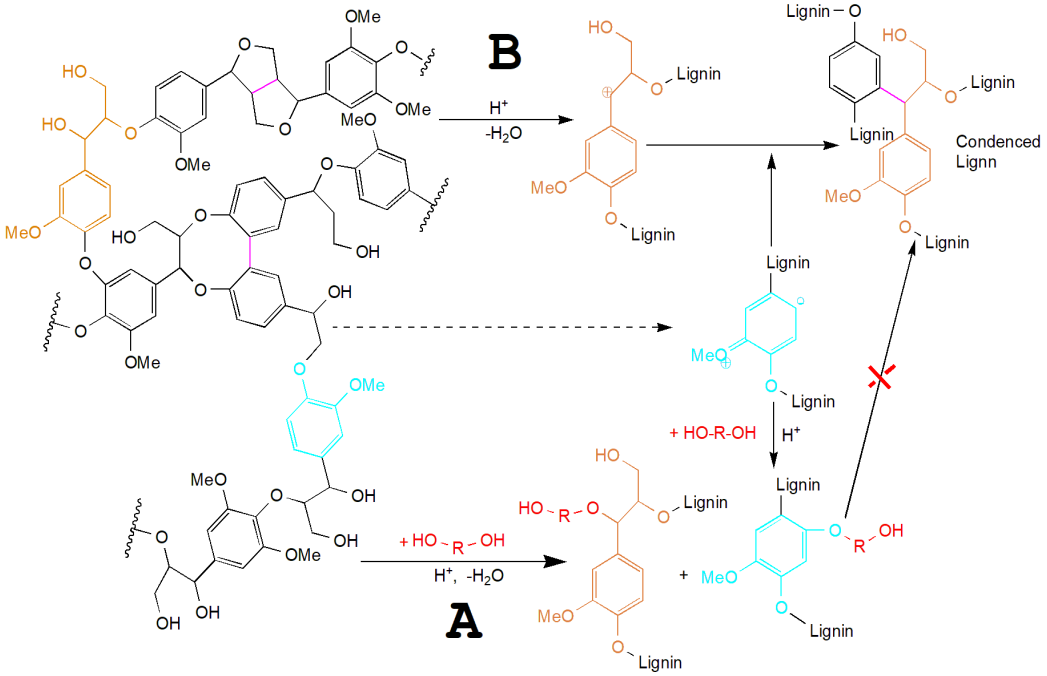

Fig. 7. Lignin extraction in normal case (route B), and the lignin protection by our DES (route A).

In the aromatic region (Fig. 6), guaiacyl (G), syringyl (S), and *p*-hydroxyphenyl (H) units signals were clearly recognized in bamboo CEL. Besides, the signals of oxidized syringyl units (S'), *p*-coumaric acid (*p*-CE), and ferulic acid (FA) were also unambiguously observed. After the DES pretreatment, no distinct signals shift of the $S_{2,6}$ and G_5 was observed with increasing temperature. This result is possibly due to the quenching of the α position of the β-O-4 structure by the 1,4-BDO, thus reducing the condensation reaction [31]. As to the content of FA, which existed as the LCC linkages, it gradually decreased and finally disappeared at temperature higher than 110 °C. On the contrary, the *p*-CE was almost unaffected by the pretreatment. For more detailed information about the lignin structure changes after the DES pretreatment, the main constituents of the ratio of S/G were calculated based on

the previous study method [47]. As shown in Table 1, the contents of S and G units of bamboo CEL were 43.39/100Ar and 45.82/100Ar, respectively. With the pretreatment temperature increasing, the S/G gradually increased and reached 1.16 at 140 °C. Notably, although the protection of the 1,4-BDO, some lignin condensation was also observed during the pretreatment. Minimal lignin condensation occurred at a low reaction temperature (up to 110 °C). The condensed S unit increased from 6.35 to 9.10/100Ar (accounting for 14.07%-19.87% of the total S unit, see Table 1), and condensed G unit was barely observed. Further increasing the temperature, lignin condensation reactions were increased. Finally, at 140 °C, 29.18/100Ar condensed S unit was observed, accounting for 53.01% of the total S unit, and all the G unit was transformed into the condensed form. This result confirmed the conclusion above that our strategy could perform well at various temperatures (lower than 120 °C), which protects the lignin structure with minimal sacrifice to delignification.

Table 1. Quantification of CEL and Regenerated Lignin (results expressed as per 100 Ar).

Sample	Lignin removal (%)	Lignin yield (%)	S		G		S/G	β-O-4	β''-O-4
			Total	Condensed	Total	Condensed			
CEL	-	-	43.39	0	45.82	-	0.95	59.19	0
L90 ℃	26.35	79.00	45.13	6.35	46.91	1.14	0.96	18.83	40.23
L100 ℃	53.15	83.01	45.66	7.74	45.94	1.99	0.99	14.65	37.30
L110 ℃	70.10	86.49	45.79	9.10	47.74	2.54	0.96	9.37	37.10
L120 ℃	85.30	85.11	52.30	16.67	47.70	10.24	1.10	5.96	25.86
L140 ℃	61.69	89.18	55.0	29.18	44.95	44.95	1.16	-	-

3.2.5 Quantitative ^{31}P NMR analysis of the CEL and recovered lignins

The changes of OH groups including aliphatic, phenolic, and carboxylic acid OH before and after DES pretreatments were investigated by ^{31}P NMR. According to a previous publication, the peaks of syringyl OH and other condensed 5-substituted phenolic OH are overlapped in the NMR spectra; therefore, they are counted together as the C_5-substituted phenolic OH content [6]. As shown in Fig. 8, in CEL, the aliphatic OH group, with a content of 3.61 mmol/g representing 58.38% of the total OH groups, is dominant. After our DES pretreatment at 90, 100 and 110 ℃, the aliphatic OH contents slightly decreased but still dominate the OH content (with content of 2.85 mmol/g at 90 ℃, 2.47 mmol/g at 100 ℃ and 2.59 mmol/g at 110 ℃). It was also determined that the total phenolic OH was almost unchanged at these temperatures. These results supported the HSQC NMR result indicating that the β-O-4 structure was protected successfully during the extraction at low temperatures. Besides, the C_5 substituted units representing the condensed lignin OH varied from 0.29 (90 ℃) to 0.26 (100 ℃) and 0.32 mmol/g (110 ℃), this slight variation of C_5 substituted units also suggested that our DES could sufficiently suppress the lignin condensation. However, when the pretreatment temperature was further increased from 110 ℃ to 140 ℃, the aliphatic OH contents decreased significantly from 2.59 (110 ℃) to 1.61 (120 ℃) and 1.04 mmol/g (140 ℃), while the phenolic OH increased from 1.19 (110 ℃) to 1.49 (120 ℃) and 1.69 mmol/g (140 ℃), and the C5 substituted OH increased from 0.32 (110 ℃) to 0.55 (120 ℃) and 0.65 mmol/g (140 ℃). These results indicate the β-O-4

structure was partially ruptured which increased the relative contents of phenolic OH compared to the aliphatic OH, accompanied by more condensed reactions occurring under harsher pretreatment conditions.

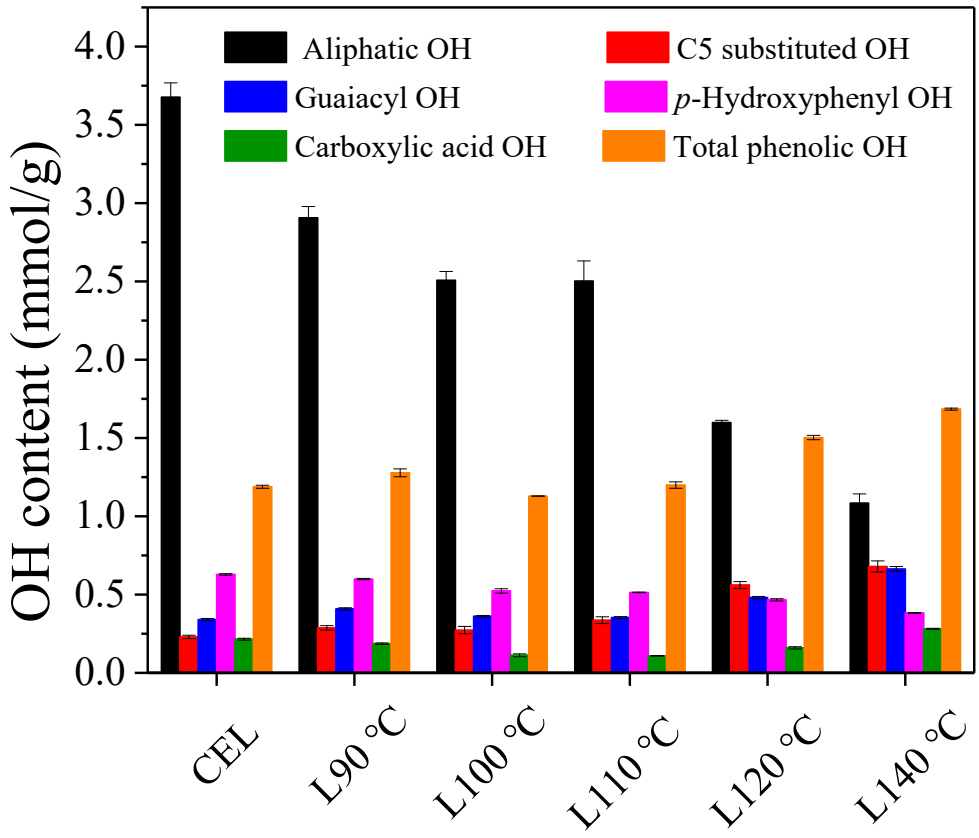

Fig. 8 Quantification of different OH groups in CEL and regenerated lignins by ^{31}P NMR.

3.3 DES reuse and mass balance

The recyclability of the DES has been deemed as a main promoter for the DES-based lignocellulose pretreatment. Our studies demonstrated that the BDO DES showed excellent recyclability, as shown in Fig. S9. After recovery and testing seven times at 120 °C for 1 h, over 90% of DES could be recovered in each recycling stage. Besides, the lignin removal and the enzymatic hydrolysis yield were also investigated. It can be seen that the lignin removal gradually decreased but still maintained at ~70% in 2-4 cycles; thereafter, it decreased

suddenly and finally was 42.78% after the 7th circulations. Nevertheless, the glucan enzymatic hydrolysis yield in all the cycles could still reach 100% even after the 7th circulation (Fig. S10).

The mass balance after DES pretreatment (based on 100 g raw bamboo) is shown in Fig. 9. The raw bamboo contains 41.00 g glucan, 12.78 g xylan, and 30.99 g lignin. After DES pretreatment, the recovery of xylan in the pretreated solid was 3.54 g which was completely hydrolyzed into xylose by enzymes. Furthermore, 0.42 g of xylan was depolymerized into xylose during cooking which was accompanied with 0.51 g acetic acid and 0.50 g furfural. As to the regenerated lignin, 18.78 g lignin was recovered and possessed well-preserved structure. Furthermore, the glucan recovery approached a theoretical maximum yield of 39.13 g, which could be enzymatically hydrolyzed into glucose (39.24 g). It is worth noted that the DES could be recovered with a 94.24% yield after separation of the solid and liquid, which will make the process sustainable and renewable.

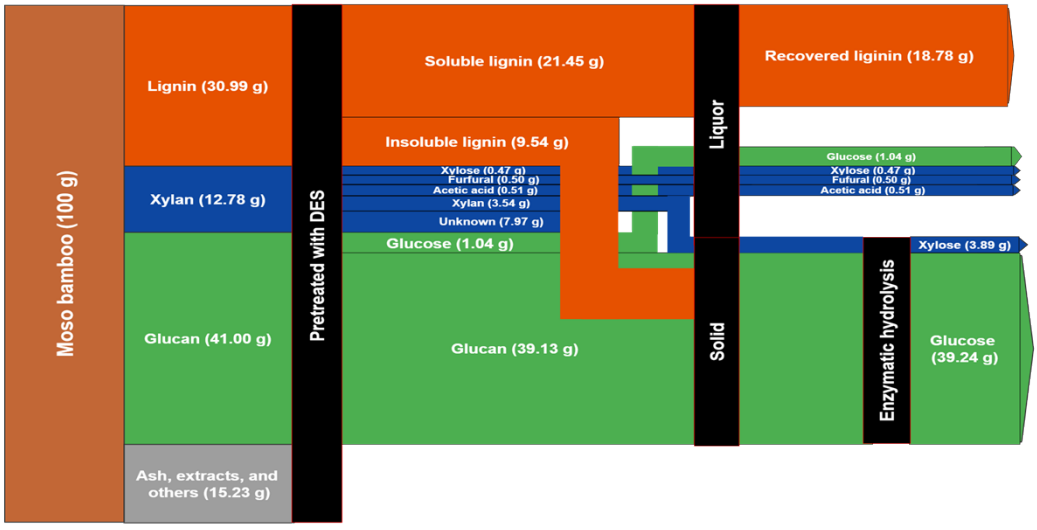

Fig. 9 Schematic diagram and mass balance of the proposed biorefinery sequence.

4. Future perspectives

Although the pretreatment approaches discussed above showed excellent ability to obtain lignins with well-preserved structures, as well as a high saccharification yield of glucan, the exploitation of hemicellulose remains to be determined. Under the acid DES, xylan is liable to degrade and generate xylose as the first production [48,49]. Further, xylose could be easily transformed into xylulose, and the xylulose normally dehydrates and generates furfural owing to the low activation energy. At more severe conditions, furfural could be transformed into furfuryl alcohol, and then produce levulinic acid through a ring-opening reaction. In addition, there is another degradation route of xylan, in which xylulose was transformed into glycolaldehyde and glyceraldehyde through anti-aldol condensation [50]. Next, the glyceraldehyde could dehydrate into methylglyoxal. At more sever condition, the methylglyoxal further transformed into methylglyoxal, formaldehyde, acetaldehyde, 1,1-dihydroxy acetone, acetic acid, lactic acid and formic acid.

In this study, a minimal amount of furfural was detected. However, a series of other hemicellulose degradation chemicals such as acetic acid, levulinic acid, and lactic acid were detected after the DES pretreatment (see Table S4), which made the conversion of hemicellulose intractable and complicated. We could not even make a mass balance of hemicellulose, which indicates that other unknown intermediate products (such as methylglyoxal, acetaldehyde, glycolaldehyde, glyceraldehyde, 1,1-dihydroxy acetone, and methylglyoxal) existed in the system. We also discovered that the accumulation of lactic acid was remarkably increased from 0 (1^{st} circulation) to 4.91 g/L (7^{th} circulation). This may be induced by the transformation of xylose to lactic acid during the $AlCl_3$ assisted DES system [50], but it only showed a very low yield in this study. Therefore, future research endeavors should be directed towards hemicellulose valorization,

575 which could help to develop a tailor-made pretreatment process for desired
576 products from the whole biomass and thus contribute to the sustainable
577 biorefinery configuration.

578 **5. Conclusions**

579 This study systematically investigated the pretreatment of bamboo using a
580 diol-based DES, reaching over 80% lignin removal while preserving almost all
581 glucan. Besides, the hydrolysis digestibility of the substrate was significantly
582 improved after the pretreatment. Importantly, the regenerated lignin possessed
583 quite an intact structure with very high β-O-4 content (31.82%-59.19%), which
584 is beneficial to the downstream lignin processing. Finally, the DES showed
585 excellent recyclability even after the seven circles.

586 **Conflicts of interest**

587 There are no conflicts to declare.

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595 **References**

596 [1] A. Mittal, R. Katahira, B.S. Donohoe, B.A. Black, S. Pattathil, J.M. Stringer,

G.T. Beckham, Alkaline Peroxide Delignification of Corn Stover, ACS Sustain. Chem. Eng. 5 (2017) 6310–6321.

[2] S.A. Rollag, J.K. Lindstrom, R.C. Brown, Pretreatments for the continuous production of pyrolytic sugar from lignocellulosic biomass, Chem. Eng. J. 385 (2020) 123889.

[3] H. Bian, M. Dong, L. Chen, X. Zhou, R. Wang, L. Jiao, X. Ji, H. Dai, On-Demand Regulation of Lignocellulosic Nanofibrils Based on Rapid Fractionation Using Acid Hydrotrope: Kinetic Study and Characterization, ACS Sustain. Chem. Eng. 8 (2020) 9569–9577.

[4] C. Liu, Y. Deng, S. Wu, H. Mou, J. Liang, M. Lei, Study on the pyrolysis mechanism of three guaiacyl-type lignin monomeric model compounds, J. Anal. Appl. Pyrolysis. 118 (2016) 123–129.

[5] L. Xu, S.J. Zhang, C. Zhong, B.Z. Li, Y.J. Yuan, Alkali-Based Pretreatment-Facilitated Lignin Valorization: A Review, Ind. Eng. Chem. Res. 59 (2020) 16923–16938.

[6] C. Huang, G. Fang, Y. Zhou, X. Du, L. Yu, X. Meng, M. Li, C.G. Yoo, B. Chen, S. Zhai, Q. Guan, Q. Yong, A.J. Ragauskas, Increasing the Carbohydrate Output of Bamboo Using a Combinatorial Pretreatment, ACS Sustain. Chem. Eng. 8 (2020) 7380–7393.

[7] H. Wu, L. Gong, X. Zhang, F. He, Z. Li, Bifunctional porous polyethyleneimine-grafted lignin microspheres for efficient adsorption of 2 , 4-dichlorophenoxyacetic acid over a wide pH range and controlled release, Chem. Eng. J. 411 (2021) 128539.

[8] X. Shen, Y. Xin, H. Liu, B. Han, Product-oriented Direct Cleavage of Chemical Linkages in Lignin, ChemSusChem. 13 (2020) 4367–4381.

[9] L. Xu, J. Zhang, Q.J. Zong, L. Wang, T. Xu, J. Gong, Z.H. Liu, B.Z. Li, Y.J. Yuan, High-solid ethylenediamine pretreatment to fractionate new lignin streams from lignocellulosic biomass, Chem. Eng. J. 427 (2022).

[10] Z.-M. Zhao, S. Zhang, X. Meng, Y. Pu, Z.-H. Liu, W.K. Ledford, S.M.K. II, B.-Z. Li, A.J. Ragauskas, As featured in : bioconversion by an alkali sterilization strategy †, Green Chem. 23 (2021) 4697–4709.

[11] M. Naebe, Y. Zhang, Lignin: A review on structure, properties, and applications as a light-colored UV absorber, ACS Sustain. Chem. Eng. 9 (2021) 1427−1442.

[12] D. He, Y. Wang, C.G. Yoo, Q.J. Chen, Q. Yang, The fractionation of woody biomass under mild conditions using bifunctional phenol-4-sulfonic acid as a catalyst and lignin solvent, Green Chem. 22 (2020) 5414–5422.

[13] L. Shuai, M.T. Amiri, Y.M. Questell-santiago, F. Héroguel, Y. Li, H. Kim, R. Meilan, C. Chapple, J. Ralph, J.S. Luterbacher, Formaldehyde stabilization facilitates lignin monomer production during biomass depolymerization, Science. 354 (2016) 329–333.

[14] J. Wen, S. Sun, T. Yuan, F. Xu, R. Sun, Structural Elucidation of Lignin Polymers of Eucalyptus Chips during Organosolv Pretreatment and Extended Deligni fi cation, Agric.Food Chem. 61 (2013) 11067−11075.

[15] M. Hassanpour, M. Abbasabadi, L. Moghaddam, F. Fuelbiol, Bioresource Technology Mild fractionation of sugarcane bagasse into fermentable sugars and β -O-4 linkage-rich lignin based on acid-catalysed crude glycerol pretreatment, Bioresour. Technol. 318 (2020) 124059.

[16] Y. Liu, N. Deak, Z. Wang, P.J. Deuss, K. Barta, H. Yu, L. Hameleers, E. Jurak, Tunable and functional deep eutectic solvents for lignocellulose valorization,

Nat. Commun. 12 (2021) 1–15.

[17] Y.T. Tan, G.C. Ngoh, A. Seak, M. Chua, Bioresource Technology E ff ect of functional groups in acid constituent of deep eutectic solvent for extraction of reactive lignin, Bioresour. Technol. 281 (2019) 359–366.

[18] C. Huang, Y. Zhan, J. Cheng, J. Wang, X. Meng, X. Zhou, G. Fang, A.J. Ragauskas, Bioresource Technology Facilitating enzymatic hydrolysis with a novel guaiacol-based deep eutectic solvent pretreatment, Bioresour. Technol. 326 (2021) 124696.

[19] W. Xing, G. Xu, J. Dong, R. Han, Y. Ni, Novel dihydrogen-bonding deep eutectic solvents : Pretreatment of rice straw for butanol fermentation featuring enzyme recycling and high solvent yield, 333 (2018) 712–720.

[20] Q. Xia, Y. Liu, J. Meng, W. Cheng, W. Chen, S. Liu, Y. Liu, J. Li, H. Yu, Multiple hydrogen bond coordination in three-constituent deep eutectic solvents enhances lignin fractionation from biomass, Green Chem. 20 (2018) 2711–2721.

[21] S. Hong, X.J. Shen, Z. Xue, Z. Sun, T.Q. Yuan, Structure-function relationships of deep eutectic solvents for lignin extraction and chemical transformation, Green Chem. 22 (2020) 7219–7232.

[22] Z. Chen, X. Bai, H. Zhang, C. Wan, Insights into Structural Changes of Lignin toward Tailored Properties during Deep Eutectic Solvent Pretreatment, ACS Sustain. Chem. Eng. 8 (2020) 9783−9793.

[23] X. Shen, J. Wen, Q. Mei, X. Chen, D. Sun, T. Yuan, R. Sun, Facile fractionation of lignocelluloses by biomass-derived deep eutectic solvent (DES) pretreatment for cellulose enzymatic hydrolysis and lignin valorization, Green Chem. 21 (2019) 275–283.

672 [24] Z.K. Wang, S. Hong, J. long Wen, C.Y. Ma, L. Tang, H. Jiang, J.J. Chen, S. Li,
673 X.J. Shen, T.Q. Yuan, Lewis Acid-Facilitated Deep Eutectic Solvent (DES)
674 Pretreatment for Producing High-Purity and Antioxidative Lignin, ACS
675 Sustain. Chem. Eng. 8 (2020) 1050–1057.
676 [25] A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton, D.
677 Crocker, Determination of structural carbohydrates and lignin in Biomass -
678 NREL/TP-510-42618, Lab. Anal. Proced. (2012) 17.
679 [26] Z. Ling, Z. Guo, C. Huang, L. Yao, F. Xu, Bioresource Technology
680 Deconstruction of oriented crystalline cellulose by novel levulinic acid based
681 deep eutectic solvents pretreatment for improved enzymatic accessibility,
682 Bioresour. Technol. 305 (2020) 123025.
683 [27] J.A. Sirviö, K. Hyypiö, S. Asaadi, K. Junka, H. Liimatainen, High-strength
684 cellulose nanofibers produced: Via swelling pretreatment based on a choline
685 chloride-imidazole deep eutectic solvent, Green Chem. 22 (2020) 1763–1775.
686 [28] K.H. Kim, T. Dutta, J. Sun, B. Simmons, S. Singh, Biomass pretreatment using
687 deep eutectic solvents from lignin derived phenols, Green Chem. 20 (2018)
688 809–815.
689 [29] Z. Chen, W.D. Reznicek, C. Wan, Aqueous Choline Chloride: A Novel Solvent
690 for Switchgrass Fractionation and Subsequent Hemicellulose Conversion into
691 Furfural, ACS Sustain. Chem. Eng. 6 (2018) 6910–6919.
692 [30] L. Yang, Y. Ru, S. Xu, T. Liu, L. Tan, Bioresource Technology Features
693 correlated to improved enzymatic digestibility of corn stover subjected to
694 alkaline hydrogen peroxide pretreatment, Bioresour. Technol. 325 (2021)
695 124688.
696 [31] C. Dong, X. Meng, C.S. Yeung, H.Y. Tse, A.J. Ragauskas, S.Y. Leu, Diol

pretreatment to fractionate a reactive lignin in lignocellulosic biomass biorefineries, Green Chem. 21 (2019) 2788–2800.

[32] Q. Chu, R. Wang, W. Tong, Y. Jin, J. Hu, K. Song, Improving Enzymatic Saccharification and Ethanol Production from Hardwood by Deacetylation and Steam Pretreatment: Insight into Mitigating Lignin Inhibition, ACS Sustain. Chem. Eng. 8 (2020) 17967–17978.

[33] T.R. Mota, D.M. Oliveira, R. Simister, C. Whitehead, A. Lanot, D. Wanderley, C.A. Rezende, S.J. Mcqueen-, L.D. Gomez, Bioresource Technology Design of experiments driven optimization of alkaline pretreatment and saccharification for sugarcane bagasse, Bioresour. Technol. 321 (2021) 124499.

[34] D. He, Y. Wang, C.G. Yoo, Q.-J. Chen, Q. Yang, The fractionation of woody biomass under mild conditions using bifunctional phenol-4-sulfonic acid as a catalyst and lignin solvent, Green Chem. 22 (2020) 5414–5422.

[35] C.S. Lancefield, I. Panovic, P.J. Deuss, K. Barta, N.J. Westwood, Pre-treatment of lignocellulosic feedstocks using biorenewable alcohols: Towards complete biomass valorisation, Green Chem. 19 (2017) 202–214.

[36] F.H.B. Sosa, D.O. Abranches, A.M. Da Costa Lopes, J.A.P. Coutinho, M.C. Da Costa, Kraft Lignin Solubility and Its Chemical Modification in Deep Eutectic Solvents, ACS Sustain. Chem. Eng. 8 (2020) 18577–18589.

[37] B. Soares, C. Lopes, A.J.D. Silvestre, P.C. Rodrigues, C.S.R. Freire, A.P. Coutinho, Industrial Crops & Products Wood delignification with aqueous solutions of deep eutectic solvents, Ind. Crop. Prod. 160 (2021) 113128.

[38] L. Wang, L. Xu, J. Zhang, Q. Zong, L. Wang, T. Xu, J. Gong, High-solid Ethylenediamine Pretreatment to Fractionate New Lignin Streams from Lignocellulosic Biomass High-solid ethylenediamine pretreatment to

fractionate new lignin streams from lignocellulosic biomass, Chem. Eng. J. 427 (2021).

[39] X. Shen, T. Chen, H. Wang, Q. Mei, F. Yue, S. Sun, Structural and Morphological Transformations of Lignin Macromolecules during Bio-Based Deep Eutectic Solvent (DES) Pretreatment, ACS Sustain. Chem. Eng. 8 (2020) 2130−2137.

[40] J.D. Zwilling, X. Jiang, F. Zambrano, R.A. Venditti, H. Jameel, O.D. Velev, O.J. Rojas, R. Gonzalez, Understanding lignin micro- And nanoparticle nucleation and growth in aqueous suspensions by solvent fractionation, Green Chem. 23 (2021) 1001–1012.

[41] J.L. Wen, S.L. Sun, T.Q. Yuan, R.C. Sun, Structural elucidation of whole lignin from Eucalyptus based on preswelling and enzymatic hydrolysis, Green Chem. 17 (2015) 1589–1596.

[42] J. Wen, B. Xue, F. Xu, R. Sun, A. Pinkert, Unmasking the structural features and property of lignin from bamboo, Ind. Crop. Prod. 42 (2013) 332–343.

[43] W. Lan, M.T. Amiri, C.M. Hunston, J.S. Luterbacher, Protection Group Effects During a , g -Diol Lignin Stabilization Promote High-Selectivity Monomer Production Angewandte, Angew.Chem. Int.Ed. 57 (2018) 1356–1360.

[44] C. Cai, K. Hirth, R. Gleisner, H. Lou, X. Qiu, J.Y. Zhu, Maleic acid as a dicarboxylic acid hydrotrope for sustainable fractionation of wood at atmospheric pressure and ≤100 °C: mode and utility of lignin esterification, Green Chem. 22 (2020) 1605–1617.

[45] Y. Liang, W. Duan, X. An, Y. Qiao, Y. Tian, H. Zhou, Bioresource Technology Novel betaine-amino acid based natural deep eutectic solvents for enhancing the enzymatic hydrolysis of corncob, Bioresour. Technol. 310

747 (2020) 123389.
748 [46] A. Romaní, E.S. Morais, P.O. Soares, M.G. Freire, C.S.R. Freire, A.J.D.
749 Silvestre, L. Domingues, Bioresource Technology Aqueous solutions of deep
750 eutectic systems as reaction media for the sacchari fi cation and fermentation of
751 hardwood xylan into xylitol, Bioresour. Technol. 311 (2020) 123524.
752 [47] Y. Wang, X. Meng, K. Jeong, S. Li, G. Leem, K.H. Kim, Y. Pu, A.J.
753 Ragauskas, C.G. Yoo, Investigation of a Lignin-Based Deep Eutectic Solvent
754 Using p -Hydroxybenzoic Acid for Efficient Woody Biomass Conversion, ACS
755 Sustain. Chem. Eng. 8 (2020) 12542–12553.
756 [48] L. Bui, H. Luo, W.R. Gunther, Y. Román-Leshkov, Domino Reaction
757 Catalyzed by Zeolites with Brønsted and Lewis Acid Sites for the Production
758 of γ-Valerolactone from Furfural, Angew. Chemie. 125 (2013) 8180–8183.
759 [49] T.M. Aida, Y. Sato, M. Watanabe, K. Tajima, T. Nonaka, H. Hattori, K. Arai,
760 Dehydration of d-glucose in high temperature water at pressures up to 80 MPa,
761 J. Supercrit. Fluids. 40 (2007) 381–388.
762 [50] M. Spangsberg, S. Shunmugavel, E. Taarning, Conversion of Sugars to Lactic
763 Acid Derivatives Using Heterogeneous Zeotype Catalysts, Science. 328 (2010).