

## Supplementary data

### Short-time deep eutectic solvents pretreatment for enhanced enzymatic saccharification and lignin valorization

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#### Keywords

DESs pretreatment; Short time; Enzymatic hydrolysis; Lignin; Recover and recycle;

## **1. Materials and methods**

### **1.1. Materials**

Corncoobs were provided by a local farm in Jinan, Shandong Province, China. The lignocellulosic samples were milled and sieved to 40-60 mesh and exhaustively extracted with toluene/ethanol (2:1 v/v). The extraction-free samples were oven-dried for 12 h at 45 °C and stored in a sealed bag at room temperature. The cellulase used in this work was bought from Shanghai Youtell Biochemical., Ltd. (Shanghai, China) and its filter paper activity was 145 FPU/g. Benzyltrimethylammonium chloride (BTMAC), Benzyltriethylammonium chloride (BTEAC), and lactic acid (LA) were purchased from Beijing Blue Yi Chemical Products Co., Ltd (Beijing, China). All other chemicals were analytical grade and used directly without further purification.

### **1.2. DESs preparation**

Two different DESs are prepared: BTMAC/LA and BTEAC/LA systems at molar ratios of 1:2. The mixtures were heated at 60 °C and stirred at 400 rpm for 1 h to form a homogeneous and transparent liquid. The obtained DESs were cooled down to room temperature in a desiccator with silica gel for avoiding moisture absorption.

### **1.3. DESs pretreatment process**

The pretreatment process was comparatively conducted in a sealed stainless steel autoclave. 1 g of raw corncob was pretreated with 20 mL of DESs at different temperature (100 °C, 120 °C, 140 °C) with stirring at 200 rpm for 2 h. After reaction, the solid phase and liquid phase were separated by centrifuging the mixed solutions at 10000 rpm for 10 min. The recovered solids were washed with water and ethanol

(2:1 v/v) to obtain regenerative cellulose until the supernatant was colorless. Cellulosic residues were vacuum dried overnight at 60 °C for 12 h. The washing liquor (water and ethanol) was recovered and recycled by using a Heidolph Hei-VAP Advantage in vacuo rotary evaporator (Germany) at 40 °C for 2 h, following by heating at 60 °C for 2 h. All assays were performed in triplicate. Lignin in the liquid was recovered through precipitation in neutral water and then freeze-dried overnight.

#### **1.4. Quantitative measurement of soluble carbohydrates in the liquid phases**

The concentrations of glucose, xylose, HMF, and furfural in the liquid phase were directly detected by a HPLC system (Agilent, 1260) equipped with a Bio-Rad HPX-87P ion-exclusion column. Degassed 0.01 N dilute H<sub>2</sub>SO<sub>4</sub> solution was used as the mobile phase with a flow rate of 0.6 ml/min, while the temperature in the column was kept constant at 50 °C. All assays were carried out in triplicate.

#### **1.5. Composition and structure determination of solid residues**

The chemical components of the untreated and pretreated corncob samples were determined by using the methods of Laboratory Procedure (LAP) by the National Renewable Energy Laboratory <sup>1</sup>. FTIR analyses were accomplished by using a Nicolet iN10 FTIR spectrophotometer at a resolution of 4 cm<sup>-1</sup> resolution and 128 scans per sample. X-ray patterns were observed from a diffractometer Bruker D8 Advance (Bruker AXS, Germany) equipped with monochromatic Cu K $\alpha$  radiation ( $k = 0.154$  nm) under a voltage of 40 kV and a current of 40 mA with a wave length of 1.79 Å. All samples were analyzed in continuous scan mode with the  $2\theta$  ranging from 5° to 40°,

with 0.02° per second of scanning rate. Surface morphology changes of the cellulosic residue were observed by a field emission scanning electron microscope (FE-SEM) operated at 5 kV acceleration voltages. Thermogravimetric analysis (TG) curves of the samples were obtained on a thermal analyzer (SDT Q600, TA Instrument). Samples (5-10 mg) were placed on an Al<sub>2</sub>O<sub>3</sub> crucible, and the ramp rate was 10 °C/min from 25 to 600 °C using nitrogen (flow rate 50 mL/min) as the flushing gas. TG, differential scanning calorimetry (DSC), and derivative thermogravimetric analysis curves of BTMAC/LA and BTEAC/LA systems from 25 to 300 °C was executed with the methods same to those for the cellulose-enriched fractions as mentioned above. The DESs (BTMAC/LA and BTEAC/LA) were cooled at a rate of 5 °C/min and the melting points were taken as the temperature at which the first liquid began to form. X-ray Photoelectron Spectroscopy (XPS) was conducted to detect the changes of elements in the pretreated samples.

#### **1.6. Enzymatic hydrolysis**

Enzymatic hydrolysis experiments of untreated and pretreated corncobs were conducted in 25 mL stoppered Erlenmeyer flask at 48 °C in a shaking air bath at 120 rpm for 72 h. 0.3 g of samples (2%, wt/v) were suspended into 15 mL of 50 mM sodium acetate buffer (pH 4.8) with 15 FPU/g substrate of cellulase containing endoglucanases, exoglucanases, and β-glucosidases. 0.2 mL of supernatant was taken periodically to monitor the saccharification kinetics, followed by deactivating enzymes in -50 °C for 5 min and centrifuging at 4000 rpm for 5 min. Then the supernatant was collected and filtered through a 0.45 μm syringe filter. The released

soluble glucose was measured by a high performance liquid chromatography (HPLC) system (Agilent, 1260) equipped with the Aminex HPX-87H organic acid column (Bio-Rad) at 323 K, and with 5 mM H<sub>2</sub>SO<sub>4</sub> at a flow rate of 0.6 ml/min. Three replicate tests were carried out in all enzymatic hydrolysis experiments and average values were presented.

### **1.7. DESs recover and recycle**

After separation of solid phase and liquid phase, all the filtrates were collected to obtain lignin by deionized water precipitation. The residual liquid fractions was separated using a Heidolph Hei-VAP Advantage in vacuo rotary evaporator (Germany) at 60 °C for 2 h, which was sufficient to remove almost all water from the BTMAC/LA solvent system. Then, the recovered BTMAC/LA solvent were used to treat corncob at 140 °C and the treatment procedure was the same as mentioned above. In each cycles, the enzymatic efficiency of cellulose-enriched samples was utilized to evaluate the DESs recycling performance. All assays were carried out in triplicate.

### **1.8. Characterization of lignin**

The sugars contents of lignin fraction were determined by a one-step hydrolysis with H<sub>2</sub>SO<sub>4</sub> at 105 °C for 2.5 h and analyzed by high-performance anion-exchange chromatography (HPAEC) system (Dionex ICS3000, USA). The weight-average (*M<sub>w</sub>*) and number-average (*M<sub>n</sub>*) molecular weights were conducted by gel permeation chromatography (GPC) with an ultraviolet detector (UV) at 280 nm on a PL-gel 10mm Mixed-B 7.5mm ID column. The column was operated at ambient temperature and a flow rate of 0.5 mL min<sup>-1</sup> was maintained. Then, the *M<sub>w</sub>* and *M<sub>n</sub>* were calculated

according to the calibration with a series of polystyrene standards and polydispersity index ( $M_w/M_n$ ) was calculated. FTIR spectra and TG curves analysis was executed with the methods same to those for the cellulose-enriched fractions as mentioned above.

**References:**

1. Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2008. Determination of structural carbohydrates and lignin in biomass. Lab. Anal. Proc. 1617, 1–16.

pretreatments.

**Fig. S2.** TG, DSC, and DTG curves of BTMAC/LA (a) and BTEAC/LA (b) system.

**Fig. S3.** FTIR spectra of cellulose-enriched fractions after DESs pretreatment (untreated: a; treated by BTEAC/LA at 120 °C: b; treated by BTMAC/LA at 120 °C: c; treated by BTEAC/LA at 140 °C: d; treated by BTMAC/LA at 140 °C: e;).

**Fig. S4.** X-ray diffractograms of corncob before (a) and after DESs pretreatment by BTMAC/LA at 100 °C: b, BTEAC/LA at 100 °C: c, BTMAC/LA at 120 °C: d, BTEAC/LA at 120 °C: e, BTMAC/LA at 140 °C: f, and BTEAC/LA at 140 °C: g.

**Fig. S5.** TG curves of corncob before (a) and after DESs pretreatment by BTMAC/LA at 100 °C: c, BTEAC/LA at 100 °C: d, BTMAC/LA at 120 °C: e, BTEAC/LA at 120 °C: f, BTMAC/LA at 140 °C: g, and BTEAC/LA at 140 °C: h.

**Fig. S6.** The correlation diagrams of the effect of crystallinity change and hemicellulose/lignin removal on the sugar yield for BTMAC/LA and BTEAC/LA pretreatment.

**Fig. S7.** XPS wide-scan spectra of corncob after BTMAC/LA and BTEAC/LA pretreatment at 140 °C.

**Fig. S8.** FTIR spectra of hemicellulose fractions extracted by BTMAC/LA at 120 °C: a, BTEAC/LA at 120 °C: b, BTMAC/LA at 140 °C: c, and BTEAC/LA at 140 °C: d.

**Fig. S9.** FTIR spectra of lignin fractions extracted by BTMAC/LA at 120 °C: a, BTEAC/LA at 120 °C: b, BTMAC/LA at 140 °C: c, and BTEAC/LA at 140 °C: d.

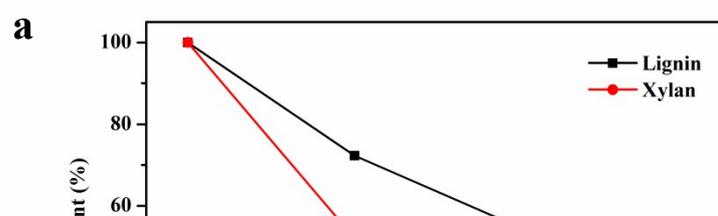
**Fig. S10.** Melting points of BTMAC/LA and BTEAC/LA.

**Table. S1.** Assignments of  $^{13}\text{C}$ - $^1\text{H}$  correlated signals in the HSQC spectra of the lignin fractions from corncob.

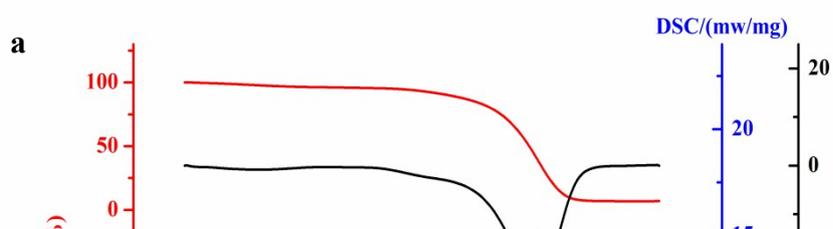
**Table. S2.** The capital costs and products profits.

**Table. S3.** Acidity (pH value) of DESs systems.

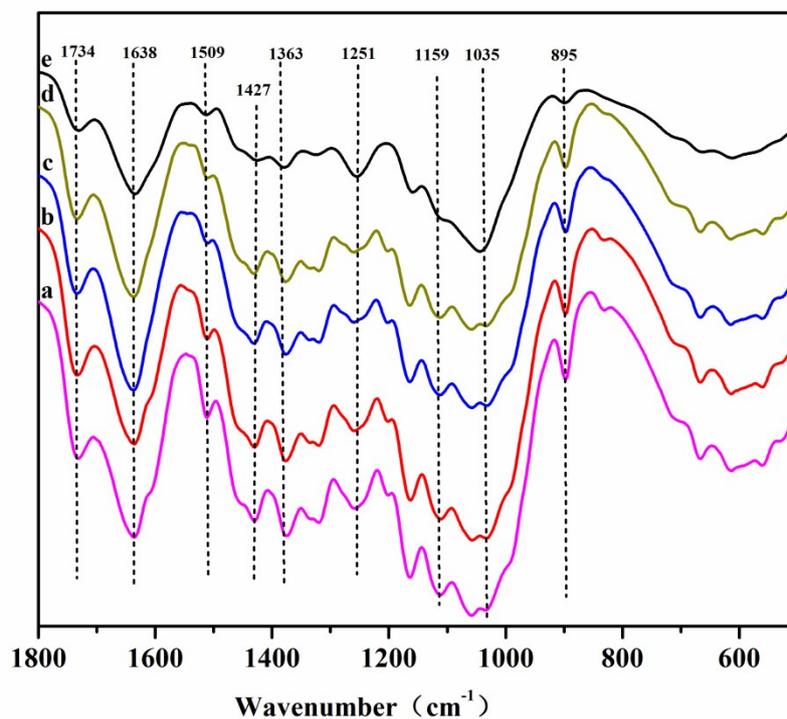
**Table. S4.** Elemental surface composition of samples pretreated by BTMAC/LA and BTEAC/LA at 140 °C was determined from XPS.



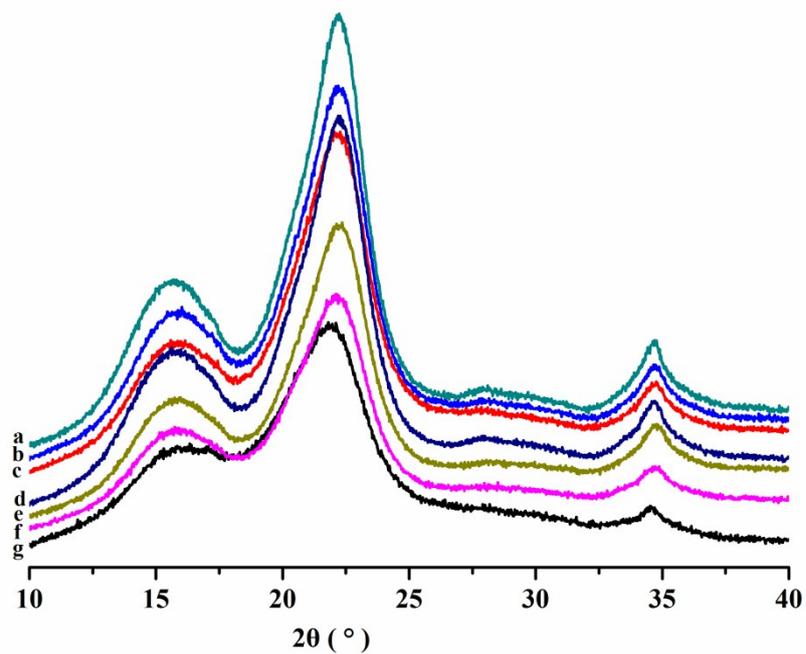
**Fig. S1.** Removal of xylan and lignin during the BTMAC/LA and BTEAC/LA systems pretreatments.



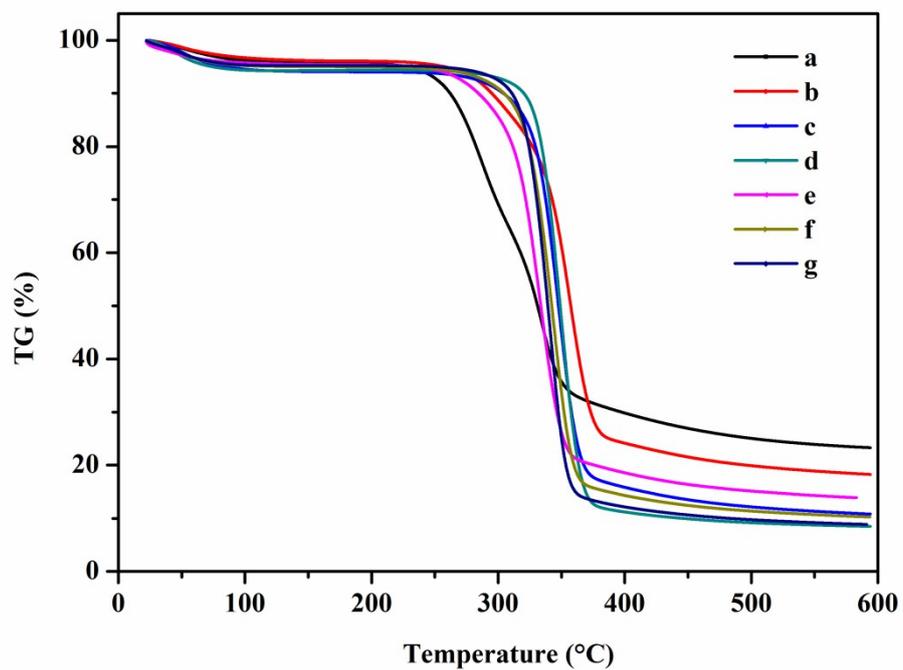
**Fig. S2.** TG, DSC, and DTG curves of BTMAC/LA (a) and BTEAC/LA (b) system.



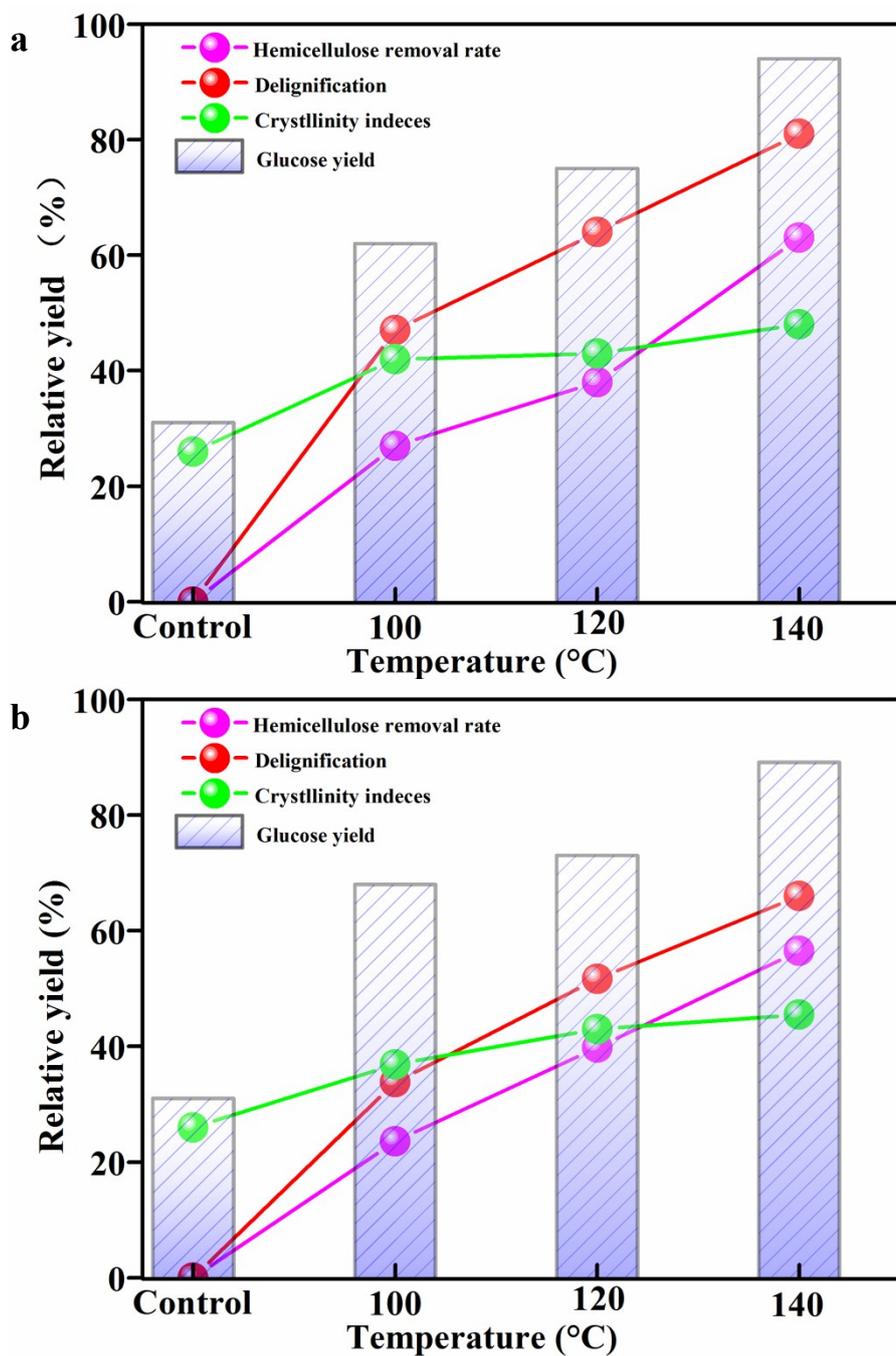
**Fig. S3.** FTIR spectra of cellulose-enriched fractions after DESs pretreatment (untreated: a; treated by BTEAC/LA at 100 °C: b; treated by BTMAC/LA at 100 °C: c; treated by BTEAC/LA at 140 °C: d; treated by BTMAC/LA at 140 °C: e).



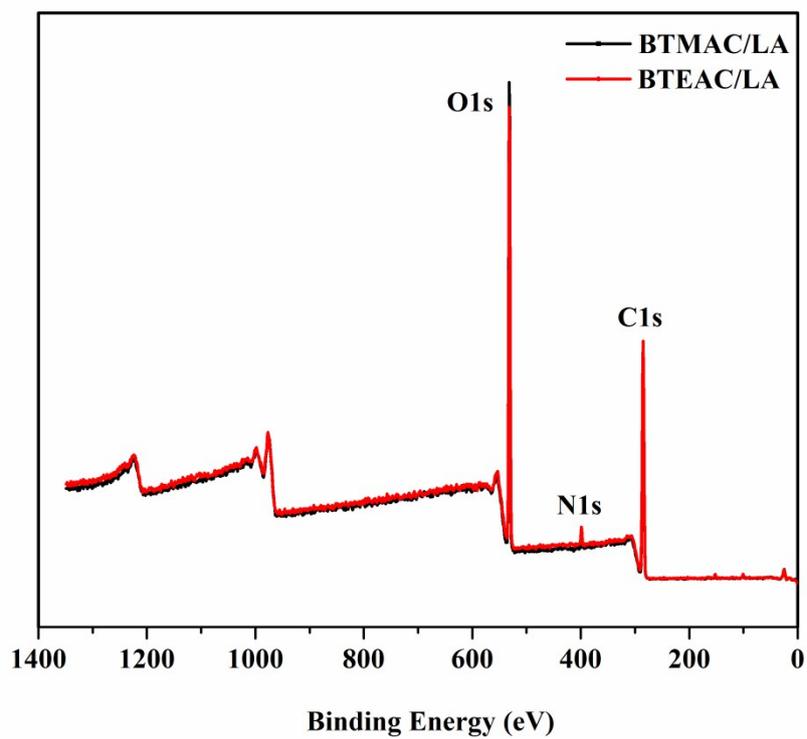
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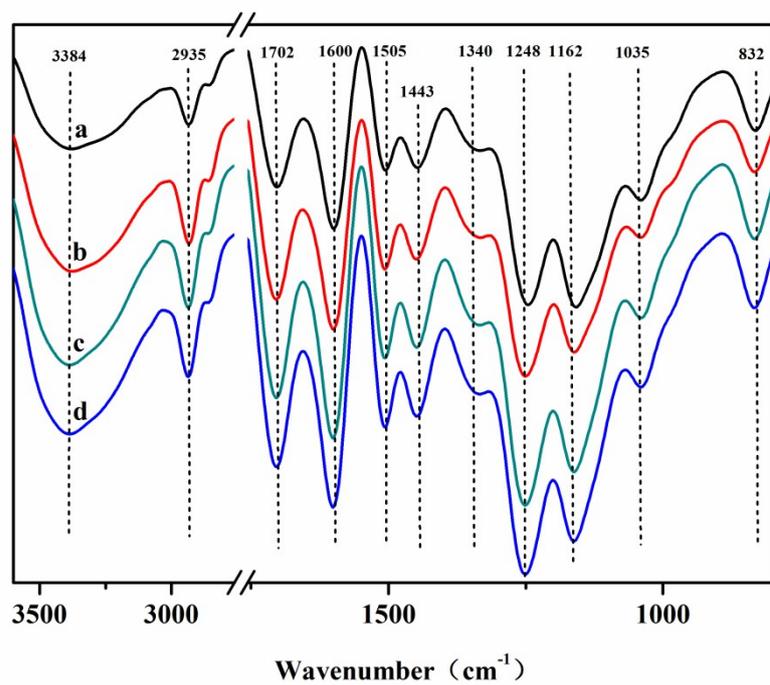
**Fig. S5.** TG curves of corncob before (a) and after DESs pretreatment by BTMAC/LA at 100 °C: c, BTEAC/LA at 100 °C: d, BTMAC/LA at 120 °C: e, BTEAC/LA at 120 °C: f, BTMAC/LA at 140 °C: g, and BTEAC/LA at 140 °C: h.



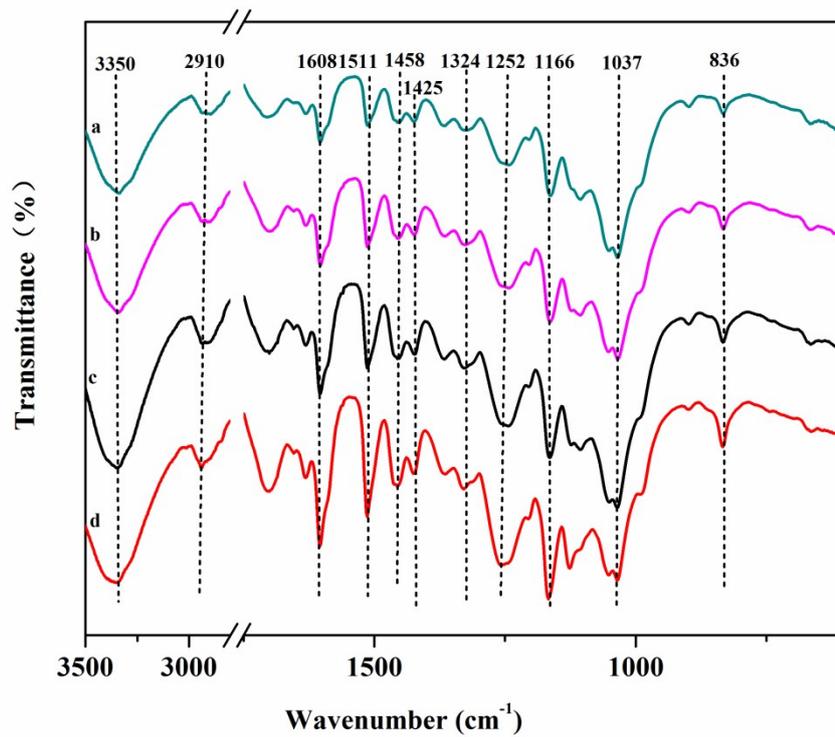
**Fig. S6.** The correlation diagrams of the effect of crystallinity change and hemicellulose/lignin removal on the sugar yield for BTMAC/LA and BTEAC/LA pretreatment.



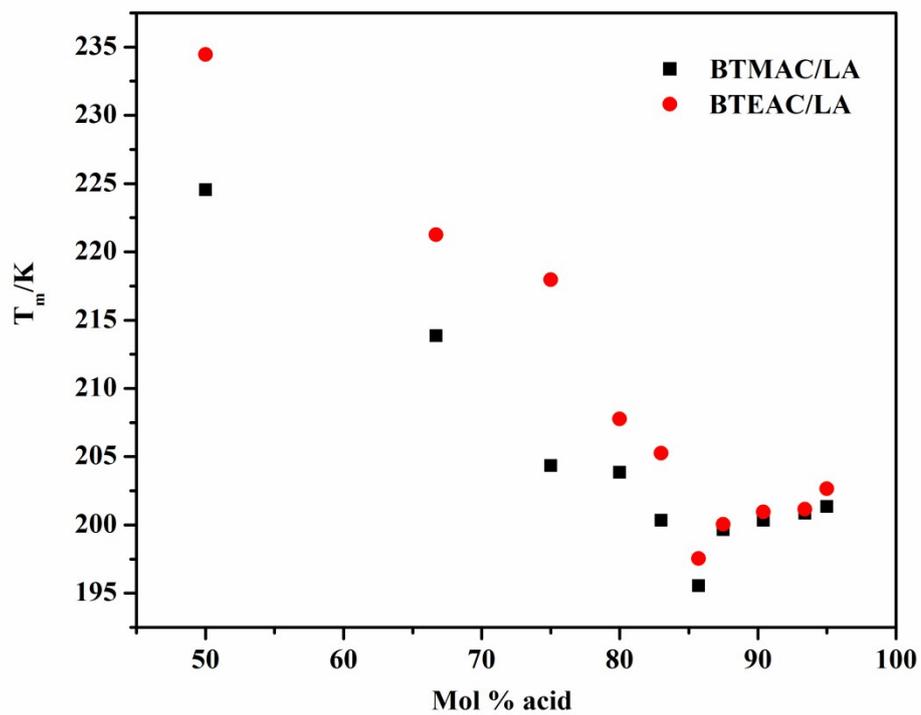
**Fig. S7.** XPS wide-scan spectra of corncob after BTMAC/LA and BTEAC/LA pretreatment at 140 °C.



**Fig. S8.** FTIR spectra of hemicellulose fractions extracted by BTMAC/LA at 120 °C: a, BTEAC/LA at 120 °C: b, BTMAC/LA at 140 °C: c, and BTEAC/LA at 140 °C: d.



**Fig. S9.** FTIR spectra of lignin fractions extracted by BTMAC/LA at 120 °C: a, BTEAC/LA at 120 °C: b, BTMAC/LA at 140 °C: c, and BTEAC/LA at 140 °C: d.



**Fig. S10.** Melting points of BTMAC/LA and BTEAC/LA.

**Table. S1.** Assignments of  $^{13}\text{C}$ - $^1\text{H}$  correlated signals in the HSQC spectra of the lignin fractions from corncob.

Labels	$\delta_{\text{C}}/\delta_{\text{H}}$ (ppm)	Assignment
-OCH <sub>3</sub>	55.5/3.73	C-H in methoxyls
A <sub><math>\gamma</math></sub>	59.8/ 3.40-3.65	C <sub><math>\gamma</math></sub> -H <sub><math>\gamma</math></sub> in $\beta$ -O-4 substructures (A)
X <sub>5</sub>	62.8/3.23	C <sub>5</sub> -H <sub>5</sub> $\beta$ -D-xylopyranoside substructures (X)
X <sub>4</sub>	75.6/3.63	C <sub>4</sub> -H <sub>4</sub> $\beta$ -D-xylopyranoside substructures (X)
X <sub>3</sub>	74.1/3.32	C <sub>3</sub> -H <sub>3</sub> $\beta$ -D-xylopyranoside substructures (X)
A' <sub><math>\gamma</math></sub> /A'' <sub><math>\gamma</math></sub>	63.8/4.21	C <sub><math>\gamma</math></sub> -H <sub><math>\gamma</math></sub> in $\gamma$ -acylated $\beta$ -O-4 substructures (A')
A <sub><math>\alpha</math></sub>	72.8/4.98	C <sub><math>\alpha</math></sub> -H <sub><math>\alpha</math></sub> in $\beta$ -O-4 substructures (A)
S <sub>2,6</sub>	104.8/6.73	C <sub>2,6</sub> -H <sub>2,6</sub> in syringyl units (S)
G <sub>2</sub>	110.8/6.96	C <sub>2</sub> -H <sub>2</sub> in guaiacyl units (G)
FA <sub>2</sub>	111.5/7.31	C <sub>2</sub> -H <sub>2</sub> in ferulate (FA)
G <sub>5</sub>	115.6/6.84	C <sub>5</sub> -H <sub>5</sub> in guaiacyl units (G)
G <sub>6</sub>	119.6/6.78	C <sub>6</sub> -H <sub>6</sub> in guaiacyl units (G)
H <sub>2,6</sub>	129.1/7.08	C <sub>2,6</sub> -H <sub>2,6</sub> in <i>p</i> -hydroxyphenyl units (H)
T <sub>3</sub>	103.6/7.01	C <sub>3</sub> -H <sub>3</sub> in triclin (T)
T' <sub>2,6</sub>	103.6/7.28	C <sub>2,6</sub> -H <sub>2,6</sub> in triclin (T)
PCA <sub>2,6</sub>	130.0/7.46	C <sub>2,6</sub> -H <sub>2,6</sub> in <i>p</i> -coumarate (PCA)
PCA <sub><math>\alpha</math></sub> /FA <sub><math>\alpha</math></sub>	145.8/7.45	C <sub><math>\alpha</math></sub> -H <sub><math>\alpha</math></sub> in <i>p</i> -coumarate (PCA) and ferulate (FA)

**Table. S2.** The capital costs and products profits.

Method <sup>a</sup>		Capital costs (\$)			Profits (\$) <sup>d</sup>			
		Solvents <sup>b</sup>	Enzyme <sup>b</sup>	Others <sup>c</sup>	Total	Sugar	Lignin	Total
BTMAC/LA corncob	for	2.17	0.35	1.14	3.66	2.54×10 <sup>-4</sup>	1.18×10 <sup>-4</sup>	3.72×10 <sup>-4</sup>
BTEAC/LA corncob	for	2.23	0.35	1.15	3.73	2.81×10 <sup>-4</sup>	1.05×10 <sup>-4</sup>	3.86×10 <sup>-4</sup>
[C <sub>2</sub> mim]OAc poplar	for	279.55	0.72	2.52	282.79	-	-	-
[EMIM]DEP wheat straw	for	127.30	0.61	0.88	128.79	-	-	-

<sup>a</sup> The economic analysis are based on the 1 g of sample in the pretreatment and enzymatic hydrolysis process.

<sup>b</sup> All the chemicals prices are based on the data from Mclean Biochemical Technology Company.

<sup>c</sup> Others including electricity cost and reactor cost in the synthesis of DESs, pretreatment, and enzymatic hydrolysis process.

<sup>d</sup> All the products prices are based on the data from the business platform ([www.alibaba.com](http://www.alibaba.com)).

**Table. S3.** Acidity (pH value) of DESs systems.

Fresh DES	pH
BTMAC/LA	1.03
BTEAC/LA	1.11

**Table. S4.** Elemental surface composition of samples pretreated by BTMAC/LA and BTEAC/LA at 140 °C was determined from XPS.

Sample	Element (at. %)			
	Cl	C	N	O
BTMAC/LA	- <sup>a</sup>	60.23	0.72	39.05
BTEAC/LA	-	60.45	2.59	36.96

<sup>a</sup> No detectable levels (below 0.01% based on dry raw materials).