Supplementary Material

Efficient separation of uncondensed lignin and high-quality cellulose from bamboo using reactive deep eutectic solvents for versatile valorization

Ruojin Shen[a], Chao Wang[a,*], Keqin Zhang[a], Changyi Zhu[a], Lupeng Shao[a,*], Kai Zhang[a], Xianhai Zeng[b], Feng Xu[a,c], Guihua Yang[a,*], Jiachuan Chen[a]

[a] *State Key Laboratory of Green Papermaking and Resource Recycling, Key Laboratory of Pulp and Paper Science & Technology of Ministry of Education, Qilu University of Technology, Shandong Academy of Sciences, Jinan 250353, China*

[b.] *State Key Laboratory of Physical Chemistry of Solid Surfaces, College of Energy, Xiamen University, Xiamen 361102, China.*

[c] *State Key Laboratory of Efficient Production of Forest Resources, Beijing Key Laboratory of Lignocellulosic Chemistry, Beijing Forestry University, Beijing 100083, China.*

*Corresponding authors

E-mail addresses: chaowang@qlu.edu.cn (C. Wang); shaolp@qlu.edu.cn (L. Shao); ygh2626@126.com (G. Yang)

1. Composition analysis

Compositional analysis of poplar samples was carried out according to standard NREL protocols.[1, 2] Accurately weighed 0.3 g of raw material was treated with 3 mL of 72% sulfuric acid. The mixture was maintained in a water bath at 30 °C for 1 h, with manual stirring from the outside to the inside every 5-10 min to ensure homogeneity. After this period, the reaction was quenched by the addition of 84 mL of deionized water. The resulting mixture was then subjected to autoclave treatment at 121 °C for 1 h. For analysis, a 5 mL aliquot was withdrawn for liquid chromatography. The remaining mixture was vacuum-filtered through a pre-weighed G3 glass filter. The solid residue was washed thoroughly with deionized water until the filtrate reached neutral pH. Finally, the filter containing the residue was dried to constant weight and weighed to determine the solid mass.

2. Analysis procedures

X-ray diffraction (XRD) analysis: X-ray diffraction (XRD) analysis was performed using an X-ray diffractometer (D8-ADVANCE, Bruker, Germany) with a scanning speed of 25 μm/min in the 2θ range of 5-50 μm.

$$C_rI(\%) = \frac{(I_{002} - I_{am})}{I_{002}} \times 100\% \quad (1)$$

I_{002} and I_{am} denote the peak intensity at 2θ = 22° in the crystalline region and the minimum intensity at 2θ = 18° in the amorphous region, respectively.[3, 4]

An appropriate amount of dried cellulose-rich residue was mixed with peroxyacetic acid at a solid-to-liquid ratio of 1:20 (w/v). The mixture was incubated in a water bath at 60 °C with continuous stirring for 30-48 h, followed by vacuum drying. Subsequent purification involved sequential washing with sodium hydroxide solution, distilled water, and acetic acid, after which the product was gradually filtered and air-dried to yield high-purity cellulose. The obtained cellulose was characterized for brightness using a spectrophotometer and for viscosity using a pulp viscometer. Brightness measurement was performed as follows: after warming up the instrument for 100 s, calibration was carried out using a standard black block and a standard white

block in sequence. The sample was then placed in the holder and measured by pressing the test button.

Viscosity and DP: To determine the viscosity and degree of polymerization (DP) of the pulp, 15 mL of water and an appropriate amount of glass beads were first added to the dispensing bottle and fully shaken for 15 min, and then the mixture was dissolved in 15 mL of copper ethylenediamine solution before adding an appropriate amount of glass beads and copper flakes for shaking. After the end of the process a holding period of 5 min was carried out in a 30 °C water bath. After applying suction to elevate the liquid, the fall time between the meniscus markers was recorded. Each sample was tested three times to calculate the average viscosity and DP.

^{31}P NMR: A homogeneous solvent mixture was prepared by combining deuterated pyridine and deuterated chloroform at a defined ratio.[5] In this mixture, the relaxation agent chromium (III) acetylacetonate was dissolved at a concentration of 11 mg/mL, and the internal standard was dissolved at a concentration of 80 mg/mL. Approximately 40 mg of dried lignin sample was accurately weighed and transferred into an NMR tube. The prepared deuterated pyridine-chloroform solvent was added to the tube, followed by the internal standard solution and the relaxation agent solution. After thorough mixing to ensure complete dissolution, the phosphorylation reagent TMDP was added. The sample was immediately analyzed using a Bruker AVANCE II 400 MHz NMR spectrometer. The phosphorylation mechanism is illustrated in the accompanying scheme.

2D HSQC NMR: The structural features of lignin extracted with DES were analyzed by 2D HSQC NMR. HSQC spectra were processed and analyzed with MestReNova 6.0.4 (MestRelab Research) with the chemical shift reference center DMSO peak.[6]

The relative contents were calculated-from-the-integrals as follows:

For the aromatic region:

$$Total\ aromantic\ rings = \frac{S_{2,6} + S'_{2,6}}{2} + S_{condensed} + \frac{G_2 + G_5 + G_6}{3} \quad (2)$$

$$Ratio\ S\ (per\ 100Ar) = (\frac{S_{2,6} + S'_{2,6}}{2 \times Total\ aromantic\ rings}) \times 100 \quad (3)$$

$$Ratio\ G\ (per\ 100Ar) = (\frac{G_2 + G_5 + G_6}{3 \times Total\ aromantic\ rings}) \times 100 \quad (4)$$

$$\frac{S}{G} = \frac{Ratio\ S}{Ratio\ G} \quad (5)$$

For-the-linkages in side chain region:

$$Ratio\ A\ (per\ 100Ar) = (\frac{A_\alpha}{Total\ aromantic\ rings}) \times 100 \quad (6)$$

$$Ratio\ GA\ (per\ 100Ar) = (\frac{GA_\alpha}{Total\ aromantic\ rings}) \times 100 \quad (7)$$

$$Total\ \beta - O - 4\ linages\ (per\ 100Ar) = (\frac{\beta - O - 4_\alpha + \alpha - alkoxylated\ \beta - O - 4_\alpha}{Total\ aronmantiv\ rings}) \times 100 \quad (8)$$

GPC: The molecular weight distribution of extracted lignin was determined via GPC. Lignin samples were first acetylated: 40 mg of lignin sample was placed in a 5 mL glass vial, and 1 mL of pyridine was added.[7] The mixture was thoroughly stirred until the sample dissolved completely. Subsequently, 1 mL of acetic anhydride was added to the solution. The vial was immediately sealed with sealing film and a rotor was attached. Place the mixture in an ultrasonic disperser to ensure thorough mixing of the reaction system. Wrap the reaction vial with aluminum foil to shield from light and stir at room temperature for 24 h. After the reaction, transfer the reaction mixture to a centrifuge tube containing ice water. The acetylated lignin gradually precipitates under low-temperature conditions. Centrifuge at 8000 rpm for 5 min, discard the supernatant, and wash the precipitate repeatedly with ice water until the washings are odorless. Finally, freeze-dry the precipitate to obtain the acetylated lignin sample.

Dissolve the acetylated lignin sample at a concentration of 2 mg/mL in chromatographic-grade THF, shaking thoroughly to ensure complete dissolution. Filter

the solution through a 0.45 μm organic filter membrane, collect the filtrate in a 1.5 mL chromatography vial, and set aside. Gel permeation chromatography (GPC) was performed on a Waters e2695 system equipped with a PL-gel MIXED-E column and a UV detector to analyze the molecular weight distribution of the acetylated lignin sample. The detection conditions were as follows: UV detector, column temperature set to 40 °C, mobile phase was chromatographic-grade THF, and flow rate was maintained at 0.6 mL/min. After sample injection, separation occurred through the column, with the UV detector recording the elution curve in real time, ultimately yielding the relative molecular mass distribution data for acetylated lignin.

Monomer quantification

Due to the difficulty in obtaining or producing a large amount of monomers, we adopted a quantitative method based on an internal standard (acetyl benzene). We dissolve the bio-oil in a predetermined amount of internal standard solution (25 mL of ethyl acetate contain 10 μL of acetyl benzene.). The depolymerization products were analyzed by gas chromatography-mass spectrometry (GC-MS) using an Agilent 7890B instrument equipped with an HP-5MS column. The GC-MS conditions were set as follows: an initial temperature of 50 °C held for 5 min, followed by a temperature ramp at 10 °C/min to 300 °C. The injection volume was 1 μL, the injector temperature was maintained at 300 °C, and the split ratio was 10:1. Compound identification was achieved by comparison with spectral libraries provided by NIST. The monomer yield was calculated based on the area of the monomer and the area of acetyl benzene in the GC-MS. The specific calculation is as follows:

$$C_{monomer} = \frac{A_{monomer}}{A_{internal\ standard}} \times C_{internal\ standard} \quad (9)$$

$$Y_{monomer} = \frac{C_{monomer} \times V}{m} \quad (10)$$

C: Concentration of the monomer (internal standard); A: The peak area of monomer (internal standard) in the GC-MS chromatogram; Y: The yield of monomer based on the weight of lignin; V: The total volume of sample; m: The weight of extracted lignin.

Mechanical Properties Testing: The mechanical properties of the specimens were qualitatively determined using a tensile testing machine (MTS SYSTEMS Co., Ltd., China). For mechanical testing, the gel bead was cut into cylindrical samples measuring 2 cm in diameter and 1.5 cm in height. Individual specimens were placed on a flat platform and compressed in the vertical direction.

Table S1 Sugar analysis of lignin samples

	Glu (%)	Xyl (%)	Ara (%)	Lignin recovery (%)
EMAL	0.81	3.38	1.17	25.05
$L_{110\text{-}10}$	0.86	0.91	5.71	90.00
$L_{110\text{-}20}$	1.04	0.78	4.80	86.00
$L_{110\text{-}30}$	0.98	0.80	5.09	65.83
$L_{110\text{-}40}$	0.77	0.86	4.57	64.42

Glu: Glucose, Xyl: Xylose, Ara: Arabinose.

Table S2 Quantification of the different hydroxyl groups in lignin fractions by quantitative ^{31}P NMR spectroscopy (mmol/g lignin)

	Aliphatic OH	G_5-OH	S-OH	G-OH	H-OH	-COOH	Total phenolic OH
EMAL	3.73	0.03	0.15	0.27	0.53	0.26	0.95
L_{110}	1.89	0.16	0.26	0.17	0.63	0.63	1.05
$L_{110\text{-}30}$	3.50	0.19	0.31	0.32	0.83	0.15	1.38

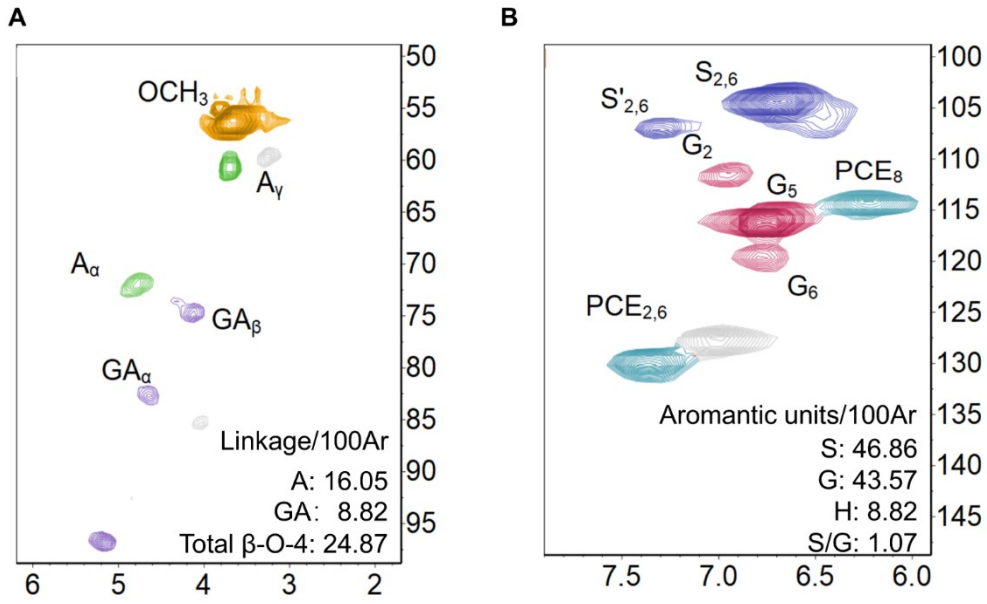

Fig. S1 (A) Side-chain region and (B) aromatic region in the 2D HSQC NMR of $L_{ChCl\text{-}GA}$.

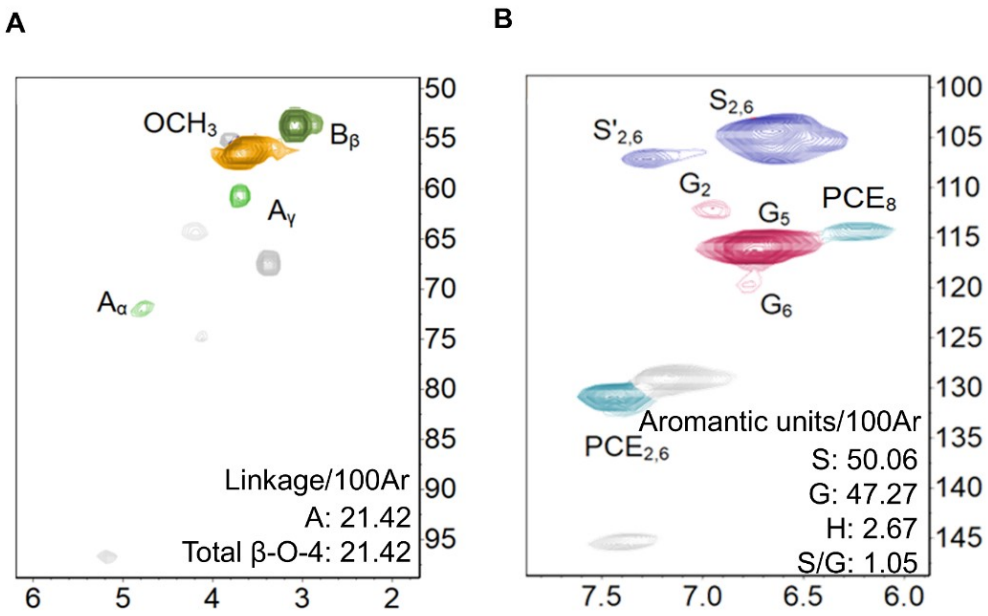

Fig. S2 (A) Side-chain region and (B) aromatic region in the 2D HSQC NMR of $L_{TBAB\text{-}LA}$.

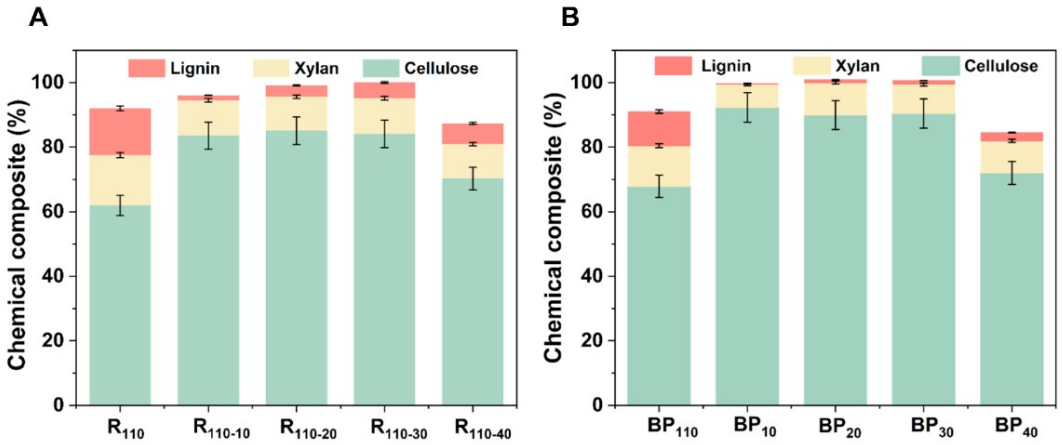

Fig. S3 Composition analysis of high-purity cellulose (A) before and (B) after bleaching.

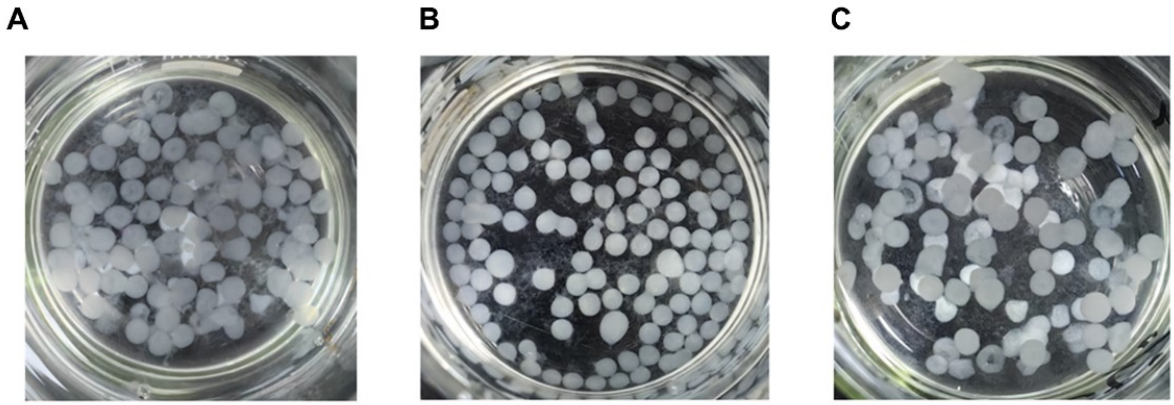

Fig. S4 (A) SA-CNF**,** (B) SA-LNPs and (C) SA-CNF-LNPs gel beads images.

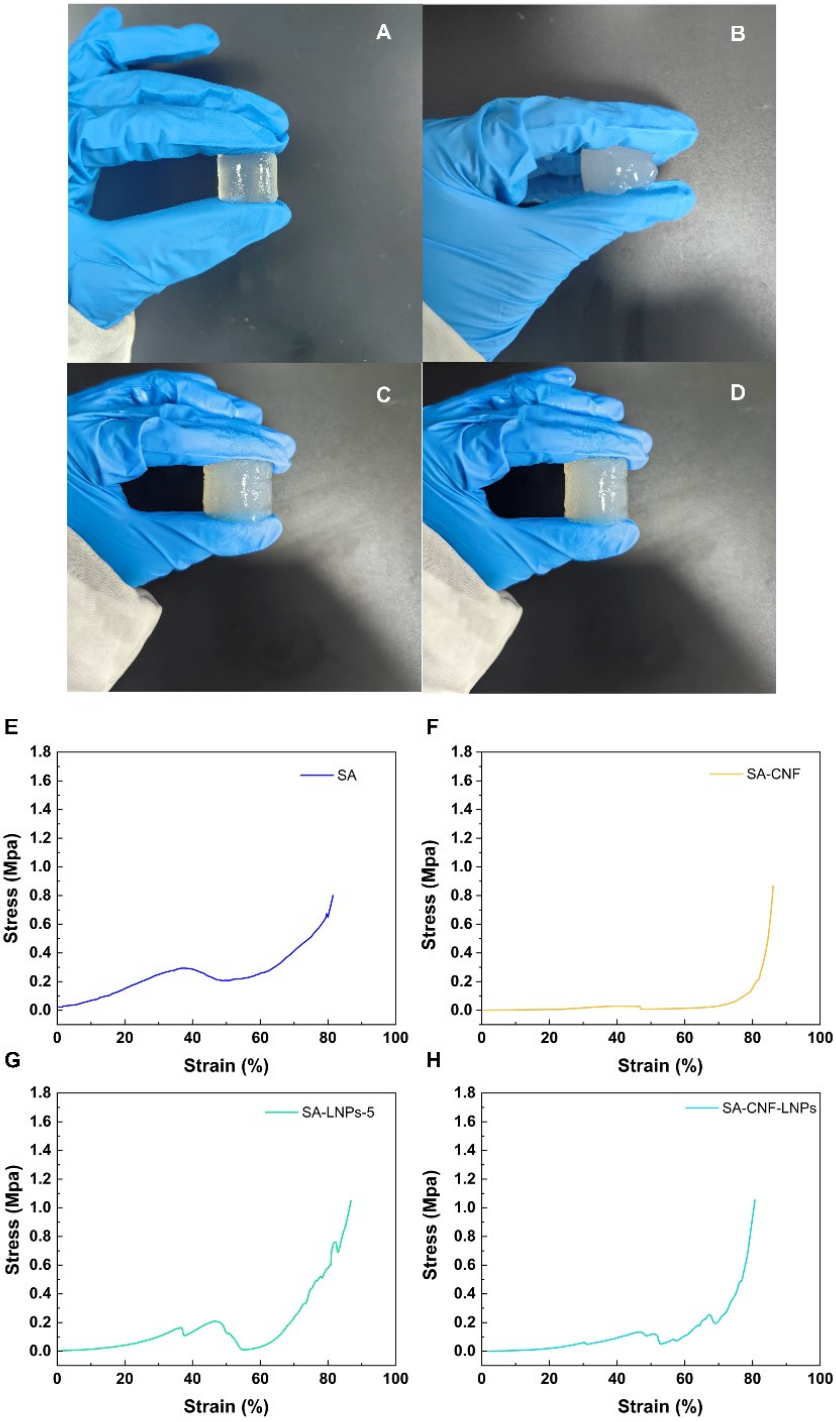

Fig. S5 (A) SA, (B) SA-CNF, (C) SA-LNPs and (D) SA-CNF-LNPs gel beads were cut into cylindrical samples images; Compression stress-strain curves of (E) SA, (F) SA-CNF, (G) SA-LNPs-5, and (H) SA-CNF-LNPs(H) at maximum load-bearing capacity.

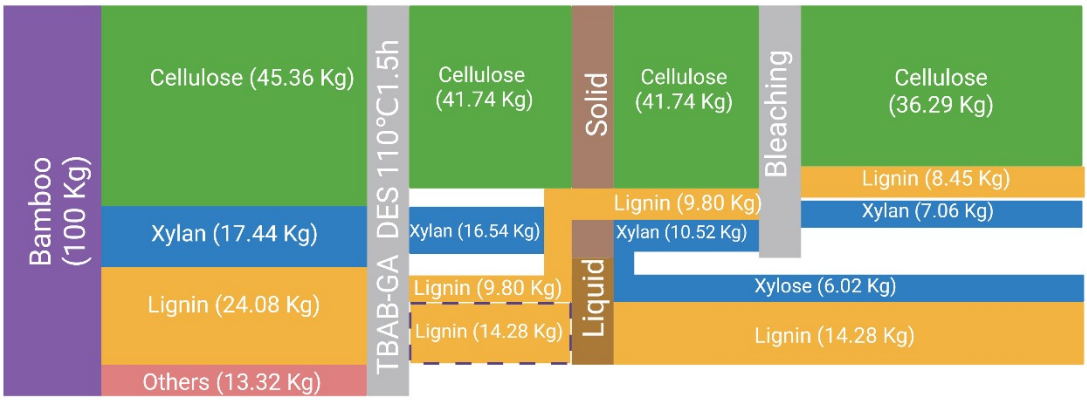

Fig. S6 Mass balance of the DES-based bamboo biorefinery process.

References

1. K. Lin, C. Wang, M. Guo, E. Aprà, R. Ma, A. Ragauskas and X. Zhang, PNAS, 2023, 120, e2307323120.
2. H. Li, X. Li, D. Li, J. Zhang, H. Nawaz, T. You and F. Xu, Bioresour Technol., 2022, 351, 126993.
3. P. Song, F. Zhou, F. Li, Z. Han, L. Wang, J. Xu, B. Zhang, M. Wang, J. Fan and B. Zhang, Carbohydr. Polym., 2021, 253, 117207.
4. L. Segal, J. Creely, A. Martin and C. Conrad, Text. Res. J., 1959, 29, 786 - 794.
5. X. Meng, C. Crestini, H. Ben, N. Hao, Y. Pu, A. Ragauskas and D. Argyropoulos, Nat. Protoc., 2019, 14, 2627-2647.
6. Z. Chen, W. A. Jacoby and C. Wan, Bioresour Technol., 2019, 279, 281-286.
7. H. Wang, B. Wang, J. Wen, T. Yuan and R. Sun, ACS Sustainable Chem. Eng., 2017, 5, 11618−11627.