Electronic Supplementary Material (ESI) for Green Chemistry.
This journal is © The Royal Society of Chemistry 2018

Supplemental Material

Biomass Pretreatment using Deep Eutectic Solvent from Lignin derived Phenols

Kwang Ho Kim[1,2], Tanmoy Dutta[1,2], Jian Sun[1,2], Blake Simmons[1,3], Seema Singh[1,2*]

[1]Deconstruction Division, Joint BioEnergy Institute, Emeryville, CA, USA

[2]Biological and Engineering Sciences Center, Sandia National Laboratories, Livermore, CA, USA

[3]Biological and Engineering Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA

*Corresponding author. Postal address: 7011 East Ave, Livermore, CA 94550; Tel: +1 510 486 7315; E-mail address: seesing@sandia.gov (Seema Singh)

Experimental

Materials

Switchgrass *(Panicum virgatum)* was provided by Dr. Daniel Putnam, University of California at Davis. All chemicals used in this work were purchased from Sigma-Aldrich (St. Louis, MO).

DES synthesis

DES were prepared by gently stirring the salt (ChCl) and the HBD at 100 °C. The solid mixtures were stirred periodically until a homogeneous liquid formed. Ten monophenolic HBDs with three different ChCl : HBD molar ratios were tested to screen the proper combination that can lead the eutectic depression (Table S1).[1]

Biomass pretreatment and enzymatic saccharification

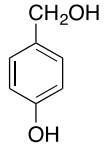
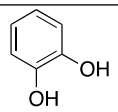
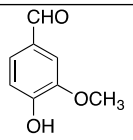
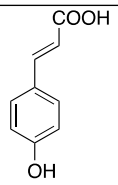
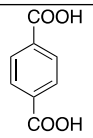
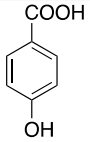
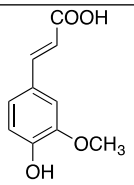
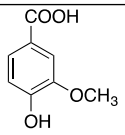
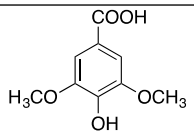
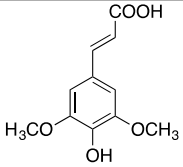
For biomass pretreatment and saccharification, a 5 wt% biomass solution was prepared by combining 0.2 g of biomass with 3.8 g of one of four DESs in a 10 mL pressure tube (Ace Glass, Vineland, NJ). The pressure tube, in triplicate, were heated in an oil bath to 160 °C with continuous stirring. After pretreatment for 3 hours, the reaction slurry was transferred to 50 mL Falcon tube and sufficient amount of ethanol water solution (2:1, V/V) was added to remove any residual DES. Enzymatic saccharification of the pretreated biomass was performed in 5 mL of 50 mM sodium citrate buffer (pH 4.8) supplemented with 10 mg protein / gram solid biomass of Cellic® CTec2 and HTec2 from Novozymes (9:1 ratio), at 50 °C and 150 rmp in a rotary incubator. After pretreatment and saccharification, the hydrolysate was separated by centrifugation.

Analytical techniques

The yield of glucose and xylose was quantified using an Agilent 1100 series high performance liquid chromatography equipped with a Bio-Rad Aminex HPX-87H ion exchange column and a refractive index detector. The mobile phase used was 4mM H2SO4 at a flow rate of 0.6 mL/min and the column was maintained at 60 °C. For 2D ^{1}H-^{13}C heteronuclear single-quantum coherence (HSQC) NMR analysis of

saccharification residues, solid material was recovered after pretreatment followed by enzymatic saccharification. Approximately 50 mg of solid residue were placed in NMR tube with 600 μL DMSO-d_6/pyridine-d_5. HSQC spectra were acquired at 25 °C using a Bruker Avance-600 MHz instrument using the "hsqcetgpsisp2.2" pulse program (ns = 200, ds = 16, number of increments = 256, d1 = 1.0 s). Assignment of the HSQC spectra is described elsewhere.[2,3]

Table S1. List of phenolic compounds for screening test.

Entry	Compound	Structure	Tm (°C)[a)]	ChCl : HBD molar ratio	
1	4-hydroxybenzyl alcohol		114 – 122	1:0.5 1:1 1:2	formed formed formed
2	catechol		100 – 103	1:0.5 1:1 1:2	formed formed formed
3	vanillin		81 – 83	1:0.5 1:1 1:2	pf pf formed
4	*p*-coumaric acid		214	1:0.5 1:1 1:2	formed formed nf
5	terephthalic acid		300	1:0.5 1:1 1:2	nf nf nf
6	4-hydroxylbenzoic acid		213 – 217	1:0.5 1:1 1:2	pf pf nf
7	ferulic acid		168 – 172	1:0.5 1:1 1:2	pf pf nf
8	vanillic acid		218 - 221	1:0.5 1:1 1:2	pf pf nf
9	syringic acid		205 – 209	1:0.5 1:1 1:2	nf nf nf
10	sinapic acid		202	1:0.5 1:1 1:2	nf nf nf

[a)]melting point of HBD, provided by vendor

nf: Not formed, pf: Partially formed

References

1. Z. Maugeri and P. D. de Maria, Novel choline-chloride-based deep-eutectic-solvents with renewable hydrogen bond donors: levulinic acid and sugar-based polyols, *Rsc Adv*, 2012, **2**, 421-425.
2. H. Kim and J. Ralph, Solution-state 2D NMR of ball-milled plant cell wall gels in DMSO-d6/pyridine-d5, *Org Biomol Chem*, 2010, **8**, 576-591.
3. J. Rencoret, A. Gutiérrez, L. Nieto, J. Jiménez-Barbero, C. B. Faulds, H. Kim, J. Ralph, Á. T. Martínez and C. José, Lignin composition and structure in young versus adult Eucalyptus globulus plants, *Plant Physiol*, 2010, pp. 110.167254.