Supporting Information

Unique Low-molecular-weight Lignin with High Purity Extracted from Wood by Deep Eutectic Solvents (DES): A Source of Lignin for Valorization

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Contents

1. Materials and Methods

1.1 Preparation of DES mixtures

DES preparation was carried out by mixing both starting materials in the solid state, followed by melting them at 65 °C for 2h in a vacuum oven as per a procedure reported elsewhere.[1] The solid mixtures were stirred periodically during this time until a homogeneous and transparent liquid was obtained. Once the liquid was formed with no evidence of solid particles, the mixture was cooled down in a desiccator to room temperature to avoid moisture absorption. (Figure S1).

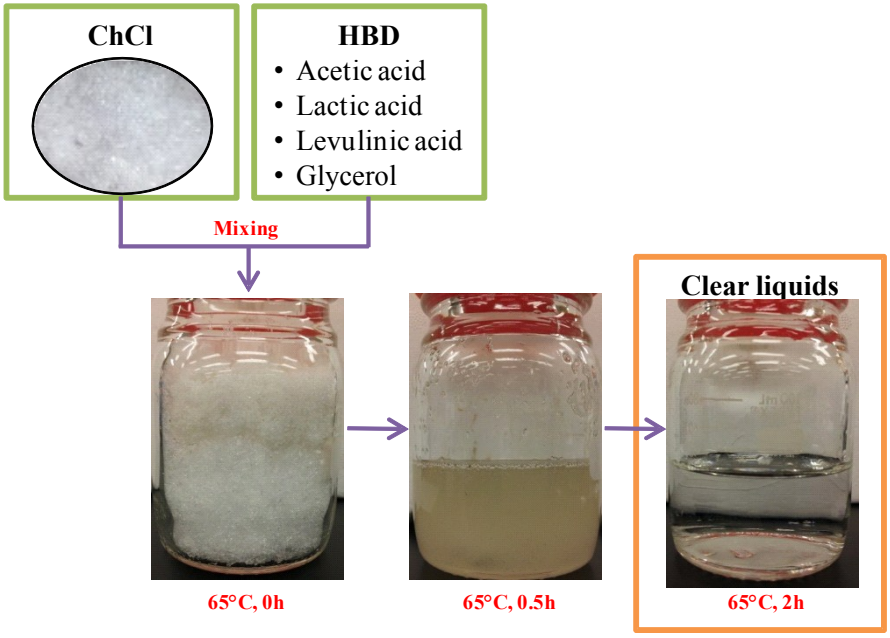


Figure S1. Homogeneous liquids formed by choline chloride and different HBD**.**

1.2 General procedure for biomass, lignin and DES separation after treatment

The procedure for biomass and DES separation after treatment includes three main steps (Figure S2). Prior to DES treatment, the samples were air-dried and milled to a specific size (mesh -40/+60). The treatments were carried out using a biomass concentration of 10 wt % (0.6 g of dry biomass and 6 g of DES) in reaction vessels.[2]

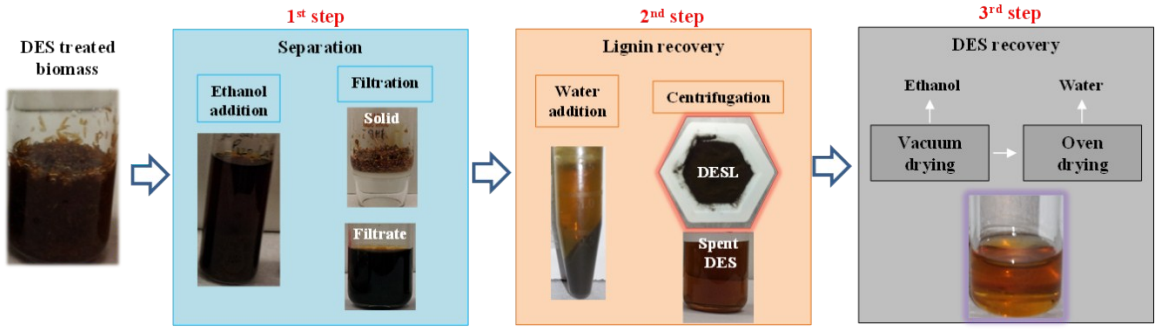


Figure S2. Procedure for pretreated solid, extracted lignin and DES recovery.

After treatment, the solid fractions and DES soluble fraction were first separated by filtration using a glass crucible with ethanol washing (1st step, Figure S2)[1]. DI water was then added to the filtrate in the second step to precipitate lignin. Lignin was collected after centrifugation and washing with additional water (water/ethanol mixture) to increase purity (2nd step, Figure S2). The ethanol was removed from the supernatant by vacuum evaporation at 65°C and the ensuing DES/water solution was then dried in the oven at 105°C for 4 hours to remove water and regenerated DES (3rd step). The composition analyses of solid fraction and collected lignin were conducted following standard NREL procedures.[3]

The solid fractions obtained from the different DES treatments and subsequent washing were shown in Figure S3.

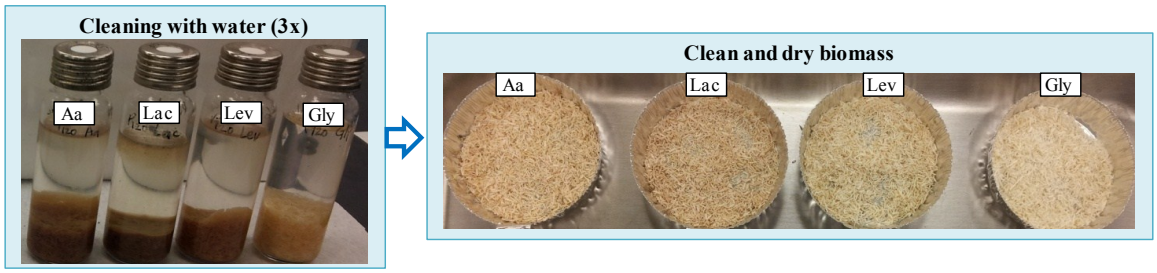


Figure S3. Solid residue cleaning procedure after DES treatment.

1.3 Lignin purity determination

Lignin purity was calculated based on the total amount of acid soluble lignin (ASL) and acid insoluble lignin (AIL) in samples determined according to standard procedures.[3b] The hydrolysate samples were analyzed by high performance liquid chromatography (HPLC) to determine the sugars and residual DES (acetic acid, lactic acid and levulinic acid) content using a Biorad Aminex H column following previous procedures.[4]

1.4 ^{13}C/HSQC NMR and molecular weight distribution analysis of DES lignin

D. fir milled wood lignin (MWL) preparation: Dioxane-HCl extracted MWL was prepared from same D. fir softwood following the previous procedure[5] with slight modification and used as a reference for DESL lignin characterization. The D. fir wood mill was ground in a laboratory vibration ball-milled for forty eight hours. The ground wood mill was then subjected to cellulase enzyme (Ctec 2 from Novozymes North American) hydrolysis for another forty eight hours to remove the residual carbohydrates. The solid residue after enzymatic hydrolysis was then washing with acetate buffer and freeze dried. The lignin in the freeze dried sample was extracted by dioxane/water/HCl for 24 hours [6] and collected as MWL.

Structural characterization of purified D. fir DES lignin (DESL) and D. fir Milled wood lignin (MWL) by the ^{13}C NMR was conducted on a Bruker 500MHz NMR spectrometer with a 90° pulse width, a 1.4 s acquisition time, and a 1.7 s relaxation delay following standard procedures for lignin analysis.[7] Both acetylated and native lignin samples were analyzed using DMSOd6 as solvent (100mg sample/0.6ml DMSOd6). A small amount of relaxation agent (Cr(aca)3, 2 mg) was added during sample preparation to facilitate the relaxation of the magnetization.[8] The 2D HSQC NMR spectra of the acetylated DESL and MWL were recorded on the same Bruker 500MHz NMR spectrometer at 25 °C using the Q-CAHSQC pulse program. Matrices of 2048 data points for the 1H-dimension and 1024 data points for the 13C-dimension were collected from 13 to -1 ppm and from 160 to 0 ppm for the 1H and 13C dimensions, respectively. Relaxation delay was set at 6 s. The lignins were dissolved in DMSO-d6 and chemical shifts were referenced to the solvent signal (2.50/39.5 ppm).

The molecular weight distribution of DESL and MWL was determined by gel permeation chromatography (GPC).[9] The lignin samples were acetylated in pyridine and acetic anhydride mixture prior to analysis. Approximately 2 mg of sample was dissolved in tetrahydrofuran and then filtered through a 0.45 um filter. A Perkin-Elmer High performance liquid chromatography (HPLC) equipped with a diode array detector (DAD) was used to conduct gel permeation chromatograph (GPC) analysis of lignin molecular weight distribution. A series of three Agilent ZORBAX PSM columns (60S, 300S and 1000S) was used to elute different lignin molecular weight fractions by tetrahydrofuran at a flow rate of 0.6 ml per minute for 60 minutes. Polystyrene standards (Alfa Aesar) with molecular weights ranging from 1,300 to 123,000 g/mol as well as veratrol (138 g/mol) were used to calibrate the molecular weight based on retention time.

2. Supplementary Results

2.1 Evaluation of solvent and solvent mixtures for separating solid fraction and DES soluble fraction after treatment and subsequent lignin precipitation from DES soluble fraction

Twelve different organic solvents were tested for solid fraction and DES soluble fraction separation and subsequent lignin precipitation (Table S1).

Table S1. Tested solvents and their interaction with DES.

Class	Solvent	DES solubility	Comments
Esters	Ethyl acetate	IS	2 phases
Aromatics	Toluene	IS	2 phases
Ketones	Acetone	SS	2 phases
Acids	Acetic acid	S	1phase, forms DES
Chlorinated	Dichloromethane	IS	2 phases, solvent on bottom
Alcohols	Methanol	S	1 phase, lower viscosity
	Ethanol	S	1 phase, lower viscosity
	Isopropanol	S	1phase, medium viscosity
	1-butanol	S	1phase, medium viscosity
	2.pentanol	S	1phase, higher viscosity
Polar	Water	S	2 phases
Mixture:	3:7	S	1 phase, lower viscosity
water/ethanol	1:1	S	2 phases negligible precipitated formation
	6:4 to 9:1	S	2 phases precipitated formation
	S=soluble	SS=slightly soluble	IS=insoluble

The selection of solvent or mixture for solid fraction and DES soluble fraction separation was based on their abilities to dissolve DES and generate a low viscosity filtrate that does not show subsequent phase separation or precipitation after overnight. DES was shown a good solubility in ethanol and methanol and produce a liquid fraction that can be readily separated by filtration. The ensuing filtrate is clear with phase separation or precipitation observed overnight.

It was also found adding water to ethanol can lead to lignin precipitation once water to ethanol ratio exceeds 1:1. DES showed a solution in these water ethanol mixture.

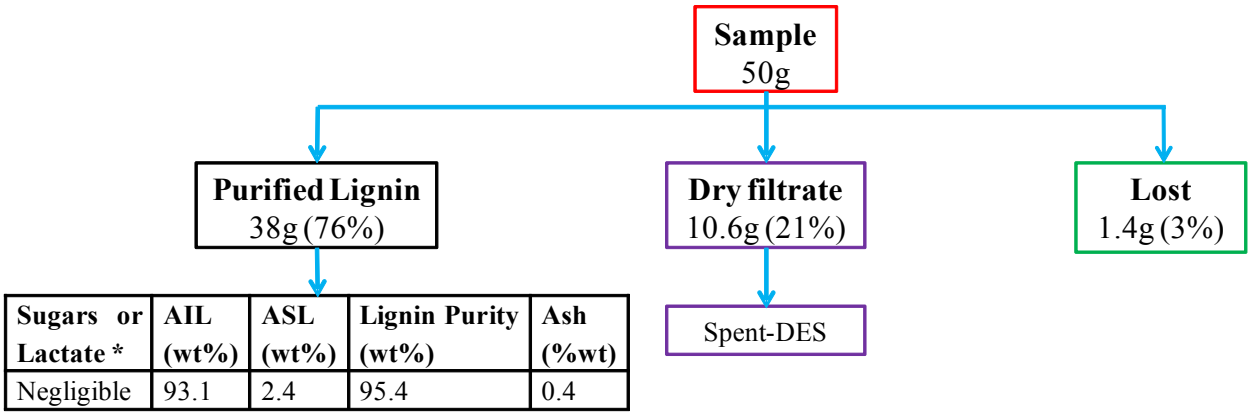
Ethanol was selected to dilute DES treated samples and assist solid fraction and DES soluble fraction separation during filtrate (1st step, Figure S2) and water/ethanol mixtures at ratios between 6:4 to 9:1 were tested for lignin precipitation and purification. A 9:1 water/ethanol mixture was chosen for purified lignin to minimize ethanol consumption.

2.2 Purifying lignin by water/ethanol washing

A 9:1 water and ethanol mixture was chosen for precipitating lignin followed by repeated washing with this mixture to achieve 95% purity. To confirm this procedure and obtain a more accurate measurement of lignin recovery yield and total mass balance (Figure S4), a large batch of purified lignin was also prepared.

In this large-batch lignin preparation, 500gram of D. fir was treated by ChCl-Lac in a 2L Parr reactor at 145°C. After the treatment lignin was precipitated following the sample procedure described in Figure S2. The yield of precipitated lignin is approximately 100 g with a purity of 78%. These results were consistent with those from tube reactor experiment. This precipitated lignin was purified by with 9:1 water/ethanol mixture under stirring for 1hour per cycle for two cycles at room temperature. After each washing cycles, the filtrate was separated and additional water/ethanol mixture was added. The lignin collected from second filtration was collected and the filtrates obtained from two washes were also collected and combined. The dry weights of purified lignin and combined filtrate were measured after oven drying. The composition of purified lignin was determined by NREL protocol [10].

The final lignin has negligible amount of residual DES or sugars with a total lignin content (acid soluble and acid insoluble lignin content of 95.4%.



Sugars or Lactate *	AIL (wt%)	ASL (wt%)	Lignin Purity (wt%)	Ash (%wt)
Negligible	93.1	2.4	95.4	0.4

Figure S4. Mass balance for DES lignin purification.

2.3 DES lignin solubility analysis

The solubility of lignin (DESL) was determined by the cloud point method[1]. In brief, 5 ml of solvent was stirred constantly at room temperature with sequential additions of 1 mg of solute (DESL) to the solvent. The addition and stirring stopped immediately once the turbidity or the presence of precipitate started to appear in the solvent. The sample was set for equilibrium for 24 h. Low, moderate and soluble were determined based on the amount of soluble can be dissolved without generating turbidity and precipitates.

Table S2. Solubility of DESL

Solvent	Solubility (mg/ml)	Solubility (wt%)	Solubility+
Ethanol	<1mg/ml	<0.1	Low
Acetone	<1mg/ml	<0.1	Low
Toluene	<1mg/ml	<0.1	Low
Hexane	<1mg/ml	<0.1	Low
Water	<1mg/ml	<0.1	Low
Ethylacetate	<5mg/ml	<0.6	Low
Methyl tert-butyl ether	<5mg/ml	<0.7	Low
Nitrobenzene	10mg/ml	0.9%	Moderate
Dioxane*	>310mg/ml	>30%	Soluble
DMSO*	>310mg/ml	>28%	Soluble
Pyridine*	>310mg/ml	>30%	Soluble

*Beyond 310 mg of lignin/ml the sample became very dark and it was hard to notice the presence of particles - if they occur - beyond this point.

+ Solubility: Below 10mg/ml is considerate low, between 10-300 mg/ml is moderate, above 300 mg/ml soluble.

2.4 GC-MS Spectra of DES depolymerization of dimeric model compounds

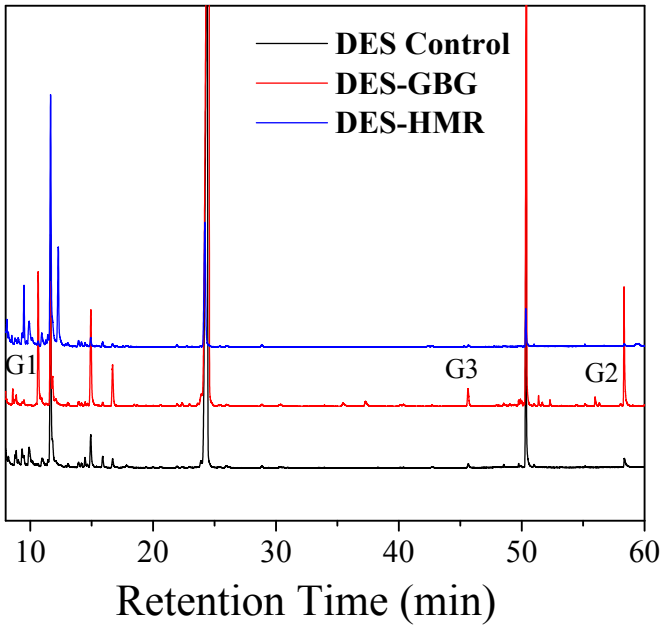


Figure S5. GC-MS spectra of ChCl-Lac treating of guaiacylglycerol-β-guaiacyl ether (GBG) and Hydromatairesinol (HMR) at 145 °C

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