Deep eutectic solvents' ability to solubilize lignin, cellulose, and hemicellulose; thermal stability; and density

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Abstract

An environmentally-friendly method to separate cellulose and hemicelluloses from lignin in recalcitrant biomass for subsequent conversion is desirable to reduce greenhouse gas generation. Easily-prepared, deep eutectic solvents (DESs) have low volatility, wide liquid range, non-flammability, nontoxicity, biocompatibility, and biodegradability. This study shows the DESs (formic acid: choline chloride, lactic acid: choline chloride, acetic acid: choline chloride, lactic acid: betaine, and lactic acid: proline) to be capable of preferentially dissolving lignin at 60 °C. Thermogravimetric analysis shows DES to be stable at typical biomass processing temperatures. Pretreating loblolly pine in one DES increased glucose yield after enzymatic hydrolysis to more than seven times that of raw or glycerol-pretreated pine. The density of DES-pretreated biomass was found to be 40% higher than the untreated pine's density.

1. Introduction

The increase in methane and carbon dioxide in the atmosphere has produced a “greenhouse effect,” seen in unpredictable weather that has jeopardized the agricultural sector (Malcolm et al., 2012; Thomson et al., 2005). New ideas are needed to mitigate disastrous climate

change. Renewable energy such as solar and wind power are intermittent, and produce only electricity. Liquid fuels will still be needed for long-haul trucking, cargo ships, and airplanes for the foreseeable future. Sustainable resources must be found to replace fossil fuels for production of the chemicals our society relies on.

The United States is a net producer of crude oil. However, if we continue to depend on fracking for our energy needs, we risk contamination of ground water, fresh water depletion, potential triggering of earthquakes, noise pollution, surface pollution, and their resulting risks to human health and the environment (Rosenberg et al., 2014). In addition, the fracking process causes uncontrolled methane releases further adding to the greenhouse effect (Howarth, 2014). Over the next 100 year time period, methane has a global warming potential per kg that is 25 times that of a kg of carbon dioxide (Stocker et al., 2013). Thus, depending on fracking as a long term energy solution will speed global warming, in addition to causing mismanagement of water resources.

Efforts to replace fossil fuels with sustainable, renewable biomass have used corn in large quantities to produce bio-ethanol. However, using food to produce fuel seems a stop-gap measure rather than a long term solution as “food vs. fuels” conflicts have increased when food stocks are designated for fuel production (Thompson, 2012). As such, biofuel production from fast growing, short rotation woody crops (SRWC) has been pursued over the last few decades and the Department of Energy’s Renewable Fuel Standard targets 36 billion gallons of renewable fuel to be blended into transportation fuel by 2022. Specifically, the DOE highlights the use of cellulosic biofuels that are derived from nonfood-based renewable feedstocks (Lynam et al., 2012a).

Loblolly pine, also known as southern yellow pine, has a rapid growth rate and is the second most common species of tree in the United States (Nix, 2016). It has significant commercial

importance in the pulp and paper industry. In North America, paper production is declining, and recycling of paper is increasing (Miranda & Blanco, 2010). Young loblolly pine trees (10-15 years old) are thus in abundant supply throughout the southern United States. This biomass could be enhanced prior to shipping to biorefineries for conversion to biofuels or other products.

If a safe, low-tech method can be discovered to separate lignin (for conversion to another bioproduct) from holocellulose and increase the residual products' density, it could be implemented at a local depot or even in the woods or fields. A cellulose- and hemicellulose-enriched product (holocellulose) could then be transported to a biorefinery, while recovered lignin could be sent to another facility for upgrading or used as a biofuel locally. Once separated, these components can be converted to valuable fuels and chemicals using available technology. Separation using a non-hazardous, green process closer to the harvesting site would reduce the volume of material to be transported, particularly if the products were denser than the original biomass. Less carbon dioxide would also be generated by transportation of decreased biomass volume. Using rural sites for such preprocessing would also stimulate rural economies that possess biomass resources.

One group of solvents with applications to lignocellulosic biomass are ionic liquids (ILs). ILs, with their extremely low vapor pressures, have novel abilities to dissolve cellulose and lignin both separately and as packaged in biomass (Lynam et al., 2012b). However, ionic liquids are expensive to produce, the synthesis of most ILs is not "green," and they are unavailable (today) in industrial quantities (Francisco et al., 2013).

Some exciting alternative types of solvents are called deep eutectic solvents (DES). DES have low volatility, wide liquid range, non-flammability, nontoxicity, biocompatibility and

biodegradability (Francisco et al., 2012). Unlike ILs, they are easily prepared from readily available materials at high purities and are low cost compared to ILs. In addition, DES typically do not inactivate enzymes, making them valuable in biofuel processing (Gorke et al., 2010). They are less sensitive to water content, making their use more feasible with wet biomass, which would be present at a field site (Francisco et al., 2012). Drying costs for biomass can be a substantial portion of pretreatment costs and, if the biomass is not first dried, transportation costs are increased (Lamers et al., 2015).

A eutectic system is a homogeneous mix of two solid-phase chemicals that forms a joint super-lattice at a particular molar ratio, called the eutectic composition. The joint super-lattice then melts at the eutectic temperature, a temperature lower than the melting points of the individual components. DES are called deep because the melting point curve has a particularly deep crevice at the eutectic point, since the eutectic temperature is much lower than the melting points of the pure substances. DES are formed by hydrogen bonding rather than the ionic bonding that ILs possess (Yiin et al., 2016).

Preparation of DES requires only gentle mixing in the proper molar ratios at temperatures of 130 °C or less (Francisco et al., 2012; Perez-Sanchez et al., 2013). Some inexpensive components used to make DES are formic acid, lactic acid, acetic acid, choline chloride, and betaine (Perez-Sanchez et al., 2013). Formic acid, lactic acid, and acetic acid are all safe food additive and can be sustainably produced from biomass (Huo et al., 2015; Reichert et al., 2015; Yang et al., 2015). Choline chloride is produced in large quantities for chicken feed and betaine is produced from sugar beets (Francisco et al., 2012; Perez-Sanchez et al., 2013). Little data is available on stability or density for DES at typical biomass pretreating temperatures of 100 °C – 160 °C.

The present work aims to prepare various DES and to determine the solubility of isolated biomass components in them. In addition, we have investigated the thermal stability of these DES. An actual recalcitrant biomass, loblolly pine, was pretreated with a DES, and the product enzymatically hydrolyzed to ascertain whether such a pretreatment enhances glucose yield. The pretreatment of loblolly pine has not been previously shown in the literature with the DESs used in this study. Fourier transform infrared spectroscopy (FTIR) of the two solid products from the separation suggested composition. Finally, density of the DES and pretreated biomass was measured to see if densification ensued.

2. Materials and methods

2.1 Materials

Loblolly pine was harvested and debarked in Alabama in 2012 and chips were acquired from the Desert Research Institute (Reno, NV, USA). It was meshed to obtain the fraction 0.6–1.2 mm in diameter. The pine particles were dried for 24 h at 105 °C prior to DES pretreatment. From fiber analysis performed using the Van Soest method, the loblolly pine particles were found to have cellulose, hemicelluloses, lignin, and neutral detergent fiber (NDF) extractives of approximately 57%, 6%, 34%, and 3%, respectively (Goering & Soest, 1970). The method used gives a precision for component percentages of $\pm 5\%$. Formic acid (reagent grade, $\geq 95\%$), lactic acid solution (reagent grade, $\geq 85\%$), acetic acid (reagent grade, $\geq 99.7\%$), choline chloride powder (BioReagent, suitable for cell culture, $\geq 98\%$), betaine ($\geq 98\%$ - perchloric acid titration), L-proline (ReagentPlus®, $\geq 99\%$) were purchased from Sigma-Aldrich Corp. (St. Louis, MO, USA). Lignin (alkali), xylan (from beechwood); cellulose (fibrous, medium), glycerol ($\geq 99.5\%$), cellulase (powder) from *Trichoderma reesei* ATCC 26921, hemicellulase (powder) from *Aspergillus niger*, cellobiase (liquid) from *Aspergillus niger*, and nylon 66 membranes of pore size 20 μm were also bought from Sigma-Aldrich Corp. (St. Louis, MO, USA). Sodium citrate

dehydrate, 99.0% min, and sodium azide, 99% min, were purchased from Alfa Aesar (Ward Hill, MA, USA).

2.2 Methods

2.2.1 Synthesis of DES

The correct mole ratios of each hydrogen bond donor and the hydrogen bond acceptor, as shown in Table 1, were vortexed in a capped 50 mL vial. The mixtures were placed in an orbital shaker at set at 60 °C and 200 RPM for 20 min. If a clear solution was not obtained, the mixture was replaced for an additional 20 min at 60 °C. All DES were then clear solutions and were stored at room temperature. They remained clear solutions overnight and those stored for longer periods remained clear indefinitely.

2.2.2 Solubility measurements of lignocellulosic components in DES

Alkali lignin, xylan from beechwood, and medium fibrous cellulose (all used as obtained from Sigma-Aldrich) were stirred into the five DES, adding 1% by mass of the lignocellulosic component initially. Xylan from beechwood was used as a representative hemicellulose, since a pure glucomannan preparation was not commercially available. The mixtures were placed in an orbital shaker set at 60 °C for 20 minutes and then observed to determine if dissolution had occurred. A clear solution indicated dissolution. If the component had dissolved, approximately 1% more was added and the mixture replaced in the orbital shaker at 60 °C for 20 more minutes. This procedure was repeated until solids were observed remaining in the solution, with the previous mass% recorded as the solubility.

2.2.3 Thermogravimetric analysis of DES

Samples between 20 and 25 mg of DES were placed into the sample chamber of an STA-6000 from Perkin Elmer (MA) under nitrogen. The samples were heated at 20 °C/min from 30 °C to 100 °C, held at 100 °C for 10 min to remove any water, and then heated to 160 °C at 10 °C/min.

2.2.4 DES pretreatment of biomass

Loblolly pine (1 g) was put into a vial with 10 g of either formic acid: choline chloride 2:1 mole ratio DES (FA:CC 2:1) or glycerol. Glycerol was used as a control to allow the same temperatures to be applied to the biomass, so that the effect of temperature alone could be determined. Glycerol's boiling point of 290 °C allowed it to be used without significant evaporation. The vials were placed into an oven set at 155 °C for 2 hours. After removal from the oven, samples were filtered at 100 °C with a 0.3 mm nylon filter to separate solid biomass residue from the DES or glycerol solvent. The pretreated loblolly pine was put into a vial that was placed in an orbital shaker (SC20XR, Torrey Pines Scientific, Inc., Carlsbad, CA, USA) with 15 ml deionized (DI) water and shaken at 200 RPM at 50 °C for 20 min. The water was decanted and the water-washing process was repeated for another 20 min at 50 °C. Finally, the water-washing process was repeated for 18 h at 50 °C to ensure that the DES was removed. Vacuum filtering with a 20 µm nylon filter separated the rinsed, pretreated loblolly pine from the water. The pretreated pine was next placed in a drying oven set at 105 °C for 24 h prior to weighing. Both pretreatments were performed in triplicate, and averages with standard error are reported. Bulk densities of the raw and pretreated pine were determined using a syringe and uniform pressure.

2.2.5 Enzymatic hydrolysis

After 24 h of drying at 105 °C, 0.1 g samples of raw and pretreated loblolly pine were placed in a 22 mL vial with 5 ml of pH 5.05 sodium citrate buffer where 100 ul of a 2% sodium azide solution was added and deionized water was added to give a volume of 10 mL total, as described in NREL's Enzymatic Saccharification of Lignocellulosic Biomass LAP 009 protocol (Selig, 2008). Cellulase was added at a concentration of 5 units per 0.1 g sample, hemicellulase was added at 50 units per 0.1 g sample, and cellobiase at a concentration of 14 units per 0.1 g sample. Samples were shaken in an SC20XR orbital shaker at 200 RPM at 50 °C. Aliquots were taken for analysis at 8 h, 18 h, 24 h, 48 h, and 72 h and filtered through a 0.45 µm syringe filter, and then stored at 4 °C prior to analysis. Glucose yield is defined as the fraction of cellulose available in the biomass that was recovered as glucose. An anhydrous factor of 0.9 was used for glucose. The formula used for glucose yield was (Eq. 1):

$$\% \text{ glucose yield} = \frac{0.9 * 10 \text{ mL volume hydrolysis} * \text{glucose concentration} \frac{\text{g}}{\text{mL}} (\text{corrected with blanks})}{\text{g pretreated substrate} * \text{fraction cellulose in pine} * \text{mass yield pretreatment}} (1)$$

2.2.6 HPLC analysis

The HPLC system (SHIMADZU, CA, USA) consisted of a system controller (SCL-10A), a liquid pump (LC-10AD), a refractive index detector (RID-6A), an auto-injector (SIL-10AD), and a column oven (CTO-10A). Aliquots were diluted 12:1 with nanopure DI water and 15 µL injected into an Aminex 87-H column from Bio-Rad, with 5 mM H₂SO₄ as the mobile phase, at 0.7 mL/min flow, at a column temperature of 55 °C. This column was calibrated for glucose. For quantitative analysis, the UV-VIS detector was utilized at 208 nm and 290 nm. Standard errors for glucose yields of FA:CC 2:1 pretreated, glycerol-pretreated and raw loblolly pine were 3%, 4%, and 5%, respectively.

2.2.7 Fourier transform infrared spectroscopy (FTIR)

A Nicolet 6700 FTIR-ATR with a SmartiTR diamond ATR (Thermo Scientific, Waltham, MA, USA) using 16 scans per sample at 4 cm^{-1} was used from 4000 to 600 cm^{-1} . FTIR analysis was performed on raw and pretreated samples, as well as the precipitate formed from ethanol addition to the used DES or glycerol. Spectra were recorded in triplicate for each sample and averaged. Peak intensities were measured from the plateaus at lower wavenumbers. The spectra for raw and pretreated biomass were normalized using the 2300 cm^{-1} plateau baseline and the 1030 cm^{-1} peak, since this peak has lignin, cellulose, and hemicelluloses components.

3. Results and discussion

3.1.1 Solubilities of lignocellulosic components in DES

Alkali lignin, xylan from beechwood, and medium fibrous cellulose were stirred into five DES at $60\text{ }^{\circ}\text{C}$ to perform an initial screening of DES's ability to dissolve biomass. Table 1 shows the maximum solubilities of each lignocellulosic component in the DES.

After separation and isolation from biomass, the components used are significantly different from those in unprocessed biomass. Alkali lignin used is different from the native lignin in biomass due to its processing for separation, it still is relevant for solubility data. Finding solvents that solubilize alkali lignin is essential for further conversion (Ni et al., 2016).

The formic acid: choline chloride 2:1 mole ratio DES (FA:CC 2:1) exhibited the highest lignin solubility and the lowest cellulose and xylan solubility. Lactic acid: choline chloride 10:1 mole ratio (LA:CC 10:1) showed the second highest lignin solubility, but higher cellulose and xylan solubilities. Acetic acid: choline chloride 2:1 mole ratio (AA:CC 2:1) showed slightly lower lignin solubility compared to LA:CC 10:1, but low cellulose and xylan solubilities. These DES based on choline chloride (CC) have been reported to enhance delignification and subsequent

enzymatic hydrolysis. For corncob (meshed to 0.177 mm to 0.841 mm) pretreated with LA:CC 10:1 at 90 °C for 24 h, glucose yield was more than 80% after enzymatic hydrolysis (Zhang et al., 2016). Corn stover (meshed to less than 0.38 mm) that was pretreated with FA:CC 2:1 at 130 °C for 2 h gave a glucose yield more than 8 times that of the control (Xu et al., 2016).

The DES lactic acid: betaine 2:1 (LA:B 2:1) and lactic acid: proline 3.3:1 (LA:P 2:1) showed high lignin solubility considering the temperature used and low cellulose and xylan solubility. Since proline is expensive compared to the other hydrogen bond acceptors, LA:P 3.3:1 would likely be less economically feasible for biomass deconstruction.

3.1.2 Thermogravimetric analysis of DES

Fig. 1 shows the thermogravimetric data from the deep eutectics investigated in the temperature range typical for biomass processing. Boiling points for formic acid and acetic acid are 101 °C and 118 °C, and ~~122 °C~~ respectively. Although some evaporation occurs at these temperatures, no boiling point is seen in this range. Lactic acid would normally be unstable at ambient pressure in this temperature range. The hydrogen bonding that occurs in DESs may prevent the formation of lactide in the DESs containing lactic acid, so that no boiling point nor degradation occurs in this temperature range. A boiling point would show a rapid decrease in mass. Thus, the deep eutectics are relatively stable in this range. This behavior suggests that ambient pressure biomass pretreatment should be possible.

3.2 Effects of DES pretreatment on loblolly pine

The DES that showed the highest lignin solubility, FA:CC 2:1, was chosen to investigate how effective DES could be on a recalcitrant biomass. The biomass chosen was the softwood loblolly

pine, since softwood biomass is known to be the most recalcitrant biomass in pretreatment before enzymatic hydrolysis (Gao et al., 2013).

3.2.1 Glucose yield from enzymatic hydrolysis of pretreated loblolly pine

The glucose yield as a percentage of the cellulose in the original biomass versus enzymatic hydrolysis time is shown in Fig. 2. The FA:CC 2:1 pretreated pine produced more than seven times as much glucose compared to the raw or glycerol pretreated pine. This suggests that FA:CC 2:1 may be effective at pretreating biomass to produce glucose for conversion to biofuels or other bioproducts. This increase in glucose yield could be partly due to crystalline cellulose disruption by DES pretreatment, in addition to removal of inhibiting lignin.

3.2.2 FTIR analysis of pretreated loblolly pine and material precipitated from the solvent by ethanol (EtOH precipitate)

Supplemental information (SI) Fig. 1a shows representative spectra for FA:CC 2:1 pretreated biomass, glycerol-pretreated biomass, and raw pine. More intense vibrations at 1056 cm^{-1} (C-O stretching vibration from cellulose and hemicelluloses), 1110 cm^{-1} (C-OH skeletal vibration of cellulose and hemicelluloses), 1160 cm^{-1} (amorphous stretching of cellulose type I and II) and 1425 cm^{-1} (native type I cellulose) for FA:CC 2:1 pretreated biomass compared to glycerol-pretreated biomass and raw pine indicate a higher cellulose concentration in the DES pretreated biomass (da Costa Lopes et al., 2013; Shi & Li, 2012; Wang et al., 2015).

One way to determine how effective a DES is in pretreating biomass in preparation for enzymatic hydrolysis is comparing FTIR peaks related to cellulose with those related to lignin. For loblolly pine, the FTIR vibration at 1425 cm^{-1} indicates native cellulose (type I), and the vibration at 1160 cm^{-1} indicates the amorphous stretching of cellulose type I and II (Shi & Li, 2012; Wang et al., 2015). Cellulose type II is disrupted from the native cellulose form (Cheng et al., 2012). The

FTIR vibration at or near 1515 cm^{-1} indicates the lignin aromatic ring skeletal stretch(Raj et al., 2015; Wang et al., 2015). Taking the ratio of the addition of the 1425 cm^{-1} and 1160 cm^{-1} peak heights and dividing them by the 1515 cm^{-1} peak height should give some measure of the amount of cellulose compared to lignin in a sample. This cellulose/ lignin measurement will be called the CL ratio (Eq. 2).

$$CL\ ratio = \frac{Peak_{1425} + Peak_{1160}}{Peak_{1515}} (2)$$

In each case, peak height is measured from the lower wavenumber plateau. If the CL ratio for a pretreatment sample is divided by the CL ratio for untreated (raw) loblolly pine, a pretreatment index (PI) can be calculated (Equation 3).

$$PI = \frac{CL\ ratio_{pretreated\ sample}}{CL\ ratio_{raw\ pine}} (3)$$

The PI should give an indication of whether a solid sample resulting from pretreatment contains more cellulose or more lignin compared to the original raw pine. Higher PI would suggest more cellulose and low PI more lignin. The PI average for two runs of FA:CC 2:1 pretreatments for recovered biomass was 5.6 ± 1.6 , suggesting that the pretreated biomass contained more cellulose than the raw pine. For two glycerol pretreatments of biomass, the PI average was 1.2 ± 0.07 , which suggests that little lignin is removed from the sample to concentrate cellulose. The high concentration of cellulose in the biomass pretreated by FA:CC 2:1 compared to that pretreated by glycerol corroborates the glucose yield results found after enzymatic hydrolysis. High concentrations of lignin is known to inhibit enzymatic hydrolysis (Rahikainen et al., 2013).

For the solids precipitated from the FA:CC 2:1 by adding ethanol, PI was 0.32 ± 0.003 . This value for PI would suggest that little cellulose remains in the precipitate compared to lignin, so that this precipitate is lignin-rich. SI Fig1b shows representative FTIR spectra for the solids precipitated from the glycerol and from the FA:CC 2:1 by ethanol addition. Very little precipitate could be obtained from the glycerol, making a good spectra for this condition difficult. The most difference among spectra can be seen in the vibrations near 1515 cm^{-1} and at 1636 cm^{-1} . These peaks for lignin can shift due to structure change from dissolution (Shi & Li, 2012). As discussed above, 1515 cm^{-1} is the lignin aromatic ring skeletal stretch (Raj et al., 2015; Wang et al., 2015). The 1636 cm^{-1} vibration indicates the C=O stretching vibration in lignin conjugated carbonyl (Shi & Li, 2012). The precipitate from FA:CC 2:1 from ethanol addition has peaks at these wavenumbers that are much larger than the other peaks, suggesting again the lignin-rich character of this substance, compared to the other biomass.

3.2.3 Mass yield and initial solvent recovery from DES pretreatment

The mass yield when loblolly pine is pretreated with glycerol is 104% ($\pm 2\%$ standard error). Glycerol apparently does not remove significant amounts of any biomass component and may cling to the residue in small amounts despite rinsing. However, when the pine is pretreated with FA:CC 2:1, the mass yield is 63% ($\pm 1\%$ standard error). The loss in mass with pretreatment suggests that lignin is being removed, since lignin protection of cellulose and lignin inhibition of enzyme reduce glucose yield (Rahikainen et al., 2013). Lignin removal is also supported by the FTIR data as discussed in section 3.2.2 above. The effectiveness of FA:CC 2:1 pretreatment is not merely due to temperature of pretreatment in solvent, since heating in glycerol did not produce noteworthy effects.

Initial solvent recovery from pretreatment involves simple vacuum filtration at ambient temperature with a coarse nylon filter. Due to the viscosity of DES at ambient temperature, solvent tends to cling to the biomass. Higher temperature filtration, which would decrease viscosity, should increase the proportion of solvent recovered. Nevertheless, 61% ($\pm 3\%$ standard error) of the initial glycerol can be recovered by filtration. Less of the FA:CC 2:1 solvent can be recovered by this method. Possibly due to the FA:CC 2:1 entering biomass pores and interacting with the biomass more than glycerol does, solvent recovery was 42% ($\pm 3\%$ standard error). More FA:CC 2:1 should be recoverable from the rinse water, but it would need to be separated from the rinse water.

The solids precipitated from the recovered solvent using ethanol (EtOH precipitate) give an idea of how much biomass component is being solubilized. For glycerol pretreatment, EtOH precipitate yield is 0.4% of the original biomass sample ($\pm 0.04\%$ standard error), indicating that little mass is removed. For FA:CC 2:1 pretreatment, EtOH precipitate yield is 11% of the original biomass sample ($\pm 0.4\%$ standard error). Since less than half the solvent was recovered for precipitation, more EtOH precipitate could likely have been recovered if higher temperature filtration had been used. As discussed in section 3.2.2, this EtOH precipitate is high in lignin content, indicating dissolution of lignin from the biomass in the FA:CC 2:1 DES.

3.2.4 Densities of raw and pretreated pine and DES

Table 2 shows the bulk densities of raw pine, glycerol-pretreated pine, FA:CC 2:1 pretreated pine, and also the densities of the DES listed in Table 1 at 24 °C and 155 °C. As expected, densities for the DES decreased with an increase in temperature from room temperature to the biomass pretreatment temperature used.

The bulk density of the FA:CC 2:1 pretreated pine is 35% higher than that of raw pine or glycerol pretreated pine. One of the major challenges in biomass pretreatment is the low density of biomass preventing its economical transport from the rural areas where it is produced to biorefineries. Pre-processing the biomass with biocompatible solvents at ambient pressure could be achieved closer to biomass harvesting areas, allowing for a denser product to be trucked for further refining to bioproducts. Based on the results shown in this manuscript, DESs have the potential to offer an alternative to existing technologies for the separation of lignin and holocellulose.

4. Conclusions

Solubility data for isolated lignin, cellulose, and hemicelluloses components in 5 DES show that lignin is preferentially dissolved at 60°C. TGA indicates these DES, despite the lower boiling points of components, are stable at typical biomass processing temperatures. Pretreatment of recalcitrant loblolly pine by FA:CC 2:1 gives a glucose yield seven times that of glycerol-pretreated or raw pine. FTIR of DES-pretreated biomass samples show diminished lignin peaks and increased cellulose peaks compared to raw or glycerol-pretreated pine, suggesting lignin removal. Density of DES-pretreated product is 40% higher than raw pine's density, allowing for more to be transported per truckload.

Supplemental Materials: SI Fig. 1 is in the Electronic Annex.

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Figure and Table Captions

Table 1 Solubility of the isolated biomass components (lignin, alkali; xylan from beechwood; cellulose, fibrous, medium) in the various DES at 60 °C.

Fig. 1 Thermogravimetric analysis of DES in biomass processing range.

Fig. 2 Glucose liberated versus enzymatic hydrolysis time. Standard error bars are shown.

Table 2 Bulk densities of raw pine, glycerol-pretreated pine, FA:CC 2:1 pretreated pine, and five DES. Averages of three measurements of biomass are reported. Standard errors for raw pine, glycerol-pretreated pine, and FA:CC 2:1 pretreated pine are $\pm 0.007 \text{ g/cm}^3$, $\pm 0.004 \text{ g/cm}^3$, and $\pm 0.02 \text{ g/cm}^3$, respectively.

Loblolly
Pine

Lignin
dissolving

Deep
Eutectic
Solvent

Enzymatic
Hydrolysis

Figure 1

Fig. 1 The

Fig 1 in blac

Note: Color Fig is for online; Black and white is for print.

Figure 2

Fig. 2 Glucose liberated versus enzymatic hydrolysis time. Standard error bars are shown.

Note: Color Figure is for online; Black and white is for print.

Table 1 Solubility of the isolated biomass components (lignin, alkali; xylan from beechwood; cellulose, fibrous, medium) in the various DES at 60 °C.

Hydrogen Bond Donor	Hydrogen Bond Acceptor	Mole Ratio	Lignin Mass Solubility (%)	Cellulose Mass Solubility (%)	Xylan Mass Solubility (%)
Formic Acid	Choline Chloride	2:1	14	<1	<1
Lactic Acid	Choline Chloride	10:1	13	<3	<5
Acetic Acid	Choline Chloride	2:1	12	<1	<1
Lactic Acid	Betaine	2:1	9	<1	<1
Lactic Acid	Proline	3.3:1	9	<1	<1

Table 2 Bulk densities of raw pine, glycerol-pretreated pine, FA:CC 2:1 pretreated pine, and five DES.

Material	Density (g/cm ³) at 24 °C	Density (g/cm ³) at 155 °C
Raw pine	0.29	-
Glycerol-pretreated pine	0.28	-
FA:CC 2:1 pretreated pine	0.39	-
FA:CC 2:1 DES	1.16	1.08
LA:CC 10:1 DES	1.19	1.02
AA:CC 2:1 DES	1.11	1.04
LA:B 2:1 DES	1.20	1.07
LA:P 2:1 DES	1.24	1.08