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Pretreatment of yellow pine in an acidic ionic liquid: Extraction of hemicellulose and lignin to facilitate enzymatic digestion

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HIGHLIGHTS

- Pine wood was treated with 1-*H*-methylimidazolium chloride.
- Treatment effectively removed lignin and hemicellulose.
- Cellulose rich fractions were hydrolyzed into glucose with cellulase.
- Demonstration of reactive delignification in ionic liquids for pretreatment.

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ABSTRACT

The acidic ionic liquid 1-*H*-3-methylimidazolium chloride can effectively pretreat yellow pine wood chips under mild conditions for enzymatic saccharification. Wood samples were treated at temperatures between 110 and 150 °C for up to 5 h in the ionic liquid and three fractions collected; a cellulose rich fraction, lignin, and an aqueous fraction. This treatment caused the hemicellulose and the lignin to be degraded and dissolved from the cell walls of the pine wood. The lignin was depolymerized and subsequently dissolved in the ionic liquid. This process occurred more quickly at higher temperatures, although at the highest temperatures tested, significant cellulose degradation also occurred. The cellulose rich fraction was saccharified using cellulase from Trichoderma viride, with longer pretreatment times at 130 °C resulting in higher glucose yields.

1. Introduction

The depletion of petroleum resources and the problem of anthropogenic climate change give an impetus to better utilize renewable sources of energy. With an annual yield of 1.37 billion tons in the US, lignocellulosic biomass has the potential to be a significant source of energy (Perlack et al., 2005). For this reason, lignocellulose has garnered significant attention as a platform for renewable energy production and a number of technologies have been developed to take advantage of biomass (Perlack et al., 2005; Holladay et al., 2007). One such technology is the fermentation of sugars derived from lignocellulose. The most common product of this fermentation is ethanol, although other products such as butanol are reported (Lin and Tanaka, 2006; Demirbas, 2009). The Energy Independence and Security Act of 2007 has required that fuel producers utilize 21 billion gallons of non-cornstarch based ethanol in fuel production in the US, a requirement which could be achieved through fermentation of cellulosically-derived saccharides (Bang, 2010).

A major hurdle in the effective utilization of biomass is lignin. Lignin is a complex biopolymer composed of phenylpropanoid units that acts as an essential glue that holds cellulose fibers together in plant cell walls (Holladay et al., 2007). The lignin biopolymer is rich in aromatics and is very resistant to biological attack, preventing the biomass from being degraded into saccharides or directly digested microbially (Davis and Sello, 2010). In order to effectively convert lignocellulose into fuel such as ethanol using currently available technology, the polysaccharides must be converted into monosaccharides that are conducive to microbial digestion. This process is most often achieved through enzymatic saccharification of cellulose through hydrolysis of the glycosidic linkages (Zhang, 2008). For this step to be completed, pretreatment of the biomass must be done to disrupt the structure of the lignocellulose to allow for enzymatic attack of the polysaccharides (Chang, 2007; Yang and Wyman, 2008; Zhang, 2008). The pretreatment and saccharification steps are the largest process hurdles in making enzymatic biorefineries economical (Yang and Wyman, 2008). Different methods, such as steam explosion, dilute acid

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hydrolysis, ammonia explosion, and ionic liquid treatment, have been explored to open the biomass structure to allow enzymatic attack (Li et al., 2007; Yang and Wyman, 2008; Kumar et al., 2009; Mora-Pale et al., 2011; Wu et al., 2011). Herein, we report a method that effectively pretreats yellow pine wood chips by depolymerizing and extracting the lignin along with the hemicellulose under mild conditions using the acidic ionic liquid 1-*H*-3-methylimidazolium chloride (HMIMCl) as both solvent and catalyst.

Ionic liquids (ILs) are a class of chemical compounds composed of anions and cations that melt at or below 100 °C and have essentially no vapor pressure. By choosing an appropriate anion/cation combination, the properties of ILs, such as viscosity, melting point, solubility, and stability can be tuned (Welton, 1999; Seddon et al., 2000). The solvent properties of some ILs allow them to either partially or completely solubilize lignocellulosic biomass (Zhu et al., 2006; Kilpeläinen et al., 2007). ILs have also received a significant amount of attention as a method to pretreat biomass through extraction of lignin and disruption of cellulose structure (Lee et al., 2009; Mora-Pale et al., 2011; Lynam et al., 2012). Added acids in ILs have also been used in the treatment of biomass both for pretreatment and saccharification purposes (Li et al., 2008, 2010). In recent studies, HMIMCl was shown to hydrolyze the ether linkages in phenolic and non-phenolic lignin model compounds and to depolymerize oak wood lignin under mild conditions, suggesting a facile method for treatment of biomass (Jia et al., 2010; Cox et al., 2011; Cox and Ekerdt, 2012). The study reported herein tests the ability of this acidic IL to pretreat biomass through reactive delignification caused by the acid catalyzed cleavage of the ether linkages in lignin. Efficacy of pretreatment has been verified via enzymatic digestion of pretreated samples into monosaccharides using cellulase enzymes. While there has been research performed using ionic liquids for pretreatment, to the knowledge of the authors, the use of an acidic ionic liquid as a combined solvent and reactive delignification catalyst is a new development in pretreatment research.

2. Methods

2.1. Materials

1-*H*-3-methylimidazolium chloride (HMIMCl, ⩾95%), 1-ethyl-3-methylimidazolium acetate (EMIMAc, ⩾90%), cellulase (⩾5 kU g^{-1}), D(+)-mannose (⩾99%), 5-(hydroxymethyl)furfural (HMF, ⩾99%), D-manitol (⩾98%), calcium carbonate (⩾99%), and citric acid monohydrate (⩾99.5%) were purchased from Sigma–Aldrich. Acetone (99.7%), sulfuric acid (96.6%), dimethylsulfoxide (99.9%), dimethylsulfoxide-d6 (⩾99.9%), and D-glucose (⩾99%) were purchased from Fisher Scientific. Yellow pine wood chips were generously donated by KIOR, Inc., and kept in a vacuum oven at 60 °C while not in use to maintain a consistent moisture level. Ionic liquids were dried at 100 °C on a Schlenk line to a pressure of 100 mTorr. All other chemicals were used without further purification.

2.2. Pretreatment

In a typical pretreatment experiment, shown in Scheme 1, yellow pine wood chips (30 mg) were added to a glass reaction vial along with of deionized water (2.5 μl) and a stir bar. IL (1.0 ml), which was melted in a boiling water bath in the case of HMIMCl, was added to the reaction vial via pipette. The vial was sealed and inserted into a Thermo Scientific Reacti-Therm heated stirred reactor set to 110, 130, or 150 °C. The samples were stirred for the prescribed time and then moved to a room temperature water bath to cool down for roughly 30 s. The sample was then mixed with a 1:1 solution of acetone and water (5 ml). The remaining solids were filtered using a 25 mm diameter glass fiber filter disk and washed twice with 1:1 acetone/water (2 × 5 ml). The washed solids were then dried overnight (at least 8 h) in a vacuum oven at 60 °C to produce Fraction 1. The acetone in the remaining liquid was then evaporated at 40 °C and using an aspirator to provide vacuum. Precipitated lignin was then filtered with a glass filter disk and washed twice with deionized water (2 × 5 ml). This solid was dried in a vacuum oven over night to yield Fraction 2. The remaining aqueous sample was Fraction 3.

In order to collect the volatile products from the treatment, the cooled sample was quenched with water instead of the 1:1 acetone/water. This quenched sample was then connected to a Schlenk line so all volatiles could be collected in a liquid nitrogen cold trap *in vacuo*. The collected liquid from the cold trap was diluted to 15 ml with deionized water and analyzed using HPLC or mass spectrometry.

2.3. Acid digestion

Fraction 1 samples were saccharified using the modified procedure published by the National Renewable Energy Lab (Sluiter et al., 2011). The solids of Fraction 1, along with the glass fiber filter disk, were loaded in a 14 ml glass pressure tube. Sulfuric acid (72 wt.%, 300 μl) was added to the pressure tube and the sample was stirred every 5–10 min at 30 °C for 60 min. After the 60 min had elapsed, deionized water (8.4 ml) was added. The tube was sealed and allowed to react in an oven set at 121 °C for 2 h, then cooled to room temperature first in air and then with room temperature water. To each tube, calcium carbonate (700 mg) was added slowly to neutralize the sulfuric acid and precipitate calcium sulfate. The residual solids (calcium sulfate, lignin, and filter disk) were filtered and the liquid was analyzed using HPLC.

2.4. HPLC/GPC analysis

HPLC analysis was carried out using a Phenomenex Rezex RPM Monosaccharide Pb2 + 300 × 7.8 mm column held at 80 °C with degassed, deionized water as an eluent and a Wyatt Optilab T-Rex differential refractive index detector. Samples were prepared by mixing liquid from the sulfuric acid saccharification (200 μl), manitol solution (6.0 mg ml^{-1}, 100 μl) as an internal standard, and deionized water (1700 μl). HPLC standards were produced by adding known amounts of glucose and mannose to pressure tubes and following the sulfuric acid saccharification procedure. The samples produced through this procedure allowed for quantification of recovered glucose and mannose and accounted for degradation of monosaccharides in the saccharification process.

GPC analysis was carried out on the same equipment as used for the HPLC using two Phenomenex Phenogel 5 μm linear/mixed 300 × 7.8 mm columns with DMSO as the eluent. Samples were prepared by adding DMSO (1.0 ml) to the filter disk with Fraction 2. This mixture was stirred until the solids from Fraction 2 had dissolved in the DMSO. The sample was then filtered and injected into the GPC without further dilution. The unreacted yellow pine lignin (5.2 mg) was dissolved in DMSO (1.0 ml) and filtered as a standard. The depolymerized lignin standard was created by subjecting EMIMAc-extracted lignin (11.9 mg) to a standard pretreatment in HMIMCl at 130 °C for 300 min.

2.5. Lignin standard and NMR analysis

Unreacted lignin was extracted from yellow pine through a modified process developed by Sun et al. (2009). Dry yellow pine wood (2 g) was added to EMIMAc (28 g) and stirred at 110 °C for