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Overcome the recalcitrance of eucalyptus bark to enzymatic hydrolysis by concerted ionic liquid pretreatment

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ABSTRACT

A novel pretreatment method-concerted ionic liquid (CIL) pretreatment was proposed and the mechanism of CIL pretreatment to overcome the recalcitrance of biomass was investigated in this study. The CIL refers to a combination of a protic ionic liquid (PIL) pyrrolidinium acetate ([Pyrr][AC]) and an aprotic ionic liquid (AIL) 1-butyl-3-methylimidazolium acetate ([BMIM][AC]) in varied proportion. In the CIL system, [Pyrr][AC] selectively dissolves lignin from biomass and [BMIM][AC] dissolves part of cellulose and hemicelluloses. Besides lignin, hemicelluloses are also an important factor of biomass recalcitrance. Optimized [BMIM][AC] fraction in the CIL pretreatment resulted in the efficient removal of lignocellulose complex while [Pyrr][AC] selectively fractionated lignin component. CILs exhibited superior efficiency in overcoming the recalcitrance of eucalyptus bark over that of the individual constituent ionic liquids. Compared to pretreatments by individual ILs of the pair, the CIL pretreatment increased the glucose yield by 30% from the cellulose of eucalyptus bark. High cellulose conversion of 91% (wt%) was achieved from enzymatic conversion of eucalyptus bark by optimizing the lignin and hemicelluloses removal.

1. Introduction

Lignocellulosic biomass, which represents a source of sufficiently abundant renewable feedstock, has led to intensified research for the production of renewable bioenergy [1]. Forest residues from harvesting and forest thinning operations are substantial sources of biomass that can be utilized for bioenergy production [2,3]. However, bioprocessing of the forest residues is typically of low efficiency due to the high lignin content [4]. Lignin constitutes a physical barrier that protects cellulose from biodegradation. Various pretreatment methods have been developed in the past several decades to overcome the biomass recalcitrance [5,6]. But most of the pretreatments involve high temperatures and pressures. The high temperature and the use of chemicals in most pretreatments resulted in the formation of various inhibitory by-products that often slow down subsequent enzymatic hydrolysis of cellulose [7]. In addition, these pretreatments are energy intensive and often unfriendly to the environment.

Recently, some ionic liquids (ILs) have emerged as a green solvent for the pretreatment of lignocelluloses. Low vapor pressure, high thermal stability, and non-flammability make ionic liquids a class of greener solvents for biomass pretreatment compares to most reported chemicals [8,9]. Different from other chemical pretreatments, the ionic liquids pretreatment do not cause the degradation of cellulose, hemicellulose and lignin. The ionic liquids just dissolve the biomass and result in much less inhibitors compared to that caused by chemical pretreatments. While there are many advantages for IL pretreatment, ILs are currently far from being used in large-scale due to insufficient efficiency and high cost [10].

Achinivu et al. reported the preparation of protic ionic liquid (PIL) by a one-step reaction from low cost acid and base reagents recently [10]. The production of the PIL is much simpler than their aprotic ionic liquid (AIL) counterparts [11,12]. In addition, this kind of PIL exhibited high selectivity for lignin extraction from biomass with little effect on the cellulose structure at a relatively low temperature (<100 °C) [10]. It is well known that lignin, which causes poor accessibility of enzymes to cellulosic substrate, is a major obstacle for biomass conversion [13,14]. The PIL is a good solvent for delignification of lignocellulose biomass, although the pretreatment by the PIL alone was not sufficient for the full enzymatic hydrolysis of the biomass to glucose, as revealed by the results of this work.

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Besides lignin, hemicelluloses are also an important recalcitrance factor for the biomass. Hemicelluloses have been recognized as potential sites for covalent cross-linking between polysaccharides and lignin [15]. The tight physical binding and chemical linkages between lignin and polysaccharides make the cell wall of biomass difficult to deconstruct [16,17]. Partial removal of hemicelluloses by an AIL may enable a PIL to penetrate through the biomass fibers and to access to the lignin for more efficient lignin removal. We hypothesize that combining AIL and PIL for concerted fractionation of lignin and hemicelluloses from native biomass may result in increased accessibility of cellulose by enzymes for enhanced saccharification of the cellulose with the highest recalcitrance reduction efficiency. Some AILs such as 1-ethyl-3-methylimidazolium acetate ([EMIM][AC]), 1-butyl-3-methylimidazolium acetate ([BMIM][AC]) and 1-butyl-3-methylimidazolium ([BMIM][Cl]) have high efficiency in hemicelluloses dissolution [8,17]. In addition, the synergistic effect between the low cost PIL as the major component and AIL as the minor component of the CIL solvent, could make the CIL pretreatment more economically attractive for large-scale application. This study was carried out to investigate a concerted ionic liquid (CIL) pretreatment strategy in overcoming the recalcitrance of biomass by selective fractionation of lignin and partially hemicelluloses from biomass; the results of the different CIL pretreatment were evaluated by a downstream bioconversion process.

The mechanism of CIL pretreatment to overcome the recalcitrance of eucalyptus bark was also investigated in this study by controlling the level of the fractionation. Although factors limiting enzymatic hydrolysis of biomass have been extensively studied [18,19], the biomass recalcitrance are still not fully understood due to the complex interactions between the plant cell wall and biomass pretreatment. Understanding the compositional changes during different pretreatment processes is critically important to improve the current bio-refinery technologies.

2. Materials and methods

2.1. Materials

The eucalyptus bark was obtained from China National Pulp and Paper Research Institute. The samples were ground using a mill (SM200 Rostfrei, Retsch, Germany) to pass through a 40-mesh (~1 mm) screen. The moisture content of eucalyptus bark was 7.72 wt% (in reference to the oven dry material). It should be noted that the ash content of the eucalyptus bark is 11.07 wt%, much higher than that of other feedstock [4]. The total amount of extractives of eucalyptus bark is also relatively high, at about 8.86 wt%. The major soluble extractives in the eucalyptus bark are mainly composed of tannins, polyphenolic compounds, fatty acids and flavonoids [20]. The high contents of both ash and extractives negatively impact on the biomass conversion process.

Pyrrolidine, of at least 99% purity, was purchased from Energy Chemistry (Shanghai, China) and used as received. High-purity glacial acetic acid, ethanol, sulfuric acid were used as-received. The ionic liquid [BMIM][AC] was purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Science.

[Pyrr][AC] was synthesized in a round-bottom flask according to Henderson's work [10]. The molar ratio of pyrrolidine and glacial acetic acid was 1:1. The flask was placed in an ice/water bath to prevent excessive heat buildup during the reaction. Stirring was continued for 24 h at room temperature to obtain the [Pyrr][AC]. Both ^{1}H and ^{13}C NMR analyses were conducted to verify the structure of the synthesized [Pyrr][AC].

Cellulase Ctec-2 (150 FPU/ml) was received from Novozymes (Beijing, China). The enzyme activity was measured according to the NREL standard protocol [21].

The Avicel and Alkali lignin were purchased from Sigma–Aldrich, China.

2.2. Thermogravimetric analysis (TGA)

Thermal analysis was performed using a thermogravimetric analyzer (STA 449F3, NETZSCH, Germany). Samples of 5 ± 0.5 mg were placed in aluminum oxide crucibles and heated from 50 to 500 °C at a rate of 10 °C/min in the presence of nitrogen (10 ml/min).

2.3. Typical procedure for the pretreatment of eucalyptus bark with ionic liquid

The IL pretreatments were carried out in 50 ml hydrothermal synthesis reactors at 5 rpm rotation; the reaction volume was 20 ml. All pretreatments were conducted at 90 °C for 10 h based on the results of Henderson's research [10]. The contents in reactors were centrifuged after pretreatment to separate the solid and the liquid. The liquid was collected to recycle the IL. The solid was washed well with ethanol and followed by water wash to make sure the IL was essentially removed [10]. The solid was collected and stored at 4 °C for moisture content analysis, chemical composition analysis and enzymatic hydrolysis. The liquid that contains IL, ethanol and water was distillated to remove water and ethanol, followed by IL recovery for further use.

2.4. Enzymatic hydrolysis

All enzymatic hydrolysis experiments were performed in 4 ml vials at 50 °C placed on a rotary shaker at 200 rpm. The reaction mixtures contained 2 ml of 50 mM sodium acetate buffer (pH 4.8), 40 mg (dry weight) biomass and the enzyme. The enzyme was Ctec2 and the enzyme loading was 20 FPU/g glucan. In addition, polyethylene glycol (PEG) 1000 was added during enzymatic hydrolysis to avoid the inhibition of ionic liquid residue on the activity of cellulase [22]. The PEG 1000 loading was 0.025 g/g substrate. All enzymatic hydrolysis experiments were conducted in duplicate and the variations are less than 2%.

2.5. Chemical compositional analysis

The chemical compositions of raw and pretreated samples were analyzed according to the procedures of NREL LAPs [23]. The concentrations of sugars in the hydrolyzed liquid were quantified by high performance liquid chromatography (HPLC) system equipped with an Aminex HPX-87H column (300 × 7.8 mm; Bio-Rad, USA) and a refractive index detector. HPLC conditions were as follows: column temperature was 65 °C, mobile phase was sulfuric acid (0.005 M, HPLC grade) with flow rate of 0.6 ml/min.

3. Results and discussions

3.1. Pretreatment of eucalyptus bark with different ionic liquids

Eucalyptus bark was pretreated by PIL [Pyrr][AC], AIL [BMIM][AC] and CILs consisting of the PIL and the AIL, separately. All the pretreatments were conducted at 90 °C and the total reaction time was kept at 10 h, unless specified otherwise. The CILs were composed of [Pyrr][AC] and [BMIM][AC] in optimized relative amount. Two methods were used to carry out the CIL pretreatment. The first one, [Pyrr][AC] and [BMIM][AC] were mixed with the biomass simultaneously to start the pretreatment. The weight ratio of [Pyrr][AC]: [BMIM][AC]: biomass was 7:3:0.5 to maintain