Catalysis Today xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Catalysis Today

journal homepage: www.elsevier.com/locate/cattod

A coupled low temperature oxidative and ionic liquid pretreatment of lignocellulosic biomass

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ARTICLE INFO

Article history:
Received 27 June 2015
Received in revised form 1 December 2015
Accepted 14 December 2015
Available online xxx

Keywords:
Ionic liquid
EMIM-Ac
Pretreatment
Acetylation
Cellulose
Lignin oxidation

ABSTRACT

An integrated pretreatment strategy consisting of a room temperature alkaline oxidation step coupled with ionic liquid (IL) incubation enables effective lignocellulosic biomass pretreatment at low temperatures (50 °C). The IL, 1-ethyl-3-methyl-imidazolium acetate (EMIM-Ac), was used in pretreatment of a lignocellulosic hardwood feedstock, poplar. Glucose and xylose yields for 24 h enzyme hydrolysis of pretreated poplar were measured to assess the efficacy of this pretreatment strategy. The proposed strategy resulted in high hydrolysis yields at a low enzyme loading of 9.5 filter paper units per gram of glucan. The low IL incubation temperatures were found to reduce undesired cellulose acetylation reactions.

1. Introduction

Lignocellulosic biomass is a renewable source of carbon, consisting of three major components: cellulose (30–45%), a highly crystalline polymer of the hexose sugar, glucose; hemicellulose (20–40%), a complex amorphous polysaccharide comprised of pentose (often primarily xylose) and hexose sugars; and lignin (5–25%), a polyphenyl-propanoid macromolecular assembly that is covalently cross-linked to hemicellulose [1]. The complex and compact structure of lignocellulosic biomass renders it largely impenetrable to water, catalysts, or enzymes used to hydrolyze its constituent polysaccharides to monomeric sugars (saccharification). Because of this, pretreatment is necessary prior to saccharification [2,3].

Effective ionic liquid (IL) incubation of lignocellulosic biomass can generate an amorphous cellulosic substrate enabling greater enzyme access for rapid hydrolysis of polysaccharides without the production of fermentation inhibitors [4]. The crystalline structure of native cellulose, commonly called cellulose I (referring to two naturally occurring allomorphs, Iα and Iβ) [5,6] is a major impediment to its hydrolysis to monomeric sugars [7]. IL pretreatment typically involves a biomass incubation step followed by IL displacement using an anti-solvent that precipitates the treated biomass. High IL incubation temperatures (up to ~160 °C) are often necessary for an effective biomass pretreatment that produces a readily hydrolyzable substrate [8–10]. However, these high temperatures can result in thermal degradation of IL and biomass and undesired cellulose derivatization reactions [11–15].

Imidazolium based ILs are effective solvents for dissolution of cellulose. Among 21 imidazolium-based ILs screened, 1-ethyl-3-methyl-imidazolium acetate (EMIM-Ac) and 1-allyl-3-methyl-imidazolium chloride (AMIM-Cl) were found to be the most effective ILs in dissolution of cellulose and woodchips [16]. In additional studies of 97 ionic liquids with a variety of cation moieties, 1-butyl-3-methyl-imidazolium chloride (BMIM-Cl), AMIM-Cl and EMIM-Ac were found to be among the best solvents for cellulose and lignocellulose [17]. However, halogenated ILs such as BMIM-Cl and AMIM-Cl pose potential corrosion issues in processing equipment. Moreover, the melting points of BMIM-Cl and AMIM-Cl are reported to be ~70 °C [18,19] and ~52 °C [20,21], significantly higher than EMIM-Ac (a liquid below room temperature). Some cholinium-carboxylate ionic liquids such as choline acetate and choline propionate are reported to have qualities similar to EMIM-Ac for pretreatment of lignocellulosic materials [22]. However, the high melting point of choline acetate (between 51–72 °C) and high

Abbreviations: AHP, alkaline hydrogen peroxide; AMIM-Cl, 1-allyl-3-methyl-imidazolium chloride; EMIM-Ac, 1-ethyl-3-methyl-imidazolium acetate; IL, ionic liquid.

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http://dx.doi.org/10.1016/j.cattod.2015.12.022
0920-5861/

viscosity of choline-based ionic liquids, ranging between 630–8500 centipoise, render these ILs less satisfactory than EMIM-Ac (160 cp) [23–25]. EMIM-Ac exhibits a high capacity for cellulose dissolution [26]. In addition to its low melting point (<−20 °C) and relatively low viscosity [27], the acetate anion is less corrosive than comparable halides [28]. These characteristics make EMIM-Ac one of the most effective solvents for IL pretreatment. However, acetylation of cellulose has been reported in EMIM-Ac at temperatures in the range used for pretreatment of biomass [12]. Acetylation of cellulose can impede enzyme hydrolysis and produce undesired acetylated glucose [29,30]. Furthermore, it can result in loss of the acetate group of EMIM-Ac and reduce the recyclability and compatibility of this ionic liquid within industrial applications. In studies presented herein, acetylation of Avicel, a crystalline cellulose model compound, was monitored for samples incubated in EMIM-Ac at high and low temperatures. Reduction in this undesired derivatization reaction was observed at decreased IL incubation temperatures.

Prior work indicated that cellulose I, found in lignin free substrates was transformed into more easily hydrolyzed cellulose II or amorphous cellulose with incubation in IL at 50 °C. In contrast, higher temperatures of ~120 °C were required to affect similar transitions in a lignocellulosic biomass [31]. This suggested that removal or partial decomposition of lignin from biomass can result in effective IL pretreatment at lower temperatures. Therefore, we employed a '*coupled method*' consisting of a room temperature alkaline hydrogen peroxide (AHP) oxidation step for partial removal of lignin, followed by IL incubation in EMIM-Ac at 50 °C. The glucose and xylose yields for 24 h enzyme hydrolysis of a pretreated lignocellulosic hardwood substrate, poplar, were measured to assess the efficacy of this pretreatment strategy using a low enzyme loading of ~9.5 filter paper units (FPU)/g of glucan.

2. Materials and methods

2.1. Materials

Poplar was provided by the National Renewable Energy Laboratory (NREL). D-xylose (99%), dimethyl sulfoxide-d_6 (99.9% D), 1-ethyl-3-methyl-imidazolium acetate (>97%), 1-allyl-3-methyl-imidazolium chloride (>97%), 1-methylimidazole (>97%), Avicel and birchwood xylan were purchased from Sigma–Aldrich. D-glucose (99+%), and hydrogen peroxide (35 wt%) were purchased from Acros Organics. Sodium hydroxide, sodium citrate dihydrate and citric acid monohydrate were purchased from Fisher Scientific. The commercial enzyme mixture, Cellic CTec2, was provided by Novozymes.

2.2. Biomass compositional analysis and quantitation of monomeric sugars

The compositional analysis of the poplar biomass samples was carried out in triplicate using NREL standard LAP 002 protocols using a concentrated and dilute acid digestion [32]. Sugar concentrations of the digests were determined via high performance liquid chromatography (HPLC), with refractive index detection using a Bio-Rad (Richmond, CA) Aminex HPX-87P carbohydrate analysis ion-exchange column. The HPLC was operated at 80 °C in an isocratic mode with a mobile phase of deionized water at 0.6 mL/min. Mixed sugar standards of known concentrations were used to generate standard curves to calculate the concentration of released sugars. Glucose and xylose released from glucan and xylan, respectively, in enzyme hydrolysis experiments were reported as a percentage of theoretical yield of monomeric sugars based on glucan and xylan analysis of untreated substrates.

2.3. Enzyme hydrolysis

Enzymatic hydrolysis of pretreated and native substrates followed NREL standard LAP 009 protocol [33] with enzyme loadings of 9.5 FPU/g of glucan (4.5 mg protein/g glucan) using a commercial cellulase enzyme mixture, Cellic CTec2 (Novozymes). The hydrolysis was run at 1% (w/v) solid loadings at 50 °C in 50 mM sodium citrate buffer, pH 4.8 for 24 h with mixing in a rotary shaker water bath. The protein concentration of Cellic CTec2 was determined using the colorimetric Bradford protein assay with bovine serum albumin standards [34]. Activity of the cellulase was measured and reported in filter paper units [35].

2.4. Biomass pretreatment

A '*coupled method*' of pretreatment consisted of a room temperature AHP oxidation step followed by IL incubation at 50 °C. Selected samples were pretreated via AHP oxidation or IL incubation alone.

2.4.1. Room temperature AHP oxidation step

For the oxidation step, native poplar was mixed with alkaline hydrogen peroxide (AHP) at 10% (w/v) biomass solid loading. Samples were incubated for 1–24 h at room temperature. The AHP solution consisted of a 25 mM NaOH solution with hydrogen peroxide added at a ratio of 12.5% (w/w) H_2O_2 with respect to native poplar. Oxidation reactions were stopped by addition of deionized water, followed by centrifugation and removal of filtrate. Samples were dried to a constant weight at 40 °C, unless otherwise noted.

2.4.2. IL incubation

For the IL incubation step, samples were mixed with 1-ethyl-3-methyl-imidazolium acetate (EMIM-Ac) at a 5–20% (w/w) loading of biomass to IL. Samples were incubated between 0.5–4 h at 50 °C (unless otherwise noted). After IL incubation, deionized water was added to the IL incubation containers to precipitate polysaccharides and displace the IL from the biomass. Samples were centrifuged, and the supernatant was removed. Precipitated biomass was repeatedly washed and centrifuged until a clear supernatant was observed. The precipitated solids (regenerated biomass) were then filtered and hydrolyzed.

2.5. ^{13}C NMR sample preparation and data acquisition conditions

Samples of Avicel, a microcrystalline cellulose model compound, were incubated in EMIM-Ac at 50 and 140 °C for 4 h with a biomass solid loading of 5% (w/w). Deionized water was added to the IL incubation containers to precipitate the Avicel and displace IL as previously described for biomass samples. Avicel samples were then dried to a constant weight under vacuum at 40 °C. The EMIM-Ac incubated Avicel samples and a control Avicel sample (not exposed to EMIM-Ac) were each separately combined with 1-allyl-3-methyl-imidazolium chloride (AMIM-Cl) at a biomass solid loading of 5% (w/w) and stirred at 30 °C until complete dissolution was visually observed. DMSO (~20% v/v) was mixed with samples in order to reduce the sample viscosity.

Liquid state ^{13}C NMR was used to detect evidence of acetylation of cellulose by EMIM-Ac: ^{13}C NMR (DMSO-d6) for acetylated cellulose (structure shown in Fig. 1) exhibited chemical shifts in ppm at: δ = 170.6 (acetate CO or C-7), 102.4 (C-1), 79.5–72.6 (C2-5), 60.2 (C-6, not substituted), 21.1 (C-8), AMIM-Cl (structure shown in Fig. 1b) at: δ = 136.8 (C-1'), 131.9 (C-6'), 123.7 (C-3'), 122.3 (C-2'), 120.0 (C-7'), 50.7 (C-5'), 36.3 (C-4'). These assignments are in agreement with those previously reported [12,36].

NMR spectra were acquired on a Bruker Avance III 600 MHz NMR spectrometer. It has a dual channel 5 mm DCH CryoProbe that was optimized for the ^{13}C sensitivity at 150.92 MHz. The data were

Please cite this article in press as: S. Vasheghani Farahani, et al., A coupled low temperature oxidative and ionic liquid pretreatment of lignocellulosic biomass, Catal. Today (2016), http://dx.doi.org/10.1016/j.cattod.2015.12.022