



Research article

An efficient process for lignin extraction and enzymatic hydrolysis of corn stalk by pyrrolidonium ionic liquids



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ARTICLE INFO

Article history:

Received 14 May 2015

Received in revised form 4 February 2016

Accepted 29 February 2016

Available online 7 March 2016

Keywords:

Ionic liquids

Corn stalk

Pretreatment

Pyrrolidonium

ABSTRACT

In recent years, lignocellulosic biomass has drawn increasingly attention as a platform for its transformation into biofuels. However, the complex structures of lignocelluloses limit their conversion into fermentable sugars or other valuable products. In this work, a series of pyrrolidonium-based ionic liquids with a simple synthetic procedure were investigated for the pretreatment of corn stalk at 90 °C for 30 min. Both ionic liquids alone and ionic liquids with deionized water were studied. Fourier transform infrared spectroscopy (FTIR) analysis, scanning electron microscopy (SEM) analysis and nuclear magnetic resonance spectroscopy (NMR) analysis were employed to determine the chemical characteristics of regenerated lignin or cellulosic feedstock. A significant yield of lignin was achieved, as high as 19.07%, which accounted for 85.94% of the original lignin of corn stalk. A high yield of reducing sugar (91.81%) was obtained from regenerated cellulosic feedstock after enzymatic hydrolysis. High yields of glucose (79.63%) and cellobiose (13.66%) were observed as determined by high performance liquid chromatography (HPLC).

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1. Introduction

With the exhaustion of fossil fuels in sight, the search for biomass as a renewable energy resource in the production of biofuels has become critically important in recent years. Lignocellulosic biomass is one of the important renewable and non-food sources for bioethanol production through its conversion into fermentable sugars [1]. Lignocelluloses mainly consist of cellulose, lignin and hemicelluloses [2]. Cellulose fibers are cross-linked to the matrix of lignin and hemicelluloses, becoming a recalcitrant structure [3], which limits the saccharification efficiency of lignocellulosic biomass [4]. Therefore, a pretreatment process is necessary to disrupt the recalcitrant structure [5–7] and then to increase the accessibility of enzymes to cellulose during hydrolysis [8,9].

In recent years, ionic liquids (ILs) have attracted much attention as pretreatment solvents for lignocelluloses [8,10,11]. They have several advantages such as low volatility, thermal stability, widely accessible temperature range, lack of flammability, and negligible vapor pressure [12–14]. The IL-pretreatment of lignocelluloses contributes to decrease the lignin content and to reduce the crystallinity of cellulose and then to increase the surface area for enzymatic hydrolysis [15]. Good results have been obtained with imidazolium-based ionic liquids. Haykir et al. reported that cotton

stalk was pretreated by 1-allyl-3-methylimidazolium chloride ([AMIM]Cl), 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium chloride ([EMIM]Cl) and 1-ethyl-3-methylimidazolium acetate ([EMIM]CH₃COO) at 150 °C for 30 min [14]. Total lignin removal was 46%, 31%, 38% and 45% (based on the lignin content) from cotton stalk, respectively. The highest biomass digestibility was obtained via [EMIM]CH₃COO-pretreatment, which was 65% after 72 h of enzymatic hydrolysis [14]. Liu et al. described that the lignin of untreated cole straw was 17.71% and the lignin was 12.35% after [BMIM]Cl-pretreatment at 145 °C for 15 min, and the yield of sugar was 31.32% after 72 h of enzymatic hydrolysis [16]. Gao et al. reported that corn stalk was pretreated by [EMIM]CH₃COO at 140 °C for 3 h [17]. 89.9% total lignin removal (based on the lignin content) from corn stalk and the yield of glucose was 91.6% after 72 h of enzymatic hydrolysis was obtained. Currently, Ninomiya et al. investigated choline acetate for the pretreatment and fractionation of bagasse at 110 °C for 16 h [18]. Almost all the carbohydrates with approximately 50% of lignin in the original bagasse were recovered as carbohydrate-rich materials and approximately 20% of the lignin (based on the lignin content) was fractionated as lignin-rich material. The cellulose saccharification was more than 95% in the case of carbohydrate-rich materials [18]. An et al. described that rice straw pretreated by cholinium arginate at 90 °C for 12 h produced 74.6% of glucose after 72 h of enzymatic saccharification and the lignin recovery was 46% in native rice straw (based on the lignin content) [19].

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Although ionic liquids including [AMIM]Cl, [BMIM]Cl, [EMIM]CH₃COO were reported widely for the pretreatment of lignocelluloses, high pretreatment temperatures and long pretreatment time were always required, unfavorable for the practical production of reducing sugars [14]. In addition, lignin regeneration was not very efficient using ionic liquids such as [AMIM]Cl, [EMIM]CH₃COOH in current studies [14], which limited the efficient enzymatic hydrolysis for lignocellulose. Novel ionic liquids should therefore be investigated.

In this work, a series of pyrrolidonium-based ionic liquids were synthesized. The pretreatment of corn stalk was carried out at 90 °C for 30 min in one of the ionic liquids including N-methyl-2-pyrrolidonium chloride ([Hnmp]Cl), N-methyl-2-pyrrolidonium methanesulfonate ([Hnmp]CH₃SO₃), N-methyl-2-pyrrolidonium hydrogen sulfate ([Hnmp]HSO₄) and N-methyl-2-pyrrolidonium dihydrogen phosphate ([Hnmp]H₂PO₄). Both ionic liquids alone and ionic liquids with deionized water were studied.

2. Experimental

2.1. Materials

All chemicals were purchased from Aladin Company (China) and used as received. The cellulase (specific activity: 10,000 U/g) from *Aspergillus niger* was supplied by Aladin Company and used as received [20]. The corn stalk, obtained from a local farm during the 2013 harvest in Harbin City (China), was ground in a cutter mill and passed through 40 mesh sieves, then dried at 40 °C for 48 h. Corn stalk consisted of 42.95% cellulose, 19.88% hemicelluloses and 22.19% lignin, which was determined according to the standard National Renewable Energy Laboratory (NREL) method [21].

Fourier transform infrared spectroscopy (FTIR) analysis was performed on a FTIR-8400S spectrometer (Shimadzu) in the range of 4000–400 cm⁻¹. Scanning electron microscopy (SEM) was performed on a S-3400N (Hitachi). Analysis of reducing sugar was performed on a UV-mini-1240 spectrometer (Shimadzu). Sugars were analyzed by HPLC (Waters 2695) equipped with an Agilent ZORBAX Carbohydrate column. Nuclear magnetic resonance (NMR) spectroscopy was conducted with a Bruker AVIII 400 MHz spectrometer at 25 °C. About 90 mg of lignin was dissolved in a mixture of deuterated DMSO/pyridine (4:1, v/v) [22].

2.2. Synthesis of pyrrolidonium-based ionic liquids

N-methyl pyrrolidone (0.1 mol) was taken into a 250 ml three-necked round bottomed flask. Sulfuric acid (0.1 mol) was added dropwise into N-methyl pyrrolidone under stirring in an ice bath. The reaction proceeded in an oil bath at 80 °C for 12 h under stirring. After that, the resulting product was washed for 3 times with 20 ml of ethyl acetate and dried in a vacuum at 60 °C for 12 h to give [Hnmp]HSO₄ [23]. The synthesis of other ionic liquids including [Hnmp]Cl, [Hnmp]CH₃SO₃ and [Hnmp]H₂PO₄ followed the similar procedure.

2.3. Determination of acidity of ionic liquids

The acidity of the pyrrolidonium-based ionic liquids was determined using Hammett method with UV-VIS spectroscopy at room temperature. 4-Nitroaniline was used as a indicator with deionized water as a solvent, the concentrations of the indicator and ionic liquids in deionized water were 0.11 mmol/L and 30 mmol/L, respectively. The acidity was measured by evaluating the protonation extent of uncharged indicator bases (named I) in a solution, in terms of the measurable ratio [I]/[IH⁺] [24,25]. The Hammett function (H₀) is defined by Eq. (1).

$$H_0 = \text{pK}(I)_{\text{aq}} + \log \frac{[I]}{[IH^+]} \quad (1)$$

where $\text{pK}(I)_{\text{aq}}$ of 4-nitroaniline is 0.99, [I] and [IH⁺] are the molar concentrations of unprotonated and protonated form of indicators in the solution, respectively.

2.4. Pretreatment of corn stalk

Corn stalk (0.25 g) was added into one of the ILs (5 g) both with and without deionized water (1 ml) in a 100 ml round bottomed flask. The mixture was stirred at 90 °C for 30 min in an oil bath. Following that, the mixture was cooled to room temperature, then 20 ml of acetone/deionized water (1/1, v/v) was added into the mixture. The remaining solid was then filtered and washed with 20 ml of acetone/deionized water (1/1, v/v), followed by 20 ml of deionized water in order to remove any residual ionic liquid. This solid was dried at 60 °C for 24 h to obtain the regenerated cellulosic feedstock (RCF). Additionally, when acetone was evaporated from the filtrate by rotary evaporation at room temperature for 30 min, the regenerated lignin precipitated out of the solution, which was washed with 20 ml of deionized water and dried at 60 °C for 24 h. The yield of lignin or RCF was determined as the ratio of the mass of lignin or RCF in the mass of the corn stalk that was subjected to the pretreatment process according to Eq. (2).

$$\text{Lignin or RCF (\%)} = \frac{\text{lignin or RCF (mg)}}{\text{corn stalk subjected to pretreatment (mg)}} \quad (2)$$

2.5. Determination of reducing sugar

After lignin was removed, the filtrate was neutralized to pH 7.0 by the addition of aqueous solution of sodium hydroxide. The reducing sugar was measured by the 3,4-dinitrosalicylic acid (DNS) method [26]. The concentration of reducing sugar was calculated by employing a standard curve prepared using glucose, and the absorbance of glucose was measured at 540 nm using a UV-mini-1240 Shimadzu spectrophotometer. The yield of reducing sugar during the pretreatment process was determined as the ratio of the mass of reducing sugar in the mass of the corn stalk that was subjected to the pretreatment process according to Eq. (3).

$$\text{Reducing sugar (\%)} = \frac{\text{reducing sugar (mg)}}{\text{corn stalk subjected to pretreatment (mg)}} \quad (3)$$

2.6. Enzymatic hydrolysis

Enzymatic hydrolysis was performed in a 50 ml conical flask on the shaker of 150 rpm at 50 °C for 72 h with a biomass loading of 1% (g/ml) and 0.02 g of cellulase in 0.1 M sodium citrate buffer (pH 4.8). The enzymatic reaction was stopped by shaking the mixture at 100 °C for 10 min. The reducing sugars were measured by 3,4-dinitrosalicylic acid (DNS) method as described by Miller [26]. The yields of glucose and cellobiose were measured by HPLC. The yields of reducing sugar, glucose and cellobiose after enzymatic hydrolysis were determined as the ratio of the mass of reducing sugar or glucose or cellobiose in the mass of the regenerated cellulosic feedstock (RCF) that was subjected to enzymatic hydrolysis according to Eq. (4). The conversion of RCF was calculated according to Eq. (5).

$$\text{Reducing sugar or glucose or cellobiose (\%)} = \frac{\text{reducing sugar or glucose or cellobiose (mg)}}{\text{RCF subjected to enzymatic hydrolysis (mg)}} \quad (4)$$

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