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Effect of [Emim]Ac pretreatment on the structure and enzymatic hydrolysis of sugarcane bagasse cellulose

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ABSTRACT

Effect of ionic liquid pretreatment on enzymatic hydrolysis of cellulose was investigated in terms of the changes in the chemical and physical structure of the preparation. In this case, original cellulose isolated from sugarcane bagasse was subjected to ionic liquid ([Emim]Ac) dissolution at a mild temperature (90 °C) followed by regeneration in water and subsequently hydrolyzed by commercial cellulases. The original and regenerated cellulose were thoroughly characterized by XRD, FT-IR, CP/MAS ^{13}C NMR, and SEM. It was found that the original cellulose experienced an increase in glucose content from 80.0–83.3% to 91.6–92.8%, a decrease in the degree of polymerization from 974–1039 to 511–521, a crystal transformation from cellulose I to cellulose II, as well as an increase of surface area during the pretreatment. The results suggested that pretreatment led to effective disruption of cellulose for subsequent enzyme hydrolysis as evidenced by a high glucose conversion yield of 95.2%.

1. Introduction

The rapid consumption of fossil fuel resources has motivated researchers to pursue renewable and sustainable sources (Farrell et al., 2006). Lignocellulosic biomass has been recognized as a potential renewable feedstock for bioconversion into biofuels and value-added chemicals (Lee, Doherty, Linhardt, & Dordick, 2009; Richard, 2010). Lignocelluloses are mainly composed of cellulose, hemicelluloses and lignin. Among them, cellulose is the most abundant component and has a great potential in the conversion into bio-fuels, chemicals and materials (Pandey et al., 2000). It is a linear homopolymer of β-(1 → 4)-linked glucopyranose repeating units, consisting of amorphous and highly structured crystalline regions (Pinkert, Marsh, Pang, & Staiger, 2009). The crystalline structure of native cellulose fibers, called cellulose I, consists of parallel chains align side-by-side via hydrogen bonding in flat sheets (Nishiyama, Sugiyama, Chanzy, & Langan, 2003). After mercerization or regeneration, cellulose I is transformed into cellulose II. Cellulose II comprises a two-chain monoclinic unit cell in which the cellulose chains are stacked with opposite polarity, in an antiparallel chain packing (Wada, Ike, & Tokuyasu, 2010).

Due to the stiffness of the molecule and the close packing of the chains via numerous intermolecular and intramolecular hydrogen bonds (Azubuike, Rodríguez, Okhamafe, & Rogers, 2011), cellulose is difficult to dissolve in conventional solvents and to hydrolyze into fermentable sugars (Arantes & Saddler, 2011). Therefore, pretreatment is a necessary step to disrupt the tight packing arrangement of cellulose fibrils in the crystalline domains, for enhancing enzymes accessibility to cellulose during hydrolysis. Dissolution of cellulose by cellulose solvents is considered as one of the easiest methods for disrupting cellulose structure. Generally, cellulose solvents can be divided into derivative solvents and non-derivative solvents. The derivative solvents dissolve cellulose by conversion of the cellulose into a soluble transient derivative or intermediate, whereas the non-derivative solvents dissolve cellulose by disrupting the hydrogen bonds without the formation of a derivative (Liebert, 2010). In order to improve the reactivity of cellulose, numerous non-derivative solvents, such as N-methyl-morpholine-N-oxide (NMMO), concentrated phosphoric acid, and ionic liquids (ILs), have been used to pretreat cellulose for enzymatic hydrolysis (Kuo & Lee, 2009). Among them, ILs have recently emerged as promising non-derivative solvents for the dissolution of lignocelluloses. It has been reported that 1-allyl-3-methylimidazolium-chloride ([Amim]Cl) is the most effective ionic liquid for dissolving wood

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chips, and 1-ethyl-3-methylimidazolium-acetate ([Emim]Ac) has good dissolving capability for cellulose (Zavrel, Bross, Funke, Büchs, & Spiess, 2009). Overall, extensive effort has been devoted to direct dissolution of lignocellulosic material and separation of the constitutive major biopolymers. However, a clear separation of cellulose can not be attained from the direct dissolving process (Azubuike et al., 2011). After the IL pretreatment of the feedstock, the cellulose in the raw material is much more prone to degradation by cellulase (Dadi, Schall, & Varanasi, 2007). However, with respect to the investigation on the cellulose, the starting materials adopted in many studies are commercial products, such as Avicel and cotton, which have different properties from the cellulose in natural lignocellulosic biomass.

Up to now, there is a little information available on the chemical and physical changes of cellulose after IL pretreatment and their impact on the subsequent hydrolysis. As a consequence, the current research is specifically focused on this aspect. In this study, cellulose was isolated from sugarcane bagasse, a typical agricultural lignocellulosic material, and subsequently pretreated with ionic liquid [Emim]Ac. Enzymatic hydrolysis of the ionic liquid pretreated cellulose and the original cellulose was compared to investigate the correlation between changes in cellulose structure and digestibility. The chemical and physical changes between the original and reconstituted cellulose were thoroughly characterized by X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FT-IR), solid polarized/magic angle spinning (CP/MAS) ^{13}C NMR, and scanning electron microscopy (SEM). The intrinsic viscosity and molecular weight of the original and regenerated cellulose were also determined. The relationship between the structural changes and the enzymatic hydrolysis allow us to better understand the mechanism involved in the enzymatic hydrolysis resulting from IL [Emim]Ac pretreatment and to further improve the efficiency of the process.

2. Experimental

2.1. Materials

Sugarcane bagasse was collected in a sugar factory (Guangzhou, China). It was air dried, milled, screened to obtain the particles of 40–80 mesh, and then stored until used. The ionic liquid [Emim]Ac (≥98.5%) was purchased from Lanzhou Institute of Chemical Physics, Lanzhou, China. Celluclast 1.5 L (cellulase) and Novozyme 188 (β-glucosidase) were provided by Novozymes (China) Investment Co. Ltd.

2.2. Isolation of cellulose from sugarcane bagasse

The dried sugarcane bagasse powder was first delignified with sodium chlorite in acidic solution (pH 3.8–4.0, adjusted by acetic acid) at 75 °C for 2 h. The holocellulose was then extracted with 10% KOH to remove hemicelluloses with a solid to liquor ratio of 1:20 (g ml^{-1}) for 10 h at 20 °C, 30 °C, 40 °C, and 50 °C, respectively. The remaining solids, considered to be original cellulose, were filtrated and washed thoroughly with water until the filtrate was neutral, and dried in an oven at 55 °C for 16 h.

2.3. Cellulose dissolution and regeneration

Ionic liquid [Emim]Ac (10.0 g) was added to a dried flask containing 0.3 g of the original cellulose. The mixture was then placed into an oil bath heated to 90 °C and the dissolution proceeded with vigorous magnetic stirring (700 rpm) for 6 h, under an inert atmosphere of nitrogen. After the reaction, distilled water was poured into the flask under stirring, and the precipitates were recovered as regenerated cellulose. The resulting precipitates were washed with distilled water at 60 °C several times to remove IL and then freeze-dried. The cellulose preparations were considered to be regenerated cellulose IL20, IL30, IL40, and IL50, respectively, according to the corresponding crude cellulose. The scheme for the treatment process of sugarcane bagasse is illustrated in Fig. 1.

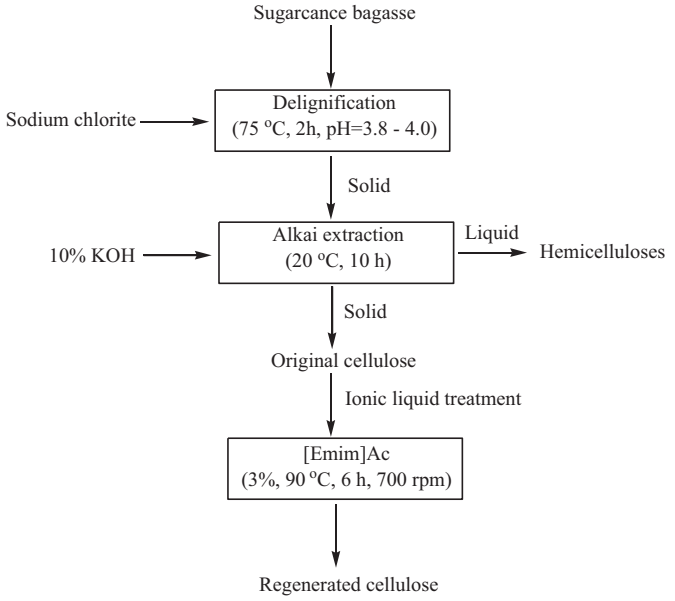

Fig. 1. Schematic illustration of the experimental procedure.

2.4. Enzymatic hydrolysis of cellulose samples

Enzymatic hydrolysis was performed at 50 °C in 0.05 M citrate buffer (pH 4.8) with 2% original and regenerated cellulose substrate in a 25 ml conical flask. The enzyme loadings were 35 FPU/g (cellulase) and 40 CbU (β-glucosidase) in relation to the dry weight of cellulose substrates. Samples were periodically withdrawn, and glucose concentration was determined by a HPAEC system. All reactions were carried out in triplicate.

2.5. Analysis methods

The viscosity-average degree of polymerization (P) of the cellulose preparations was determined by the cupri-ethylene-diamine (CED) method as described elsewhere (Wang, Jiang, Xu, & Sun, 2009) and calculated using the following equation:

$$P^{0.90} = \frac{1.65\,[\eta]}{\text{ml g}^{-1}} \quad (1)$$

The molecular weight of the cellulose was then calculated, using P multiplying by 162, the molecular weight of an anhydroglucose.

The carbohydrate compositions of the original and regenerated cellulose preparations were analyzed by high-performance anion exchange chromatography (HPAEC) (Dionex, ICS 3000, USA) equipped with a CarboPac PA 20 analytical column and an amperometric detector. The glucose yield was calculated as follows:

$$\text{Glucose yield (\%)} = \frac{\text{Released glucose weight} \times 0.9}{\text{Cellulose substrate weight} \times \text{content of glucose}} \times 100 \quad (2)$$

The X-ray powder diffraction pattern of the original and regenerated cellulose preparations was measured using an XRD-6000 instrument (Shimadzu, Japan) with a Cu Kα radiation source (λ = 0.154 nm) at 40 kV and 30 mA. Samples were scanned from