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Cholinium ionic liquid/cosolvent pretreatment for enhancing enzymatic saccharification of sugarcane bagasse

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ABSTRACT

In order to reduce the amount of choline acetate ([Cho][OAc]), (a less expensive, more biodegradable and biocompatible ionic liquids) used in the pretreatment of sugarcane bagasse, [Cho][OAc] combined with organic solvents pretreatments were attempted. The glucose yields after a 72-h enzymatic saccharification of sugarcane bagasse pretreated with [Cho][OAc] combined with organic cosolvents dimethyl sulfoxide (DMSO), *N*-methyl-2-pyrrolidone (NMP), or ethylene glycol (EG) at a weight ratio of 1:1 keeping at 130 °C for 180 min were approximately equal to those from the bagasse treated with [Cho][OAc] alone. The result indicated that the combination use of the cosolvents made it possible to reduce 50% of amount used of [Cho][OAc]. The enhancement effect of the [Cho][OAc]/cosolvent pretreatment on enzymatic saccharification could be attributed to an equal or greater effect on removal of hemicelluloes and lignin compared to the [Cho][OAc] treatment alone, although a lower effect on reduction in cellulose: which was supported by componential and X-ray diffraction (XRD) analyses. The bagasse pretreated with [Cho][OAc]/DMSO (1:1 w/w) used in simultaneous saccharification and fermentation using *Saccharomyces cerevisiae* BA11, and ethanol was produced with a corresponding to conversion ratio of 69.9%.

1. Introduction

Pretreatment is a requisite step for the production of biofuels or chemicals from lignocellulose through saccharification and fermentation (Mai et al., 2014). Lignocellulose is the complex association of cellulose-lignin-hemicelluloses; it is difficult to break down and offers limited accessibility to enzymes and microorganisms (Auxenfans et al., 2014; Perez-Pimienta et al., 2013). The aim of the pretreatment of lignocellulose is to increase the accessibility of cellulase to cellulose by removing lignin and hemicelluloses, increasing the surface area of cellulose, and decreasing the cellulose crystallinity (Uju et al., 2012; Yoshida et al., 2008; Silveira et al., 2015).

Ionic liquid (IL) pretreatment has received much attention since certain types of ILs were discovered to be able to dissolve cellulose (Swatloski et al., 2002). ILs are organic salts, usually composed of an organic cation and an inorganic anion, and existing in liquid form below 100 °C (Nasirpour et al., 2014; Cheng et al., 2014). ILs have unique properties such as high thermal stability, high ionic conductivity, miscibility, water stability, density, viscosity, polarity, non-flammable, and non-volatility (Vancov et al., 2012; Ninomiya et al., 2013b; Hallett and Welton, 2011). Besides, ILs dissolve polar and non-polar organic, inorganic, and polymeric compounds including cellulose, hemicellulose, and lignin, in addition, boiomass itself under mild conditions (Costa Lopes et al., 2013; Ninomiya et al., 2013b; Olivier-Bourbigou et al., 2010). Sun et al. (2009) reported that southern yellow pine could be almost completely dissolved in imidazolium-IL at 110 °C for 16 h. Pretreatment using ILs, which have these interesting characteristics, for lignocellulosic biomass has been widely studied, and the high effectiveness has been clarified. Silva et al. (2011) reported that almost 100% cellulose conversion of sugarcane bagasse after a 48-h saccharification time was achieved by the pretreatment using imidazolium-IL at 120 °C for 120 min. Moreover, Ujum Nakamoto et al. (2013) reported that the short time (10 min) pretreatment using pyridinium ILs caused 71% cellulose conversion of bagasse after a 24h-saccharification.

We used choline acetate ([Cho][OAc]), which is a completely bioderived IL, for pretreatment of lignocellulose. [Cho][OAc] has important advantages, such as being less expensive, more biodegradable, and more biocompatible compared to highly effective ILs such as imidazolium-IL (Ninomiya et al., 2015). In our previous study, we demonstrated that IL pretreatment using [Cho][OAc] at 130 °C for 180 min enabled 98.7% of the cellulose

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of pretreated bagasse to convert into glucose after 72 h of enzymatic saccharification (Asakawa et al., 2015). In addition, the energy profit ratio (Okajima and Sako, 2014; Khawkomol et al., 2013), which is defined as the energy production/energy requirement during pretreatment for ethanol production, was higher for this pretreatment than for other pretreatments, such as comminution, microwave irradiation, and alkaline treatment (Asakawa et al., 2015). These results indicate that IL pretreatment using [Cho][OAc] is energy-saving and cost-effective, and may be expected to overcome the economic drawbacks of the conventional pretreatment method. However, although [Cho][OAc] is relatively cheap among ILs, [Cho][OAc] is nevertheless expensive as a reagent for pretreatment, and a reduction of cost will be necessary for practical use. Furthermore, the ILs/biomass solution is highly viscous, which makes it difficult to handle and difficult to increase biomass loading (Mood et al., 2013; Fu and Mazza, 2011; Wang et al., 2015).

In order to overcome these problems, this work proposes pretreatment combining [Cho][OAc] with organic solvent. Pretreatments using various organic solvents have been reported, and they have the following advantages: (1) cellulose is recovered as solids with only minor degradation, (2) lignin and hemicelluloses dissolve into organic solvent, which increases the surface area of cellulose, (3) organic solvent can be easily recovered by distillation and recycled (Zhang et al., 2016).

To evaluate the efficiency of pretreatment combining [Cho][OAc] with organic solvent as cosolvent, hydrolyzabilities of the sugarcane bagasse pretreated with mixtures of [Cho][OAc] and several polar organic solvents were demonstrated. In addition, componential analysis of [Cho][OAc]/cosolvent-pretreated bagasse, X-ray diffraction (XRD) analysis of the pretreated microcrystalline cellulose, and ethanol fermentation of the pretreated bagasse were performed.

2. Materials and methods

2.1. Materials

The sugarcane bagasse was kindly provided by Kyuyo Sugar Industry (Okinawa, Japan). The raw bagasse was ground by a cutter mill (D3V-10; Osaka Chemical Co., Ltd., Osaka, Japan) for 1 min, and sieved to obtain 100–500 μm fractions, which were then used in all the experiments. The moisture content and the chemical composition of the raw bagasse were 3.6%, and 38.9% cellulose, 28.5% hemicelluloses, 2.5% acid soluble lignin (ASL), 26.4% acid insoluble lignin (AIL), and 3.7% others. Microcrystalline cellulose (Avicel PH-101) and [Cho][OAc] (melting point of 85 °C, ≧95.0) were obtained from Sigma-Aldrich Japan Co. LLC. (Osaka, Japan). *N*,*N*-Dimethylacetamide (DMA, >98.0%), *N*,*N*-dimethylformamide (DMF, >99.5%), dimethyl sulfoxide (DMSO, >99.0%), *N*-methyl-2-pyrrolidone (NMP, >97.0%), ethylene glycol (EG, >99.5%), and glycerin (G, 99.0%) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). The commercial enzyme mixture, Meicelase (derived from Trichoderma viride; 224 FPU/g: β-glucosidase activity, 264 IU/g) was provided by Meiji Seika Pharma Co. Ltd. (Tokyo, Japan). In the simultaneous saccharification and fermentation (SSF) experiments, *Saccharomyces cerevisiae* BA11 (Bio Acdemia CO., Ltd., Osaka, Japan) was used as an ethanol production strain.

2.2. Pretreatment combining [Cho][OAc] IL with cosolvent

1.5 g of the bagasse or Avicel was placed in a 300-ml eggplant flask with 4.5 g of [Cho][OAc]/cosolvent (1:1 w/w) and incubated in an oil bath at 130 °C for 180 min. The contents of the flask were mixed at 30 min after the beginning of heating and then every 60 min until the end of the heating period. Following incubation, 135 mL of distilled water was added, and the residue was separated from the mixture by filtration, which was then washed thoroughly four times with an equal volume of distilled water. The residue on the filter paper was collected and stored at 4 °C for subsequent enzymatic saccharification. For recovery measurement and compositional analysis, the pretreatment was conducted as described above, and the residue after washing was dried overnight at 105 °C ± 3 °C. The recovery ratio was calculated using the following equations:

$$\text{Recovery ratio}(\%) = \{\text{Residue after pretreatment (g)}/\text{Sample before pretreatment (g)}\} \times 100$$

2.3. Componential analysis

The ASL, AIL, cellulose, and hemicellulose contents of untreated and pretreated bagasse were determined as follows (Asada et al., 2015). 3 mL of 72% (w/w) H_2SO_4 was added to 0.2 g of the sample, and the solution was left at room temperature for 4 h. The mixture was then diluted to 4% (w/w) H_2SO_4 and autoclaved at 121 °C for 60 min. The ASL content was determined from the UV absorbance of this hydrolyzed solution at 205 nm using an absorption coefficient of 110 L g^{-1} cm^{-1} (TAPPI UM-250). The cellulose content was determined based on the monomer (glucose) content in the hydrolyzate. The glucose content was determined using HPLC equipment with a refractive index detector (RID-10A; Shimdzu Co., Ltd., Kyoto, Japan) with an Aminex column (HPX-87H; Bio-Rad, Richmond, U.S.A.) at 65 °C, with 5.0 mM H_2SO_4 as the mobile phase at 0.6 mL/min. The hemicellulose content was determined by subtracting the cellulose content from the holocellulose content. The holocellulose content was determined using the phenol–sulfuric acid method. The insoluble residue in the hydrolysate, i.e., AIL (high molecular weight lignin), was washed, dried to constant weight at 105 °C ± 3 °C, and weighed. All the analytical determinations were performed in triplicate, and the means were calculated.

2.4. X-ray diffraction (XRD) analysis

The XRD patterns of the treated Avicel were obtained using the X-ray diffractometer (Multiflex; RIGAKU, Yamanashi, Japan). The samples were scanned in the 2θ range of 5–45° with the step size of 0.2° at 40 kV and 40 mA at a temperature of 25 °C. The crystallinity index (CrI) was determined by multiple peak separation method. Crystalline peak (2θ = 22.1–22.5°) and amorphous peak (2θ = 20.2–21.0°) were separated using the software package JADE7 (MDI, Livermore, USA) and RaspWin (HT Soft Lab, Kuala Lumpur, Malaysia), and the CrI was calculated by the following equations:

$$\text{CrI}(\%) = (S_c/S_t) \times 100$$

where S_c and S_t were the crystalline area and the total area (the crystalline area + the amorphous area), respectively.

2.5. Enzymatic saccharification

Enzymatic saccharification of untreated and pretreated bagasse was performed in a 110-mL vial containing 0.2 g of the sample, 0.02 g of enzyme (Meicelase), and 10 mL of 100 mM sodium acetate buffer (pH 5.0). The reaction condition was at 50 °C on a rotary shaker at 140 rpm for 72 h. The glucose concentration was determined at specific time intervals using an enzymatic determination glucose assay kit (Autokit Glucose; Wako Pure Chemical Industries Ltd., Osaka, Japan). The saccharification ratio and glucose yield were calculated using the following equations: