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Comparison of choline acetate ionic liquid pretreatment with various pretreatments for enhancing the enzymatic saccharification of sugarcane bagasse

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ABSTRACT

In this study, ionic liquid (IL) pretreatment using choline acetate ([Cho][OAc]), which is a completely bioderived IL, for enhancing the enzymatic saccharification of lignocellulose was investigated. To evaluate [Cho][OAc] IL pretreatment, its effects on sugarcane bagasse composition, its subsequent enzymatic saccharification, and the energy profit ratio (EPR) were compared with those of various pretreatments, such as comminution, microwave irradiation, and alkaline treatment. After 72 h of enzymatic saccharification using bagasse pretreated with [Cho][OAc] at 110 °C for 360 min, 0.355 g of glucose per 1 g of raw bagasse was obtained (i.e., 98.7% of the cellulose content of the pretreated bagasse was converted into glucose), and maximum EPR was achieved in these pretreatment conditions.

1. Introduction

Lignocellulose is the most abundant organic material on earth, and it is generally unutilized, except as a feedstock in the production of useful materials, such as biofuels and bioplastics (Palonen et al., 2004; Qiu et al., 2012). Initially, cellulose needs to be converted into glucose during the production of these materials. However, this is difficult because the cellulose in the lignocellulose has a crystalline structure, which is covered by the robust and complex structure of lignin and hemicellulose (Uju et al., 2013). Numerous pretreatments have been used to increase the accessibility of cellulose for cellulase enzymes during enzymatic saccharification, i.e., by increasing the surface area of cellulose, decreasing the cellulose crystallinity, and removing lignin and/or hemicellulose (Yoon et al., 2012; Li et al., 2010b). Biological (Sasaki et al., 2011), physical (Zheng et al., 2014), chemical (Masarin et al., 2013), and physicochemical (Asada et al., 2011) pretreatments have been reported; however, they are all affected by problems, such as long residence time, high energy requirements, or high cost of the solvents used for processing and recycling (Ortiz and Oliveira Jr, 2014). Thus, the development of an innovative pretreatment method is necessary to improve the commercial viability of this process.

Studies of pretreatment using ionic liquids (IL) have continued to rise since midazolium ILs were discovered by Swatloski et al., 2002 to be able to dissolve cellulose. ILs are capable of dissolving hemicellulose, lignin, and cellulose in biomass, thereby allowing the removal of hemicellulose and lignin and reduction the crystallinity of cellulose (Uju et al., 2012; Li et al., 2010a). Thus, the accessibility of cellulase is greatly improved, which increases saccharification rate and the sugar yield (Zheng et al., 2014; Dadi et al., 2006). In addition, it is been suggested that ILs could be recycled and reused because they have low melting points, and they are also nonvolatile and thermally stable (Uju et al., 2013; Ohno and Fukuya, 2009).

In particular, imidazolium ILs have been widely studied, thereby demonstrating their powerful effects on enhanced enzymatic saccharification (Silva et al., 2011). However, imidazolium ILs are derived from petroleum; thus, they have disadvantages, such as low biodegradability, cytotoxicity, and high cost (Ninomiya et al., 2013c; Datta et al., 2010). In the present study, choline acetate ([Cho][OAc], Fig. 1) was focused on as an alternative to imidazolium ILs. [Cho][OAc] consists of cholinium cations and acetate anions, and the former are derived from choline chloride, which is part of the vitamin-B complex, the latter are derived from intercellular metabolites (Ninomiya et al., 2013a). That is, [Cho][OAc] is a completely bioderived IL, which is more biodegradable, bio-

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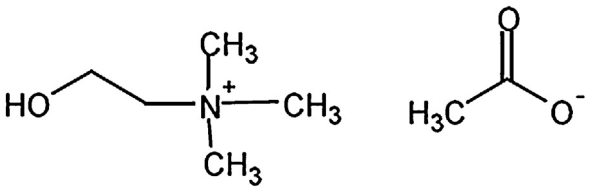

Fig. 1. Choline acetate.

compatible, and less expensive than imidazolium ILs (Ninomiya et al., 2015; Boething et al., 2007). Furthermore, Ninomiya et al. (2013c) reported that [Cho][OAc] pretreatment facilitated almost 100% cellulose conversion using kenaf powder after 48-h enzymatic saccharification, which is comparable with the results obtained with 1-ethyl-3-methylimidazolium acetate. However, few studies have assessed the use of [Cho][OAc] as a pretreatment for lignocellulose, which remains to be fully elucidated.

In the present study, the effects of IL pretreatment using [Cho][OAc] was compared with various methods, such as mechanical comminution, microwave irradiation, and alkaline treatment. To determine the most effective pretreatment method, component analysis of the pretreated lignocellulose, monitoring the subsequent enzymatic saccharification, and calculation of the energy profit ratio (EPR) with each different pretreatment were carried out. To calculate EPR, the theoretical ethanol yield was estimated based on the glucose yield obtained from enzymatic saccharification. Sugarcane bagasse was selected as the lignocellulosic material, which is an agricultural residue that is unutilized except for producing steam and electricity in sugarcane processing plants (Martín et al., 2002); thus, a more beneficial use of this substrate is desirable.

2. Materials and methods

2.1. Raw material

The sugarcane bagasse was kindly provided by Kyuyo Sugar Industry (Okinawa, Japan), and it was used as the lignocellulosic material. The raw bagasse was ground for 1 min and passed through a 500-μm sieve to obtain particles with a uniform size, which were then used in all the pretreatment tests, except the comminution pretreatment.

2.2.1. IL pretreatment using [Cho][OAc]

[Cho][OAc] with a melting point of 85°C was purchased from Sigma–Aldrich Japan Co., LLC. (Osaka, Japan). 1.5 g of the untreated bagasse was placed in a 300-ml eggplant flask with 1.5, 3.0, and 4.5 g of [Cho][OAc] and incubated in an oil bath at 90 °C, 110 °C, and 130 °C for 60, 180, and 360 min, respectively. Mixing was applied at 30 min after the beginning of heating and then every 60 min. Following incubation, 135 ml of distilled water was added, and the residue was separated from the mixture by filtration, which was then washed thoroughly with an equal volume of distilled water, four times. The residue on the filter paper was collected and stored at 4 °C for subsequent enzymatic saccharification. Before analyzing the composition and obtaining gravimetric measurements, a part of the residue on the filter paper was dried overnight at 105 °C ± 3 °C.

2.2.2. Specific heat capacity determination by differential scanning calorimetry

To calculate the energy requirement for pretreatment using [Cho][OAc], the specific heat capacity of [Cho][OAc] was determined by differential scanning calorimetry (DSC) (DSC6220; Hitachi High-Tech Science Corporation, Tokyo, Japan). The measurements were performed three times under identical conditions. The first measurement was performed with two empty crucibles, the second with the test sample ([Cho][OAc]) and the third with the reference substance (Al_2O_3). The resulting heat flux curves are the basis for determining the value of the specific heat capacity C_P, which was calculated from the following relationship (Przeliorz et al., 2014):

$$C_{p_s}(T) = \frac{HF_{sample} - HF_{blank}}{HF_{ref.} - HF_{blank}} \times \frac{m_{ref.}}{m_{sample}} \times C_{p_{ref.}}(T)$$

where C_{p_s} is the specific heat capacity of the test sample, J/g K; HF is the heat flux, respectively, for test sample (sample), empty crucibles (blank), and reference substance (ref.), μV; m is the mass of the sample and the reference substance, g; and $C_{p_{ref.}}$ is the heat capacity of the reference substance, J/g K.

The measurements were performed using 3.5 mg of the samples sealed in stainless steel crucibles and in the temperature range of 25–130 °C at a heating rate of 10 °C/min under N_2. As the heat capacity of the reference substance (Al_2O_3), the specific heat capacity of α-Al_2O_3 (JIS K 7123) was used.

2.3. Mechanical comminution

A SAMPLE MILL (a rod mill with two pots (250 ml) and a power consumption of 750 W; TI-300, CMT Co., Ltd., Fukushima, Japan) was used for the mechanical comminution pretreatment. Approximately 40 g (dry weight) of raw bagasse was placed in each pot, which was then ground for 10, 20, 30, or 60 min. The pretreated samples were stored at room temperature for enzymatic saccharification and dried overnight at 105 ± 3 °C to analyze their compositions.

2.4. Microwave irradiation

Microwave irradiation pretreatment was performed at 2.45 GHz using an Initiator+8 (maximum temperature of 200 °C; Biotage Japan Co., Ltd., Tokyo, Japan) with a 30-ml reaction vial. First, 0.9 g (dry weight) of untreated bagasse was suspended in 16.2 ml of distilled water and irradiated at 150 °C, 170 °C, or 200 °C for 1, 3, 5, or 10 min with stirring. Furthermore, 81 ml of distilled water was added, and the residue was separated from the mixture by filtration, before washing several times with 81 ml of distilled water. The residue on the filter paper was collected and stored at 4 °C for subsequent enzymatic saccharification. A part of the residue on the filter paper was dried overnight at 105 °C ± 3 °C to analyze the composition and to obtain gravimetric measurements.

2.5. Alkaline pretreatment

In this pretreatment, 1.5 g (dry weight) of untreated bagasse was suspended in 30 ml of sodium hydroxide (NaOH) at concentrations of 0.25%, 0.5%, 1.0%, or 3.0% (w/v), and the mixture was heated in an autoclave at 121 °C for 5, 15, 30, or 60 min. After cooling to room temperature, the mixture was neutralized with acetic acid and stirred by a magnetic stirrer for 1 h. The residue was separated by centrifugation and washed with distilled water, several times. The residue was collected and stored at 4 °C for subsequent enzymatic saccharification. A part of the residue on the filter paper was dried overnight at 105 °C ± 3 °C before analyzing the composition and obtaining gravimetric measurements.

2.6. Component analysis of untreated and pretreated bagasse

The acid-soluble lignin (ASL), acid-insoluble lignin (AIL), cellulose, and hemicellulose contents of untreated and pretreated bagasse were determined as follows.