



Efficient saccharification by pretreatment of bagasse pith with ionic liquid and acid solutions simultaneously



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ABSTRACT

Hydrolysis of hemicellulose and disruption of cellulose during pretreatment process are conducive to the following cellulase hydrolysis performance. In this work, bagasse pith was first pretreated by 1-butyl-3-methylimidazolium chloride ([BMIM]Cl) solution containing 0–1.2% hydrochloric acid (HCl) and 30% water. The water (30%) added into the acidic ionic liquid (IL) solutions led to an increase in the biomass loading up to a biomass/IL solutions ratio of 1:10 (wt.%). Hemicellulose was hydrolyzed to reducing sugars by HCl and cellulose was dissolved by [BMIM]Cl. In this process, 76.9% of hemicellulose conversion and 95% of cellulose recovery were obtained. The pretreated bagasse pith was then followed by hydrolysis with commercially available enzymes. The effects of pretreatment temperature, reaction time and acid concentration on cellulase hydrolysis of pretreated bagasse pith were investigated. Pretreatment of bagasse pith with [BMIM]Cl solutions containing 1.0% HCl at 120 °C for 30 min resulted in the glucose concentration of 92.3 g/l and yield of 94.5% after 72 h of cellulase hydrolysis. The maximum total reducing sugars yield reached to 89.9% after pretreatment and cellulase hydrolysis.

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1. Introduction

Lignocellulosic biomass are renewable and abundant energy resource in nature, which have complex structures and composition of three main components: cellulose, hemicellulose and lignin [1,2]. Cellulose is a linear polymer of glucose containing amorphous and crystalline structures [3,4]. The cellulose chain is made up of glucose units joined together by β -1,4 glycosidic bonds. Individual cellulose chains are held together by strong hydrogen bonds that make cellulose a highly crystalline polymer [5]. Hemicellulose is an amorphous chain of polysaccharides containing sugar residues and it protects the cellulose fibrils from enzymatic deconstruction [6]. Cellulose and hemicelluloses can be hydrolyzed to producing sugar. Lignin without sugar-based structure is an amorphous polymer made up of aromatic derivatives [7]. The complex structure of lignocellulosic biomass makes it difficult to be hydrolyzed and thus leads to low sugar yield in cellulase hydrolysis [8,9].

Pretreatment is an essential step to increase the sugar yield from cellulase hydrolysis of pretreated biomass as it may disrupt the crystalline structure of cellulose, removes hemicellulose or breaks the carbohydrate-lignin complex [10–12]. Numerous

pretreatment methods have been developed, including biological, physical, physicochemical, and chemical processes [13–15]. Various pretreatment methods have their advantages as well as drawbacks. In the past few years, many ionic liquids (ILs) have received much attention because of the ability to dissolve cellulose under moderate conditions [16–18]. ILs exhibit special properties such as high thermal stability [19], nearly complete non-volatility and recyclability [20]. Compared to other pretreatment methods, the digestibilities of biomass can be enhanced effectively and a high glucose yield can be achieved after biomass pretreatment with ILs. 1-ethyl-3-methylimidazolium acetate ([EMIM]Ac) was widely studied by researchers. Qiu et al. [21] reported that a glucose yield of beyond 90% could be obtained after pretreatment of energy cane bagasse with [EMIM]Ac. Ana da Silva et al. [17] reported that [EMIM]Ac was selected among six ILs as it significantly enhanced bagasse enzymatic saccharification rate and yield. Auxenfans et al. [22] found that the enzymatic saccharification of [EMIM]Ac-pretreated sawdust can be significantly increased. Pretreatment of agave bagasse using [EMIM]Ac was very effective in enhancement of enzyme kinetics according to the work of Perez-Pimienta et al. [23]. Furthermore, other various ILs have been used in different types of biomass pretreatment presented different effects on dissolution of lignocellulose. Sun et al. [24] investigated the structural comparison and enhanced enzymatic hydrolysis of

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eucalyptus cellulose via pretreatment with different ILs. They found that crystallinity index (CrI) of cellulose via [AMIM]Cl pretreatment was significantly decreased from 70.2% to 31.2%, which enabled the cellulase enzymes easier access to cellulose. Spronsen et al. [25] reported that [BMIM]Cl was more efficient in the dissolution of straw than [EMIM]Ac at the same pretreatment conditions. The current state-of-the-art on the fractionation of lignocellulosic biomass in ILs was reviewed [26] and concluded that ILs had potential to be used in the biomass pretreatment.

In previous studies, although ILs have been extensively studied by virtue of its particular advantages, there are some drawbacks restrict their application in biomass pretreatment. Some researchers had to use a low biomass/ILs ratio of 1:20 (wt.%) due to the high viscosities of ILs [27,28]. ILs are so expensive that limit their extensive use for pretreatment of lignocellulose. Cruz et al. [29] studied the impact of high biomass loading of switchgrass on IL pretreatment in order to improve the biomass loading. Weerachanchai et al. [16] used [EMIM]Ac to pretreat cassava pulp residue and obtained a high sugar conversion of more than 90% but it required a long pretreatment time of 24 h. Researchers reported that [BMIM]Cl has the ability to dissolve cellulose and it is relatively cheap because of its simple synthesis compared to [EMIM]Ac [30].

Despite ILs have the ability to dissolve cellulose, the dissolution of hemicellulose in ILs is rather difficult and it still remains in the biomass during the pretreatment process [31,32]. The presence of hemicellulose in biomass will reduce the efficiency of the following cellulase hydrolysis. Moreover, hemicellulose can be hydrolyzed by acid. Therefore, a combined biomass pretreatment method using IL and acid was developed. The pretreatment method was divided into two steps: biomass was first pretreated by acid for a period of time to remove hemicellulose and then the residue was treated by IL. Uju et al. [33] found that first pretreatment of pine biomass with peracetic acid followed by [EMIM]Ac pretreatment resulted in 70% cellulose conversion in 1 h. Work of Auxenfans et al. [4] showed that combination pretreatment of dilute acid and IL led to an efficient lignocellulosic biomass deconstruction. Jiang et al. [28] used sulfuric acid to remove hemicellulose and the remained solid residue after acid-pretreatment was further pretreated by [AMIM]Cl to decrease the crystallinity of biomass. The above pretreatment process was not simultaneous and it may lead to the complexity of the approach. Qing et al. [34] investigated pretreatment of biomass with different types of acids and ILs simultaneously and concluded that HCl and [MMIM]DMP were the most effective combination to improve cellulase hydrolysis conversion. Zhang et al. [35] studied pretreatment of sugarcane bagasse with acid and IL solution in order to improve glucan digestibility of cellulase hydrolysis. They also investigated the pretreatment of fresh biomass with recycled acidified IL solution on influence of enzymatic hydrolysis.

Hemicellulose is a polysaccharide fraction representing, in general, 15–35% of lignocellulosic biomass which can be hydrolyzed to producing sugars. Hydrolysis of hemicellulose is an important step to increase the total sugars yield. Nevertheless, it was neglected in most ILs pretreatment processes. The focus of this work is to develop an effective pretreatment method which can make full use of biomass to maximize sugars yield. In this process, the bagasse pith was pretreated by HCl and [BMIM]Cl solutions simultaneously. Hemicellulose was hydrolyzed to reducing sugars by HCl and cellulose was dissolved in [BMIM]Cl. The disrupted cellulose recovered is conducive to the following cellulase hydrolysis. A certain amount of water (30%) was added into the IL and acid solutions to decrease the viscosities of IL. Thus a high bagasse pith/IL ratio of 1:10 (wt.%) was used to reduce the IL solvent costs and allow for increased biomass loading.

2. Methods

2.1. Raw materials

The bagasse pith (waste of sugar manufacturing) was obtained from Guangxi province, China. The air-dried bagasse pith was milled and sieved to obtain 40–80 mesh fractions. The collected bagasse pith was stored at room temperature in a sealed container. The components of bagasse pith were measured according to the National Renewable Energy Laboratory (NREL) procedure [36]. Its main composition and content were shown in Table 1.

2.2. Chemicals and enzyme

1-Butyl-3-methylimidazolium chloride (99%) used in the experiment was purchased from Chengjie Chemical Co. Ltd., Shanghai. Hydrochloric acid (36%) was purchased from Lingfeng Chemical Reagent Co. Ltd., Shanghai. Cellulase from *Trichoderma reesei* was purchased from Sigma–Aldrich Co. Ltd., Shanghai. Anhydrous sodium acetate (99%) and acetic acid needed to prepare the buffer solution were purchased from Xinran Industry Co. Ltd., Shanghai. Phenol, sodium potassium tartrate, sodium hydroxide and 3,5-dinitrosalicylic acid for DNS analysis were purchased from Sinopharm Chemical Reagent Co., Ltd, Shanghai.

2.3. Bagasse pith pretreatment

A 2.0 g of bagasse pith and 20.0 g of [BMIM]Cl solutions containing the required amounts of HCl and 30% water were added into a 250 ml flask. The flask was sealed with a lid to prevent water loss and immersed in an oil bath. The mixture of bagasse pith and [BMIM]Cl solutions were stirred with a magnetic stirrer and the reaction temperature (80–130 °C) was controlled by using an oil bath. Pretreatment was carried out with an agitation speed set at 400–500 rpm for 10–90 min. The pretreated biomass was recovered from the ionic liquid solutions by adding deionized water to precipitate its pith. First, 20 ml deionized water was added into the reaction mixture. The precipitated bagasse pith was filtered and then washed with the second 50 ml deionized water to remove excess ionic liquid. The regenerated bagasse pith was then dried prior to cellulase hydrolysis. All experiments of bagasse pith were conducted in duplicate. The concentration of reducing sugars obtained from the first washed pretreatment hydrolysate was measured by DNS (3,5-dinitrosalicylic acid) assay [37]. The dried components of regenerated residue after pretreatment were analyzed according to the NREL. The overall experimental design of this study is summarized in Fig. 1.

2.4. Cellulase hydrolysis

The pretreated bagasse pith was hydrolyzed with a loading of 20 FPU/g substrate. Acetate buffer solution of pH 4.8 was used to buffer the mixture of bagasse pith and cellulase enzymes. The cellulase hydrolysis was conducted at 50 °C for 72 h [38]. Samples with a volume of 0.2 ml were taken at 24, 36, 48, 60, 72 h to determine the reducing sugar by high performance liquid chromatography (HPLC) equipped with evaporative light scattering detector PL-ELS2100 (Polymer laboratories, British) using ZORBAX-NH2 column (150 mm × 4.6 mm, 5 μm, Agilent, USA) operated at 40 °C. All experiments were performed in duplicate.

2.5. Scanning electron microscopy (SEM) and X-ray powder diffraction (XRD)

The morphology of bagasse pith before and after pretreatment was examined with scanning electron microscope

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