

# The study of factors affecting the enzymatic hydrolysis of cellulose after ionic liquid pretreatment

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## ABSTRACT

Cellulose resource has got much attention as a promising replacement of fossil fuel. The hydrolysis of cellulose is the key step to chemical product and liquid transportation fuel. In this paper a series of chloride, acetate, and formate based ionic liquids were used as solvents to dissolve cellulose. The cellulose regenerated from ILs was characterized by FTIR and X-ray powder diffraction. From the characterization and analysis, it was found that the original close and compact structure has changed a lot. After enzymatic hydrolysis, different kinds of ionic liquids (ILs) have different yields of the reducing sugar (TRS). They are 100%, 90.72%, and 88.92% from 1-butyl-3-methylimidazolium chloride ([BMIM]Cl), 1-ethyl-3-methylimidazolium acetate ([EMIM][OAc]), 1-butyl-3-methylimidazolium formate ([BMIM][HCOO]) respectively after enzymatic hydrolysis at 50 °C for 5 h. The results indicated that the yields and the hydrolysis rates were improved apparently after ILs pretreatment comparing with the untreated substrates.

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## 1. Introduction

Cellulose is a class of biomass which widely exists in the world. Cellulose materials can be converted to a range of important industrial chemicals and can serve as a source of carbon based feedstock for ethanol fuel (Samayam & Schall, 2010). Generally, cellulose materials include three components: cellulose, hemicellulose and lignin. Cellulose is the key component of energy crops, agricultural residues and woods (Datta et al., 2010). It is a highly crystalline polymer of D-anhydroglucopyranose units joined together in long chains by  $\beta$ -1,4-glycosidic bonds (Miller, 1959; Swatloski, Spear, Holbrey, & Rogers, 2002; Zavrel, Bross, Funke, Büchs, & Spiess, 2009). It is extremely difficult to dissolve cellulose in water and most common organic solvents as the result of the tight hydrogen-bonding network (Hermanutz, Gähr, Uerdingen, Meister, & Kosan, 2008; Xu, Wang, & Wang, 2010) and van der Waals interactions (Atalla & VanderHart, 1984; Datta et al., 2010). That leads to notorious resistance of cellulose to hydrolysis finally.

In order to solve the problem, many methods, including biological, physical, chemical and physicochemical processes have been studied. But few of them appeared efficient. For example, the cellulose after ball-milling treatment is still an insoluble material with  $\beta$ -1,4-glycosidic bonds but has large parts of noncrystalline regions which can be confirmed by XRD and CP/MAS  $^{13}\text{C}$  NMR (Zhao et al.,

2005). The drawbacks of these methods have been noticed and categorized (Zhao et al., 2009).

Therefore, an effective pretreatment method seems necessary. In fact, the final purpose of pretreatment is to make the cellulose susceptible to hydrolysis. The appearance of the ionic liquids supplies a promising pretreatment routine. Ionic liquids (ILs) are a type of environmental friendly molten salts, most of which have the virtues of excellent solvency, low melting point, nonvolatility and designability. The physical and chemical properties of ILs can be adjusted to different reactions by choosing different cations, anions and substituents (Swatloski et al., 2002; Xu et al., 2010). Due to these unique qualities, ILs are considered as a kind of promising alternatives to traditional solvents. In 2002, Rogers and coworkers firstly reported [C<sub>4</sub>mim]Cl could dissolve cellulose efficiently (Swatloski et al., 2002). Subsequently, more ILs were discovered to possess the superiority in dissolving cellulose materials. From these studies, it was discovered that the polymerization and crystallinity degree of the treated cellulose declined seriously by FTIR analysis (Liu & Chen, 2006) and X-ray powder diffraction analysis (Dadi, Varanasi, & Schall, 2006). Further more, ILs pretreatment could avoid the problems of excessive residence time, abundant energy consuming and equipment corruption. So, cellulose pretreatment with ionic liquid has become the hotspot in recent years. Dadi et al. pretreated the cellulose with [C<sub>4</sub>mim]Cl and the initial enzymatic hydrolysis rate was approximately 50-fold higher for regenerated cellulose compared to untreated cellulose (Dadi et al., 2006). Ramakrishnan hydrolyzed the cellulose regenerated from N-methyl morpholine oxide to get 60% TRS conversion rate which

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was three times as much as the untreated cellulose after enzymatic hydrolysis for 5 h (Ramakrishnan, Collier, Oyetunji, Stutts, & Burnett, 2010). Liu got 70.37% and almost 100% yield of TRS respectively from the wheat straw and steam-exploded wheat straw both of which were regenerated from [BMIM]Cl (Liu & Chen, 2006). Zhao et al. also reported that the D-glucose yield from switchgrass by ILs pretreatment could reach 96% after enzymatic hydrolysis for 24 h (Zhao, Baker, & Cowins, 2010). These results indicate that the ILs are favorable in enzymatic hydrolysis process. But why the ILs can be used to pretreat the cellulose well? Which factors affect the yield of glucose and the hydrolysis rate? Some problems still need further research. This study aims to investigate the impact of ILs on enzymatic hydrolysis and some other influencing factors. It will be helpful for choosing the proper solvent and the optimal conditions for enzymatic hydrolysis process.

## 2. Experimental

### 2.1. Materials

N-methylimidazole (99.0%) was purchased from Yancheng Kalechem Co., Ltd (Jiangsu, China). Amberlyst A-26 (OH) ion exchange resin was supplied from J&K chemical (Beijing, China). Cellulase from *Trichoderma reesei* (cellulase activity was 7.5 FPU/mg) was purchased from Imperial Jade Bio-Technology Co., Ltd (Ningxia, China). Cellulose microcrystalline (average particle size 50  $\mu\text{m}$ ) was purchased from Acros (NJ, USA).

### 2.2. Ionic liquid preparation

[BMIM]Cl and [AMIM]Cl were prepared according to the methods described in previous literatures (Huddleston, Willauer, Swatloski, Visser, & Rogers, 1998; Zhang, Wu, Zhang, & He, 2005). Carboxylic based ionic liquid was prepared by the ion exchange resin (Amberlyst-26 OH form) following these steps: Firstly, the ion exchange resin was loaded with the desired anion on the resin. A 10 wt% formic acid solution was flushed through a column which contained 100 g resin. About 300 ml solution was acquired to achieve complete substitution of the OH ions. Then the column was flushed with an aqueous solution of the chloride or bromine salt of desired cations which resulted in rapid displacement of the target anions. After the reaction completed, the aqueous solution containing the formic based ionic liquid was concentrated and dried. Residual chloride or bromide ions were determined by an ion selective electrode until the ions content in the formic based ionic liquid was less than 20 ppm. [Bmim]Cl (5.0 g) was dissolved in 50 ml distilled water and added to a column loaded with formic anions. The eluted was concentrated and dried under vacuum at 90 °C over 24 h. The same procedure can be carried out to get [EMIM][HCOO], [BMIM][OAc] and [EMIM][OAc].

### 2.3. Buffer and DNS solution preparation

The citrate buffer (pH 4.8, 0.05 mol/l) was prepared by mixing citric acid (conc. 4.01 g/l) and sodium citrate (conc. 8.56 g/l) in distilled water. The DNS solution was prepared according to an IUPAC method: 1416 ml distilled water, 10.6 g 3,5-dinitrosalicylic acid, 19.8 g sodium hydroxide, 306 g potassium sodium tartrate, 7.6 ml phenol melted at 50 °C, and 8.3 g sodium metabisulfite.

### 2.4. Cellulose dissolution and regeneration

Cellulose (1.0 g) was added into a certain amount of ILs and stirred for 20 min at a controlled temperature until they formed a transparent solution. The cellulose was regenerated by adding anti-solvent into the solution with vigorous stirring for 30 min.

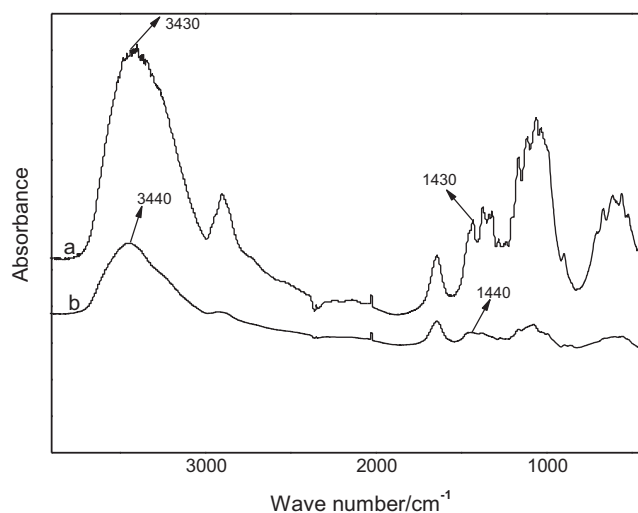


Fig. 1. The FTIR spectrum of the regenerated cellulose from [BMIM]Cl and the original cellulose. a, Original cellulose; b, regenerated cellulose.

The regenerated cellulose was collected by a vacuum filtration and washed with anti-solvent. In view of previous study, the residual ILs would affect the yield of the reducing sugar to different degree (Zhao et al., 2009). Therefore, the regenerated cellulose needed to be washed thoroughly.

### 2.5. Enzymatic hydrolysis of cellulose

A suspension solution of 1.0 g cellulose and 0.015 g cellulase were added into 150 ml critic buffer. The enzymatic hydrolysis was carried out in a water bath. At a time interval, the reaction solution 1.0 ml was withdrawn to a test tube and 3.0 ml DNS solution was added. Then the test tube was sealed and placed in the boiling water for 15 min. The test tube was cooled to room temperature in an ice bath. The reducing sugar (TRS) yield was determined by DNS method using glucose as a standard (Miller, 1959). The enzymatic hydrolysis rate was calculated as follows:

$$\text{Enzymatic hydrolysis rate (\%)} = \frac{\text{reduced sugar} \times 0.9}{\text{sample weight}} \times 100$$

## 3. Results and discussion

### 3.1. Characterization of regenerated cellulose

The FTIR spectrum of the cellulose regenerated from [BMIM]Cl and the original cellulose is shown in Fig. 1. In curve a, the peak at 1430  $\text{cm}^{-1}$  can be assigned to bending vibration of  $\text{CH}_2$ . This band is strong in crystalline cellulose, and weak in amorphous cellulose (Nelson & O'Connor, 1964). So, the curve b reveals obviously that the crystallinity of the regenerated cellulose is much lower than the original cellulose. The broad absorption in the range from 3000  $\text{cm}^{-1}$  to 3500  $\text{cm}^{-1}$  is due to stretching vibration of intermolecular O–H forming among cellulose molecules. Comparing curve b to a, the broad absorption gets much weaker, which may suggest the intermolecular hydrogen bonds are broken greatly. Hermetically sealed construction of cellulose tends to amorphism which can supply higher accessible surface areas and more binding sites for enzyme than the untreated cellulose. The declined crystallinity and the incompact structure of regenerated cellulose could be the important reason of the increase of the hydrolysis rate.

The X-ray diffraction patterns of the cellulose films are shown in Fig. 2. The original cellulose is cellulose I as indicated by the

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