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Full Length Research Paper

Enhancement of the enzymatic hydrolysis of wheat straw by pretreatment with 1-allyl-3-methylimidazolium chloride ([Amim]Cl)

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The enhancement of the enzymatic hydrolysis of lignocellulose by pretreatment is a key difficulty in biomass utilization. Wheat straw was treated with 1-allyl-3-methylimidazolium chloride ([Amim]Cl), and the morphology, enzymatic hydrolysis, recovery and the content of the composition of the treated wheat straw were investigated. Significant morphological change was observed when the wheat straw was heated at 150°C. When the temperature increased from 125 to 150°C, the glucose yield after enzymatic hydrolysis increased from 0.13 to 0.23 g/g (within 2 h). When the wheat straw was treated at 150°C for 6 h, the recovery of solid was only 23.9%, and the contents of cellulose, hemicellulose and lignin were 46.7, 10.8 and 24.2% respectively. It was concluded that heating with [Amim]Cl is an efficient pretreatment method; too intense treatment condition would induce degradation of wheat straw and the suitable condition of pretreatment is 150°C and 2 h.

Key word: Ionic liquid, [Amim]Cl, wheat straw, pretreatment, cellulose, cellulase, enzymatic hydrolysis.

INTRODUCTION

Lignocellulose is the most abundant renewable biomass on earth. The utilization of lignocellulosic feedstocks such as forest and agricultural residues in environmental friendly way has been a highly active research area. For example, wheat straw has been seen as an important feedstock of cellulosic ethanol and other potential products (Chen and Qiu, 2010; Li and Chen, 2007; Taherzadeh and Karimi, 2008; Howard et al., 2003).

Since the digestibility of native cellulose is hindered by some structural and compositional factors, pretreatment is necessary to achieve enzymatic degradation in production of ethanol and some other products with lingo-cellulose (Alvira et al., 2010).Many methods, including milling, steam explosion, irradiation and treatment with acid, have been introduced for pretreatment of lignocellulosic materials (Taherzadeh and Karimi, 2008). Ionic liquids (IL) are compounds composed entirely of ions, which are liquids at room temperature. Because of the negligible vapor pressure and high thermal stability (Li et al., 2010; Pârvulescu and Hardacre, 2007), it can be used as the reaction media for many processes (Castner et al., 2010; Dreyer and Kragl, 2008; Ma et al., 2010).

Recently, some scientists and engineers have found that some ILs are valued solvent of cellulose, and they have attempted to treat the lignocellulose material with these liquids (Tadesse and Luque, 2011). For example, Liu and Chen (2006) found that the hydrolysis rate of wheat treated with [Bmim]Cl and microwave could reach 70.37%; Rogers et al. (2007) reported that not only cellulose, but also, wood can be dissolved in some ILs. Fort et al. (2007), Swatloski et al. (2002) and Dadi et al. (2006) reported that the initial enzymatic hydrolysis rates

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were approximately 50-fold higher for regenerated cellulose (from [C_4mim]Cl) as compared to untreated cellulose. Li et al. (2009) reported that the yield of reducing sugars from wheat straw pretreated with 1-ethyl-3-methyl imidazolium diethyl phosphate ([Emim]DEP) reached 54.8% after being enzymatically hydrolyzed. 1-Allyl-3-methylimidazolium chloride ([Amim]Cl) is IL designed and synthesized by Ren et al. (2003) and Zhang et al. (2005). They found that the [Amim]Cl is a good solvent for cellulose (Zhang et al., 2005). When cellulose is dissolved and regenerated from IL solution, the native structure would breakdown (Li et al., 2009), and [Amim]Cl might be used to treat the wheat straw and other lignocellulosic materials to enhance enzymatic hydrolysis.

In this study, wheat straw was heated in [Amim]Cl. The morphology, enzymatic hydrolysis, recovery and the content of the composition of the treated wheat straw were investigated.

MATERIALS AND METHODS

Wheat straw was harvested from a local farmer in the suburb of Xinle, Hebei Province, China. The straw was comminuted to about 40 meshes with a plant disintegrator and extracted with ethanol/benzene (1/2, v/v) for 8 h in a Soxhlet apparatus. The extracted wheat straw flour was dried for 4 h at 105°C immediately before use.

[Amim]Cl was synthesized using the method described by Ren et al. (2003) and Zhang et al. (2005). 1-Methylimidazole and allyl chloride (molar ratio 1:1.25) were added to a round-bottomed flask, heated at 55°C, and stirred for 8 h. The product was vacuum distilled to remove un-reacted chemicals reagent and water.

Penicillium decumbens cellulase was provided by the Ningxia Cellulase Preparation Plant. Filter paper activity (FPA) was determined to be 110.0 IU/ml (Miller, 1959).

Treatment with [Amim]Cl

Ten grams of [Amim]Cl and 0.5 g of wheat straw was heated in a round-bottomed flask at 100, 125 and 150°C and gently stirred with a magnetic stirrer. Then, 20 ml deionized water was added. The mixture was filtrated by millipore filtration (0.22 μm), and the obtained solid was washed with deionized water for three times.

Enzymatic hydrolysis

The obtained solid after treatment with [Amim]Cl was dried at 105°C for 4 h. The dried solid was mixed with a sodium acetate buffer of pH 4.8 (20 ml buffer per g solid) and the *P. decumbens* cellulase (30 IU per g solid). Then, the mixture was incubated in a rotary shaker at 50°C and 170 rpm for 48 h. After the enzymatic hydrolysis, the glucose and xylose concentrations were assayed by high performance liquid chromatography (HPLC).

Compositional analysis

Cellulose and hemicellulose of all samples were determined based on the standard NREL procedure No. 002 (NREL, 1996; Zhang et al., 2010). The sample was treated with 72% (v/v) sulphuric acid at 30°C for 1 h, followed by 4% dilute acid (w/w) at 121°C for 45 min. The glucose and xylose in the hydrolysate were determined by HPLC (Agilent, US) equipped with a refractive index detector (RID agilent, US) and an Aminex HPX-87H column (Bio-Rad). 5 mmol/L H_2SO_4 was used as the mobile phase. The flow rate of the mobile phase and the column temperature were maintained at 0.6 ml/min and 65°C. The cellulose and hemicellulose contents were calculated from glucose and xylose contents multiplied by conversion factors of 0.90 and 0.88, respectively (Zhang et al., 2010). The residual solid of hydrolysis was filtrate with a sand core filter (G 3) washed three times with deionized water. The washed solid was dried at 105°C for 4 h and then, burned at 575°C for 4 h with a muffle furnace. The content of lignin was calculated from the difference of the weight between before and after burning.

RESULTS AND DISCUSSION

Morphology of the wheat straw treated with [Amim]Cl

The wheat straw was heated in [Amim]Cl at 100 and 150°C. The morphology was observed with a micro-scope. Figure 1 shows that when the treatment time was 2 h at 100°C (Figure 1b), no significant change was found; when the heating time was 6 h (Figure 1c), the outline of the particles became blurred. At 150°C, the morphological change of the straw particles was more obvious after heating. When the treatment time was only 1 h (Figure 1d), the straw became curved, and when the heating time was extended to 6 h (Figure 1f), most of the particles disappeared.

[Amim]Cl, a good solvent of cellulose, was designed by Zhang et al. (2005). They found that 14.5 weight % cellulose (dissolved pulp) or 8.0 wt % cotton linter can be dissolved in [Amim]Cl). When compared with the pure cellulose, it was difficult for the native lignocellulose to be dissolved by [Amim]Cl. However, Figure 1 shows that the straw can not be dissolved within 6 h, though the temperature was higher than 100°C. The reason might be that compact structure of wheat straw hinders the penetration of the IL.

The figures suggested that temperature is a critical factor of the pre-treatment with [Amim]Cl. High tempe-rature (≥150°C) induces significant changes in the morphology of wheat straw.

Influence of treatment temperature on sugar yield of the enzymatic hydrolysis of wheat straw

Wheat straw was treated with [Amim]Cl at 100, 125 and 150°C, and then regenerated with water. The recovered solid was hydrolysed with cellulase. The glucose and xylose yields were calculated and are shown in Figure 2.

Figure 2 shows that the glucose yield of recovered solid increased with the treatment time at any temperature, and the yield on 150°C was much higher than those on

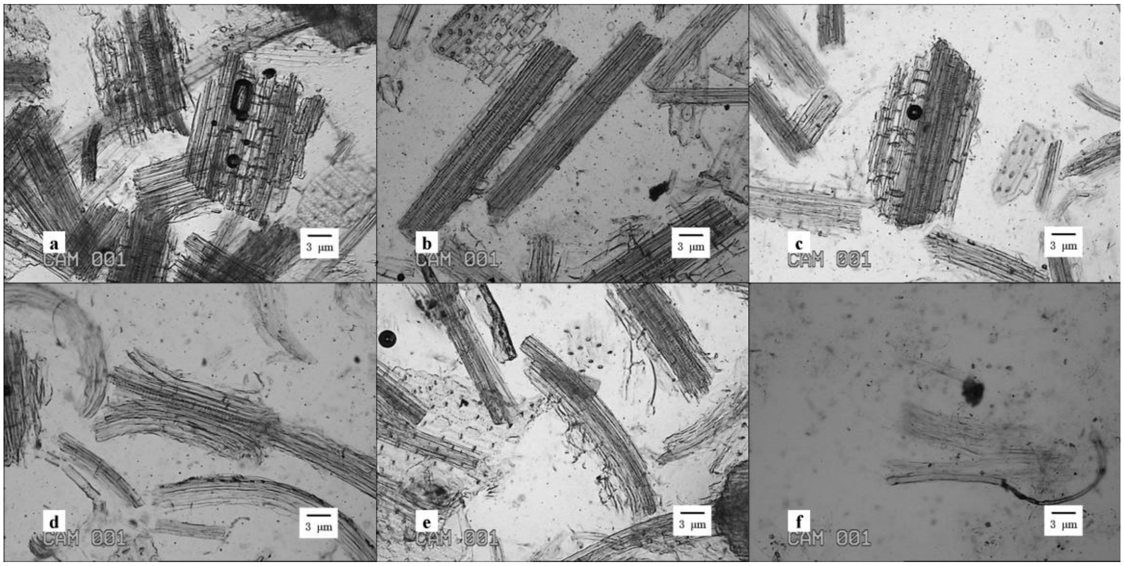


Figure 1. Morphological changes of wheat straw during the treatment with [Amim]Cl. a, 100°C for 1 h; b, 100°C for 2 h; c, 100°C for 6 h; d, 150°C for 1 h; e, 150°C for 2 h; f, 150°C for 6 h.

100 and 125°C (Figure 2a). The xylose yield showed similar trend (Figure 2b).

Li et al. (2009) investigated the pretreatment of wheat straw with [Emim]DEP. They found that when the temperature increased from 70 to 100°C, the hydrolytic effect increased considerably. In our study, the substantial increase stopped between 125 and 150°C. The difference might lie in the different solubility of the compounds of wheat straw in [Emim]DEP and [Amim]Cl. Since the content of cellulose in the treated wheat straw (heated for 2 h) was 38% (Figure 4), the maximum hydrolysis rate was 55.2%. Based on our findings, the effect was similar to the treatment of steam explosion at 1.5 MPa for about 10 min.

The yields of glucose and xylose in Figure 2 suggest that when [Amim]Cl was used to treat wheat straw, high temperature (≥150°C) was more effective than low temperature.

Influence of treatment temperature on recovery rate of wheat straw

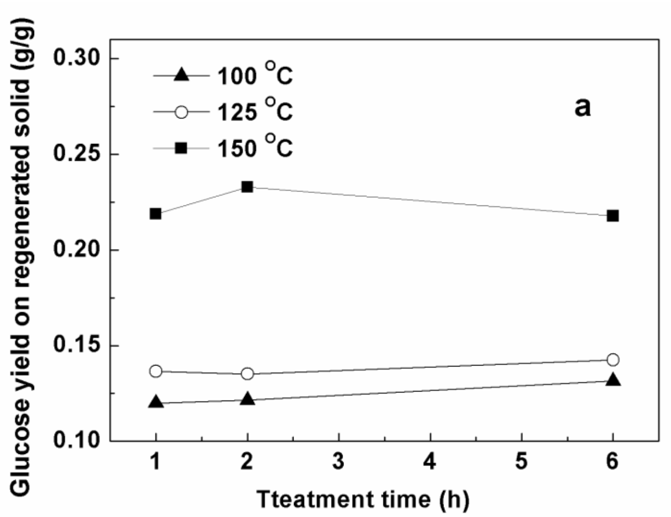
When wheat straw was heated in [Amim]Cl, high temperature resulted in not only the dissolution of cellulose but also the degradation of some compounds (Zhang et al., 2005). To study the influence of treatment temperature on the degradation of wheat straw, we heated the wheat straw at 100, 120 and 150°C in [Amim]Cl, and then regenerated it with deionized water. The recovery was calculated and is shown in Figure 3.

It is found that when the treatment temperature was lower than 125°C, there was only little difference between before and after 6 h treatment, while when the temperature was increased to 150°C, the recovery decreased to 23.9%. The data of the recovery in Figure 3 suggested that too intense condition would induce the degradation of wheat straw, though the treatment with [Amim]Cl can enhance enzymatic hydrolysis. The appropriate condition was at 150°C for 2 h.

Since high temperature induced the decrease of the recovery of the wheat straw, it composition which is easy to be degraded should be investigated. In this study, wheat straw was treated with [Amim]Cl at 150°C. The contents of the compositions of the recovered solid were analysed.

Figure 4 shows that when the wheat straw was treated with [Amim]Cl for 2 h at 150°C, all the content of the three compounds (cellulose, hemicellulose and lignin) increased slightly. The untreated wheat straw contained 35.1% cellulose, 22.7% hemicellulose and 16.1% lignin, while when the wheat straw was heated for 2 h, the contents of cellulose, hemicellulose and lignin were 38.4, 24.2 and 18.3%, respectively. Figure 3 shows that the straw was not significantly degraded when the straw was heated for 2 h, the reason for the increase in the contents of the three compounds might be that some other compounds such as pectin were degraded.

When the wheat straw was treated for 6 h, the contents of cellulose, hemicellulose and lignin were 46.7, 10.8 and 24.2%. Because the recovery was only 23.9% when wheat straw was treated at 150°C for 6 h, all the three components in wheat straw were partly degraded in the condition. Since only the content of hemicellulose reduced, it was the most easily degradable component. The data in Figure 4 indicate that too intense condition



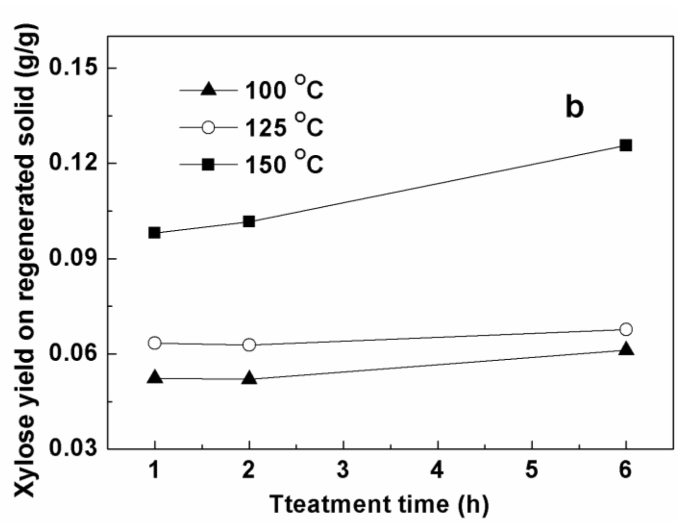


Figure 2. Influence of treatment temperature on the enzymatic hydrolysis of straw. a, Glucose yield; b, xylose yield.

would result in the degradation of the compounds of straw (cellulose, hemicellulose and lignin), though enzymatic hydrolysis can be enhanced by treatment with [Amim]Cl, and the most easily degradable compound was the hemicellulose.

Conclusions

Wheat straw was heated in [Amim]Cl to enhance the enzymatic hydrolysis of cellulose. The morphology, enzymatic hydrolysis rate, the recovery and the content of the composition of the recovered solid were investigated. Significant morphological change was observed when the wheat straw was heated at 150°C. When the temperature increased from 125 to 150°C, the glucose yield of enzymatic hydrolysis was increased from 0.13 to 0.23 g/g. When the wheat straw was treated at 150°C for 6 h, the recovery of solid was only 23.9% because all the three main components of wheat straw (cellulose, hemicellulose and lignin) were partly degraded. It was concluded that treatment with [Amim]Cl is an efficient pretreatment method to enhance the enzymatic hydrolysis of wheat straw; too intense treatment condition would induce degradation of wheat straw and the most easily degradable component is hemicellulose. Heating at 150°C for 2 h is a suitable condition for the pretreatment.

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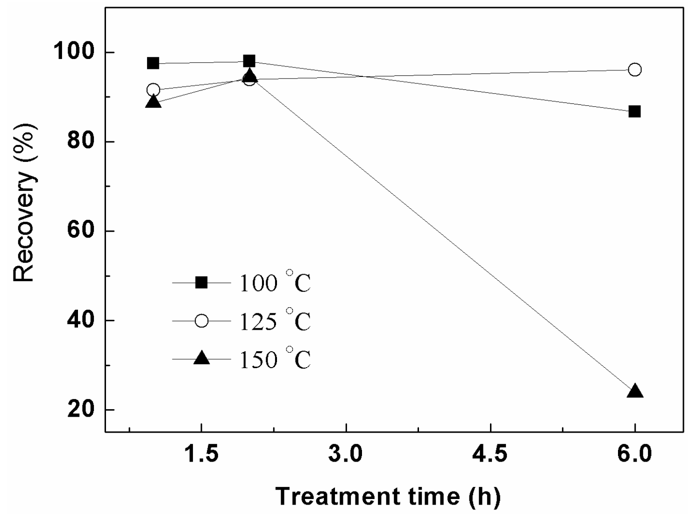

Figure 3. Recovery of wheat straw after treatment with [Amim]Cl and regeneration with water.

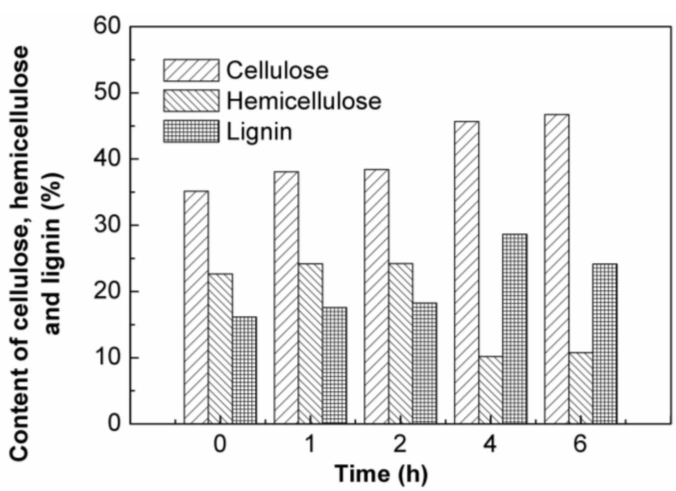

Figure 4. Content of cellulose, hemicellulose and lignin in wheat straw treated with [Amim]Cl at 150°C.

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