SIMULTANEOUS SACCHARIFICATION AND FERMENTATION OF CELLULOSE TO ETHANOL BY IONIC LIQUID PRETREATMENT

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The production of ethanol by simultaneous saccharification and fermentation (SSF) of cellulose, pretreated with an ionic liquid (IL), 1-n-butyl-methylimidazolium acetate ([C_4mim][CH_3COO]), was studied in the present work. The experimental results showed that the degree of polymerization and crystallization of cellulose decreased by ionic liquid pretreatment. Under the pretreatment temperature of 90 °C, pretreatment time of 2 h and ethanol as anti-solvent, the SSF rate of the regenerated cellulose increased by 180%, as compared to that of untreated cellulose. Therefore, ionic liquid pretreatment would be a promising raw material pretreatment technology for the cellulosic ethanol production.

Keywords: cellulose, ionic liquid, fermentation, ethanol

INTRODUCTION

The development of lignocellulose as a raw material to produce bioethanol is one of the main targets for the national energy technology of China. It is known that ethanol fermentation by starches is a mature technology; however, cellulosic biomass is more difficult and more expensive to convert into ethanol than starches based on current technologies. The high degree of polymerization and crystallinity of lignocelluloses are the main reasons. Owing to these structural characteristics, the pretreatment is an essential step for the fermentation of lignocellulose. The traditional pretreatment technologies include physical methods (mechanical crushing and radiation, etc.), chemical methods (acid, alkali and organic solvents, etc.), physicochemical methods (steam explosion and supercritical fluid), the biological method (white-rot bacteria, etc.) and others.[1] However, these pretreatment methods have several drawbacks, for example, special equipment is needed for the extreme operating conditions and many byproducts are produced causing environmental problems.[2]

In 2002, Swatloski and co-workers[3] found for the first time that ionic liquid 1-n-butyl-3-methyl-imidazolium chloride ([C_4mim]Cl) can dissolve cellulose with high solubility. Since then, the use of ionic liquid pretreatment of cellulose has become a hot topic for the related research and development. Some powerful ionic liquids, such as 1-allyl-3-methylimidazolium chloride,[4] 1,3-dialkylimidazolium formates,[5] 1-ethyl-3-methylimidazolium acetate,[6] 1-ethyl-3-methyl imidazolium diethyl phosphate[7] etc., have been reported. Dadi *et al.*[8] discovered that after pretreatment by [C_4mim]Cl, the saccharification rate of the regenerated cellulose significantly increased. Xie *et al.*[9] and Bodirlau *et al.*[10] got the same results by using different ionic liquids. At present, the research regarding the fermentation of cellulose pre-treated by ionic liquid is in its infant stage. Therefore, in the present work, 1-n-butyl-3-methylimidazolium acetate ionic liquid [C_4mim][CH_3COO] was synthesized, which has a lower viscosity and higher capacity to dissolve cellulose compared with [C_4mim]Cl,[11] and then was used to study the effect of cellulose pretreatment on ethanol fermentation.

EXPERIMENTAL

Materials

Microcrystalline cellulose was purchased from Shanghai Hengchang Reagent Factory. Cellulase (1200 FPA/g) was supplied by Beijing Shuangxuan Microbe Culture Medium Products Factory. Commercial ethanol instant active dry yeast (*Saccharomyces cerevisiae*) was obtained from Angel Yeast Co., Ltd. (Wuhan, China). Medium composition: yeast extract 3 gL^{-1}, KH_2PO_4 5 gL^{-1}, $(NH_4)_2SO_4$ 2 gL^{-1}, $MgSO_4$ 0.2 gL^{-1} and $CaCl_2$ 0.1 gL^{-1}; the reagents used were of analytical grade.

Experimental methods

Preparation of [C_4mim][CH_3COO]

[C_4mim][CH_3COO] was prepared from [C_4mim]Cl (Henan Lihua Pharmaceutical Co., Ltd.) using anion exchange resin, and the procedure described in literature[12] was closely followed.

Ionic liquid pretreatment of cellulose

Microcrystalline cellulose (5%, w/w) was added into [C_4mim][CH_3COO] and processed at a constant temperature (80, 90, or 100 °C) for a constant time (1, 2, or 3 h). After that, the precipitant (such as water, ethanol, etc.) was added to the mixture, and centrifugal separation was carried out after as the cellulose was precipitated. The regenerated cellulose was used for fermentation experiments after being washed repeatedly by water and then dried under reduced pressure. The ionic liquid was recovered by drying in vacuum at 80 °C and then was recycled in the next dissolution processes.

Simultaneous saccharification and fermentation of cellulose

Culture medium, water and a certain amount of cellulose were mixed, the pH was adjusted to 4.6, and the mixture was cooled to room temperature after being sterilized at high temperature for 20 min. The cellulase (33.3 FPA/g cellulose) and dry yeast (2 gL^{-1}) were added into the medium. Simultaneous saccharification and fermentation (SSF) was carried out at 37 °C, and timed sampling was performed to determine the concentration of glucose and ethanol in the mash.

Detection methods

Concentrations of glucose were determined by the DNS method[13] and those of ethanol by gas chromatography.

RESULTS AND DISCUSSION

Changes in cellulose structure by ionic liquid pretreatment

The dissolution process of cellulose was observed through the polarizing microscope, and pictures were taken by a connected digital camera. It can be seen from

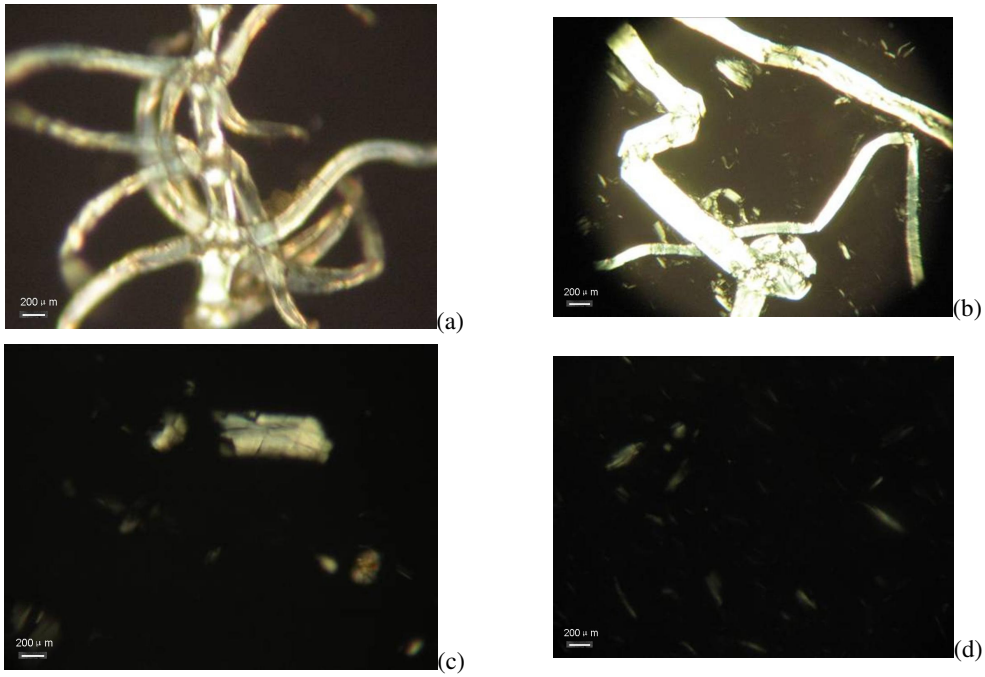

Figure 1: Polarization microscope images of cellulose dissolved in [C_4mim][CH_3COO] at different time periods; (a) 15 min, (b) 30 min, (c) 45 min, (d) 60 min

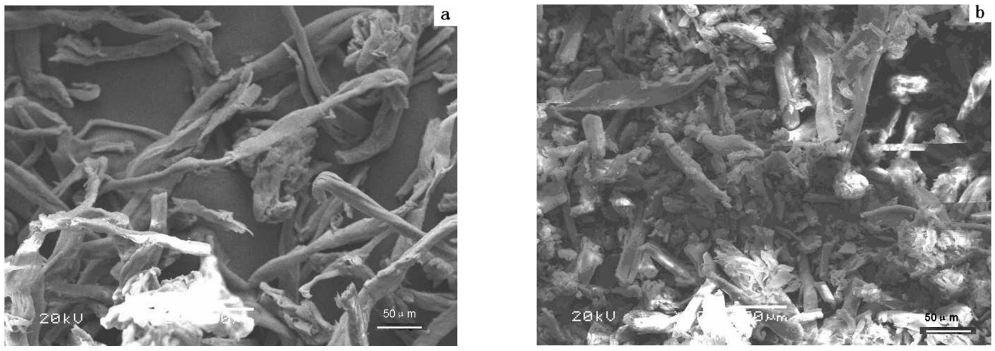

Figure 2: SEM images of original and regenerated cellulose (×300); (a) original cellulose; (b) regenerated cellulose

Figure 1 that as time proceeded, the chain-like structure of cellulose was slowly opened and its crystalline structure was broken. After about 1 h, the cellulose was completely dissolved in $[C_4mim][CH_3COO]$ and the polarizing microscope showed a black field of view.

Figure 2 shows scanning electron micrographs of the original cellulose and regenerated cellulose. As evident from this figure, the long chain of the regenerated cellulose was significantly shortened and the particle surface became rough after the ionic liquid pretreatment. During the process of dissolution and regeneration in the ionic liquid, the degree of polymerization and the crystallinity of cellulose were reduced, which are favorable for the following saccharification and fermentation.[14]

Optimization of cellulose concentration for enzymatic hydrolysis

Substrate concentration is an important parameter in the enzymolysis and fermentation process. High concentrations of substrate can conserve water, get higher ethanol concentrations in the mash and save energy consumption of distillation. Figure 3 compares the enzymolysis rate of cellulose under different substrate concentrations over time. It can be seen that with the extension of the enzymolysis time, the enzymolysis rate gradually slowed down and finally glucose concentration tended to a constant, and the enzymolysis rate eventually decreased with increasing substrate concentration due to the influence of product inhibition. In the SSF process, the glucose obtained by enzymolysis can be used by the yeast as soon as it has been generated, which permits to get rid of the effects of product inhibition. Figure 3 also shows that the final glucose concentration along with the initial substrate concentration first increased and then had a decreasing trend, and reached a maximum when the substrate concentration was 250 gL^{-1}. However, the high substrate concentration would result in a high viscosity that may limit the action of the enzyme and cause its inactivation. Thus, in the following SSF experiments, the initial substrate concentration of 250 gL^{-1} was used.

Simultaneous saccharification and fermentation of cellulose by ionic liquid pre-treatment

Effect of pretreatment temperature

The changes in the concentrations of ethanol and residual glucose in the SSF process of the regenerated cellulose at different pretreatment temperatures are shown in Figures 4 and 5, respectively. The results indicate that the SSF rate and the overall ethanol yield of cellulose pretreated by the ionic liquid $[C_4min][CH_3COO]$ were all significantly higher than that of untreated cellulose. Relative to the pretreatment of cellulose at 80 °C and 100 °C, the best results of SSF of regenerated cellulose were obtained at 90 °C, and the efficiency of the saccharification and the concentration of ethanol were highest at this temperature. After 36 h fermentation of cellulose pretreated at 90 °C, the concentration of ethanol was as high as 98.3 gL^{-1}, reaching the level of 84 h fermentation of the untreated cellulose, and the fermentation rate increased by 1.3 times. The residual glucose concentrations in the SSF process were all at extremely low levels and the concentration of residual glucose in the fermentation process was even lower after the pretreatment by ionic liquids.

Effect of pretreatment time

The changes in the concentrations of ethanol and residual glucose in the SSF process of the

regenerated cellulose at different pretreatment times (pretreatment temperature of 90 °C) are shown in Figures 6 and 7, respectively. It can be seen that as the fermentation time proceeded, the ethanol concentration gradually increased, and the corresponding residual glucose concentration decreased. The SSF rate and the overall ethanol yield of cellulose pretreated by $[C_4min][CH_3COO]$ were all significantly higher than that of the untreated cellulose. After 2 h pretreatment, finally, the ethanol concentration was the highest and the residual glucose concentration was the lowest in the fermentation mash.

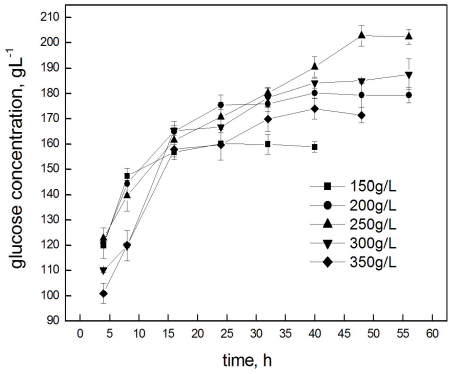

Figure 3: Effect of cellulose concentrations on saccharification

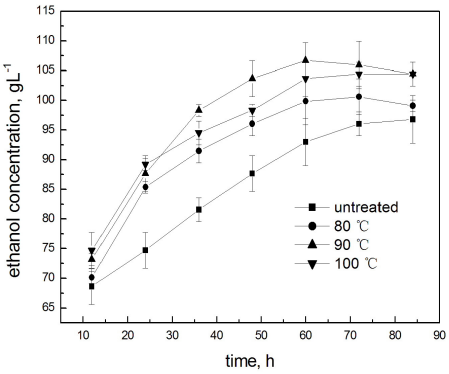

Figure 4: Time courses of ethanol concentration in SSF of cellulose with different pretreatment temperature

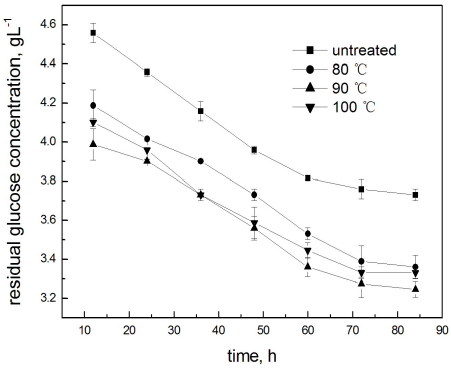

Figure 5: Time courses of residual glucose concentration in SSF of cellulose with different pretreatment temperature

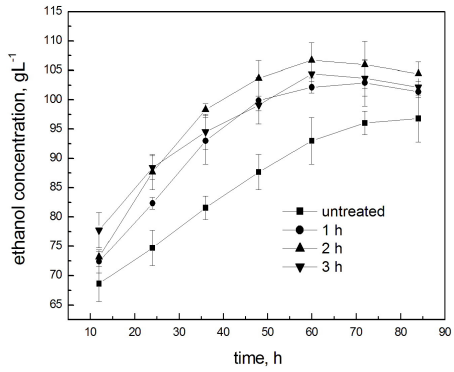

Figure 6: Time courses of ethanol concentration in SSF of cellulose with different pretreatment time

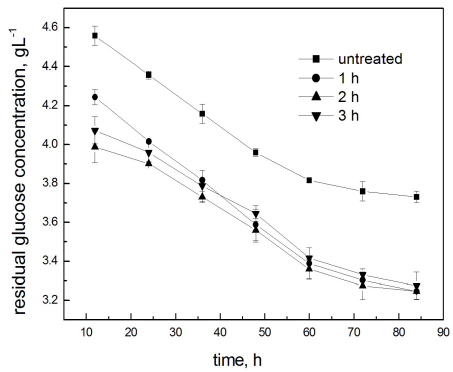

Figure 7: Time courses of residual glucose concentration in SSF of cellulose with different pretreatment time

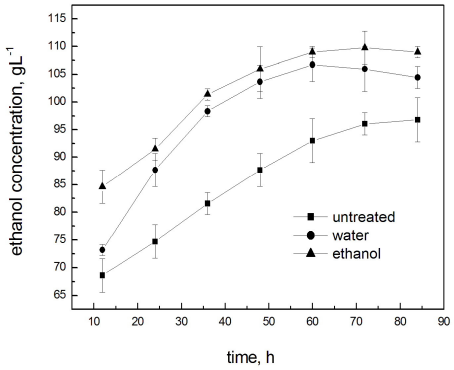

Figure 8: Time courses of ethanol concentration in SSF of cellulose with different anti-solvent

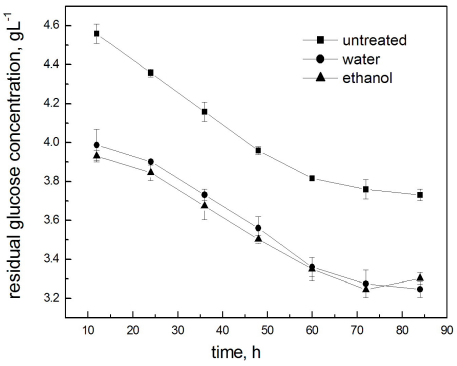

Figure 9: Time courses of residual glucose concentration in SSF of cellulose with different anti-solvent

Effect of precipitants

The effects of different precipitants on the concentrations of ethanol and residual sugar in the SSF process of the regenerated cellulose are illustrated in Figures 8 and 9, respectively. It may be noted that the SSF of cellulose was significantly improved after ionic liquid pretreatment. Compared to water as precipitant, the SSF efficiency of the regenerated cellulose was higher when ethanol was used as the precipitant. After the 2 h pretreatment at 90 °C, the ethanol concentration produced from 30 h SSF of the regenerated cellulose was equal to that of the 84 h fermentation of the untreated cellulose with ethanol as precipitant. The average fermentation rate was 2.8 times that of the untreated cellulose.

CONCLUSION

This study demonstrated a significant decrease in the degree of crystallinity and polymerization of cellulose after ionic liquid pretreatment. Under the pretreatment temperature of 90 °C, pretreatment time of 2 h and ethanol as anti-solvent, the ethanol production rate from SSF of the regenerated cellulose increased by 180%, as compared to that of untreated cellulose. Therefore, it may be concluded that the ionic liquid pretreatment is a promising raw material pretreatment technology for the cellulosic ethanol production.

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