



## Effect of Steam Explosion and Ionic Liquid Pretreatment Technology on the Enzymatic Hydrolysis of Corn Stalk

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The enzymatic hydrolysis of corn stalk could be increased *via* the methods of steam explosion and ionic liquid (IL)[Bmim]Cl as a pretreatment step. The corn stalk was enzymatic hydrolyzed for 24 h, the experimental results revealed that the yield of reducing sugars from corn stalk treated with a steam pressure of 2.6 MPa for 90 sec and combined with [Bmim]Cl treatment can reach 961.66 mg/g (dry sample). Steam explosion can also enhance the content of cellulose in the corn stalk and make the ionic liquid treatment efficient. The corn stalk treated was characterized by FTIR, XRD and SEM and the results showed that the crystallinity of corn stalk was changed from crystalline to amorphous pattern.

**Key Words:** Corn stalk, Steam explosion, Ionic liquid, Enzymatic hydrolysis.

### INTRODUCTION

Lignocellulosic materials are abundant on earth, 10-50 billion tons was almost produced every year<sup>1</sup>. With the depletion of world's fossil fuel resources and the aggravation of environment, the conversion of energy production from the lignocelluloses has driven considerable attention<sup>2,3</sup>. Moreover, the energy production from renewable lignocelluloses through burning will not make the CO<sub>2</sub> exhausted redundantly to the atmosphere, because these CO<sub>2</sub> can be recycled into new plants through photosynthetic fixation<sup>4</sup>. So lignocelluloses have been considered as potential sustainable feedstocks for energy production and using this energy production is beneficial for the world environment<sup>5,6</sup>. Enzymatic hydrolysis was regarded as the most promising technology to break down lignocelluloses and make it to fermentable reducing sugars. But the enzymatic hydrolysis is difficult to lignocelluloses because it's the high order structure and crystallinity. For improving the hydrolysis efficiency, pretreatment considered as the most effective and versatile method to reduce the cellulose crystallinity and increase the lignocelluloses porosity<sup>7</sup>.

The pretreatment treatments were mainly physical and chemical method or incorporating both methods were also effects<sup>8</sup>. Physical technologies include mechanical milling, steam explosion and hydrothermolysis and chemical techno-

logies were mainly acid and alkali process, such as H<sub>2</sub>SO<sub>4</sub> and NaOH, respectively<sup>9</sup>. Among several pretreatment processes, steam explosion displayed many attractive features, such as lower environmental contamination, lower cost, less hazardous process, *etc.* So steam explosion had more potential for energy efficiency and more complete recovery of lignocelluloses<sup>10</sup>. One of the most driven highlight of steam explosion about the structure and behaviour of lignocellulose was the extensive solubility in neutral or alkali solvents<sup>11</sup>. In recent years, a new treatment by non-derivatizing media for lignocellulose, such as ionic liquids (ILs) could increase yield of enzymatic hydrolysis. Ionic liquids could dissolve and regenerate lignocellulose, such as bagasse, corn stalks, wheat and wood and cellulose regenerated were found essentially amorphous and porous<sup>12-14</sup>. The features of ionic liquids process were easy to operate, less energy demanding and more environmentally friendly. As a novel technology, ionic liquids process had been gained more and more attention in past, but this process has been inefficient for application to lignocelluloses pretreatment mainly due to its compact crystal structure. For enhancing the process effect, an advanced technology is urgent.

In this paper, a method combined steam explosion and ionic liquid [Bmim]Cl was designed for the pretreatment of corn stalk. Steam explosion was employed to remove the hemicellulose and lignin from corn stalk but not cellulose<sup>15</sup>. Mean-

while, the structure of corn stalk was destroyed by steam explosion progress which can make the [Bmim]Cl treatment more efficient. So our combined method has been based on the structure damage by the steam explosion to make the [Bmim]Cl solubilization efficient and corn stalk treatment by [Bmim]Cl to enhance enzymatic hydrolysis.

## EXPERIMENTAL

The corn stalks used in this experiment was collected from farm in the suburb of Chong qing, China. The corn stalks were air-dried at room temperature and milled to a particle size to pass through a 60-mesh screen using a laboratory hammer mill. Ionic liquid 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) were purchased from Lanzhou Institute of Chemical Physics, Chinese Academy of Science, China. Cellulase was obtained from Ningxia Imperial Jade Bio-Technology Co. Ltd., China. All chemicals used in the experiment, unless otherwise noted, were reagent grade and obtained from the commercial source.

**Steam explosion and fractions process:** Steam explosion pretreatment was carried out in a batch pilot unit equipped with a 400 mL reaction vessel and maximum 4.0 MPa pressure. The corn stalk (80 g) was steam exploded for a predetermined pressure in the range of 1.4-3.0 MPa and a predetermined time in the range of 30-180 s. Then the samples were washed with 4 × 500 mL deionized hot water. The samples were dried at 105 °C and then were refluxed for 1 h at 160 °C in 70 % (v/v) ethanol medium<sup>16</sup>. The samples were washed with 4 × 500 mL deionized water and then dried at 105 °C for 12 h.

**Ionic liquid [Bmim]Cl treatment:** The mixture of 1 g corn stalk sample after steam explosion and fractions process and 19 g [Bmim]Cl was stirring for 4 h at 120 °C under vacuum condition. Then the alcohol (60 mL) was rapidly added to the mixture to regenerate the corn stalk under vigorously stirring condition. The regenerated corn stalk was filtered and washed with 4 × 250 mL deionized water and dried at room temperature under vacuum condition.

**Enzymatic hydrolysis of [Bmim]Cl treated corn stalk:** 0.1 g corn stalk treated by [Bmim]Cl and 2.44 mL sodium citrate buffer (pH 4.8) were added in the 5 mL tube, then cellulase concentration of 60 FPU/g substrate was added in the mixture. Enzymatic hydrolysis of corn stalk was performed at 50 °C for 24 h in a rotary shaker. According to the formula (1), the efficiency of enzymatic hydrolysis could be calculated.

$$\text{Reducing sugar yield} = \frac{\text{Reducing sugar weight}}{[\text{BMIM}]\text{Cl treated corn stalk weight}} \quad (1)$$

Reducing sugars weight was calculated based on DNS method<sup>17</sup>.

**Detection method:** Cellulase activity was determined by the standard filter paper assay and expressed as filter paper units per gram of glucan (FPU)<sup>18</sup>. The compositions of the corn stalks were measured according to the method of Van Soest<sup>19</sup>. The FTIR spectra of samples were recorded on Perkin-Elmer GX spectrometer (Perk-Elimer, USA) in KBr discus. XRD patterns of samples were collected using high-resolution XRD-6000 diffractometer (Shimadzu, Japan) with CuK<sub>α</sub> as radiation source ( $\lambda = 0.15418$  nm) at 40 kV and 30 mA over

the interval 5°- 40°/2θ. The surface morphology of samples was examined by a TESCAN VEGA2 (Tescan, Czech) SEM. The samples was fixed on a brass stub using double-sided tape and then gold coated in a vacuum by a SC7620 sputter coater (Emitch, England). Then the SEM pictures were taken at an excitation voltage of 10 kV.

## RESULTS AND DISCUSSION

**Optimization of steam explosion conditions:** The steam explosion pressure was varied from 1.4 to 3.0 MPa. After steam explosion and fractions process, the corn stalks were treated by [Bmim]Cl then hydrolyzed in a batch system. As seen in Fig. 1, the reducing sugar from the [Bmim]Cl treated corn stalks appeared to be significantly affected by this variation in steam pressure from 2.2 to 2.6 MPa and largely unaffected by this variation in steam pressure from 1.4 to 2.2 MPa, although yield of reducing sugars appeared to be slightly lower for the 3.0 MPa sample. The optimum pretreatment temperature was selected at 2.6 MPa.

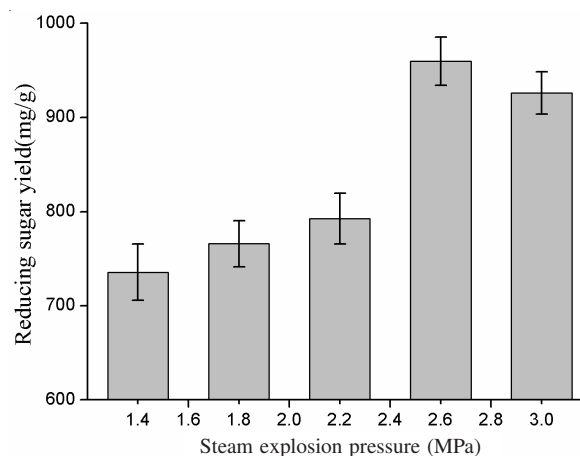


Fig. 1. Effect of steam explosion pressure

The steam explosion time was varied from 30 s to 180 s. The corn stalk samples treated by [Bmim]Cl were also hydrolyzed in a batch system after steam explosion and fractions process. As can be seen in Fig. 2, corn stalk steam exploded for 90 s, the yield of reducing sugars can reach 961.66 mg/g. The steam explosion time appeared to have little effect on the yield of reducing sugars from 120 s to 180 s. So the optimum steam explosion time was 90 s.

Based on the above experimental results, the optimum steam explosion conditions were chosen as follows; the corn stalk samples were steam exploded with a steam pressure of 2.6 MPa for 90 s. After fractions process, corn stalk samples were treated by [Bmim]Cl subsequently hydrolyzed for 24 h, the reducing sugars yield could reach 961.66 mg/g, but only 248.60 mg/g was produced from the untreated corn stalk.

**FTIR spectra and chemical composition analysis:** According to the method of Van Soest, the compositions of untreated corn stalk and the samples treated by steam explosion and fractions process (2.6 Mpa, 90 sec) were showed in the Table-1. The content of cellulose in the corn stalk increased significantly, it can revealed that more substrate was provided for enzymatic hydrolysis. The comparison of FTIR spectras

of untreated (c), treated by steam explosion and fractions process (2.6 Mpa, 90 sec); (b) and [Bmim]Cl treated; (a) corn stalk are shown in Fig. 3. The absorption band at  $897\text{ cm}^{-1}$  assigned as C-O-C stretching at the  $\beta$ -(1-4)-glycosidic linkage is weak and broad in cellulose I, strong and sharp amorphous. The absorption band at  $1430\text{ cm}^{-1}$  assigned to the  $\text{CH}_2$  scissoring motion is strong in cellulose I, very weak in the amorphous cellulose. Hence, the region in the  $1500\text{-}700\text{ cm}^{-1}$  of FTIR spectra was employed to characterize the structure of corn stalk<sup>20,21</sup>. The stretching vibration intensity at  $897\text{ cm}^{-1}$  in (c) was weaker than stretching vibration intensities of (a) and (b). And the absorption intensity at  $1430\text{ cm}^{-1}$  decreased from (c) to (a). So the spectra shown in Fig. 3 indicate that the structure of untreated corn stalk was transformed from the cellulose I into amorphous after being treated by [Bmim]Cl.

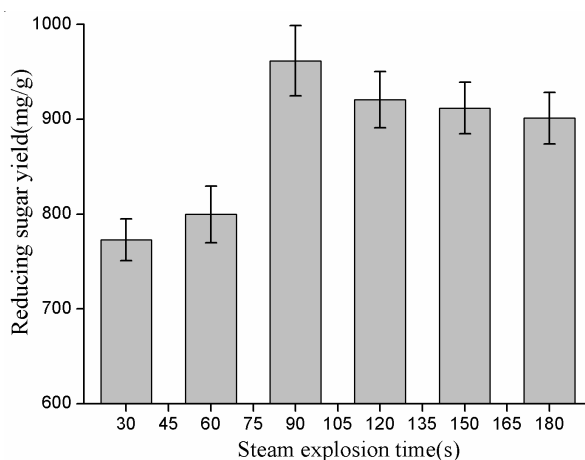


Fig. 2. Effect of steam explosion time

TABLE-1  
COMPOSITION OF UNTREATED CORN STALK AND  
THE SAMPLE TREATED BY STEAM EXPLOSION  
AND FRACTIONS PROCESS (2.6 Mpa, 90 SEC)

	Cellulose (g)	Hemi-cellulose (g)	Lignin (g)	Others (g)
Untreated corn stalk 1.000 g	$0.308 \pm 0.002$	$0.315 \pm 0.003$	$0.077 \pm 0.001$	$0.298 \pm 0.002$
Steam explosion treated (2.6 Mpa, 90 sec) and fractions process 1.000 g	$0.507 \pm 0.003$	$0.255 \pm 0.001$	$0.078 \pm 0.002$	$0.160 \pm 0.003$

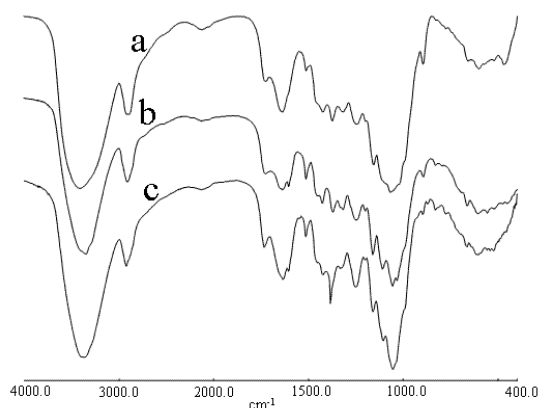


Fig. 3. FTIR spectra of [Bmim]Cl treated (a) steam explosion treated and fractions process (2.6 Mpa, 90 sec) (b) untreated (c) corn stalk

**Crystallinity analysis:** XRD has been extensively used for the investigation of crystallinity of cellulose fibers<sup>10</sup>. The XRD spectra of samples treated by steam explosion and fractions process (2.6 Mpa, 90 sec) (a), untreated (b) and [Bmim]Cl treated (c) were shown in Fig. 4. The (a) and (b) have typical cellulose I diffraction angles around  $14.9^\circ$  and  $22.6^\circ$ . For the corn stalk treated by [Bmim]Cl, diffraction angles around  $14.9^\circ$  and  $22.6^\circ$  disappeared, a quite flat diffraction pattern was obtained, this indicates an amorphous structure<sup>22</sup>. During the steam explosion and fractional process, the hemicellulose and other materials in the corn stalk were removed, the relative content of cellulose increased. So the crystalline polymorphism was not destroyed. But crystalline structure of corn stalk was transformed into amorphous after treatment by [Bmim]Cl. Probably, during the regeneration process, the rapid precipitation with alcohol prevented the dissolved corn stalk from restructuring into its original crystalline structure<sup>23,24</sup>. As consequence, the regenerated corn stalk with amorphous structure could provide more surfaces for enzymes to attack on. So it could conclude that the intact structure was disrupted by [Bmim]Cl treatment and resulted in a transformation from the cellulose I into amorphous.

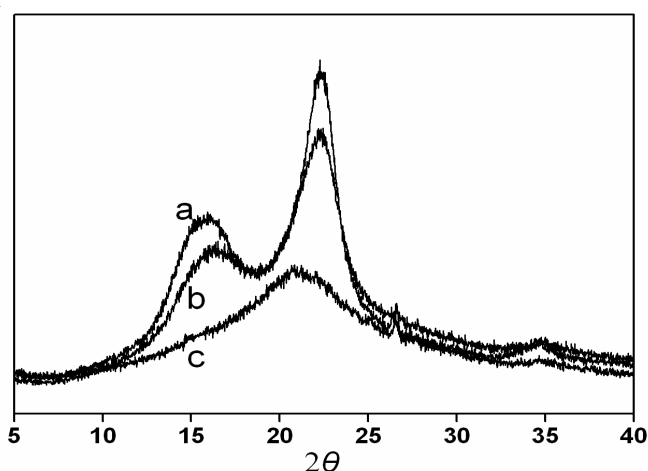


Fig. 4. X-ray diffraction spectra of samples treated by steam explosion and fractions process (2.6 Mpa, 90 sec) (a), untreated (b) and [Bmim]Cl treated (c)

**Morphological analysis:** The SEM micrographs of corn stalk untreated (a), samples treated by fractions process (b) and [Bmim]Cl treated (c) are shown in Fig. 5. From the (a) in Fig. 5, the SEM micrograph displayed an orderly and compact structure and the surface of original sample is flat and smooth. After the steam explosion and fractions process, we could see a lost structure of (b), which could be consistent with the removal of hemicelluloses and lignin. It is also clear that the samples have a rough surface and a long and linear shape. It indicated that the (b) still have crystalline structure and this rough surface can make the [Bmim]Cl treatment more efficient. After being treated by the [Bmim]Cl, the SEM micrograph of (c) showed holes on the surface of the corn stalk. More accessible external and internal surface area of corn stalk was attainable, providing more surfaces for enzymes to attack on. This structure dramatically improved the reducing sugar yield of enzymatic hydrolysis. It is also exhibited that

[Bmim]Cl treatment destroyed the corn stalk crystalline structure and changed it into amorphous pattern.

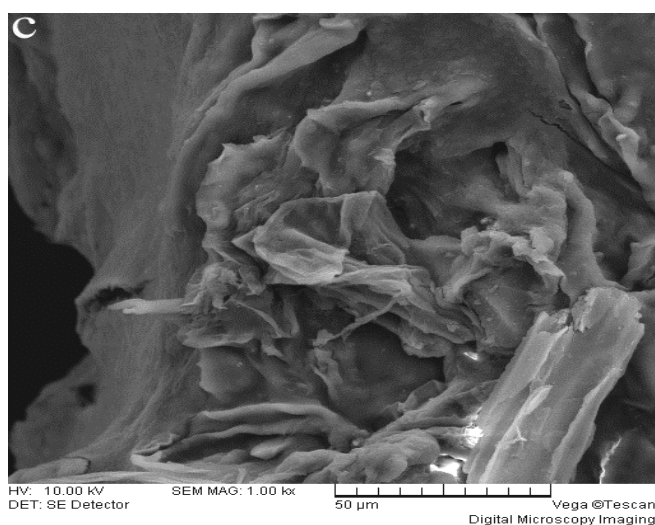
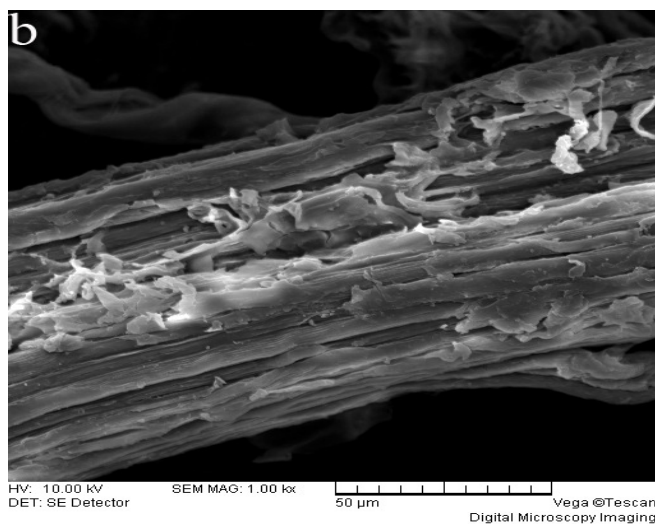
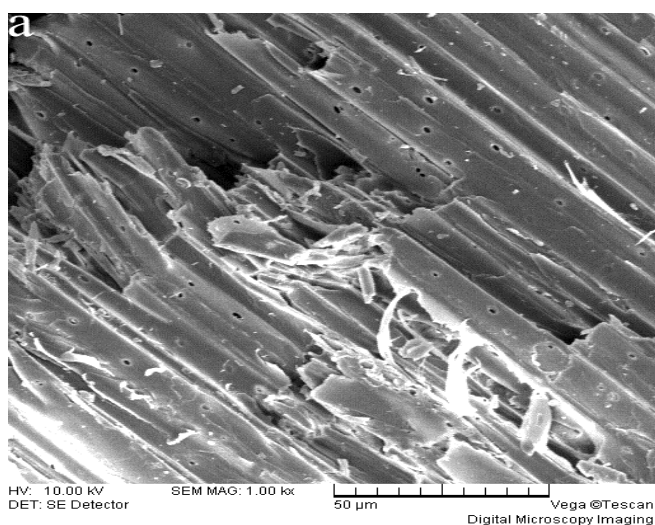


Fig. 5. SEM micrographs of untreated (a), steam explosion and fractions process (2.6 Mpa, 90 sec) (b) and [Bmim]Cl treated (c) corn stalk

## Conclusion

Reducing sugars yield could be significantly enhanced during enzymatic hydrolysis of corn stalk treated by [Bmim]Cl and steam explosion. After optimizing the steam explosion parameters, the reducing sugars yield of corn stalk treated by [Bmim]Cl can reach 961.66 mg/g, which increased by 3.87 times compared to untreated corn stalk. The results of chemical composition analysis showed that the content of cellulose in the corn stalk increased after steam explosion and fractions process, but the crystalline polymorphism was not destroyed. The crystal structure changed from cellulose I to amorphous after treatment by [Bmim]Cl, which was indicated by the FTIR, XRD and SEM analysis. The structure changing is the main reason for the reducing sugars yield increasing during enzymatic hydrolysis of corn stalk.

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## REFERENCES

1. A. Alfaro, A. Rivera, A. Perez, R. Yáñez, J.C. García and F. López, *Bioresour. Technol.*, **100**, 440 (2009).
2. I. Kilpeläinen, H. Xie, A. King, M. Granstrom, S. Heikkinen and D.S. Argyropoulos, *J. Agric. Food. Chem.*, **55**, 9142 (2007).
3. R.K. Sukumaran, R.R. Singhanian, G.M. Mathew and A. Pandey, *Renew. Energ.*, **34**, 421 (2009).
4. E. Araque, C. Parra, J. Freer, D. Contreras, J. Rodríguez, R. Mendonça and J. Baeza, *Enzyme Microb. Technol.*, **43**, 214 (2008).
5. A. Carroll and C. Somerville, *Annu. Rev. Plant Biol.*, **60**, 165 (2009).
6. G.W. Huber, S. Iborr and A. Corma, *Chem. Rev.*, **106**, 4044 (2006).
7. R.P. Chandra, R. Bura, W.E. Mabee, A. Berlin, X. Pan and J.N. Saddler, *Adv. Biochem. Eng./Biotechnol.*, **108**, 67 (2007).
8. T.A.D. Nguyen, S.J. Han, J.P. Kim, M.S. Kim, U.K. Oh and S.J. Sim, *Int. J. Hydrogen. Energ.*, **33**, 5161 (2008).
9. N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple and M. Ladisch, *Bioresour. Technol.*, **96**, 673 (2005).
10. K. Wang, J.X. Jiang, F. Xu and R.C. Sun, *Polym. Degrad. Stab.*, **94**, 1379 (2009).
11. M. Ibrahim and W.G. Glasser, *Bioresour. Technol.*, **70**, 181 (1999).
12. C. Li, Q. Wang and Z.K. Zhao, *Green Chem.*, **10**, 177 (2008).
13. S. Zhu, Y. Wu, Q. Chen, Z. Yu, C. Wang, S. Jin, Y. Ding and G. Wu, *Green Chem.*, **8**, 325 (2006).
14. L.Y. Liu and H.Z. Chen, *Chin. Sci. Bull.*, **51**, 2432 (2006).
15. H.Z. Chen and L.Y. Liu, *Bioresour. Technol.*, **98**, 666 (2007).
16. L.Y. Liu and H.Z. Chen, *Technology of Steam Explosion: Principles and Applications*, Chemical Industry Press, Beijing, p.2/60 (2007).
17. G.L. Miller, *Anal. Chem.*, **31**, 426 (1959).
18. T.K. Ghose, *Pure Appl. Chem.*, **59**, 257 (1987).
19. H.K. Goering and P.J. Van Soest, *Forage Fibre Analysis Apparatus, Reagents, Procedure and Some Applications*, Agricultural Handbook 379, ARS USDA, Washington, DC, p.1/20 (1970).
20. M.L. Nelson and R.T. O'Connor, *J. Appl. Polym. Sci.*, **8**, 1311 (1964).
21. C.H. Kuo and C.K. Lee, *Carbohydr. Polym.*, **77**, 41 (2009).
22. K. Wang, J.X. Jiang, F. Xu and R.C. Sun, *Bioresour. Technol.*, **100**, 5288 (2009).
23. Q.J. Li, Y.L. Yin and R.X. Su, *Acta Chim. Sin.*, **67**, 88 (2009).
24. Q. Li, Y.-C. He, M. Xian, G. Jun, X. Xu, J.-M. Yang and L.-Z. Li, *Bioresour. Technol.*, **100**, 3570 (2009).