INFLUENCE OF STEAM EXPLOSION PRETREATMENT ON THE COMPOSITION AND STRUCTURE OF WHEAT STRAW

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Steam explosion pretreatment of wheat straw can solubilize a significant portion of the hemicellulosic component and enhance the enzymatic digestibility of the remaining cellulose for fermentation into ethanol. In this work, wheat straw was pretreated by steam explosion using different steam temperatures and retention times, and the chemical compositions of the raw and steam-exploded wheat straw were analyzed. Results showed that the content of hemicellulose decreased sharply at higher steam temperatures and longer retention times; however, the content of lignin changed inconspicuously. After pretreatment, the characteristics of the straw fiber were investigated by studying their proportion of microfibrils, SEM, and FTIR. To assess the differences among various pretreatment parameters, the concentration of the reducing sugar and glucose conversion were determined. The highest reducing sugar concentration and glucose conversion were achieved at the explosion conditions of a pretreatment temperature of 220 ºC and a residence time of 3 min.

Keywords: Wheat straw; Steam explosion; Structure properties; Enzymatic hydrolysis

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INTRODUCTION

One of the greatest challenges for society in the 21st century is meeting the fast-growing demand for energy for transportation, heating, and industrial processes, and to provide raw material for the industry in a sustainable way (Wang *et al*. 2009).

Lignocellulosic materials, including non-food cultures and wastes from agriculture or forestry, are considered to be a sustainable environmental alternative for producing bioethanol (Jurado *et al*. 2009). In many countries, including the USA, wheat straw is an abundant by-product from wheat production. It consists mainly of three major components: cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are structural carbohydrates that can be depolymerized through enzymatic hydrolysis to fermentable sugars for ethanol production. It has been well known that cellulose in the lignocellulosic complex has the potential to be used as an energy source in industrial biotechnology processes (Zhang *et al*. 2008; Xu *et al*. 2011).

Pretreatment is necessary to achieve reasonable rates and yields in the enzymatic hydrolysis of biomass. To open up the lignocellulosic structure to wide microbial degradation, expensive energy-demanding pretreatment processes are required. In the pretreatment area, the reduction of power for milling is one of the technological improvements that can result in substantially lower ethanol production costs (Ballesteros *et al*. 2002). Steam explosion is one of the most attractive pretreatment processes due to its low use of chemicals, low energy consumption, and efficient biomass disruption characteristics. Compared with the processes using mechanical size reduction prior to chemical pretreatment with large liquid-to-wood solid ratios, steam explosion is relatively energy efficient (Zhu and Pan 2010). With steam explosion, optimal solubilization, and degradation of hemicellulose can generally be achieved by either high temperature and short residence time (270 ºC, 1 min) or lower temperature and longer residence time (190 ºC, 10 min) (Sheldon and William 1996; Saha 2003). The final structure of the biomass presents larger pores and higher surface area than it did before treatment (Alfani *et al*. 2000).

Enzymatic hydrolysis presents several advantages when compared with acid hydrolysis, including lower equipment costs (as hydrolysis is conducted at mild conditions) and higher glucose yields without sugar-degradation products, which may affect ulterior fermentation (Cara *et al*. 2007).

In the present study, wheat straw was used as a raw material. To further enhance the accessibility and cellulose content of the solid substrate, steam explosion pretreatment was investigated. With this method, the substrate, wheat straw, was loaded into a pressure vessel and heated by steam injection for a defined time-temperature period. At the end of this period of heat treatment, a pressure drop was suddenly made. Steam-exploded wheat straw was characterized to study its chemical and morphological properties. The optimum pretreatment conditions were defined, and enzymatic hydrolysis of steam-exploded straw was also investigated.

EXPERIMENTAL

Raw Material and Enzyme

In this work, wheat straw was first harvested from a local farm in Dezhou, Shandong Province, China, and then stored in a sheltered area before using. The moisture content of the straw was 6.92%. For steam explosion pretreatment, the wheat straw was chopped into pieces measuring between 5 and 10 cm by a crop chopper.

Cellulase was purchased from Zaozhuang Jienuo Biotech. Corp., China, with a filter paper activity (FPA) of 43.5 FPU/g. All other chemicals used in this study were of analytical grade.

Steam Explosion Pretreatment of Wheat Straw

Steam explosion of the raw material was carried out in a 7-L reaction vessel (BL-08, Beijing Forestry University, China) designed to reach a maximum operating pressure of 3.5 MPa. 500 g of wheat straw was used for each pretreatment experiment. The reactor was heated with saturated steam. The temperature inside the reactor was monitored during the pretreatment procedure. After treatment at a given temperature and residence time, the steam-treated straw was released from the vessel by rapid despressurization, causing the material to expand into a stainless steel cyclone. The procedure of steam explosion pretreatment was evaluated at different temperatures (200 and 220 ºC) and time periods (3 and 6 min). The final pretreated straw samples were stored at 4 ºC until further use.

Characterization of Pretreated Wheat Straw

Scanning electron micrograph (SEM)

SEM (JSM-6380LV, FEI, Czech) was used for the microstructural analysis of the steam-exploded wheat straw samples with gold coating. All images were taken at an accelerating voltage of 30 kV.

Proportion of microfibrils analysis

Length and proportion of microfibrils after steam explosion were measured using a Fiber Tester (912, Lorentzen & Wettre, Sweden). Approximately 2 g of the dry solids were dispersed and used for analysis.

Fourier transform infrared (FTIR) spectroscopy

Fourier transform infrared (FTIR) spectra were recorded on a FTIR-650 spectrometer (Tianjin Gangdong Sci. & Tech. Development Co., Ltd., China) in order to determine the changes in functional groups that may have been caused by the treatments. Prior to the analysis, 3.5 to 4.0 mg of the fibers mixed with 350 mg of KBr were ground together. The resultant suspensions were pressed into transparent pellets and analyzed in transmittance mode within the range of 4000 to 400 cm^{-1}.

Enzymatic Hydrolysis

The washed water-insoluble residue of the pretreated wheat straw was determined by the total reducing sugar and glucose concentration in the hydrolyzate. The experiment for enzymatic hydrolysis was carried out in a 250 mL conical flask, and the enzyme loading was 25 FPU/g substrate. The experiment was performed in a sodium acetate buffer (pH of 4.8) at 50 ºC on an incubator shaker (HNY-100B, Tianjin Honour Instrument Co., Ltd, China) at 160 rpm for 60 h and at 2% (w/v) pretreated material concentration (Cui *et al*. 2011).

Analytical Methods

The compositions of the raw and whole steam-exploded wheat straw were determined using the following TAPPI standards: T 429 cm-01 for cellulose content, T 223 cm-01 for hemicellulose content, and T 222 om-02 for lignin content. The characteristics of fiber fines after steam explosion were determined using SEM, fiber testing, and FTIR. The reducing sugar concentration was determined using dinitrosalicylic acid reagent (Miller 1959).

Glucose concentration in the samples was measured by a Biosensing analyzer (SBA-40C, manufactured by Biology Institute of Shandong Academy of Sciences, China). The glucose conversion was calculated as the ratio of the glucose released during enzymatic hydrolysis divided by potential glucose content in the pretreated material expressed by percentage.

Statistical Analyses

All experiments were performed in duplicate, and average values were reported. Experimental errors, which were calculated as the relative standard deviation, are shown by the error bars in the figures.

RESULTS AND DISCUSSION

Chemical Compositions

Chemical compositions of the untreated and whole steam-exploded wheat straw are shown in Table 1.

Table 1. Chemical Compositions of Untreated and Steam-exploded Wheat Straw (% of Dry Weight)

Sample	Cellulose	Hemicellulose	Lignin
Straw untreated	36.14 ± 0.96	23.16 ± 0.69	17.74 ± 0.31
200 ºC, 3 min	47.35 ± 1.15	20.12 ± 0.52	15.42 ± 0.26
200 ºC, 6 min	57.68 ± 1.27	18.62 ± 0.47	19.15 ± 0.49
220 ºC, 3 min	63.59 ± 1.32	12.36 ± 0.18	20.95 ± 0.56
220 ºC, 6 min	55.23 ± 1.21	9.65 ± 0.09	21.67 ± 0.62

In the current model of the structure of lignocellulose, cellulose fibers are embedded in a lignin-polysaccharide matrix. Various pretreatment conditions were available to fractionate, solubilize, hydrolyze, and separate cellulose, hemicellulose, and lignin components. As shown in Table 1, the cellulose content increased with an increase in temperature and time duration; however, at a temperature of 220 ºC and duration time of 6 min, the cellulose content decreased to 55.23%, probably because the higher severity conditions caused β-glycosidic bonds between D-glucosyl groups to be destroyed, which resulted in breakage of the cellulose. Therefore, the cellulose was degraded. In relation to cellulose content, solubilization increased as pretreatment conditions were more drastic. The cellulose content in the pretreated wheat straw ranged between 47.35% and 63.59%, depending on the pretreatment severity. It was also obvious that a large amount of hemicellulose was destroyed under higher steam pressures and residence times. As expected, the steam explosion pretreatment preferentially attacked the hemicellulose components; more severe pretreatment conditions produced higher solubilization of hemicellulosic fractions (Ballesteros *et al*. 2002). The main reason for this decrease was that hemicellulose was hydrolyzed by the acetic and other acid released during steam explosion pretreatment. As the temperature and time increased, the hemicellulose content decreased from 23.16% to 9.65%. It is noteworthy that with more hemicellulose loss, the bond linking lignin and hemicellulose could be affected; therefore, the structure of lignin must be destroyed to a certain extent (Zhang *et al*. 2008). The lignin content changed slightly, however, ranging from 15.42% to 21.67%.

The moisture content of the biomass, measured during the final stage of steam explosion pretreatment, amounted to 57.67 wt.% on average.

Morphology of Wheat Straw under Various Steam Explosion Treatments

Figure 1 shows the images of wheat straw after various steam explosion treatments. It is clear that the sizes of wheat straw particles tended to be smaller at a higher steam temperature and longer retention time. More fibers were found when samples were treated at a higher temperature and for a longer time. The reason for this phenomenon was due to the following: during the steam explosion process, the rapid thermal expansion was used to terminate the reaction and it could help open up the particulate structure of the biomass. The rigid and highly ordered structure of biomass was disrupted. The separation of individual vascular cells (*i.e.* plant fibers) was performed by disruption of middle lamella lignin.

Fig. 1. Images of wheat straws before and after steam explosion pretreatment

Through observing the surface morphology, it can be seen that the raw wheat straw had obvious fibrous structure, which is the common characteristic of most biomass. SEM pictures of the wheat straw after steam explosion were used to investigate the structure of these fibers. From Fig. 2, we could easily find that after steam explosion pretreatment, the wheat straw was broken into some large fragments and appeared in porous structure; however they were still fibrous. These pictures clearly demonstrate that more fibers burst open with an increase in temperature and extended retention time; the amount of long fibers decreased while the amount of short fibers increased.

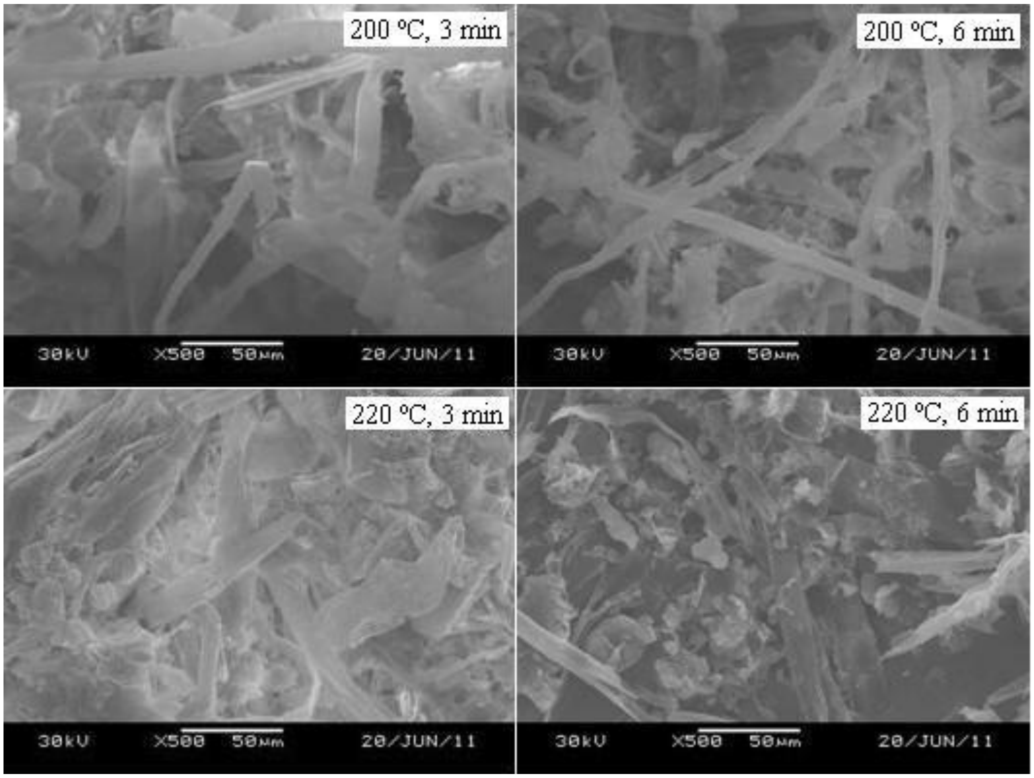

Fig. 2. SEM images of microfibrils after steam explosion

Figure 3 illustrates the length distribution of wheat straw fibers after the steam explosion pretreatment.

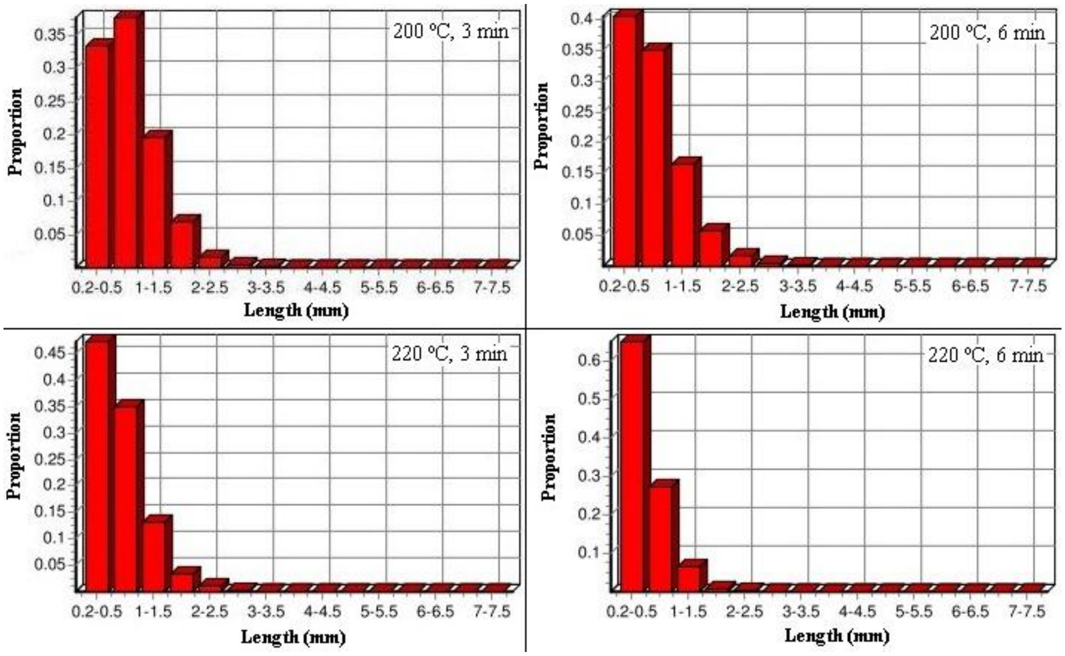

Fig. 3. Proportion of microfibrils after steam explosion

As can be seen in Fig. 3, the proportion of coarse particles decreased, while the small particles and fiber bundles increased with increasing severity of steam explosion treatment. When reaction temperature reached 200 ºC and retention time was 3 min, the fiber length ranged from 0.2 mm to 2.5 mm. As a whole, fiber length came out between 0.5 and 1.0 mm, with the proportion being approximately 37%. As retention time and reaction temperature increased, more and more fiber fines burst into fragments between 0.2 and 0.5 mm in length. At the steam explosion pretreatment condition of 220 ºC and 6 min, the proportion of fiber fines (0.2 to 0.5 mm) counted for about 65%. Many small fragments with loose structure can be observed after steam explosion, which means that action of pretreatment gave rise to a substantial change in porous structure and broke down some fibrous structures simultaneously. Because of the existence of porous structure, the enzyme could penetrate into cellulose and contact the interior structure of cellulose in the biomass more easily, which would enhance the yield of the enzymatic hydrolysis.

Results summarized in Figs. 1 to 3 indicate that pretreated wheat straw disintegrated into fibers or fiber bundles through steam explosion during discharge. The cellulose became more porous and loose, and the length of fiber became short. The change of fiber morphology was very meaningful to the following enzymatic hydrolysis for the better absorption of enzyme.

FTIR

Untreated and steam-exploded wheat straw fibers were analyzed using FTIR to examine the changes that occurred in the chemical constituents of the fibers before and after steam explosion. Figure 4 shows the FTIR spectrum of the raw wheat straw fibers and steam-exploded wheat straw fibers. The peaks at 3399 cm^{-1} correspond to O-H stretching band, which was due to the vibrations of the hydrogen-bonded hydroxyl group, whereas those around 2950 to 2900 cm^{-1} were due to the stretching of C-H (Khalil *et al*. 2001; Xiao *et al*. 2001; Sun *et al*. 2005; Kaushik and Singh 2011). The hydrophilic tendency of raw wheat straw fibers and steam-exploded fibers was reflected in the broad absorption band in the 3700 to 3100 cm^{-1} region, which was related to the –OH groups presented in their main components. The peak at 2261 cm^{-1} was attributed to SO_2 absorption; the intensity of this peak almost disappeared, which indicated that most of SO_2 became degraded when wheat straw was pretreated by steam explosion. The peak at 1735 cm^{-1} in the raw wheat straw was attributed to either the acetyl and uronic ester groups of the hemicelluloses or the ester linkage of the carboxylic group in the ferulic and *p*-coumaric acids of the lignin and/or hemicelluloses (Alemdar and Sain 2008; Jonoobi *et al*. 2009). It can be seen from Fig. 4 that the peak almost disappeared in the spectra of the steam-exploded wheat straw, which indicated that most of the hemicellulose was removed during pretreatment. The peak at 1652 cm^{-1} may be due to the bending mode of the absorbed water and some contributions from carboxylate groups.

Aromatic skeleton vibrations occurred at 1639 and 1425 cm^{-1}. The absorbance for these bands appeared at similar intensities for the different lignin fractions, suggesting that the aromaticity properties of different samples remained the same (Zhang *et al*. 2010). The peaks at 1373 cm^{-1} represented C-H asymmetric deformation. The intensity of the peak at 1249 cm^{-1} decreased sharply after steam explosion treatment indicating the removal of hemicelluloses (Kaushik and Singh 2011; Kaushik *et al*. 2010). The peaks in the region of 1200 to 1059 cm^{-1} represent the C-O stretch band and deformation bands in the cellulose, lignin, and residual hemicellulose (Sun *et al*. 2005). Furthermore, the peaks that vibrated at 898 cm^{-1} in all samples are assigned to the typical structure of cellulose (due to β-glycosidic linkages of glucose ring of cellulose, which are symmetric in polysaccharides) (Jonoobi *et al*. 2009; Troedec *et al*. 2008).

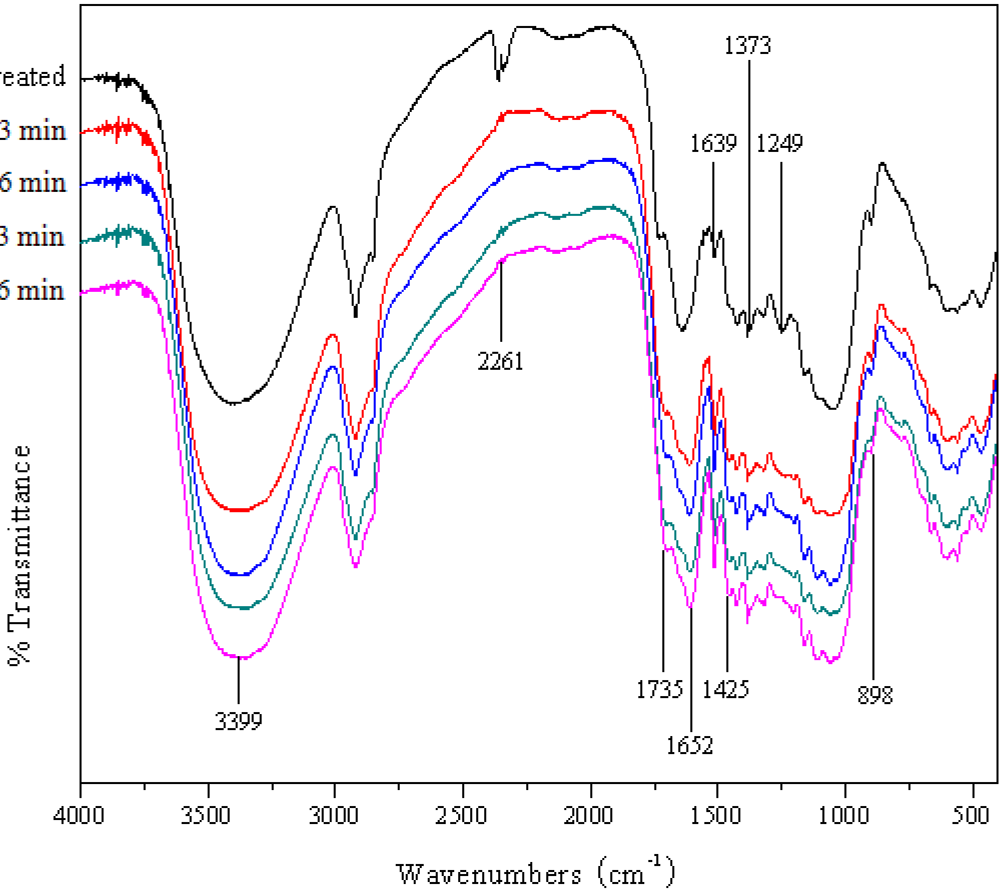

Fig. 4. FTIR spectra of wheat straw fibers (untreated and steam exploded)

Enzymatic Hydrolysis

The rate and extent of enzymatic hydrolysis of lignocellulose was highly dependent on the solids loading, enzyme loading, hydrolysis periods, and structural features of the substrate (Zhu *et al*., 2008; Maache-Rezzoug *et al*. 2011; Samaniuk *et al*. 2011). After the steam explosion pretreatment, the wheat straw became more susceptible to enzyme attack. A mass of available surface area on the lignocellulose tremendously reduced the amount of enzyme required for digestion (Yang *et al*. 2010). Figure 5 shows the effect of different explosion conditions on reducing sugar concentration and glucose conversion from the enzymatic hydrolysis of the pretreated wheat straw.

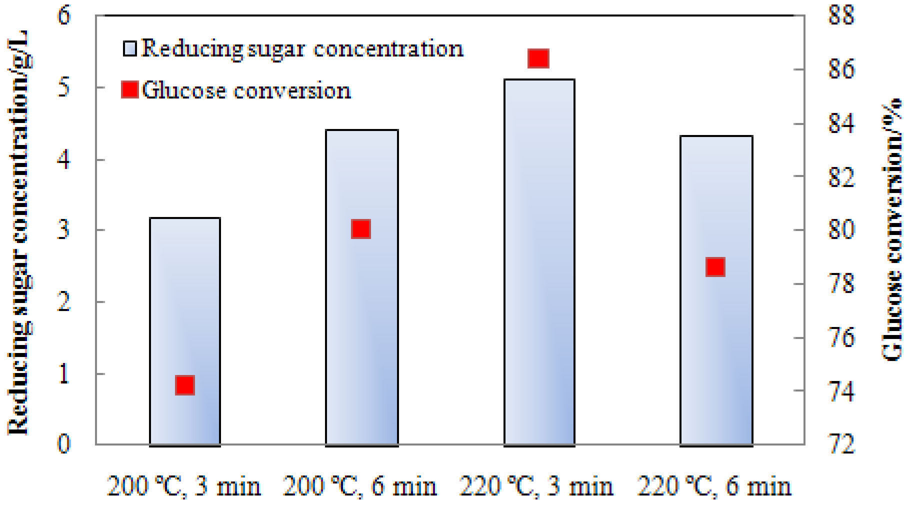

Fig. 5. Effect of different explosion conditions on enzymatic hydrolysis

From Fig. 5, it can be easily found that the reducing sugar concentration and the glucose conversion increased as the conditions of steam explosion became more severe. As mentioned above, these harsh conditions dissolved a greater fraction of hemicellulose, increasing accessibility of the enzymes to cellulose, and as a consequence, a higher reducing sugar was produced after the enzymatic hydrolysis (Saha *et al*. 2005).

As shown in Fig. 5, the highest sugar concentration was 5.12 g/L, achieved at the explosion condition of 220 ºC and 3 min, which was approximately 2 g/L higher than that achieved at the explosion condition of 200 ºC and 3 min. When the steam explosion condition was 220 ºC and 6 min, however, the reducing sugar concentration experienced a decreasing trend, which decreased to 4.33 g/L. Similar to the change tendency of reducing sugar concentration, the glucose conversion increased with increasing severity of pretreatment condition. The highest glucose conversion was 86.42% under the pretreatment condition of 220 ºC and 3 min. The reason for this is that firstly, under more severe conditions, a part of the cellulose was solubilized together with the hemicellulose. Secondly, the severe conditions could also cause degradation of the physical and chemical properties of the cellulose. Finally, under harsh conditions, the lower enzymatic digestibility of lignocelluloses may also be observed after steam explosion (Varga *et al*. 2004; Zabihi *et al*. 2010); therefore, the optimum effect of steam explosion should be carried out at 220 ºC for 3 min.

CONCLUSIONS

1. This study documented important changes in the composition and structure of raw wheat straw and steam-explosion pretreated wheat straw.
2. The results revealed that the cellulose content increased from 36.14% to 63.59%. A large amount of hemicellulose was lost, however, with the hemicellulose content decreasing from 23.16% to 9.65% at a higher treatment temperature and longer

retention time. SEM, fiber testing, and FTIR illuminated the findings that the small particles and fiber bundles increased, and the cellulose became more porous and loose, which indicated that the cellulose had higher specific surface under severe explosion conditions.

3. In the enzymatic hydrolysis process, the highest reducing sugar concentration and glucose conversion were 5.12 g/L and 86.42%, respectively, which was achieved at the explosion conditions of 220 ºC and 3 min of treatment time.

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