

Enzymatic hydrolysis of pretreated soybean straw

Zhong Xu^{a,c}, Qunhui Wang^{b,d,*}, ZhaoHua Jiang^a, Xue-xin Yang^c, Yongzhen Ji^b

^aDepartment of Applied Chemistry, Harbin Institute of Technology, 150001, China

^bDepartment of Environmental Science and Engineering, Harbin Institute of Technology, 202 Haihe Road, Nangang District, Harbin 150090, China

^cDepartment of Environmental Engineering, Harbin University of Commerce, 150076, China

^dDepartment of Environmental Engineering, University of Science and Technology Beijing, 100083, China

Received 9 February 2004; received in revised form 2 June 2006; accepted 10 June 2006

Available online 20 September 2006

Abstract

In order to produce lactic acid, from agricultural residues such as soybean straw, which is a raw material for biodegradable plastic production, it is necessary to decompose the soybean straw into soluble sugars. Enzymatic hydrolysis is one of the methods in common use, while pretreatment is the effective way to increase the hydrolysis rate. The optimal conditions of pretreatment using ammonia and enzymatic hydrolysis of soybean straw were determined. Compared with the untreated straw, cellulose in straw pretreated by ammonia liquor (10%) soaking for 24 h at room temperature increased 70.27%, whereas hemicellulose and lignin in pretreated straw decreased to 41.45% and 30.16%, respectively.

The results of infrared spectra (IR), scanning electron microscope (SEM) and X-ray diffraction (XRD) analysis also showed that the structure and the surface of the straw were changed through pretreatment that is in favor of the following enzymatic hydrolysis. maximum enzymatic hydrolysis rate of 51.22% was achieved at a substrate concentration of 5% (w/v) at 50 °C and pH 4.8 using cellulase (50 fpu/g of substrate) for 36 h.

© 2006 Elsevier Ltd. All rights reserved.

Keywords: Soybean straw; Ammonia liquor; Pretreatment; Enzymatic hydrolysis

1. Introduction

Materials including lignocellulosic such as agricultural residues, food processing wastes, wood, municipal solid wastes and wastes from pulp and paper industry are considered as low cost and abundant raw materials for bioconversion into sugars which can be fermented to lactic acid [1–3].

In lignocellulosic materials cellulose, a linear polymer of glucose is associated with hemicellulose and surrounded by lignin seal. Lignin, a complex three-dimensional (3D) polyaromatic matrix prevents enzymes from accessing some regions of the cellulose polymers. Crystallinity of the cellulose further impedes enzymatic hydrolysis [4,5].

The pretreatment of lignocellulosics is primarily employed to increase the accessible surface area of cellulose to enhance the conversion of cellulose to glucose. Ammonia has a number of desirable characteristics as a pretreatment reagent. It has high selectivity for reacting with lignin compared with other carbohydrates. Its high volatility makes it easy to recover and reuse. The common methods for degradation of cellulose to glucose are acid hydrolysis and enzymatic hydrolysis. Enzymatic hydrolysis can not only economize energy on account of the relatively mild reaction conditions, but also avoid using toxic and corrosive chemicals. Some lignocellulosic materials like wheat straw, wastepaper, etc., have been used for lactic acid production [6,7], but there is no report to the best of our knowledge on utilization of soybean straws for lactic acid production.

Soybean is widely cultivated in many areas and the production of soybean straw was 25 million tons in China in 2000 [8]. The large quantity of soybean straws annually cannot be used effectively and are generally burnt

*Corresponding author. Department of Environmental Science and Engineering, Harbin Institute of Technology, 202 Haihe Road, Nangang District, Harbin 150090, China. Tel./fax: +86 451 641 8414.

E-mail address: wangqh59@hit.edu.cn (Q. Wang).

in the fields causing environmental pollution. Therefore, soybean straws, as lignocellulosics, afford a renewable and low-cost raw material for the production of fermentable sugars.

In order to produce lactic acid, from soybean straw including fermentable sugars, which is a raw material for biodegradable plastic production [2,3,9], the optimal conditions of pretreatment using ammonia and enzymatic hydrolysis of soybean straw were determined. Changes in chemical characteristics, physical properties and cellulose microstructure for pretreated soybean straw were also observed.

2. Materials and methods

2.1. Materials

Soybean straw used in the present study was supplied by the Environmental Engineering Laboratory of Harbin Institute of Technology. It was smashed to 140 mesh and was used in experiments. The initial composition of soybean straw was determined to be 24.99% cellulose, 11.91% hemicellulose and 17.64% lignin.

α -cellulose was purchased from Sigma Company. Cellulase was obtained from the Wuxi Company of enzyme making of China. Its filter enzyme activity was 126 FPU/mL.

2.2. Pretreatment of soybean straws

Powdered soybean straw was pretreated by ammonia before enzymatic hydrolysis. Ammonia liquor (2–20%) pretreatment of the straw was carried out in a flask at room temperature for 0–40 h. Solid:liquid ratio was maintained at 0.1 g/ml. Solid was recovered by filtration and then washed repeatedly with distilled water until the wash water turned to pH 7.0. Subsequently this straw was dried at 70 °C to have constant weight in oven. The pretreated straw was used as the substrate for enzymatic hydrolysis.

2.3. Enzymatic hydrolysis

The pretreated straw was hydrolyzed using cellulase in stoppered Erlenmeyer flasks. The hydrolysis was performed in 0.1 M citrate buffer (pH 4.8) at 150 rpm at 50 °C for 36 h under shaking. To determine the effectiveness of different factors such as pretreatments, substrate concentration, temperature and time of enzymatic hydrolysis, enzyme to substrate ratio was maintained at 30 FPU/g of substrate. The effect of substrate concentration (2–20% w/v), enzyme loading (5–70 FPU/g substrate), temperature (40–55 °C) and hydrolysis time (6–48 h) on enzymatic hydrolysis of soybean straw was also studied. The percentage of hydrolysis of soybean straws was

calculated according to the following formula [10]:

$$\text{Hydrolysis rate (\%)} = \frac{\text{Reducing sugar} \times 0.9}{\text{Cellulose} + \text{hemicellulose (in substrate)}} \times 100$$

2.4. Analytical methods

Filter-paper activity (FPA) of cellulase was measured according to the standard procedure recommended by the Commission Biotechnology, IUPAC [11] and expressed in filter paper units (FPU). One unit of enzyme was defined as the amount of enzyme capable of producing 1 μ mole of reducing sugars in 1 min.

Cellulose, hemicellulose and lignin content were determined according to the procedures of Goering and Van Soest [12]. Reducing sugars were determined by the DNS method [13].

Crystallinity of the soybean straw was determined by X-ray diffraction (XRD) using a diffractometer (Rigooku DMAX-RB) operated at 45 kV and 50 mA. The samples were scanned at 1°/min from $2\theta = 10^\circ$ to 30° . Infrared spectra were measured by a AVATAR60 FT-IR spectrometry. The microscope pictures of the samples (scanning electron microscope (SEM)) were taken by a HITACHI S-570 SEM.

3. Results and discussion

3.1. Pretreatment of soybean straw by ammonia liquor and smash

Figs. 1–3 show, respectively, the effect of ammonia liquor concentrations (2–20% w/v), pretreated time (0–40 h) and smash size on the enzymatic hydrolysis of soybean straw. When soybean straw was milled to pass through 140 mesh size and pretreated by ammonia liquor

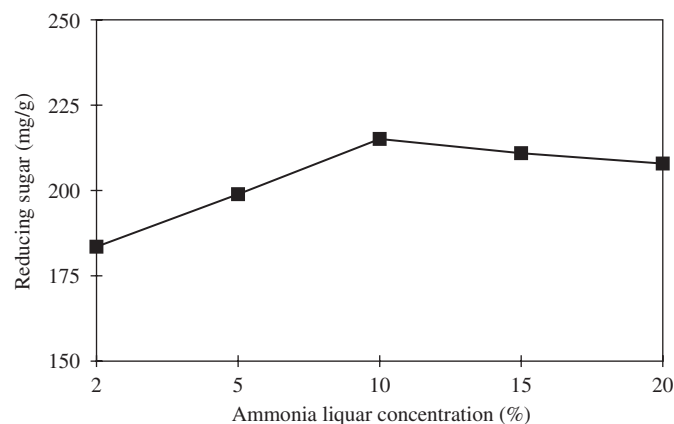


Fig. 1. Effect of ammonia liquor concentration on the enzymatic hydrolysis of soybean straw. Enzyme loading 30 FPU/g, substrate concentration 5% (w/v), pH 4.8, temperature 50 °C, pretreated time 36 h.

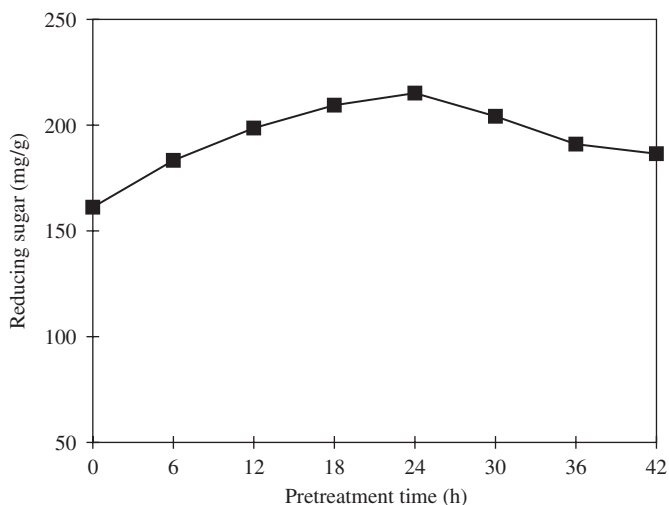


Fig. 2. Effect of ammonia liquor pretreated time on the enzymatic hydrolysis of soybean straw. Enzyme loading 30 FPU/g, substrate concentration 5% (w/v), pH 4.8, temperature 50 °C, ammonia liquor concentration 10%.

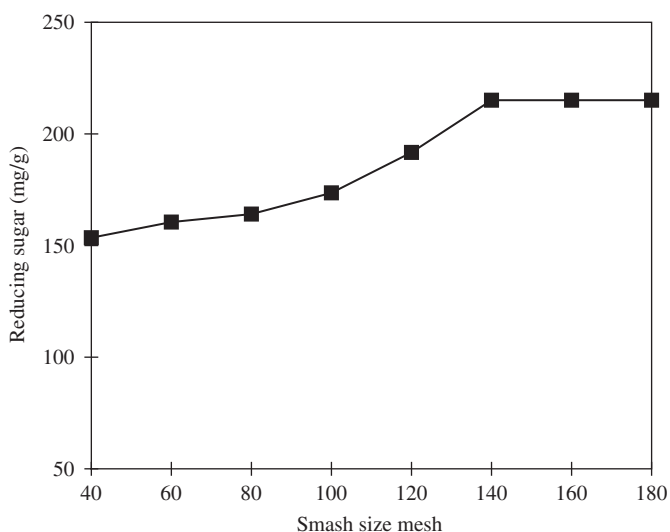


Fig. 3. Effect of different smash size on the enzymatic hydrolysis of soybean straw. Enzyme loading 30 FPU/g, substrate concentration 5% (w/v), pH 4.8, temperature 50 °C, ammonia liquor concentration 10%, pretreated time 36 h.

at 10% concentration for 24 h at room temperature, the hydrolysis effect was best.

3.2. Chemical characteristics of pretreated soybean straw

Optimally pretreated soybean straw has been analyzed for chemical components. Pretreated soybean straw contains 42.55% cellulose, 6.97% hemicellulose and 12.32% lignin. Therefore, compared with the chemical components in the untreated straw, it is clear that pretreatment increased cellulose 70.27%, and decreased hemicellulose and lignin 41.45% and 30.16%, respectively.

3.3. Physical properties and cellulose microstructure of pretreated soybean straw

The chemical composition was not the sole factor influencing the enzymatic hydrolysis. Physical properties and cellulose microstructure were among the potential factors influencing enzymatic hydrolysis. One frequently cited property was soybean straw crystallinity. The XRD of pretreated, untreated soybean straw and α -cellulose was measured (Fig. 4), showing that crystallinity of soybean straw actually decreased with time development of ammonia liquor pretreatment. The content of cellulose increased due to partly removal of lignin and hemicellulose.

Infrared spectroscopy is frequently used for investigating the structure of constituents and chemical changes in lignocellulosic materials. Pandey [21] reported specific band positions of each constituent in his study of chemical structure of wood. Soybean straw, being a grass species, has two types of lignin (guaiacyl and syringyl lignin). According to the report, the lignin characteristic peaks were observed at 1238 cm^{-1} (C–O of guaiacyl ring), 1315 cm^{-1} (C–O of syringyl ring), and 1504–1630 cm^{-1} (aromatic skeletal vibration). The FTIR spectra of two pretreated samples and untreated soybean straw are shown in Fig. 5. The band intensities at all lignin peaks (1504–1630 cm^{-1}) of the untreated sample were higher than those of all pretreated samples. This again proves the delignification effect of the ammonia liquor pretreatment.

Because a large fraction of hemicellulose and lignin was removed by pretreatment, there was some physical changes in the straw. For this reason, SEM pictures of pretreated and untreated soybean straw were produced (Figs. 6 and 7). The untreated soybean straw exhibited rigid and highly ordered fibrils, while the fibers of pretreated samples by ammonia liquor were distorted. The microfibrils were also separated from the initial connected structure and fully exposed, thus increasing the external surface area and the porosity.

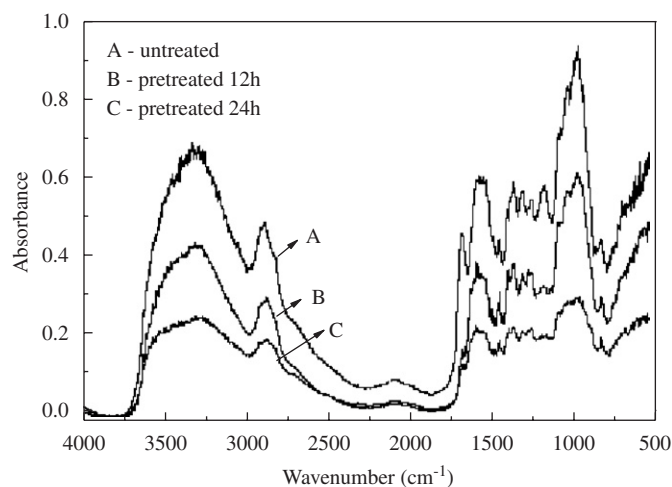


Fig. 4. FTIR spectra of ammonia liquor pretreated soybean straw.

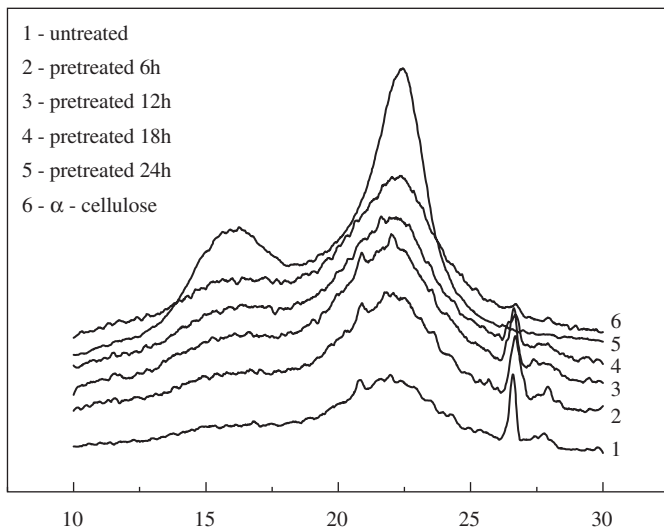


Fig. 5. The XRD diagram of ammonia liquor pretreated soybean straw.

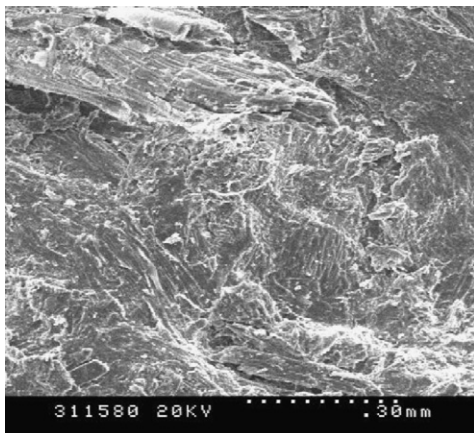


Fig. 6. SEM photograph of untreated soybean straw.

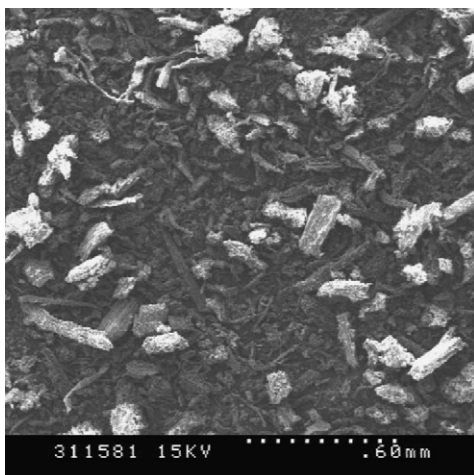


Fig. 7. SEM photograph of pretreated soybean straw.

3.4. Enzymatic hydrolysis

Enzymatic hydrolysis of pretreated soybean straw was carried out by cellulase. The various parameters such as hydrolysis time, substrate concentration, temperature and enzyme loading were optimized to achieve maximum hydrolysis rate of the pretreated soybean straw.

3.4.1. Effect of time on the enzymatic hydrolysis

The hydrolysis of pretreated soybean straw was carried out at a range of 6–48 h and the results are presented in Fig. 8. The hydrolysis of soybean straw yielded 437.2 mg reducing sugars per gram cellulose and hemicellulose for 36 h, i.e., hydrolysis rate was 44%. The reducing sugars as well as percent hydrolysis rate decreased as soon as prolong the time after optimum. This behavior might due to the inhibition of the enzyme action by the accumulated hydrolysis products [14].

3.4.2. Effect of substrate concentration on the enzymatic hydrolysis

The results of the effect of substrate concentration (2–20% w/v) on enzymatic hydrolysis were shown in Fig. 9. The hydrolysis rate increased up to substrate concentration of 5%. Maximum hydrolysis rate of 43.73% was achieved at substrate concentration of 5%. Further increase in the substrate concentration decelerated the rate of hydrolysis. Stirring difficulties, reduction of the aqueous movable phase and end product inhibition might hinder the enzymatic hydrolysis at higher substrate concentration [14,15].

3.4.3. Effect of temperature on the enzymatic hydrolysis

The hydrolysis of pretreated soybean straw was carried out at temperature ranging from 40 to 55 °C (Fig. 10). The initial hydrolysis rate increased with enhancing temperature, and maximum hydrolysis rate was observed at 50 °C.

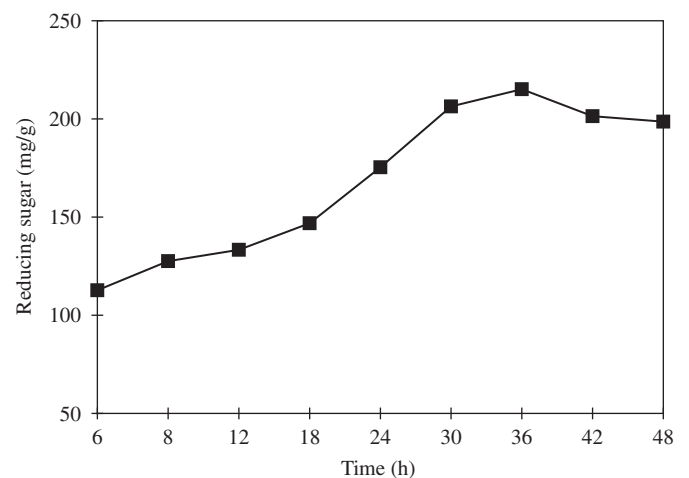


Fig. 8. Effect of hydrolysis time on the enzymatic hydrolysis. Enzyme loading 30 FPU/g, substrate concentration 5% (w/v), temperature 50 °C, pH 4.8.

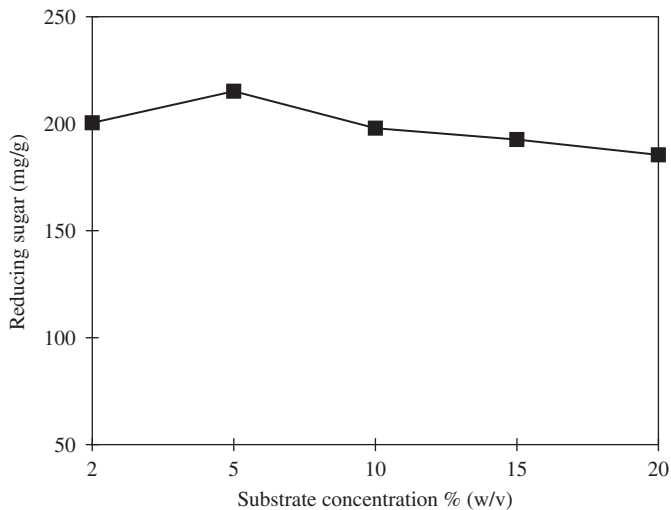


Fig. 9. Effect of substrate concentration on the enzymatic hydrolysis. Enzyme loading 30 FPU/g, temperature 50 °C, pH 4.8, time 36 h.

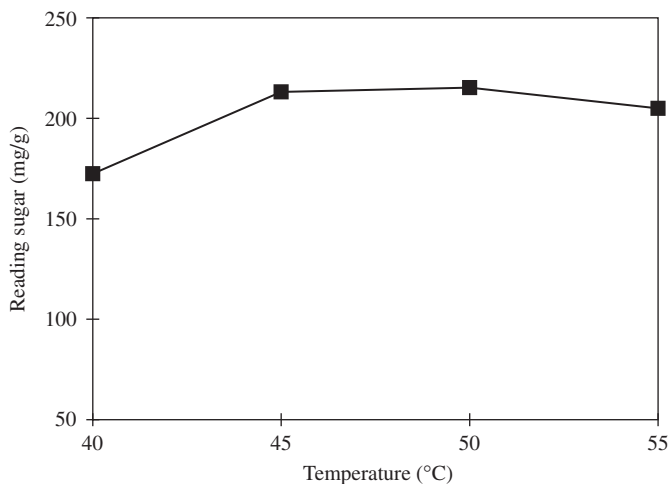


Fig. 10. Effect of temperature on the enzymatic hydrolysis. Enzyme loading 30 FPU/g, substrate concentration 5% (w/v), pH 4.8, time 36 h.

Then hydrolysis rate decreased when temperatures exceeded 50 °C. This result could be attributed to the thermal inactivation of endoglucanase I and cellobiohydrolase I [16,17]. The temperature of 50 °C was also found to be optimum for enzymatic hydrolysis of different lignocellulosic materials [15,18–20].

3.4.4. Effect of enzyme loading on the enzymatic hydrolysis

The effect of cellulase loading (5–70 FPU/g of substrate) on the hydrolysis of pretreated soybean straw had been studied and the results were presented in Fig. 11. The rate of hydrolysis increased with increase in the enzyme loading. Maximum hydrolysis rate (51.22%) was observed at the enzyme loading 50 FPU/g substrate. Slow decrease in reducing sugars afterwards might be due to the less adsorption efficiency for higher enzyme loading than for diluted ones or saturation of the cellulose surface with enzyme [10,18].

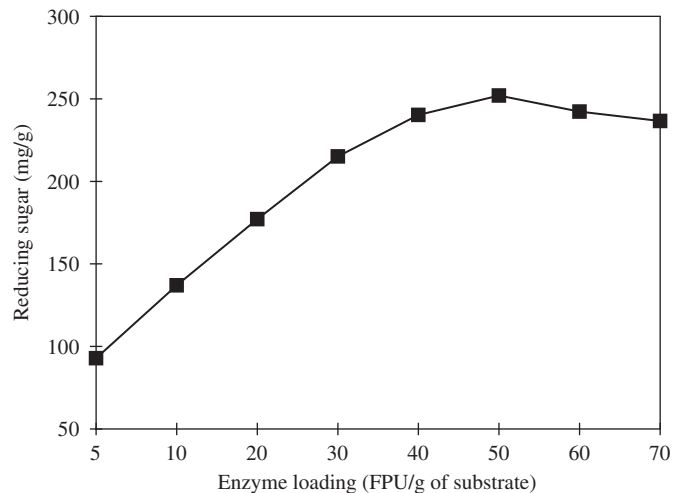


Fig. 11. The effect of enzyme loading on the enzymatic hydrolysis. Substrate concentration 5% (w/v), temperature 50 °C, pH 4.8, time 36 h.

4. Summary and conclusions

This study examined the effects of ammonia liquor pretreatment on the enzymatic hydrolysis. Among the conditions employed, the optimum condition of the pretreatment was that soybean straw smashed (140 mesh) was soaked in ammonia liquor (10%) for 24 h at room temperature. Compared with the untreated straw, cellulose in pretreated straw increased 70.27%, while hemicellulose and lignin decreased 41.45% and 30.16%, respectively. The result of FTIR analysis also confirmed that lignin was partly removed by ammonia liquor pretreatment. The SEM pictures indicated that the straw structure was deformed and fibers exposed by the pretreatment. The XRD spectra showed decrease of crystallinity with increase of pretreated time, in favor of following enzymic hydrolysis. Furthermore, in order to increase hydrolysis rate of soybean straw, the influence factors during the enzymic hydrolysis of soybean straw had been studied. The optimum hydrolysis of soybean straw was that the 5% (w/v) pretreated soybean straw was treated with cellulase of 50 FPU/g of substrate at 50 °C and pH 4.8 for 36 h. When pretreatment and hydrolysis as prescribed, the hydrolysis rate was 51.22%. The higher hydrolysis rate also indicated that ammonia liquor pretreatment is effective for improving the enzymic hydrolysis of soybean straw. The factor of the hydrolysis after ammonia liquor pretreatment depends on the enzyme loading. Future work will focus on increasing the efficiency of the enzymes.

Acknowledgements

The project was supported by National Science Foundation of China (50278024), and by National Science Foundation of Hei Longjiang Province (E0316).

References

- [1] Wang QH, Narita J, Xie WM, Ohsumi Y, Kusano K, Shirai Y, et al. Effects of anaerobic/aerobic incubation and storage temperature on preservation and deodorization of kitchen garbage. *Bioresource Technology* 2002;84:213–20.
- [2] Wang QH, Narita J, Nanqi Ren, Ohsumi Y, Kusano K, Shirai Y, et al. Effect of pH adjustment on preservation of kitchen garbage used for producing lactic acid. *Water, Air, and Soil Pollution* 2003;144:405–18.
- [3] Wang QH, Zhong Xu, Ling-hui Meng, Xiao-hong Sun, Xu-ming Wang. Production of lactic acid from kitchen garbage. *Journal of Harbin Institute of Technology (English)* 2003;10:195–9.
- [4] Weil J, Westgate P, Kohlmann J, Ladisch MR. Cellulose pretreatments of lignocellulosic substrates. *Enzyme Microbial Technology* 1994;16:1002–4.
- [5] Bothast RJ, Saha BC. Ethanol production from agricultural biomass substrates. *Advances in Applied Microbiology* 1997;44:261–86.
- [6] Arvid Garde, Gunnar J, Anette SS, Birgitte KA. Lactic acid production from wheat straw hemicellulose hydrolysate by *Lactobacillus pentosus* and *Lactobacillus brevis*. *Bioresource Technology* 2002;81:217–23.
- [7] Schmidt S, Padukone N. Production of lactic acid from wastepaper as acellulosic feedstock. *Journal of Industrial Microbiology & Biotechnology* 1997;18:10–4.
- [8] Dai-ze Huo. The utilizing status and prospects of the crop straw resources in China. *Resource Development and Market* 2000;16: 19–20.
- [9] Wang QH, Yamabe K, Narita J, Morishita M, Ohsami Y, Kusano K, et al. Suppression of growth of putrefactive and food poisoning bacteria by lactic acid fermentation of kitchen garbage. *Process Biochemistry* 2001;37:351–7.
- [10] Soto ML, Dominguez MJ, Lema JM. Enzymatic saccharification of alkali treated sunflower hulls. *Bioresource Technology* 1994;49:53–9.
- [11] Ghose TK. Measurement of cellulose activities (Recommendations of Commission on Biotechnology IUPAC). *Pure and Applied Chemistry* 1987;59:257–68.
- [12] Goering HK, VanSoest PJ. Forage fiber analysis. *Agriculture handbook*. Agricultural Research Services, United States Department of Agriculture, No. 379, 1970.
- [13] Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugars. *Analytical Chemistry* 1959;31:426–8.
- [14] Lee YH, Fan LT. Kinetic studies of enzymatic hydrolysis of insoluble cellulose: analysis of the initial rates. *Biotechnology and Bioengineering* 1982;24:2383–406.
- [15] Szczodrak J. The enzymatic hydrolysis and fermentation of pretreated wheat straw to ethanol. *Biotechnology and Bioengineering* 1987;32:771–6.
- [16] Dominguez JM, Acerbal C, Limenez J, Mata-I-de-la, Macarron R, Castillon MP. Mechanisms of thermoinactivation of endoglucanase I from *Trichoderma reesei* QM 9414. *Biochemical Journal of London* 1992;287:583–8.
- [17] Jimenez J, Dominguez JM, Castillon MP, Acerbal C. Thermoinactivation of cellobiohydrolase I from *reesei* QM 9414. *Carbohydrate Research* 1995;268:257–66.
- [18] Vlaseenko EYu, Ding H. Enzymatic hydrolysis of pretreated rice straw. *Bioresource Technology* 1997;59:109–19.
- [19] Krishna SH, Prasanthi K, Chowdary GV. Ayyanna pretreated sugarcane leaves to ethanol. *Process Biochemistry* 1998;33:825–30.
- [20] William EK, Mark TH. Using lime pretreatment to facilitate the enzymic hydrolysis of corn stover. *Biomass and Bioenergy* 2000;18:189–99.
- [21] Pandey KK. A study of chemical structure of softwood and hardwood. *Journal of Applied Polymer Science* 1999;71:1969–75.