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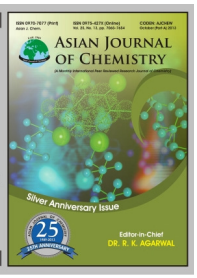

Production of Bioethanol from Pretreated Rape Straw: Focus on Low Energy Input in Distillation Process

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For ethanol production, distillation is a necessary process, but it is a high energy-consuming process. In the distillation process, a low initial ethanol concentration requires a high energy input. In this work, pretreated rape straw was used to produce bioethanol after enzymatic hydrolysis. In order to improve the initial concentration of bioethanol, a high solids content was used both in enzymatic hydrolysis and the fermentation process. The results showed that the glucose and bioethanol concentration reached 76.2 and 46.0 g/L, respectively, after enzymatic hydrolysis and fermentation when the solids content was fixed at 25 % (w/w). Compared to the solids content at 10 % (w/w), the glucose and bioethanol concentration increased 124 and 105 %, respectively. In addition, the time series analysis method was used to estimate the bioethanol yield of a city in southwest China.

Key Words: Bioethanol production, Pretreated rape straw, Distillation process, Low energy input, Time series analysis method.

INTRODUCTION

Bioethanol, as a liquid fuel by the fermentation of renewable biomass, is important from the viewpoint of global environmental protection[1]. Its advantage over biogas is that it is a liquid fuel that can readily be integrated into existing fuel supply systems and directly substitute fossil fuels in the transportation sector[2]. Currently, starch and sugar base materials are the primary raw materials for ethanol production worldwide. They are easily decomposed into glucose, which is then fermented to ethanol. However, the starch and sugar to ethanol industry draws its feedstock from a food stream and is quite mature with little possibility of process improvements[3,4]. In order to solve these problems, alternative feedstocks are needed for ethanol production such as wastes or agricultural residues (lignocellulosic materials) which include straw, wood and bagasse.

Lignocellulose is the most abundant organic material on earth and is also a promising raw material for bioenergy production[5,6]. Bioethanol production from lignocellulose requires at least four steps *i.e.*, pre-treatment, enzymatic hydrolysis, fermentation and distillation. Among the four steps, distillation is a huge energy-consuming process. According to the former research, the initial concentration of ethanol has a significant effect on the energy consumption during distillation process, increasing the ethanol concentration in the feed to the distillation reduces the production costs considerably[7,8]. When the ethanol concentration in the feed below 4 wt. %, the energy will increase sharply during distillation process[9]. In previous research, most enzymatic hydrolysis and fermentation processes were carried out at lower than 10 % solids content[5,10]. Under this condition, the theoretical concentration of ethanol from fermentation is lower than 3 % (w/w). Therefore, the final concentration of ethanol is much lower, which will require a lot of energy for the distillation process.

In this work, the final ethanol concentration produced from pretreated rape straw increased when the solids content of enzymatic hydrolysis and fermentation was increased, which decreased the energy input of the following distillation process. In addition, the time series analysis method was used to estimate the bioethanol yield of a city in southwest China to prove the advantages of this research.

EXPERIMENTAL

Raw rape straw was pretreated with 1 % (w/w) diluted sulphuric acid at 180 ºC for 10 min[10]. The remaining solid was then washed by distillated water to neutralization and dried in preparation for pretreatment.

The commercial cellulase enzyme used in this study was celluclast 1.5 L. β-glucosidase Novozym 188 was also used in this study for the hydrolysis of pretreated rape straw. The yeast of *Saccharomyces cerevisiae* was obtained from a local super market.

Enzymatic hydrolysis: Enzymatic hydrolysis was carried out at a solids loading of 10, 15, 20, 25 and 30 % dry matter. All experiments were done in duplicate. Hydrolysis was performed at 52 ºC and pH 5.0 with an enzyme loading of 0.11 g celluclast 1.5 L/g cellulose and 0.05 g β-glycosidase/g cellulose for 48 h. Samples were taken at regular intervals.

Bioethanol fermentation: When the enzymatic hydrolysis was finished, the hydrolyzate was cooled to room temperature and 10 g yeast/1 liquid was added. The fermentation was carried out in 100 mL Pyrex flasks. These flasks were equipped with yeast locks filled with glycerol for the release of produced CO_2 and then incubated at 37 ºC for a period of time. Samples were taken at certain time intervals. The concentrations of sugars and ethanol were determined by high performance liquid chromatography (HPLC).

Analytical method: The chemical composition of the remaining solid after pretreatment by diluted sulphuric acid was determined using the standard Laboratory Analytical Procedures for Biomass Analysis provided by the National Renewable Energies Laboratory[11-13].

The released sugar monomers in the hydrolyzate, as well the concentration of ethanol, were determined by HPLC (Agilent) using a column (BioRad Aminex HPX-87H, 300 mm × 7.8 mm) at 64 ºC and 4 mM H_2SO_4 as eluent at a flow rate of 0.6 mL min^{-1}.

Estimated ethanol production: The yields of rape straw and bioethanol production for 2012 and 2013 were estimated based on the yields of rape straw from a city in southwest China between 2007 and 2011.

RESULTS AND DISCUSSION

Composition of remaining solid after pretreatment by diluted sulphuric Acid: In order to calculate the hydrolysis rate and the yield of bioethanol, the composition of the remaining solid after pretreatment was analyzed and the results are shown in Table-1.

TABLE-1
COMPOSITION OF RESIDUAL SOLID AFTER PRETREATMENT

Component	Dry weight (%)
Cellulose	56.9
Glucose	63.2
Hemicellulose	7.8
Xylose	8.7
Klason lignin	22.7
Ash	7.7
Others	4.9

Table-1 showed that the cellulose content in the pretreated rape straw improved by 20 % compared with the raw material (data not shown) because of the hydrolysis of hemicelluloses during pretreatment.

The theoretical yield of ethanol was based on the assumption that all glucose found in the remaining solid after pretreatment could be converted into ethanol, with a theoretical yield of 0.51 g ethanol/g glucose. According to the glucan content, the theoretical yield of ethanol was estimated to be 32.2 g ethanol/100 g dry matter.

Effect of different solids contents on enzymatic hydrolysis: During enzymatic hydrolysis, high solids content means a high concentration of glucose, which inhibits hydrolysis. Therefore, finding suitable solids content is very important for decreasing the cost of the process.

Fig. 1 shows that the final concentration of glucose increases with an increase of solids content. The final concentration of glucose was 34.0, 49.3, 62.4, 76.2 and 83.5 g/L, when the solids content was 10, 15, 20, 25 and 30 %, respectively. The results showed that the hydrolysis rate was the highest during the first 6 h for all solids contents. The hydrolysis rate was 3.08 g glucose/L h during the first 6 h for 10 % solids content, but it decreased to 0.37 g/L h during the following 42 h. The reason for the decrease in hydrolysis rate was the accumulation of glucose and cellbiose during enzymatic hydrolysis. Previous research proved that cellbiose is a product of cellulose hydrolysis that significantly inhibits enzymatic hydrolysis. The results of chromatogram analysis showed that cellbiose could accrete on tryptophan residues, which were close to the active position of cellobiohydrolase. This created the "steric effect," which inhibited the molecular chain of cellulose from entering the active position. Moreover, combined with cellbiose, the molecular conformation of cellobiohydrolase made it difficult for the microfibril to be apart from the cellulose, forming "invalid adsorption"[14,15].

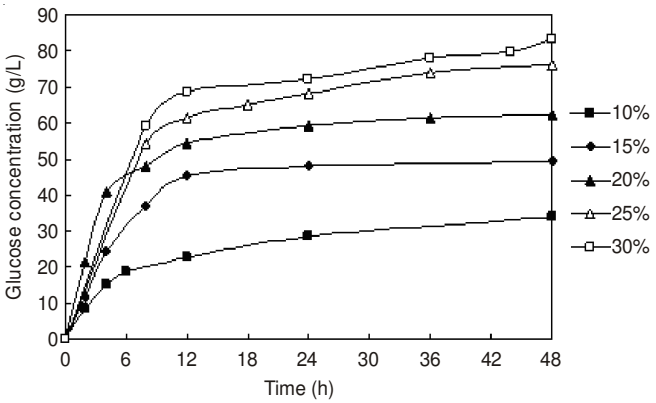

Fig. 1. Effects of different solids contents on glucose concentration

Fig. 2 shows the effects of different solids contents on glucose recovery. The results showed that increasing the solids content decreases the glucose recovery. When the solids content varied in a suitable range from 10-25 %, the glucose recovery was not affected remarkably (70-73 %). Meanwhile, when the solids content was increased to a certain degree, the glucose recovery was affected significantly. Although the final glucose concentration for 30 % solids content was the highest out of all the experiments, the lowest glucose recovery proved that it was not a good choice for enzymatic hydrolysis. During enzymatic hydrolysis, higher solids content within a certain range accelerated the hydrolysis rate because high solids content can support greater contact of enzymes with substrate. However, with a further increase of the solids content, the volume of the liquid decreased which was a disadvantage for the diffusion of the reactant and caused enzymatic hydrolysis inhibition.

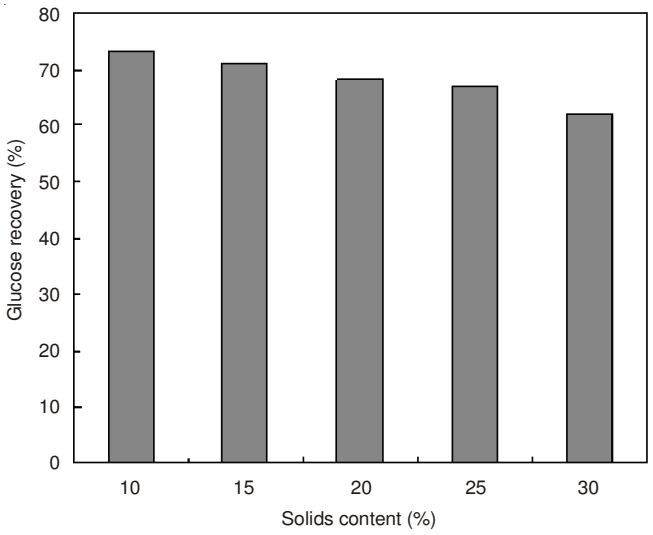

Fig. 2. Effects of different solids contents on glucose recovery

Effect of different solids contents on fermentation process: After enzymatic hydrolysis, fermentation was carried out to produce bioethanol. A high glucose concentration caused a high concentration of bioethanol. Fig. 3 shows the effects of different solids contents on ethanol concentration. When the solids content was fixed at 10, 15, 20, 25 and 30 %, the final glucose concentration after enzymatic hydrolysis was 34.0, 49.3, 62.4, 76.2 and 83.5 g/L, respectively. According to the transformation coefficient, the theoretical bioethanol concentration would be 17.3, 25.1, 31.8, 38.9 and 42.6 g/L, respectively. The final bioethanol concentration for all solids contents actually exceeded the theoretical values. The values were 22.4, 31.5, 43.1, 46.0 and 49.7 g/L, respectively. The reason for this phenomenon was that during the fermentation process, enzymatic hydrolysis was still occurring so that some remaining solid was hydrolyzed during fermentation, which caused higher bioethanol concentrations compared with the theoretical values. Because of the difference in temperature between enzymatic hydrolysis and fermentation, the hydrolysis rate of cellulose was low during the fermentation process.

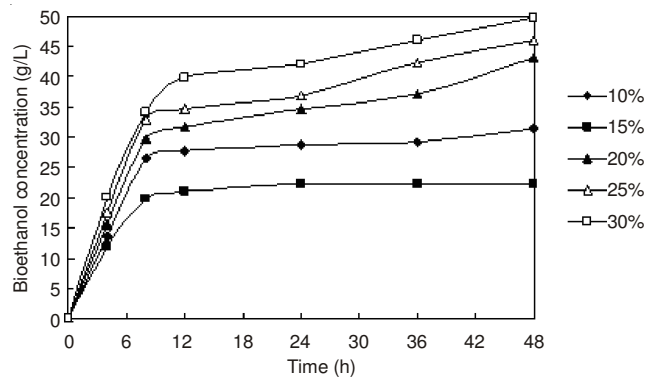

Fig. 3. Effects of different solids contents on ethanol concentration

During the fermentation process, the concentration of bioethanol accumulated. However, a high concentration of bioethanol can inhibit fermentation, which decreases the fermentation rate. Fig. 3 showed that during the first 8 h, the production rate of bioethanol was very high, which was 2.49, 3.31, 3.71, 4.10 and 4.28 g/L h when the solids content was fixed at 10, 15, 20, 25 and 30 %, respectively. After 8 h, the production rate of bioethanol became slower. Yeast and other ethanol producers are known to suffer from ethanol inhibition. Kadar *et al.*[16] reported that *S. cerevisiae* ATCC 26602 could be adapted to higher concentration of 5.2 vol % (*ca.* 50 g/L) final ethanol concentration. Cazetta *et al.*[17] used *Z. mobilis* to ferment molasses and a high ethanol concentration of 55.8 g/L could be obtained. In this research, the final ethanol concentration was lower than the yeast.

Fig. 4 showed the concentration of glucose and xylose during fermentation when the solids content was fixed at 10 %. The results showed that most of the glucose was consumed by yeast in the first 8 h, which was consistent with the results of bioethanol production process. After 8 h, hydrolysis was still occurring along with fermentation, although the rate was very slow due to the lower optimum temperature of fermentation (37 ºC) compared to hydrolysis (52 ºC). The xylose concentration was stable during fermentation, which showed that this yeast could not use xylose.

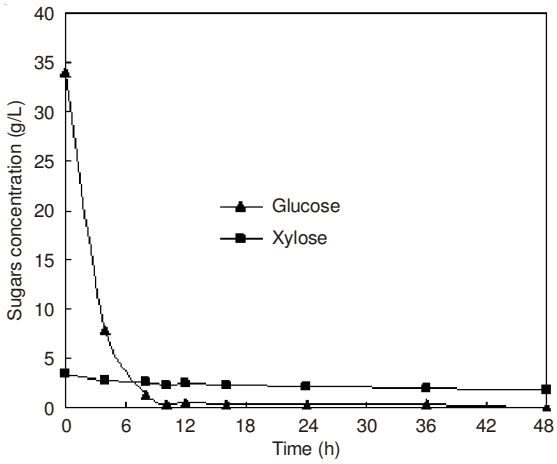

Fig. 4. Concentration of glucose and xylose during fermentation (10 % solids content)

Estimation of bioethanol yield: A simple mass balance was made for each step and is shown in Fig. 5. During the pretreatment process, 26.4 % of the solids were dissolved and 138 kg xylose/1000 kg raw material and 50.5 kg glucose/1000 kg raw material were released. Following pretreatment, a 48 h enzymatic hydrolysis and fermentation process at 25 % solids content was carried out; 135.4 kg bioethanol was obtained, which related to a 65 % theoretical yield.

Fig. 5 concluded that 1000 kg raw rape straw could produce 116.6 kg bioethanol. The yield of rape straw from a city in southwest China between 2007 and 2011 is shown in Fig. 6.

The regression analysis method was used and the relationship of the yield of rape straw from 2007 to 2011 is shown in eqn. 1.

$$y = 583.44x^4 - 7000x^3 + 28917x^2 - 43500x + 46000 \quad (1)$$

From eqn. 1, the yield of rape straw from 2012 and 2013 could be estimated at 46000 and 48000 tons. According to the results of this research, 5363.6 and 5596.8 tons of bioethanol would be obtained.

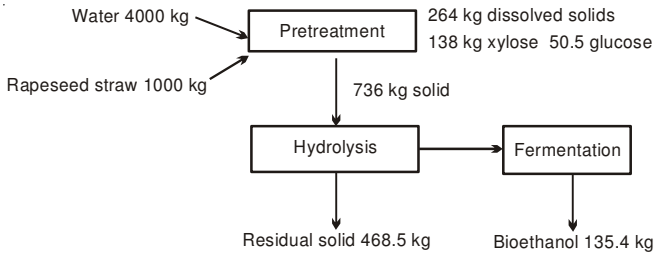

Fig. 5. Mass balance for pretreatment, hydrolysis and fermentation for rape straw (25 % solids content)

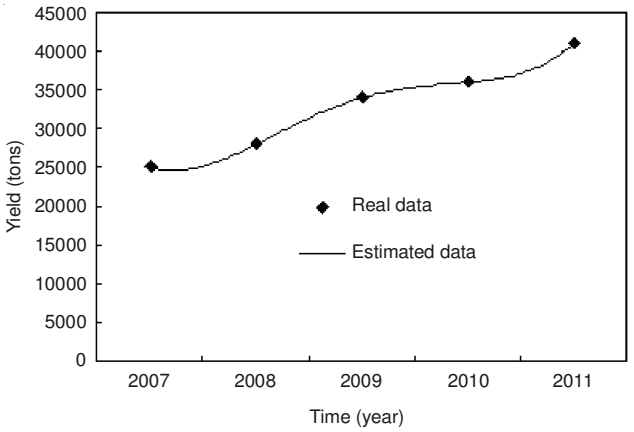

Fig. 6. Relationship of the yield of rape straw and time (year)

Conclusion

The production of bioethanol from lignocellulose is a popular topic in the bioenergy field. In bioethanol production, distillation is a high energy-consuming process because the final bioethanol concentration is lower than 3 % after fermentation. In this work, enzymatic hydrolysis and fermentation were carried out at 25 % solids content, which resulted in a bioethanol concentration of 46 g/L. The higher bioethanol concentration will decrease the energy input of the distillation process. Based on the yields of rape straw from 2007-2011, the regression analysis method was used and the yields of rape straw from 2012 and 2013 were estimated, which were used to calculate the bioethanol yield. According to this estimation, 5363.6 and 5596.8 tons of bioethanol would be obtained. An increase in bioethanol production with lower energy input would certainly be a big step forward to the reservation of natural energy resources and sustained economic development.

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REFERENCES

1. M. Han, S.K. Moon, Y. Kim, Y. Kim, B. Chung and G.W. Choi, *Biotechnol. Bioprocess. Eng.*, **14**, 606 (2009).
2. D. Karakashev, A.B. Thomsen and I. Angelidaki, *Biotechnol. Lett*., **29**, 1005 (2007).
3. M.H. Han, S.K. Moon and Y. Kim, *Biotechnol. Bioprocess. Eng*., **14**, 606 (2009).
4. G.Y. Wei, G. Wa, I.H. Jin, S.Y. Yoo, J.H. Lee, C.H. Chung and W.J.W. Lee, *Biotechnol. Bioprocess. Eng*., **14**, 828 (2009).
5. X.B. Lu, Y.M. Zhang, J. Yang and Y. Liang, *Chem. Eng. Technol*., **30**, 938 (2007).
6. Y. Zhao, Y. Wang, J.Y. Zhu, A. Ragauskas and Y. Deng, *Biotechnol. Bioeng*., **99**, 1320 (2008).
7. Y. Yang, K. Boots and D. Zhang, *Sustainability*, **4**, 92 (2012).
8. A. Wingren, M. Galbe and G. Zacchi, *Biotechnol. Prog.,* **19**, 1109 (2003).
9. K. Ohgren, O. Bengtsson, M.F. Gorwa-Grauslund, M. Galbe, B. Hahn-Hagerdal and G. Zacchi, *J. Biotechnol.,* **126**, 488 (2006).
10. X.B. Lu, Y.M. Zhang and I. Angelidaki, *Bioresour. Technol.*, **100**, 3048 (2009).
11. National Renewable Energy Laboratory (NREL), NREL, Golden, CO, USA (1994).
12. National Renewable Energy Laboratory (NREL), NREL, Golden, CO, USA (1996).
13. National Renewable Energy Laboratory (NREL), NREL, Golden, CO, USA (1998).
14. S.B. Lee, K.H. Park and J.F. Robyt, *Carbohydr. Res.*, **331**, 13 (2001).
15. P. Väljamäe, V. Sild, G. Pettersson and G. Johansson, *Eur. J. Biochem.*, **253**, 469 (1998).
16. Z. Kadar, S.F. Maltha, Z. Szengyel, K. Reczey and W.D. Laat, *Appl. Biochem. Biotechnol.*, **136-140**, 847 (2007).
17. M.L. Cazetta, M.A.P.C. Celligoi, J.B. Buzato and I.S. Scarmino, *Bioresour. Technol*., **98**, 2824 (2007).