

Optimum Bioconversion Process of Wheat Straw to Glucose: Ethanol Pretreatment

Lanfeng Hui,* Huangwei Xiong, and Kunlun Du

The research aimed at optimizing the conditions of pretreatment of wheat straw and of enzymatic hydrolysis of the cellulose (ethanol pulp). The ethanol pretreatment process involving acid-catalytic and enzymatic hydrolysis were evaluated for bioconversion of wheat straw to glucose. The influence of the independent process variables on cellulose yields, cellulose contents, lignin contents, and the rate of lignin removed were analyzed over a broad range by the response surface methodology (RSM). The results of the factorial experiment showed that the significant external factors affecting acid-catalytic ethanol pretreatment of wheat straw were ethanol concentration, maximum temperature, acid dosage, and time at maximum temperature. By analyzing the response surface plots, the optimum process parameters for pretreatment were obtained as follows: ethanol concentration 65%, maximum temperature 180 °C, acid dosage 1.2%, and time at maximum temperature 60 min. Pulps with residual klason lignin ranging from 9.27% to 13.56% (w/w) were prepared from wheat straw using the acid-catalytic ethanol pretreatment process and were evaluated for bioconversion using enzymatic hydrolysis of the cellulose fraction to glucose. The effects of temperature, pH value, time of enzymolysis, and cellulase dosage on the hydrolysis yield of cellulose were separately examined.

Keywords: Response surface methodology; Wheat straw; Acid-catalytic ethanol pretreatment; Cellulase; Enzymatic hydrolysis

Contact information: Tianjin Key Laboratory of Pulp & Paper, Tianjin University of Science and Technology, Tianjin 300222, China; *Corresponding author: hlfeng@tust.edu.cn

INTRODUCTION

During the past few decades, global warming has emerged as a major political and scientific issue. It is likely that this is due primarily to increased emissions of greenhouse gases formed by burning fossil fuels, concurrent with increased energy consumption (Dickerson and Johnson 2004). Various studies have shown that ethanol or ethanol-blended transportation fuels produce less harmful emissions, while the production of ethanol from biomass has the advantage of displacing transportation fuels derived from oil with a fuel obtained from a renewable resource (Bergeron 1996; Galbe and Zacchi 2002). Lignocellulose is an abundant and renewable feedstock. There is an estimated annual worldwide production of 10 to 50 billion dry tonnes, accounting for about half of the global biomass yield (Claassen *et al.* 1999).

Wheat straw is an important source of lignocellulosic biomass. However, in China, it is typically incinerated for power generation or used to make other low value-added products.

Pretreatment is an important stage for practical cellulose conversion processes. It is required to change the structure of cellulosic biomass and to make cellulose more

accessible to the enzymes, so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with high yield. Various pretreatment options have been used to fractionate, solubilize, hydrolyze, and separate cellulose, hemicellulose, and lignin moieties. These different processes usually exploit a combination of chemical and physical treatments that serve to render the lignocellulosics more receptive to subsequent enzymatic hydrolysis (McMillan 2011).

Hydrolysis of cellulose to glucose can be achieved using either inorganic acids or cellulolytic enzyme. Chemical hydrolysis of biomass is relatively efficient and economic. However, it generates fermentation inhibitors (Leathers 2003). On the other hand, enzymatic hydrolysis, despite its relatively slow rate, is a biocompatible and environmentally friendly option. Thus enzyme hydrolysis is more attractive than others. The research described below is concerned with the evaluation of the organosolv process for the bioconversion of wheat straw. In this study, an acid-catalytic ethanol pretreatment process was used to pretreat the wheat straw. The process parameters were studied by using response surface methodology (RSM). Enzymatic hydrolysis was used to convert the cellulose to glucose.

EXPERIMENTAL

Materials and Methods

Pretreatment

The wheat straw used in this study was collected from the field at the harvest of the wheat crop growing at the farm of Hebei province, China. After being stored for three months, the wheat straw was cut into smaller pieces of approximately 30 mm length and sieved using screen of 14 mesh size. The cooking liquor was prepared by mixing water with industrial ethyl alcohol (concentration, 93% to 95%) from the market.

Wheat straw pulp was attained by acid-catalytic ethanol pretreatment process in a 15 L cooking reactor. A range of processing conditions (ethanol concentration, 45% to 65%; maximum temperature, 175 to 195 °C; time at maximum temperature, 45 to 75 min; sulfuric acid dosage 0.8% to 1.2%; the ratio of liquor to wood, 6:1) were chosen to provide pulps with residual lignin contents varying from 9.27% to 13.56% (w/w).

Design of experiments

In this study, a Box-Behnken design (BBD) was used to evaluate the main and interaction effects of the factors: ethanol concentration (X1), cooking temperature (X2), catalyst dosage (X3), and time at maximum temperature (X4). The range and levels of the variables investigated are given in Table 1, whereas the experimental designs are presented in Table 2. A polynomial quadratic equation was fitted to evaluate the effect of each independent variable to the response:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{i < j} \beta_{ij} x_i x_j + \varepsilon \quad (1)$$

In this expression Y is the response; x_i and x_j are the coded independent variables, and β_0 , β_i , β_{ii} , and β_{ij} are intercept, linear, quadratic, and interaction constant coefficients,

respectively. Response surfaces and contour plots were developed using the fitted quadratic polynomial equation obtained from regression analysis.

Table 1. Independent Variables Used in the BBD and Actual Factor Levels Corresponding to Coded Factor Levels

Independent Variables	Code	Actual factor level at coded factor levels		
		-1	0	1
ethanol concentration (%)	X1	45	55	65
maximum temperature (°C)	X2	175	185	195
catalyst dosage (%)	X3	0.8	1.0	1.2
time at max. temperature (min)	X4	45	60	75

Table 2. Experimental Design

Run	X1	X2	X3	X4
1	45	175	1.0	60
2	45	195	1.0	60
3	65	175	1.0	60
4	65	195	1.0	60
5	55	185	0.8	45
6	55	185	0.8	75
7	55	185	1.2	45
8	55	185	1.2	75
9	45	185	1.0	45
10	45	185	1.0	75
11	65	185	1.0	45
12	65	185	1.0	75
13	55	175	0.8	60
14	55	175	1.2	60
15	55	195	0.8	60
16	55	195	1.2	60
17	45	185	0.8	60
18	45	185	1.2	60
19	65	185	0.8	60
20	65	185	1.2	60
21	55	175	1.0	45
22	55	175	1.0	75
23	55	195	1.0	45
24	55	195	1.0	75
25	55	185	1.0	60
26	55	185	1.0	60
27	55	185	1.0	60
28	45	175	1.0	60
29	45	195	1.0	60

Enzymatic hydrolysis

Commercial cellulase was provided by Novozyme (Tianjin, China). Cellulase activity was determined using the filter paper assay and was expressed as filter paper activity (FPA). Batch hydrolysis experiments were routinely conducted at 2% (w/w) cellulose consistency in 50 mL of 100 mM sodium acetate buffer, pH 4.8. Reaction mixtures were incubated at 160 rpm in a rotary shaker and sampled periodically for sugar analysis using SBA-40C Biosensing analyzer.

Analytical Methods

The lignin content of the wheat straw before and after pretreatment was determined using the following TAPPI standards: T 17 wd-70 for cellulose content, T 222 om-02 for lignin content.

The following equation was used to evaluate the cellulosic yield:

$$\text{Cellulose yield (\%)} = \frac{\text{Cellulose content of pretreated solids (\%)} \times \text{Yield}}{\text{Cellulose content of material (\%)}} \times 100\% \quad (2)$$

RESULTS AND DISCUSSION

Ethanol Pretreatment

The response surface plots of each of the two factors and their interactive effect on the cellulose contents, cellulose yield, lignin contents, and the rate of lignin removal were obtained by use of an SAS package (Fig. 1(a-d) and Fig. 2(a-d)). The four independent variables designated as X1, X2, X3, and X4, respectively, are ethanol concentration (X1), cooking temperature (X2), catalyst dosage (X3), and time at maximum temperature (X4).

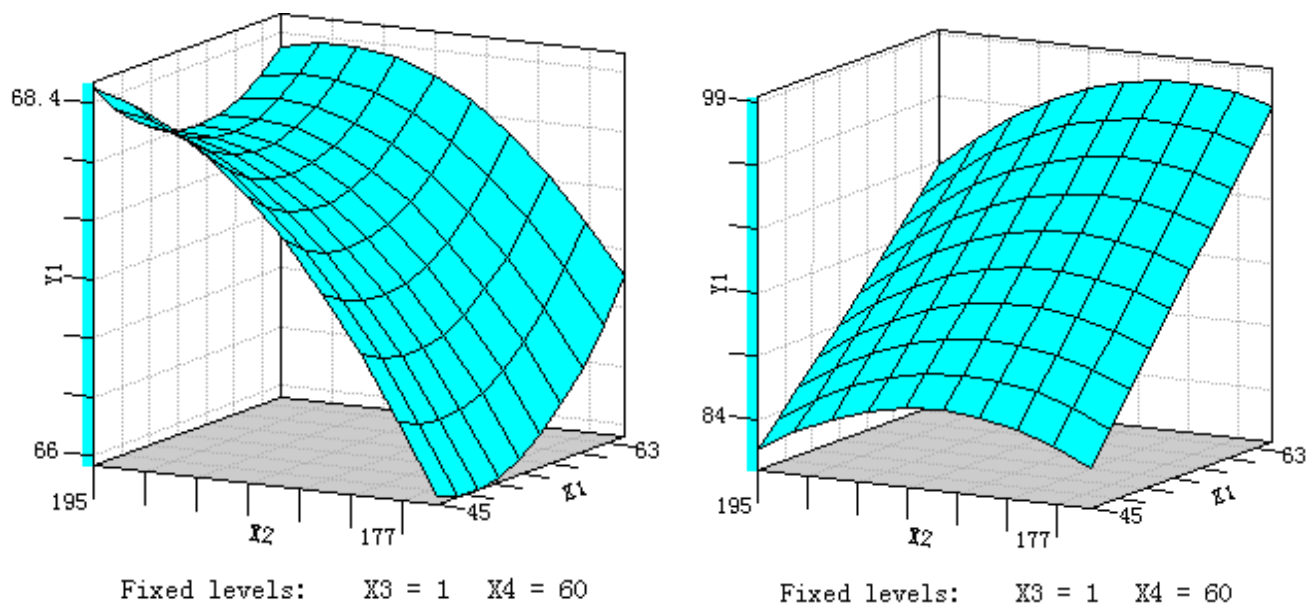


Fig. 1. (a) Response surface of ethanol concentration (X1) vs. maximum temperature (X2) on the cellulose contents

Fig. 1. (b) Response surface of ethanol concentration (X1) vs. maximum temperature (X2) on the cellulose yield

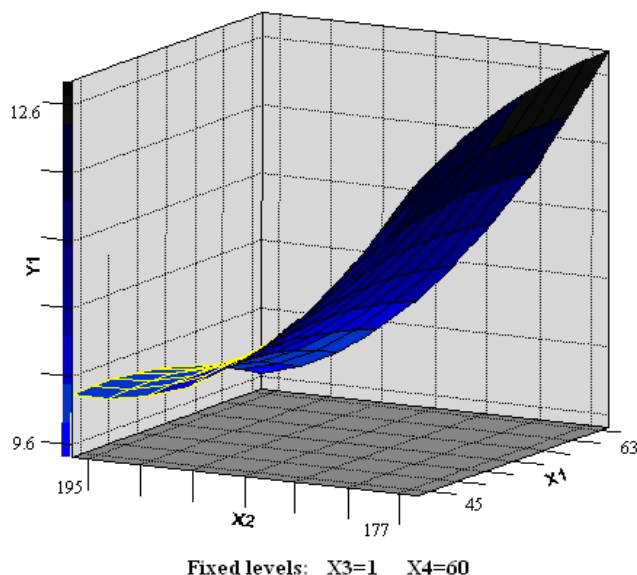


Fig. 1. (c) Response surface of ethanol concentration (X1) vs. maximum temperature (X2) on the lignin contents

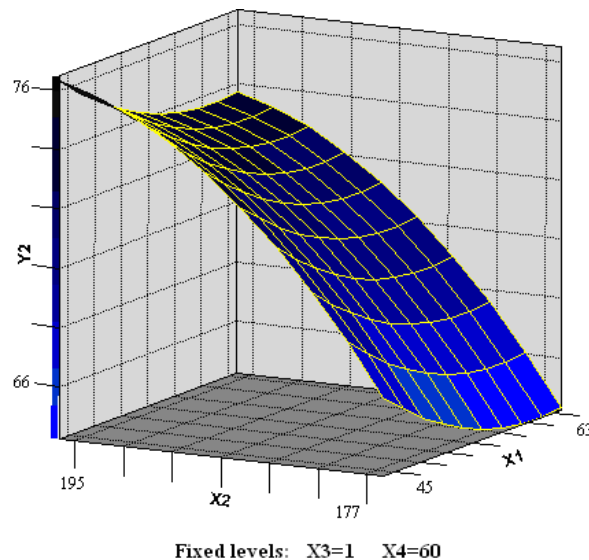


Fig. 1. (d) Response surface of ethanol concentration (X1) vs. maximum temperature (X2) on the extent of lignin removed

Effect of maximum temperature and ethanol concentration

From Fig. 1(a-d), it follows that changes in the cellulose contents, cellulose yield, lignin contents, and the rate of lignin removed with maximum temperature and ethanol concentration can be estimated by keeping acid dosage and time at maximum temperature constant. Thus, with a center acid dosage (1.0%) and a center time at maximum temperature (60 min) the maximum temperature and the ethanol concentration both had a great impact on all of the response variables.

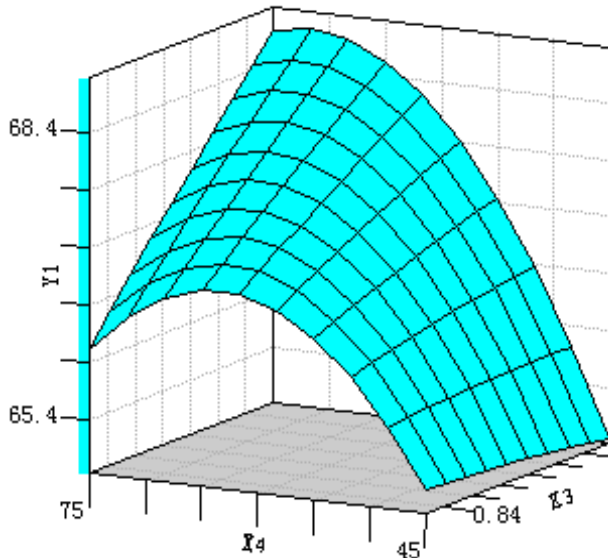
As Fig. 1 shows, the cellulose contents, cellulose yields, and the rate of lignin removal increased and the lignin contents decreased quickly at the beginning of the increasing maximum temperature. However, this tendency of cellulose yield stopped and started to go to in the opposite direction when the maximum temperature reached approximately 180 °C. However the cellulose contents increased with the maximum temperature. All the figures above show that the higher the ethanol concentration was, the better the values of respond variables were. So the ethanol concentration was determined at 65% as an optimum condition in the next experiment. In this case it could be supposed to accelerate the removal of lignin and the dissolution of carbohydrate.

From the foregoing, it thus follows that all the responding variables depended markedly on ethanol concentration and maximum temperature. Accordingly, the optimum maximum temperature and ethanol concentration were judged to be 180 °C and 65%, respectively.

Effect of time at maximum temperature and acid dosage

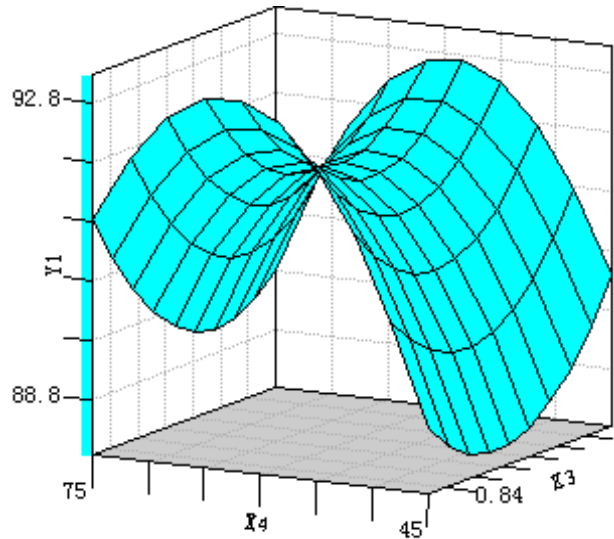
From Fig. 2, it follows that changes in the cellulose contents, cellulose yield, lignin contents, and the extent of lignin removed with acid dosage and time at maximum temperature can be estimated by keeping ethanol concentration and maximum temperature constant. Thus, with a center maximum temperature (185 °C) and a center

ethanol concentration (55%), the acid dosage and time at maximum temperature had little effect on all of the response variables.



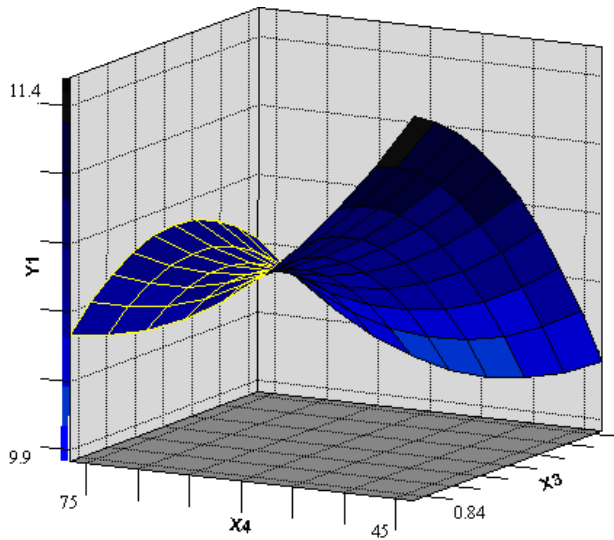
Fixed levels: $X_1 = 55$ $X_2 = 185$

Fig. 2. (a) Response surface of acid dosage (X_3) vs. time at maximum temperature (X_4) on the cellulose contents



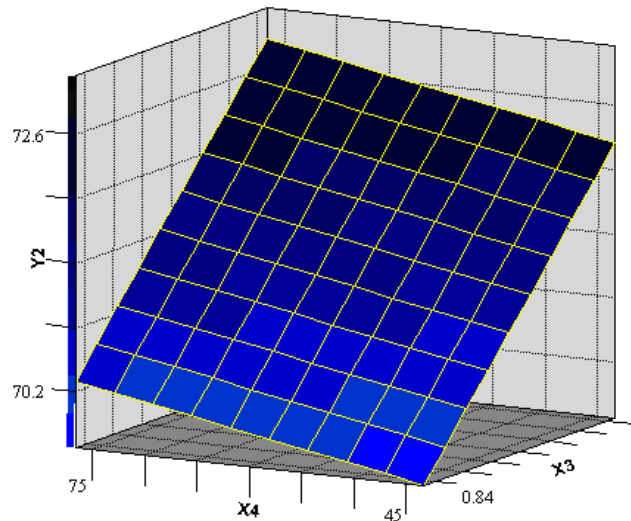
Fixed levels: $X_1 = 55$ $X_2 = 185$

Fig. 2. (b) Response surface of acid dosage (X_3) vs. time at maximum temperature (X_4) on the cellulose yield



Fixed levels: $X_1=55$ $X_2=185$

Fig. 2. (c) Response surface of acid dosage (X_3) vs. time at maximum temperature (X_4) on the lignin contents



Fixed levels: $X_1=55$ $X_2=185$

Fig. 2. (d) Response surface of ethanol concentration (X_3) vs. maximum temperature (X_4) on the extent of lignin removed

Figure 2(a) shows that the amount of removed lignin increased with increasing time, but the relation of cellulose contents and the cellulose yield showed a different trend. As time increased, the cellulose contents and cellulose yield increased quickly until arrived the maximum value at 60 h. Then the cellulose contents and cellulose yield

decreased. But the effect of acid dosage was quite different with the time at maximum temperature. The cellulose contents and cellulose yield went up with increasing acid dosage.

From the foregoing, it thus follows that all the dependent variables were affected inconspicuously by changes in time at maximum temperature and acid dosage. A long duration and high acid dosage decreased the cellulose yield and the cellulose contents owing to the dissolution of carbohydrates. Thus, the optimum time at maximum temperature and acid dosage were judged to be 1.2% and 60 min, respectively.

In conclusion, the optimum process parameters for pretreatment were obtained as follows: ethanol consistency 65%, maximum temperature 180 °C, acid dosage 1.2%, and time at maximum temperature 60 min, by analyzing the response surface plots. In such a case, ethanol pulps with high cellulose contents and low lignin contents were attained, which benefited the subsequent hydrolysis process.

Enzymatic Hydrolysis of Ethanol Pulp

Effect of temperature and pH on the hydrolysis yield

Temperature and pH are important factors in the enzymatic hydrolysis process, not only affecting the speed of the reaction of enzymatic hydrolysis, but also having great impact on the enzymatic activity. Therefore, the preliminary experiments were performed in order to optimize hydrolysis condition regarding temperature and pH (Fig. 3). The hydrolysis yields of the pretreated wheat straw fibers in Fig. 3 were obtained at 50 °C, 2%(w/w) consistency with varying pH and temperature, at 27 IU /g cellulose.

The highest hydrolysis yield (47%) was achieved at 50 °C. At all different pH values tested, pH 4.5 produced a higher hydrolysis yield. Therefore, in the subsequent experiments, the optimal hydrolysis conditions, pH 4.5, and temperature 50 °C were used for hydrolysis of the pretreated wheat straw fiber slurry.

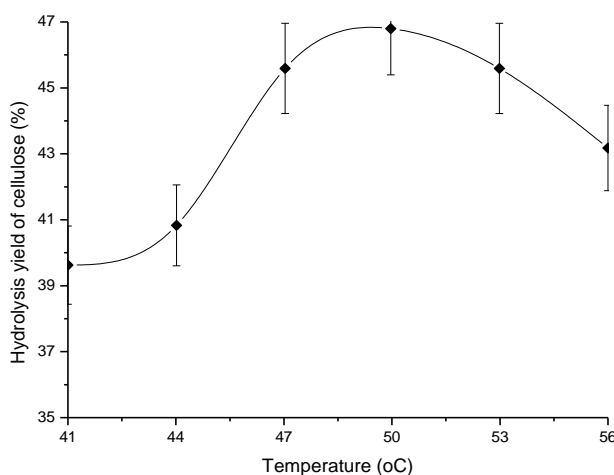


Fig. 3. (a) The effect of temperature on hydrolysis yield of cellulose

Effect of enzyme loading on the hydrolysis yield

For an industrial process to be more attractive, it is preferable to hydrolyze high dry mass content without separation of the filtrate from the solids, and in doing so to obtain a high ethanol concentration, thus reducing the cost of subsequent distillation. Therefore, the effect of enzyme loadings on hydrolysis yield of wheat straw was

assessed. The hydrolysability of the pretreated wheat straw fiber solids, performed at 2% (w/w) consistency with varying enzyme loadings (9, 18, 27, 36, 45, and 54 IU /g cellulose), is shown in Fig. 4.

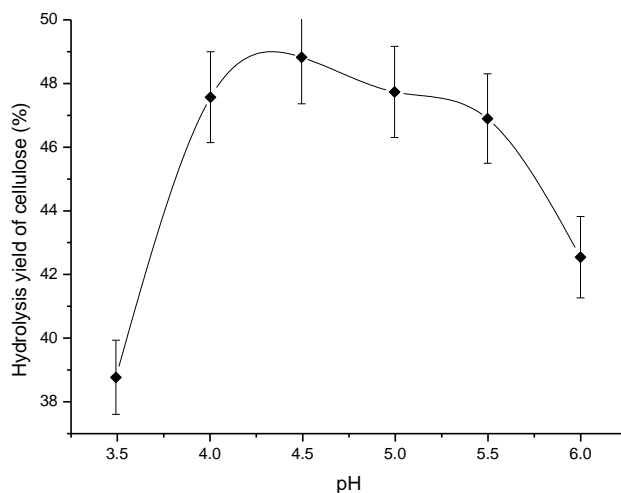


Fig. 3. (b) The effect of pH values on hydrolysis yield of cellulose

Figure 4 shows that the hydrolysis yield of cellulose increased with increasing enzyme dosage, especially in the initial phase. However, the hydrolysis yield almost remained constant when the enzyme loading was more than 27 IU /g cellulose. Thus it seemed that it is reasonable to enhance the yield and rate of the hydrolysis to a certain extent by increasing the dosage of the cellulase in the process. Unfortunately, this might significantly increase the cost of the overall process (Gregg and Saddler 1996). Therefore, in the case of enzymatic hydrolysis of pretreated wheat straw fiber solids, it is possible to use as little as 27 IU /g cellulase loading to achieve high hydrolysis yield (55%).

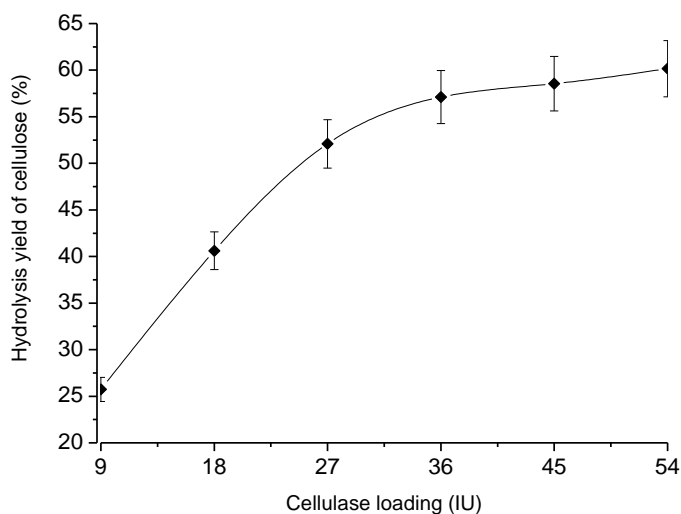


Fig. 4. The effect of enzyme loading on hydrolysis yield of cellulose

Effect of cellulose consistency on the hydrolysis yield

To produce higher glucose concentrations, hydrolysis yields at different cellulose consistency were examined under 27 IU/g cellulose loading (Fig. 5). Due to the high viscosity of the reactants at higher consistency (more than 2%), glass balls were added to facilitate mixing.

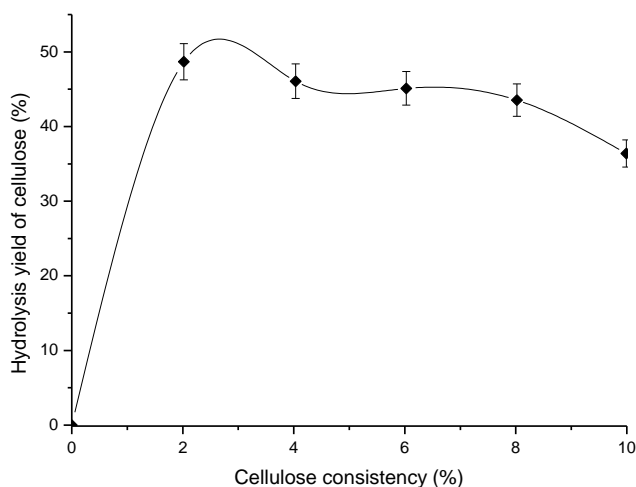


Fig. 5. The effect of cellulose consistency on the hydrolysis yield of cellulose

As observed by Schwald *et al.* (1989) and Gregg and Saddler (1996), both the batch enzymatic hydrolysis yields and the initial hydrolysis rate were influenced by the concentration of the substrate. As Fig. 5 shows, maximum hydrolysis yield (52%) was achieved after about 24 h using 27 IU/g cellulose. After that, the hydrolysis decreased gradually at 4.0% to 8.0% consistency. When the cellulose consistency reached 10%, the hydrolysis yield decreased rapidly. Thus there is an inverse relationship between the concentration of the substrate and the enzymatic hydrolysis yield at high consistency (more than 2%).

However, economic studies have shown that substrate concentrations have a major impact on process costs (Nguyen and Saddler 1991). Accordingly, to make the biomass to ethanol process economically feasible, *i.e.*, increasing the sugar concentration available for further fermentation process, higher solids concentrations should be used during the enzymatic hydrolysis process.

Effect of time on the hydrolysis yield

The effect of hydrolysis time on hydrolysis yield of wheat straw fiber was also investigated. The pretreated wheat straw fiber was subjected to enzymatic hydrolysis at 27 IU /g cellulose for 70 h (Fig. 6).

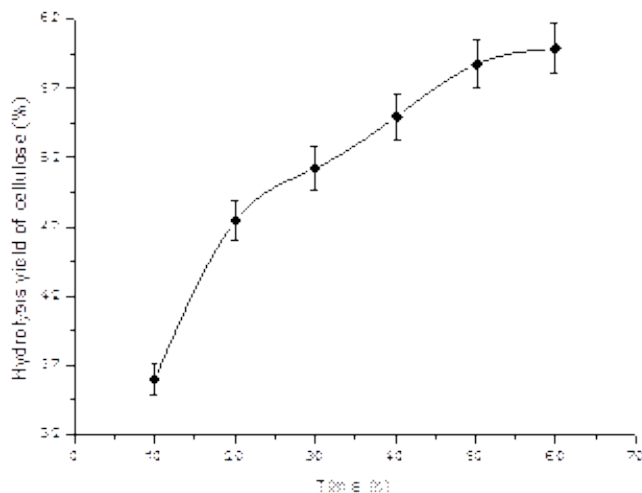


Fig. 6. The effect of time on the hydrolysis yield of cellulose

One of the factors affecting enzymatic hydrolysis of lignocellulosic biomass, apart from enzyme inactivation due to denaturation, end-product inhibition, or unproductive binding of active enzyme to the substrate, is substrate transformation (Eriksson *et al.* 2002). Increased substrate recalcitrance has been proposed to explain the declining rates of hydrolysis. Easily hydrolyzed parts of the cellulose substrate might be hydrolyzed in the early stage, leaving recalcitrant parts for later stages. The results (Fig. 6) indicated that the hydrolysability of the wheat straw fiber solids increased with the increasing of hydrolysis time, reaching almost maximum hydrolysis yield (60%) for 27 IU/g cellulose loading at 50 h. The extended hydrolysis time did not markedly improve hydrolysability of the slurry during the increase of substrate recalcitrance.

To conclude, the optimal reaction conditions found by measuring the glucose contents of were pH 4.5, temperature 50 °C, time 50 h, and an enzyme loading of 27 filter paper units of cellulose/g cellulose. With such process variables, the maximum hydrolysis yield of cellulose (60%), namely 87% glucose conversion yield, was attained.

CONCLUSIONS

Bioethanol was obtained from acid-catalysis of wheat straw. During the treatment, fiber with high cellulose contents and low lignin contents were obtained, employing a relatively short time at maximum temperature (about 60 min) and low cooking temperature (about 180 °C) under the condition of acid dosage (1.2%) and of ethanol consistency (65%) used. The pulp samples were shown to be susceptible to attack by a commercial cellulase preparation. Approximately 87% of the total cellulose in the untreated wheat straw was recovered as monomeric glucose after hydrolysis of the solid fraction using a low enzyme loading (27 filter paper units of cellulose/g cellulose), pH 4.5, temperature 50 °C, and time of hydrolysis 50 h.

ACKNOWLEDGMENTS

The financial support for this project by the National Nature Science Foundation of China (NSFC, No. 31000283, 21076160 and 31270631) and Tianjin Municipal Science and Technology Commission (Grant No. 12ZCZDZX01100) are gratefully acknowledged.

REFERENCES CITED

- Bergeron, P. (1996). In: *Handbook on Bioethanol*, Taylor & Francis, Washington, DC.
- Claassen, P. A. M., van Lier, J. B., Contreras, A. M. L., van Niel, E. W. J., Sijtsma, L., Stams, A. J. M., de Vries, S. S., and Weusthuis, R. A. (1999). "Utilisation of biomass for the supply of energy carriers," *Appl. Microbiol. Biotechnol.* 52, 741-755.
- Dickerson, P., and Johnson, P. (2004). "World population information," the Population Reference Bureau, Washington, DC.
- Eriksson, T., Karlsson, J., and Tjerneld, F. (2002). "A model explaining declining rate in hydrolysis of lignocellulose substrates with cellobiohydrolase I (Cel7A) and endoglucanase I (Cel7B) of *Trichoderma reesei*," *Applied Biochemistry and Biotechnology* 101, 41-60.
- Galbe, M., and Zacchi, G. (2002). "A review of the production of ethanol from softwood," *Appl. Microbiol. Biotechnol.* 59, 618-628.
- Gregg, D. J., and Saddler, J. N. (1996). "Factors affecting cellulose hydrolysis and the potential enzyme recycle to enhance the efficiency of an integrated wood to ethanol process," *Biotechnol. and Bioengineering* 51, 375-383.
- McMillan, J.D., Jennings, E. W., Mohagheghi, A., and Zuccarello, M. (2011). "Comparative performance of precommercial cellulases hydrolyzing pretreated corn stover," *Biotechnol Biofuels* 4, 29
- Leathers, T. D. (2003). "Bioconversions of maize residues to value-added coproducts using yeast-like fungi," *FEMS Yeast Research* 3, 133-140.
- Nguyen, Q. A., and Saddler, J. N. (1991). "An integrated model for the technical and economic evaluation of an enzymatic biomass conversion process," *Bioresource Technol.* 35, 275-282.
- Schwald, W., Breuil, C., Brownell, H. H., Chan, M., and Saddler, J. N. (1989). "Assessment of pretreatment conditions to obtain fast complete hydrolysis on high substrate concentrations," *Applied Biochem. and Biotechnol.* 20/21, 29-44.

Article submitted: January 16, 2014; Peer review completed: March 3, 2014; Revised version received and accepted: April 8, 2014; Published: April 15, 2014.