

## STUDY ON THE OPTIMUM PROCESS OF ACID-CATALYTIC ETHANOL PRETREATMENT OF CHINESE TRIPLOID POPLAR TO ENHANCE SUGAR RECOVERY BY HYDROLYSIS

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Organosolv pretreatment was applied to Chinese triploid poplar using the acid-catalysis ethanol method to enhance sugar recovery in enzymatic hydrolysis. A Box-Behnken design (BBD) was used to optimize the effect of temperature, ratio of wood to liquor, ethanol concentration, and holding time on the pretreatment process. All variables except for lignin content were found to significantly affect the cellulosic yield as well as crystallinity index (Crl). Quadratic polynomial equations were used to model on the cellulosic yield, Crl, and lignin content for a regression analysis, using response surface methodology (RSM). The optimum process of organosolv pretreatment of Chinese triploid poplar was found to be: temperature: 185 °C, ratio of wood to liquor: 1 to 4.2, ethanol concentration: 45%, and holding time: 41 min. The solid pretreated under optimum process conditions was evaluated for bioconversion using enzymatic hydrolysis of the cellulose fraction to glucose. Approximately 87% of the total cellulose in the poplar was recovered as monomeric glucose after hydrolysis of the solid fraction.

*Keywords:* Chinese triploid poplar; Organosolv pretreatment; RSM; Enzymatic hydrolysis

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### INTRODUCTION

Cellulosic ethanol, which has been the subject of significant technological progress (Lynd et al. 1996), is produced from lignocellulose biomass, which is a low-cost, plentiful feedstock that does not have the ethical concerns associated with the use of potential food resources (Rass-Hansen et al. 2007). Utilization of such agricultural residues for industrial products has potential advantages. It is even more important for countries that lack abundant wood fiber (Rainey et al. 2009).

The Chinese triploid poplar is characterized as fast-growing, moisture-loving, and shade-intolerant medium to large trees with a short life span. The chemical composition of poplar wood is characterized by its high cellulose content (over 50%) and low lignin content (about 20% or less), making it an attractive biomass for fermentable sugar production (Balatinecz and Kretschmann 2001).

Over the years a number of different methods, including un-catalyzed steam explosion, liquid hot water, and dilute acid have been developed for the pretreatment of

lignocellulosic biomass (Liu and Wyman 2005). The general idea of these methods is to remove or alter the hemicellulose or lignin, resulting in an increase of the surface area (Mosier et al. 2005). Additionally, most of these pretreatment methods require high-temperature or high-pressure reactions and the application of chemicals that may be toxic to the enzymes or the fermentative microorganisms. The removal of these toxic substances is always costly and complicated.

Pretreatment processes utilizing organic solvents as cooking media have a long history and have achieved commercial or pre-commercial scale (Dahlmann and Schroeter 1990; Schroeter et al. 1991). The promise of value-added products from dissolved lignin and hemicelluloses has captured researchers' attention. Ethanol has been used to separate the wood components in order to study them (Sousa et al. 1986). Aqueous ethanol penetrates easily into the structure of freshly cut softwoods and hardwoods (Kleinert 1975a). This results in practically uniform delignification. The fibre separation on ethanol-water pulping is related to the removal of both lignin and hemicellulose fractions. Today it is one of the most promising organic pretreatment chemicals. Generation of high-quality lignin and other potentially valuable co-products (e.g., furfural and acetic acid) from woody feedstocks may be critical to otherwise marginally cost-effective biorefinery schemes for fuel ethanol production (Pan et al., 2005). In many ethanol pretreatments studies small amounts of acids such as sulfuric acid have been used as catalysts (Liu et al. 2008). Delignification of wood by ethanolysis involves cleavage of lignin-lignin or lignin-carbohydrate linkages, or both, and is catalyzed by the presence of hydrogen or hydroxyl ions in the pulping liquor. Ethanol pretreatment has been shown to improve the efficiency of enzymatic hydrolysis of wood (Holtzapple and Humphrey 1984; Skachkov et al. 1991). Therefore, it is safe to believe that the organosolv pretreatment is a unique and promising process. However, there have been few reports on acid-catalytic ethanol pretreatment recently.

RSM is a statistical technique for the modeling and optimization of multiple variables. RSM determines optimum process conditions by combining experimental designs with interpolation by first- or second-order polynomial equations in a sequential testing procedure (Ferreira et al. 2009). RSM has already been successfully applied for the optimization of bioprocesses such as pretreatments (Li et al. 2010; Ma et al. 2009).

Temperature, ratio of wood to liquor, ethanol concentration, and holding time are primary factors of acid-catalysis ethanol pretreatment (Liu et al. 2008). The main factors that influence the enzymatic hydrolysis of cellulose in lignocellulosic feedstocks can be divided in two groups: enzyme-related and substrate-related factors. Substrate-related factors limiting enzymatic hydrolysis are directly connected to the pretreatment employed and mainly studied in this work. Cellulosic yield and lignin content are the main features affecting enzymatic saccharification (Liu et al. 2008).

Crystallinity is believed to be an important feature affecting enzymatic saccharification of cellulose (Kumar et al. 2009). In order to systemically study the optimum process of organosolv pretreatment of Chinese triploid poplar, BBD was applied in this study to investigate the effects of factors on the cellulosic yield, as well as crystallinity index and lignin content. Changes of main chemical components and hydrolysis for pretreated material were also determined to evaluate the influence of organosolv pretreatment.

## EXPERIMENTAL

### Material

The Chinese triploid poplar used in this experiment was supplied by Beijing University of Forest. The size of the wood chips was about 30mm×25mm×10mm, and these were used after being stored for a month.

### Organosolv Pretreatment

Acid-catalysis with ethanol was used for organosolv pretreatment. The cooking liquor was prepared by mixing water with industrial alcohol (concentration, 95%). Sulfuric acid (98%) was used as catalyst, with a dosage of 0.8%. All chemical reagents were analytically pure (Tianjin North Tianyi chemical reagents factory). 100 g (oven dry) of material was filled manually into each 1.5 L vessel of a four-vessel, rotating digester made by Shaanxi University of Science & Technology, Machinery works. After cooking, the vessels were cooled to room temperature in a water bath. Solids and liquor were then separated using nylon mesh. The pulp was washed with 400 mL aqueous ethanol (65%). The pulp was then washed 3 times with water. The washed pulp was homogenized in a pulp washer (L&W, Sweden) for 10 min and passed through a laboratory flat screen with 0.35 mm slits to remove rejects (non-defiberized woodchips and knots). The screened pulp comprises the solids fraction.

### Design of Experiments

The range and levels of the independent variables studied were selected based on a preliminary study and are listed in Table 1. All variables at zero level constitute the center points, and the combination of each of the variables at either its lowest (-1.0) or highest (+1.0) level with the other variables at zero level constitute the axial points. Ranges of processing conditions mentioned above were chosen to provide pulp samples. In this study, a Box-Behnken design (BBD) was used to evaluate the main and interaction effects of the factors: temperature (A), ratio of wood to liquor (B), ethanol concentration (C), holding time (D) on the cellulosic yield, as well as CrI and lignin content. The range and levels of the variables investigated are given in Table 1, whereas the experimental designs with the observed responses and values for cellulosic yield, CrI, and lignin content are presented in Table 2. 29 experiments were performed according to Table 4 to optimize the parameters. Among them, 5 replications were at center points (Run 5, 11, 18, 21 and 24). 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  $\beta_0$ ,  $\beta_i$ ,  $\beta_{ii}$ , and  $\beta_{ij}$  are intercept, linear, quadratic, and interaction constant coefficients, respectively. Response surfaces 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 variable	Code	Actual factor level at coded factor levels		
		-1	0	+1
Temperature (°C)	A	165	175	185
Ratio of wood to liquor	B	1:4	1:6	1:8
Ethanol concentration (%)	C	45	55	65
Holding time (min)	D	30	45	60

**Table 2.** Experimental Design and Results

Run	A	B	C	D	Lignin content	Yield	CrI	Cellulosic yield
					(%)	(%)	O'KI	(%)
1	185	1:6	55	30	7.31	44.15	1.670	80.37
2	175	1:6	65	60	7.25	40.96	1.715	74.38
3	175	1:8	65	45	7.33	43.42	1.694	79.89
4	175	1:4	45	45	7.88	44.81	1.709	76.07
5	175	1:6	55	45	8.18	45.83	1.719	81.03
6	165	1:6	55	45	10.72	42.44	1.733	70.70
7	185	1:6	45	45	6.57	46.41	1.763	82.64
8	165	1:6	45	45	10.64	35.91	1.651	59.53
9	175	1:4	65	45	9.86	42.17	1.719	74.27
10	175	1:6	45	30	8.89	38.97	1.722	67.31
11	175	1:6	55	45	8.43	44.49	1.683	78.41
12	175	1:6	45	30	9.41	43.95	1.680	79.20
13	165	1:6	55	60	9.36	42.74	1.658	72.71
14	185	1:6	65	45	6.97	40.44	1.664	75.33
15	175	1:8	45	45	8.05	37.64	1.715	66.37
16	185	1:4	55	45	6.84	44.27	1.682	79.63
17	165	1:6	55	30	10.06	38.27	1.739	65.28
18	175	1:6	55	45	8.59	45.71	1.712	80.43
19	185	1:6	55	60	5.97	41.41	1.738	76.04
20	165	1:6	65	45	10.57	44.71	1.739	78.16
21	175	1:6	55	45	8.12	44.64	1.706	78.84
22	175	1:4	55	30	8.56	43.80	1.739	75.59
23	175	1:8	55	60	7.29	42.04	1.700	76.20
24	175	1:6	55	45	7.64	44.51	1.708	78.78
25	175	1:4	55	60	8.02	39.66	1.687	69.30
26	175	1:6	45	60	7.54	43.55	1.678	76.00
27	175	1:8	55	30	7.89	38.07	1.704	68.20
28	185	1:8	55	45	6.14	42.80	1.720	79.61
29	165	1:8	55	45	8.92	37.89	1.631	66.08

## Statistical Analysis

The response surfaces of the variables inside the experimental domain were analyzed using Design Expert software (Version 7.0, Stat-Ease Inc., Minneapolis, CA, USA). The analysis of variance (ANOVA) was used to estimate the statistical parameters.

## Analytical Methods

The lignin content of the polar before and after pretreatment was determined using the following TAPPI standards: T 17 wd-70 for cellulose content, T 223 cm-01 for pentosans content, T 222 om-02 for lignin content, and T 211 om-02 for ash content.

An equation was used to evaluate the cellulosic yield:

$$\text{cellulosic yield(\%)} = \frac{\text{cellulose content of pretreated solids(\%)} \times \text{yield}}{\text{cellulose content of raw material(\%)}} * 100\% \quad (2)$$

Fourier transform infrared (FT-IR) spectra of polar were obtained on an FT-IR spectrophotometer (FTIR-650, Tianjin Gangdong Sci.&Tech. Development Co., Ltd) using a KBr (Tianjin JinKe Institute of fine chemical Industry) disc. FTIR spectroscopy methods were used to investigate structural changes on materials and pretreated solids. In the scope of spectral analysis CrI had also been calculated by comparing two different band ratios:

$$\text{O'KI} = \frac{I_{1429} \text{ cm}^{-1}}{I_{893} \text{ cm}^{-1}} \quad (3)$$

The band at 1429  $\text{cm}^{-1}$  appears to be due to  $\text{CH}_2$  symmetrical bending and responds to changes in the environment of the  $\text{C}_6$  group, such as formation or breaking of an intra-molecular H-bond involving  $\text{O}_6$ . The band at 893  $\text{cm}^{-1}$  is the anomeric group frequency in cellulose and hemicellulose and is assigned due to motions of atoms attached to  $\text{C}_1$ . It is sensitive to changes in the cellulose lattice type and amorphous cellulose (Nelson and O'Connor, 1964). In addition to visual examination of the spectra, Lateral Order Index ( $I_{1430}/I_{897}$ ), which gives information about changes of crystallinity, was calculated.

## Enzymatic Hydrolysis

Commercial cellulase was provided by Zaozhuang Jienuo Biotech. Corp., China, with a filter paper activity (FPA) of 43.5 FPU/g. Batch hydrolysis experiments were routinely conducted at 2% (w/w) cellulose consistency in 50 mL 100 mM sodium acetate buffer, pH 4.8, temperature 50 °C, and an enzyme loading of 25 filter paper units of cellulose/g cellulose. Reaction mixtures were incubated at 160 rpm in a rotary shaker and sampled periodically for sugar analysis using Bio-sensing analyzer (SBA-40C, manufactured by Biology Institute of Shandong Academy of Sciences, China). Hydrolysis data are averages from duplicate experiments. The conversion of glucose was calculated with the

following equation:

$$\text{conversion of glucose(\%)} = \frac{\text{content of glucose(mg)}}{\text{cellulose content of pretreated material(mg)}} * 100\% \quad (4)$$

## RESULTS AND DISCUSSION

### Chemical Composition of the Material

The amounts of the main components of the raw material are listed in Table 3. The chemical composition of poplar wood is characterized by its high cellulose content (50.18%) and low lignin content (19.75%), making it an attractive biomass for hydrolysis.

**Table 3.** Chemical Composition of the Material

Component	Content %
Ash	0.514
Cellulose	50.18
Lignin	19.75
pentosans	20.64

### Regression Analysis

Further optimization of the organosolv pretreatment was achieved by employing BBD of experiments. Data were analyzed using Design Expert software to yield analysis of variance (ANOVA), regression coefficients, and regression equation.

The polynomial equation, describing the cellulosic yield as a simultaneous function of the temperature (A), ratio of wood to liquor (B), ethanol concentration (C), and holding time (D) is presented as Eq. (5)

$$Y_1 = 79.50 + 5.10 * A - 0.77 * B + 2.78 * C + 0.72 * D + 1.15 * A * B - 6.48 * A * C - 2.94 * A * D + 3.83 * B * C + 3.57 * B * D - 3.38 * C * D + 2.69 * A^2 - 3.21 * B^2 - 2.31 * C^2 - 3.38 * D^2 \quad (5)$$

Table 4 shows the ANOVA for the fitted model. The model F value of 61.21 and the values of probability (P) > F (<0.0001) implies that the model was significant. The p-value of the adjusted model was lower than 0.0001, indicating that the model is statistically valid. And the p-values of A, C, A<sup>2</sup>, B<sup>2</sup>, C<sup>2</sup>, D<sup>2</sup>, AC, AD, BC, BD, and CD were all lower than 0.0001 demonstrating that they were the most significant factors, compared to the other ones, influencing the response. B, D, and AB were also significant factors with p-value lower than 0.05.

The coefficient of determination R<sup>2</sup> for the model was 96.78%. The high R<sup>2</sup> value specifies that the model obtained will be able to give a convincingly good estimate of response of the system within the range studied. The lack of fit test, which was not significant for the model, showed that the model satisfactorily fitted the data.

**Table 4.** Analysis of Variance (ANOVA) for Regression Equation

Source	df	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	14	925.85	66.13	61.21	< 0.0001
A	1	311.75	311.75	288.53	< 0.0001
B	1	7.07	7.07	6.54	0.0228
C	1	92.46	92.46	85.57	< 0.0001
D	1	6.28	6.28	5.82	0.0302
AB	1	5.28	5.28	4.89	0.0442
AC	1	168.16	168.16	155.63	< 0.0001
AD	1	34.60	34.60	32.03	< 0.0001
BC	1	58.71	58.71	54.33	< 0.0001
BD	1	51.06	51.06	47.25	< 0.0001
CD	1	45.68	45.68	42.27	< 0.0001
A <sup>2</sup>	1	46.99	46.99	43.49	< 0.0001
B <sup>2</sup>	1	66.97	66.97	61.98	< 0.0001
C <sup>2</sup>	1	34.56	34.56	31.99	< 0.0001
D <sup>2</sup>	1	74.11	74.11	68.59	< 0.0001
Residual	14	15.13	1.08		
Lack of Fit	10	9.78	0.98	0.73	0.6876
Pure Error	4	5.35	1.34		
Total	28	940.98			
R-square Adj.			96.78%		

CrI is described as a simultaneous function of the temperature (A), ratio of wood to liquor (B), ethanol concentration(C), and holding time (D) in Eq. (6)

$$Y_1 = 1.70 + 7.163 \times 10^{-3} \times A - 8.75 \times 10^{-3} \times B - 2.25 \times 10^{-3} \times C - 6.5 \times 10^{-3} \times D + 0.035 \times A \times B - 0.047 \times A \times C + 0.037 \times A \times D - 7.75 \times 10^{-3} \times B \times C + 0.012 \times B \times D - 0.02 \times C \times D \quad (6)$$

Table 5 gives the ANOVA for the fitted model. The model F value of 17.72 implies that the model was significant. And the p-values of A, AB, AC, AD were all lower than 0.0001. B, C, and CD were also significant factors with p-value lower than 0.05.

The coefficient of determination, R<sup>2</sup> for the model was 83.90%. The lack of fit test was not significant for the model.

**Table 5.** Analysis of Variance (ANOVA) for Regression Equation

Source	df	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	10	0.024	$2.37 \times 10^{-3}$	17.72	< 0.0001
A	1	$6.16 \times 10^{-4}$	$6.16 \times 10^{-4}$	4.61	0.0456
B	1	$9.19 \times 10^{-4}$	$9.19 \times 10^{-4}$	6.88	0.0173
C	1	$6.07 \times 10^{-5}$	$6.08 \times 10^{-5}$	0.45	0.5086
D	1	$5.07 \times 10^{-4}$	$5.07 \times 10^{-4}$	3.80	0.0672
AB	1	$4.90 \times 10^{-3}$	$4.90 \times 10^{-3}$	36.68	< 0.0001
AC	1	$8.74 \times 10^{-3}$	$8.74 \times 10^{-3}$	65.45	< 0.0001
AD	1	$5.55 \times 10^{-3}$	$5.55 \times 10^{-3}$	41.55	< 0.0001
BC	1	$2.40 \times 10^{-4}$	$2.40 \times 10^{-4}$	1.80	0.1966
BD	1	$5.76 \times 10^{-3}$	$5.76 \times 10^{-4}$	4.31	0.0525
CD	1	$1.56 \times 10^{-3}$	$1.56 \times 10^{-3}$	11.68	0.0031
Residual	18	$2.40 \times 10^{-3}$	$1.34 \times 10^{-4}$		
Lack of Fit	14	$1.67 \times 10^{-3}$	$1.19 \times 10^{-4}$	0.65	0.7575
Pure Error	4	$7.32 \times 10^{-4}$	$1.84 \times 10^{-4}$		
Total	28	0.026			
R-square Adj.			83.90%		

Lignin content is described as a simultaneous function of the temperature (A), ratio of wood to liquor (B), ethanol concentration(C), and holding time (D) in Eq. (7)

$$Y=8.24-1.71*A-0.52*B+0.15C-0.56*D \quad (7)$$

**Table 6.** Analysis of Variance (ANOVA) for Regression Equation

Source	df	Sum of Squares	Mean Square	F Value	p-value Prob > F
Model	4	42.19	10.55	45.53	< 0.0001
A	1	34.92	34.92	150.72	< 0.0001
B	1	3.27	3.27	14.10	0.001
C	1	0.28	0.28	1.19	0.2859
D	1	3.73	3.73	16.10	0.0005
Residual	24	3.11	0.17		
Lack of Fit	20	2.59	0.18	1.92	0.2789
Pure Error	4	0.53	0.13		
Total	28	47.75			
R-square Adj.			86.41%		

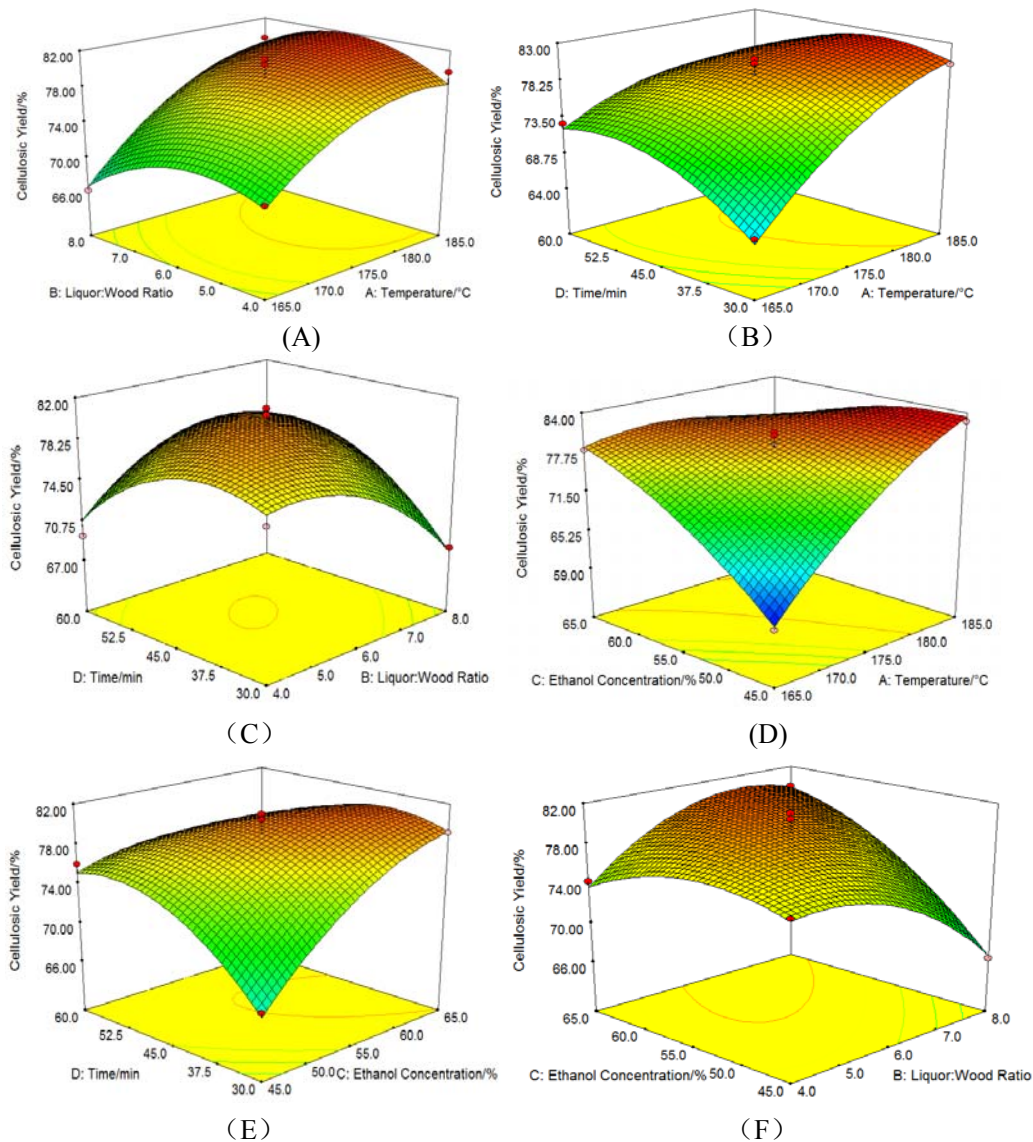
The polynomial equation is a linear form, quadratic, and interaction constant coefficients are not significant. Table 6 reports the ANOVA for the fitted model. The model F value of 61.21 implies that the model was significant. And the p-values of A



were all lower than 0.0001. B, D, and BC were also significant factors with p-value lower than 0.05. The coefficient of determination,  $R^2$  for the model was 86.41%. The lack of fit test was not significant for the model.

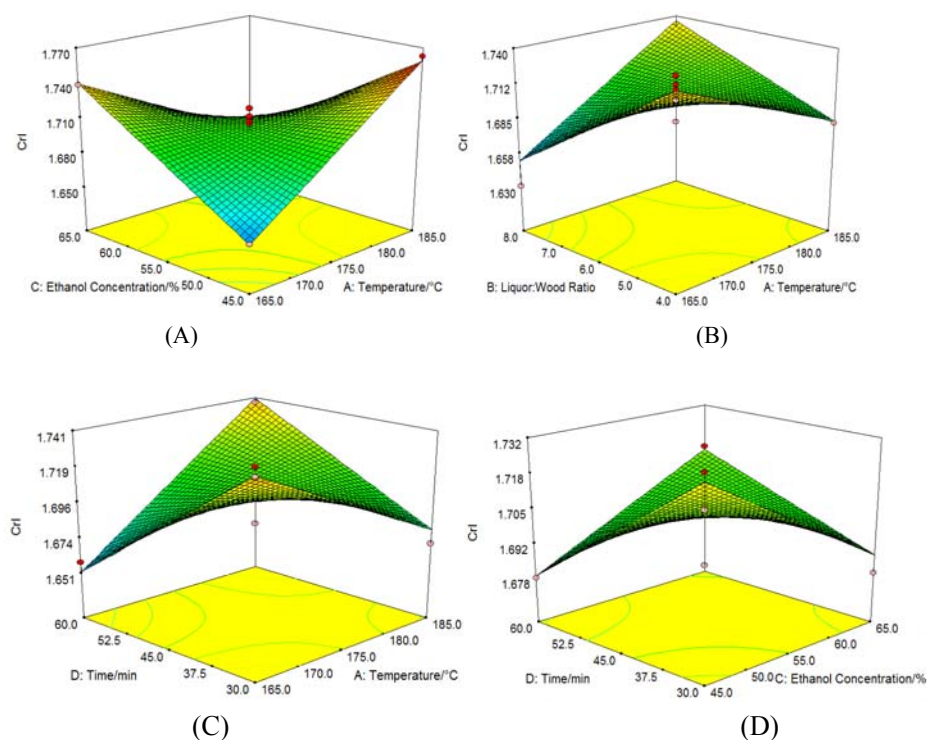
### Model Analysis

The 3D response surfaces and the 2D contour plots of the responses using Eq. (2) for the cellulosic yield are respectively shown in Fig. 1 (A-E). To depict the interactive effects of independent variables on responses, two variables were varied in certain ranges while others were kept constant. Similarly, corresponding to Figs. 1 (A-E), the interactive effect between each two factors significantly influenced the cellulosic yield.



**Fig. 1.** 3D response surface displaying relative effect of two variables on cellulosic yield. Central point: Temperature 175 °C, Liquor: Wood Ratio 1:6, Ethanol Concentration 55%, Time 45 min.

As shown in Fig. 1 (A), within the temperature range investigated, 165-185 °C, the cellulosic yield increased with increasing temperature, and the ratio of wood to liquor reached a maximum yield at 185 °C when ratio of wood to liquor reached 1:6. High temperature promoted delignification and removal of hemicellulose. Proper ratio of wood to liquor promoted penetration of ethanol, which was also helpful for delignification. But further increase in ratio of wood to liquor beyond 1:6 resulted in a decrease of cellulose yield because of dissolution of carbohydrate. The holding time showed a similar effect on cellulosic yield, and a maximum cellulose yield was observed at 41 min (Fig. 1 (B)). Longer time also led to dissolution of carbohydrate. As Fig. 1(D) shows, maximum cellulose yield was observed at 45% when the temperature was 185 °C. Low ethanol concentration, which generates higher hydrogen ion concentration (lower pH value) at the same dosage of H<sub>2</sub>SO<sub>4</sub>, promotes acid-catalyzed cleavage of  $\beta$ - and  $\alpha$ -ether linkages in lignin (McDonough et al. 1993; Goyal et al. 1992), while higher temperature also leads to a decrease of cellulosic yield due to dissolution of carbohydrate (Kabel et al. 2007). Similarly, corresponding to Figs. 1(C), (E), and (F), the interactive effect between BC, BD and CD significantly influenced the cellulose yield. It supports previous studies that proper increasing severity of biomass-pretreatment could enhance cellulose yield (Liu et al. 2008). With the conditions becoming more severe, the removal of lignin and the dissolution of carbohydrate were accelerated.



**Fig. 2.** 3D response surface displaying relative effect of two variables on crystallinity index while other at center level. Central point: Temperature 175 °C, Liquor: Wood Ratio 1:6, Ethanol Concentration 55%, Time 45 min.

Various pretreatments can change cellulose crystal structures by disrupting inter- and intra-chain hydrogen bonding of cellulose fibrils (Mosier et al. 2005). Bands at  $4000\text{ cm}^{-1}$ ,  $2995\text{ cm}^{-1}$ ,  $2900\text{ cm}^{-1}$ ,  $1430\text{ cm}^{-1}$ ,  $1375\text{ cm}^{-1}$ , and  $900\text{ cm}^{-1}$  are especially sensitive to crystalline and amorphous regions. The band at  $1429\text{ cm}^{-1}$  is assigned as “crystalline” and the band at  $897\text{ cm}^{-1}$  as “amorphous” (Nelson and O’Connor 1964).

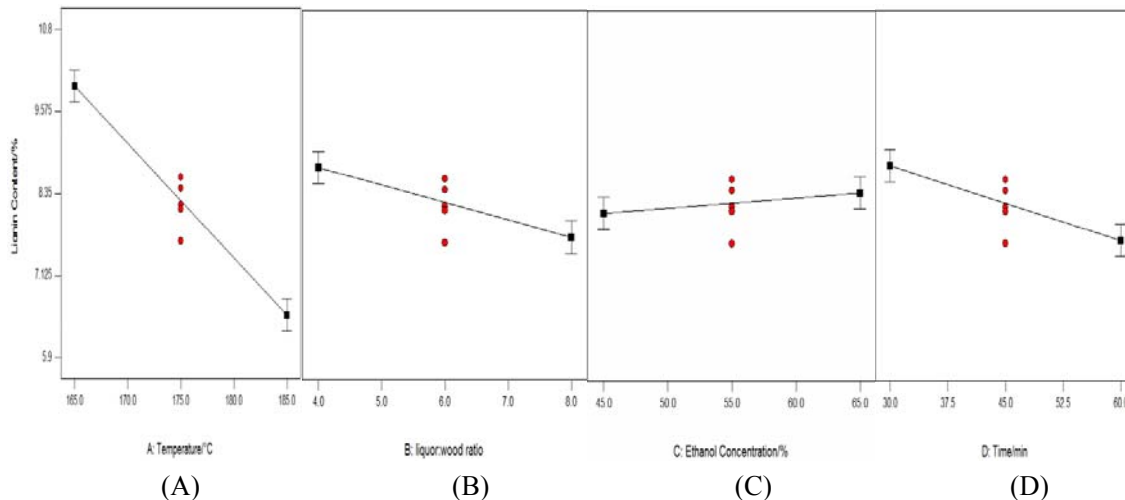
The features of recovered poplar solids after acid-catalysis ethanol pretreatment were examined using Lateral Order Index and also compared to the corresponding untreated samples. Results show that sample obtained after the pretreatment had been altered significantly. The CrI for all samples was calculated, and the results are summarized in Table 2. The CrI of untreated material was 1.484, and after the pretreatment there was an observed increase in the CrI. This increase in CrI after pretreatment is consistent with the results reported by (Kumar et al. 2009), suggesting that the amorphous cellulose breaks down.

Relative effects of two variables on CrI are displayed by 3D response surface in Fig. 2. The CrI increased quickly with the increasing maximum temperature, but this tendency went in the opposite direction when the ethanol concentration reached approximately 55% (Fig. 2(A)). Amorphous cellulose broke down with the temperature increasing, and the CrI became higher. But with the removal of lignin and hemicellulose, there was a degradation of the cellulose that led to the CrI decreasing. Broadening of the band at  $897\text{ cm}^{-1}$  reflects a higher amount of disordered structure. If a relative shift in the band at  $1430\text{ cm}^{-1}$  from higher to lower frequencies is observed, this is a sign of the degradation processes of the cellulose (Kavkler et al. 2011). This decrease in cellulose crystallinity indicates that the recovered product is highly amorphous and therefore has an increase in cellulose surface accessibility and would theoretically enable more efficient cellulose hydrolysis. As shown in Fig. 2 (B), extending the ratio of wood to liquor and increasing temperature led to a decrease of CrI. Interestingly, when both of them were raised higher (beyond  $175\text{ }^{\circ}\text{C}$  and 1:6), the CrI increased again. Figure 2 (C&D) have the same situation with Fig. 2 (B). An explanation is that with the increase of ratio of wood to liquor or time, crystalline cellulose was converted to amorphous cellulose, which led to a decrease of CrI. When the temperature went higher, both the crystalline and amorphous cellulose broke down, though amorphous cellulose had a faster rate of decomposition, leading to the increase of CrI according to Equation (3).

As Fig. 3(A) shows, the lignin content decreased very fast as the temperature became higher. There was a less significant dropping trend with the increasing of the ratio of wood to liquor (Fig. 3(B)) and holding time (Fig. 3(D)), because higher temperature, larger ratio of wood to liquor, and longer time promote delignification. The slight increase in lignin yield in Fig 3(C) is probably due to promotion of lignin condensation reactions leading to reduced defibrillation. Another explanation is that the rate of defibrillation is faster than that of delignification. These might be the reasons the ethanol concentration and quadratic, and interaction constant coefficients are not significant.

Though less lignin content improved the efficiency of enzyme hydrolysis, a more severe condition led to a drop of cellulosic yield.

Based on an overall consideration of cellulosic yield, CrI, and lignin content, and taking both costs and efficiency into consideration, optimum process of organosolv pretreatment was extracted by design expert software through a graphical optimization.



**Fig. 3.** Effect of processing conditions on lignin content.

### Optimization and Confirmation Experiments

Through a graphical optimization by design expert software, optimum process of organosolv pretreatment of poplar was found to be: temperature: 185 °C, ratio of wood to liquor: 1 to 4.2, ethanol concentration: 45% and holding time: 41 min. Under these conditions, confirmation experiments were conducted in three replicates. The observed mean cellulosic yield, CrI and lignin content were found to be largely consistent with the predicted values. At the same time, the rationality and practicability of optimal conditions for organosolv pretreatment have been confirmed in this work.

**Table 5.** Verified Results of the Model Equation

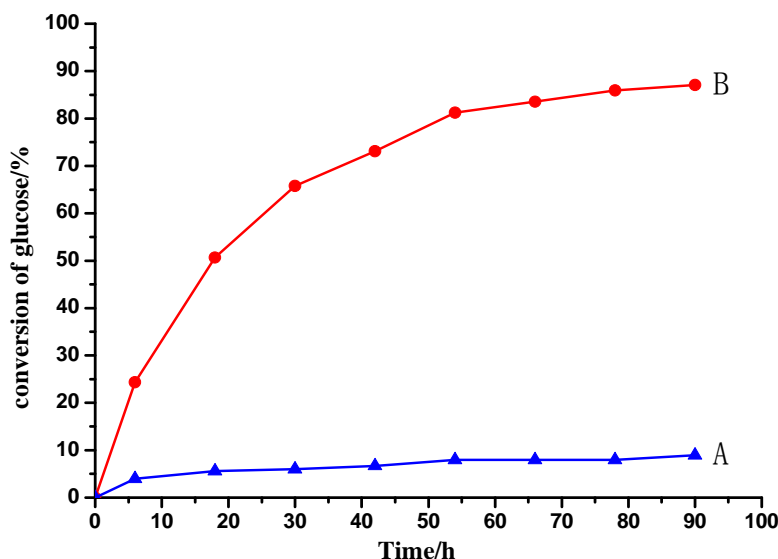
Y	cellulosic yield %	crystallinity index	Lignin content %
Predicted Value	84.14	1.726	6.99
Actual Value	82.62	1.712	7.24

### Enzyme Hydrolysis of Material and Pretreated Polar Solids

Enzymatic hydrolysis of both as-received and pretreated poplar solids under the optimum process to glucose was carried out to compare their cellulose digestibility. Figure 4 shows conversion of material and pretreated poplar to glucose at the same enzyme loading. Organosolv pretreatment poplar exhibited significantly higher saccharification kinetics, with conversion of glucose reaching 87.06% within 90 h, whereas the as-received material's was only 8.97% over the same time interval. The loss of intra- and inter-molecular hydrogen bonding resulting in amorphous cellulose provides an enhanced surface area, leading to better enzyme accessibility and increased binding sites in recovered cellulose fibers (Li et al. 2010). Lignin could also act as a major obstacle to enzyme attack on cellulose and can irreversibly adsorb cellulase. Compared

with the result of hybrid poplar (Pan et al. 2006), approximately 85% of the total cellulose in the untreated poplar was recovered as monomeric glucose after hydrolysis indicated that Chinese triploid poplar is an attractive biomass for hydrolysis by the pretreatment with lower ethanol concentration and shorter holding time.

Hydrolysis results clearly showed that soluble sugars were released faster and to a greater extent following the organosolv pretreatment. Additional reductions in enzyme loading, hydrolysis time, pH, and time are essential to promote the economy of biorefineries and need to be optimized further.



**Fig. 4.** Comparison of enzyme hydrolysis of both material and pretreated polar solids  
A: material B: pretreated polar

## CONCLUSIONS

1. Organosolv pretreatment (acid-catalytic ethanol method) of Chinese triploid poplar resulted in a high cellulose yield, low CrI, and low lignin content. These results were obtained when employing a relatively short holding time (41 min), low cooking temperature (185 °C), ratio of wood to liquor (1:4.2), and ethanol concentration (45%) under the condition of acid dosage (0.8%). The pretreated solids under the optimum process conditions were shown to be susceptible to attack by a commercial cellulase preparation.
2. Approximately 87% of the total cellulose in the poplar was recovered as monomeric glucose after hydrolysis of the solid fraction using enzyme loading, pH 4.8, temperature 50 °C, and time of hydrolysis 90 h.

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