

Improved Saccharification of Sugarcane Bagasse with Deep Eutectic Solvent Composed of Choline Hydroxide and Glycerol

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Abstract

In this study, deep eutectic solvent (DES) consisted of choline hydroxide and glycerol (ChOH/G) was developed as pretreatment reagent for sugarcane bagasse (SCB). The influences of pretreatment parameters on enzymatic hydrolysis were analyzed, such as the quality ratio of ChOH to glycerol, pretreatment time and temperature. For the highest total reducing sugar yield (TRSY) and total reducing sugar concentration (TRSC), the pretreatment parameters were optimized by response surface method. After optimization, the TRSC was 17.36 g/L (Optimized-P1: quality ratio of 1:3, 170, 4 h). The TRSY was 0.51 g/g (Optimized-P2: quality ratio of 1:2, 150, 5 h).

Keywords

Lignocellulosic Biomass; Pretreatment; Deep Eutectic Solvent; Saccharification.

1. Introduction

The problems caused by the use of fossil energy have become the main bottleneck restricting the sustainable and healthy development of social economy, such as energy crisis and environmental pollution (Qureshi et al., 2021). Therefore, it is extremely urgent to find green and renewable new energy to replace fossil energy. Non-food lignocellulosic is a kind of green and renewable energy material, including forest residues and crop harvest residues (Abdeshahian et al., 2020). They are widely distributed on the earth, and can be used as the substrate of liquid fuel and chemicals (Gasser et al., 2015). As a raw material, non-grain lignocellulosic can greatly reduce the production cost, relieve the resource strain and reduce the emission of greenhouse gas, which has attracted extensive attention of scientific researchers. (Chatterjee et al., 2014).

However, lignocellulosic biomass has not been widely used in industry. The reason is that lignocellulosic biomass has a compact structure and complex composition, which makes it difficult to achieve component separation and efficient saccharification of enzymes (Upton and Kasko, 2015). Cellulose is the skeleton material of lignocellulosic raw material (Jørgensen et al., 2010; Tadesse and Luque, 2011). Cellulose and hemicellulose are connected by hydrogen bonds, and lignin is filled in lignocellulose (Gírio et al., 2010; Iiyama et al., 1994). The three components interact closely and are woven into a complex structure (Reddy and Yang, 2005). The complex structure greatly hinders its contact with biological or chemical reagents and impedes the degradation of cellulose substrates (Koppram et al., 2014). Hence, lignocellulosic biomass needs to be pretreated effectively to disintegrate the dense structure before it can be further fully utilized.

At present, chemical reagents are usually utilized to pretreat lignocellulosic biomass. Acid reagents can degrade lignocellulosic materials by dissolving hemicellulose and cellulose in raw materials (Jung et al., 2013). Whereas, it is easy to cause further degradation of monosaccharides and produce inhibitors (furfural and hydroxymethyl furfural) (Klinke et al., 2004). In addition, high temperature and strong acid concentration can cause lignin recondensation, which adheres to the surface of cellulose (Weil et al., 2002). The alkaline (NaOH or KOH) pretreatment can weaken the hydrogen bonds between cellulose and hemicellulose, and disrupt the ester bonds between hemicellulose and lignin, making it easier to separate lignin from carbohydrates (Rabelo et al., 2014; Silva et al., 2016). But there are many problems in alkaline pretreatment, such as the corrosive effect of high concentration of alkali on the reaction vessel, and the pollution of the flow of alkaline filtrate into the ground.

In recent years, deep eutectic solvent (DES) has been gradually regarded as pretreatment solvent due to its green pollution-free, which has great application potential in lignocellulosic biomass conversion (Yu et al., 2018). DES is a mixture of hydrogen bond donor and hydrogen bonding acceptor (Zhao et al., 2018). When DES is used for the pretreatment of lignocellulosic raw materials, lignin can be effectively removed, but the cellulose component to a large extent was retained (Francisco et al., 2012). DES can break the hydrogen bond and the ether bond in carbohydrate complexes, which forms through hydrogen bonding between lignin and hemicellulose (Li et al., 2021). For the first time, Maria Francisco demonstrated that lignin could be dissolved by malic acid/proline, and the solubility of lignin was 14.9 % when the molar ratio of malic acid/proline was 1:3 and the temperature was 100 °C (Francisco et al., 2012). Four date palm residues were pretreated with the molar ratio of choline chloride/glycerin of 1:2, and the removal rate of lignin was 22 % (Fang et al., 2017). Procentese employed choline chloride/imidazole (the molar ratio of 3:7) for the pretreatment of corncob residues, and found that the removal rate of lignin was 23.8 %, and the cellulose content in the residue was 41.1 % (Procentese et al., 2015). Pan observed that there is an order for the ability of ChCl/urea to separate from chemical excerpt was in an order of. This gradation went like alpha-cellulose (9.60 %) < hemicellulose and amorphous cellulose (16.71 %) < AIL (22.87 %) (Pan et al., 2017).

In the paper, choline hydroxide and glycerol (ChOH/G) was first proposed for the pretreatment of sugarcane bagasse (SCB). We explored the optimal conditions of DES pretreatment, including the quality ratio of ChOH to glycerol, pretreatment temperature and time.

2. Materials and Methods

2.1 Materials

Sugarcane bagasse(SCB) was afforded by sugar mills from Liuzhou, Guangxi province, China. The powder with an 100 - 200 mesh particle size was obtained by pulverization. The major raw compositions: 40.16 ± 0.40 % glucan, 23.07 ± 0.84 % xylan, and 25.23 ± 1.50 % lignin. Analytical grade choline hydroxide (43.4 % purity) was purchased from McLean Biochemical Technologies Ltd (Shanghai, China). Cellic CTec2 was bought from Sigma-Aldrich and LLC (Shanghai, China). Analytical grade glycerol (99 % purity) was bought from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China).

2.2 Methods

2.2.1 Preparation of DES

A mixture of ChOH and glycerol was stirred at 80 °C for 2 h to form the deep eutectic solvent (DES), followed by rotary evaporation at 80 °C to remove water in DES. The quality ratios of ChOH to glycerol were 1:2, 1:4, 1:6 and 1:8, named ChOH/G2, ChOH/G4, ChOH/G6, and ChOH/G8, respectively. In DES (ChOH/G), the hydrogen bond donor is glycerol and the hydrogen bond acceptor is ChOH.

2.2.2 Pretreatment with DES

This quality ratios of ChOH to glycerol at a temperature of 170 °C for 1 h for the pretreatment of the SCB were firstly investigated. Then single factor tests were carried out at the different pretreatment temperatures (130 °C, 150 °C, 170 °C and 190 °C) and time (0.5 h, 1 h, 2 h, 3 h, 4 h, 5h). The major compositions and the solid recovery of the residues were recorded to evaluate the enzymatic saccharification.

2.2.3 Response Surface Method

Response surface method (RSM) was employed to assess various conditions and parameters of the pretreatment. To get the highest TRSC and TRSY Box Behnken design (BBD) was applied to further optimize the process with combination of three conditions from three perspectives on the basis of former preliminary results. In this experiment, Design-Expert was used for the design and analysis of the response surface method. From Table 1, it was showed the detailed experimental designs of three conditions and from three perspectives.

Table 1. Code values and experimental range of variables used in RSM.

Factors	Encoded symbol	Factor levels		
		-1	0	1
Quality ratio	X ₁	1:2	1:4	1:6
Pretreatment temperature (°C)	X ₂	150	170	190
Pretreatment time (h)	X ₃	3	4	5

2.2.4 Hydrolysis with Enzyme

The SCB's hydrolysis process with enzyme was conducted. Firstly, 0.5 g SCB was weighted and put into a 50 mL serum tube including 25 mL 0.1 M citric acid or citric sodium buffer at pH 4.8. Next, 80 g/mL tetracycline and 60 g/mL nystatin were added for the aim of microorganism contamination prevention. The volume of loaded Cellulase was 40 FPU/g substrate. The experiment condition of hydrolysis was carried out at a speed of 200 rpm and 50 °C for 72 hours. Then boiling water were used to heat up the products for 10 min to let the enzymes lose activity. The products were centrifuged at a speed of 12,000 rpm for 10 min. A 0.22 µm membrane filter were used to filter supernatants before HPLC analysis of glucose and xylose components.

2.2.5 Methodology of Analysis

Two-step acid hydrolysis was used to test the composition of cellulose, hemicellulose and lignin that exist within original materials as well as processed SCB. A HPLC system (LC-15C, Shimadzu, Japan) provided with an Aminex HPX-87H column (Bio-Rad, USA) and a detector for the refractive ratio (RID-10A Shimadzu, Japan) was used to exam glucose and xylose. The remove phase exists in the column under the operation of with 5 mM H₂SO₄ at 55 °C, and a speed of 0.6 mL per minute. The glucose and xylose yields were used to determine the hydrolysis efficacy with enzyme and fermentation process. The following would include the calculation equations:

$$\text{TRSC (g/L)} = \text{Glucose concentration (g/L)} + \text{Xylose concentration (g/L)}$$

$$\text{TRSY (g/g)} = \text{TRSC (g/L)} \times \text{V (L)} / \text{Original bagasse (g)}$$

$$\text{Original bagasse (g)} = \text{Solid mass (g)} / \text{Solid recovery (g)}$$

2.2.6 Data Analysis

Whole measurements and experiments were conducted with 3 time-repeat and the data were analyzed by mean \pm standard deviation. Data was analyzed and carried out in SPSS and GraphPad Prism statistics using the method of ANOVA, which is shorted for one-way analysis of variance. The significance exists under the condition that the value is within the 95 % confidence interval ($p < 0.05$).

3. Data and Discussion

3.1 Influence of Reagent Concentration on SCB During ChOH/G Pre-test

With the pre-test incubation of SCBs with ChOH/G2, ChOH/G4, ChOH/G6 and ChOH/G8. The pretreatment proceeded under the temperature of 170 °C and time of 1h. The aim of this pre-test is to elucidate the related influence on solid recovery, which includes the major composition (glucan, xylan and lignin), TRSC, and SCB's TRSY. Table 2 and Figure 1 displayed these research outputs.

Table 2. Chemical composition of sugarcane bagasse before and after pretreatment under different conditions.

Pretreatment conditions	Solid recovery (%)	Glucan (%)		Xylan (%)		Lignin (%)	
		Content	Recovery ^a	Content	Recovery ^b	Content	Delignification ^c
170 °C, 1 h							
Raw material	100	40.16 \pm 0.40C	—	23.07 \pm 0.84C	—	25.23 \pm 1.50A	—
Glycerin	94.38 \pm 0.11A	30.17 \pm 1.85D	70.89 \pm 2.36B	22.64 \pm 1.21C	92.61 \pm 2.95A	17.9 \pm 0.42B	33.03 \pm 1.58D
ChOh/G2	67.42 \pm 0.22B	40.66 \pm 0.95C	68.25 \pm 1.60C	27.61 \pm 1.83A	80.70 \pm 3.36BC	2.95 \pm 0.08E	92.13 \pm 0.20A
ChOh/G4	63.92 \pm 0.07D	43.54 \pm 0.28B	69.29 \pm 0.44C	29.24 \pm 0.82A	81.02 \pm 0.82B	2.33 \pm 0.06E	94.09 \pm 0.14A
ChOh/G6	62.83 \pm 0.09E	57.50 \pm 0.19A	89.97 \pm 0.30A	26.23 \pm 0.31B	71.42 \pm 0.86C	5.66 \pm 0.23D	85.91 \pm 0.58B
ChOh/G8	64.90 \pm 0.16C	56.8 \pm 0.28A	91.43 \pm 0.45A	26.50 \pm 0.37B	74.54 \pm 1.03C	7.04 \pm 0.83C	81.90 \pm 2.12C
ChOh/G4, 1h							
Raw material	100	40.16 \pm 0.40C	—	23.07 \pm 0.84C	—	25.23 \pm 1.50A	—
130 °C	68.06 \pm 0.07A	49.16 \pm 1.48A	83.32 \pm 2.51A	27.05 \pm 1.03B	79.81 \pm 3.04A	5.48 \pm 0.10B	85.23 \pm 0.27C
150 °C	65.39 \pm 0.08B	42.79 \pm 1.94BC	69.67 \pm 3.17B	28.37 \pm 1.06AB	80.42 \pm 3.00A	2.65 \pm 0.06C	93.12 \pm 0.14B
170 °C	63.92 \pm 0.07C	43.54 \pm 0.28B	69.29 \pm 0.44B	29.24 \pm 0.82A	81.02 \pm 0.82A	2.33 \pm 0.06C	94.09 \pm 0.14A
190 °C	64.22 \pm 0.06C	41.45 \pm 0.28C	66.29 \pm 0.44B	28.26 \pm 0.11AB	78.67 \pm 0.32A	2.27 \pm 0.04C	94.22 \pm 0.09A
ChOh/G4, 170 °C							
Raw material	100	40.16 \pm 0.40D	—	23.07 \pm 0.84D	—	25.23 \pm 1.50A	—
0.5 h	58.26 \pm 0.11D	56.10 \pm 0.72B	81.39 \pm 1.04C	24.90 \pm 0.44C	62.87 \pm 1.10D	9.38 \pm 0.38B	78.34 \pm 0.89D
1 h	63.92 \pm 0.07A	43.54 \pm 0.28C	69.29 \pm 0.44D	29.24 \pm 0.82A	81.02 \pm 0.82A	2.33 \pm 0.06E	94.09 \pm 0.14A
2 h	61.24 \pm 0.08B	61.39 \pm 1.12A	93.61 \pm 1.71A	27.83 \pm 0.54B	73.87 \pm 1.43C	3.76 \pm 0.08D	90.87 \pm 0.18B
3 h	59.40 \pm 0.25C	56.30 \pm 0.28B	83.27 \pm 0.41C	29.64 \pm 0.27A	76.31 \pm 0.68B	5.02 \pm 0.25C	88.19 \pm 0.59C
4 h	58.11 \pm 0.08D	58.10 \pm 0.24B	84.06 \pm 0.24B	29.45 \pm 0.47A	74.18 \pm 1.19BC	5.03 \pm 0.68C	88.41 \pm 1.57C
5 h	58.22 \pm 0.04D	59.81 \pm 2.66AB	86.7 \pm 3.86B	29.20 \pm 0.38A	73.68 \pm 0.97C	4.88 \pm 0.45CD	88.74 \pm 1.04C

a Glucan Recovery = (Glucan content in pretreated SCB \times solid recovery) / Glucan content in Raw material.

b Xylan Recovery = (Xylan content in pretreated SCB \times solid recovery) / Xylan content in Raw material.

c Delignification = (lignin content in Raw material - lignin content in pretreated SCB \times solid recovery) / lignin content in Raw material.

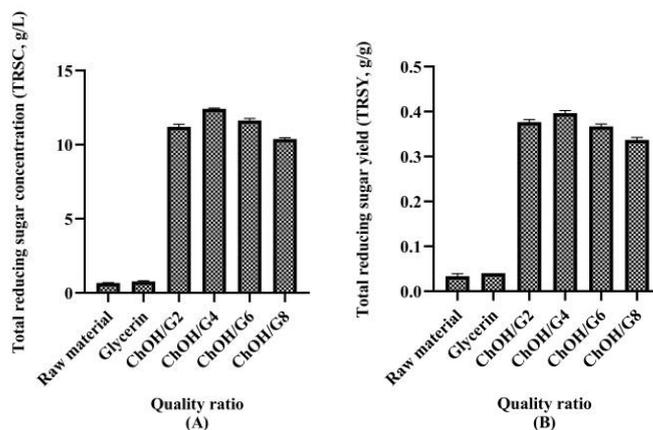


Figure 1. Enzymatic hydrolysis of SCB after pretreatment with different quality ratios of ChOH/G. Total reducing sugar content (A); Total reducing sugar yield (B). Pretreatment conditions: 170 °C, 1 h, and the solid load of 10 %. All experiments and assays were performed in duplicate (n = 3).

As displayed in Table 2, the pretreatment processes with ChOH/G had an obvious influence on the solid's recovery and major units. Besides, this improvement changes, the hydrolysis efficacy of enzyme with SCBs compared with the R-SCB. The delignification rates of ChOH/G2 and ChOH/G4 were as high as 92.13 % and 94.09 %, respectively, and there was no significant difference between them.

Figure 1 displayed the enzymatic hydrolysis effects under different quality ratios of ChOH/G. The results revealed that the TRSCs of R-SCB and glycerin group were 0.66 g/L and 0.77 g/L separately. But there was no statistical gap between these two groups ($P = 0.1009$). Considering TRSCs from ChOH/G2, ChOH/G4, ChOH/G6 and ChOH/G8 were 11.20 g/L, 12.42 g/L, 11.63 g/L and 10.38 g/L, respectively. These were obviously higher than that of R-SCB and glycerin group, as the P values less than 0.0001. The TRSC of ChOH/G4 was the highest. The TRSYs of R-SCB, glycerin group, ChOH/G2, ChOH/G4, ChOH/G6 and ChOH/G8 were 0.03 g/g, 0.04 g/g, 0.38 g/g, 0.40 g/g, 0.37 g/g and 0.34 g/g, respectively. Although almost no statistical gap of TRSY was noticed between R-SCB and glycerin group ($P = 0.1835$), the TRSYs of ChOH/G2, ChOH/G4, ChOH/G6 and ChOH/G8 were significantly increased, P values less than 0.0001. When the quality ratio of ChOH to glycerol was 1:4, the best remove speed of lignin and hydrolysis process with enzyme were observed. Therefore, the ChOH/G4 experimental group was selected for the following single-factor experiment.

3.2 Temperature's Influence on SCB Incubation with ChOH/G in Pre-test

For the changes of major lignocellulosic components after pretreatment of SCB at different temperatures, these were demonstrated in Table 2. The results indicated that significant changes occurred after pretreatment at different temperatures, and there was no obvious linear relationship between the temperature and the chemical composition of SCB. Under the condition that the temperature raised starting at 130 °C and then to 190 °C. The SCB's delignification showed an increasing trend, and the removal rates of lignin at 170 °C and 190 °C were 94.09 % and 94.22 %, respectively, between which there was no difference.

As can be in Figure 2, the results of the enzymatic hydrolysis effect at different pretreatment temperatures showed that the TRSC and TRSY of R-SCB were 0.66 g/L and 0.36 g/g, separately. For highest TRSC at 170 °C, it was 12.42 g/L, and obviously higher than that of 130 °C (10.52 g/L, $P = 0.0003$), 150 °C (10.78 g/L, $P = 0.0019$) and 190 °C (10.64 g/L, $P = 0.0004$). The TRSY at 170 °C also reached the highest (0.40 g/g), which clearly higher than that of 130 °C (0.36 g/g, $P = 0.0202$), 150 °C (0.35 g/g, $P = 0.0051$) and 190 °C (0.34 g/g, $P = 0.0034$). When the pretreatment temperature was 170 °C, it had outstanding remove speed of and effect of hydrolysis process with enzyme, so the temperature was used for the subsequent single-factor experiment.

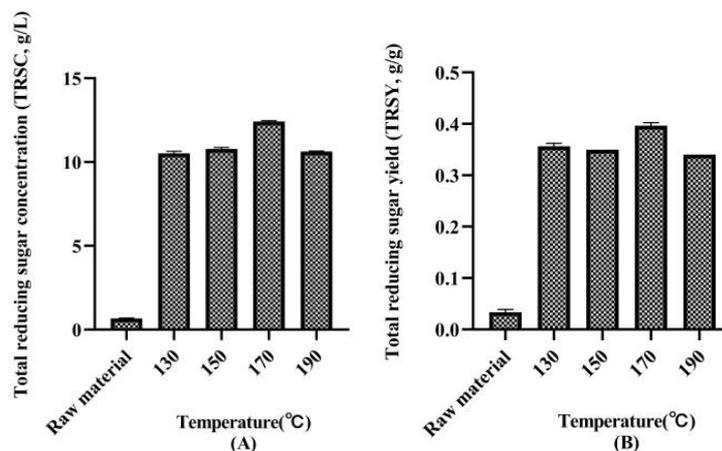


Figure 2. Enzymatic hydrolysis of SCB after pretreatment with different temperatures.

Total reducing sugar content (A); Total reducing sugar yield (B). Pretreatment conditions: ChOH/G4, 1 h, and the solid load of 10 %. All experiments and assays were performed in duplicate (n = 3).

3.3 Influence of Pre-test's Duration on SCB Incubated with ChOH/G

According to the results in Table 2, the main components of SCB changed significantly after pretreatment at different time. With the prolongation of pretreatment time, both the solid and the removal rate of lignin decreased. There were no significant differences in delignification effect among pretreatment for 3 h, 4 h and 5 h. As shown in Figure 3, the TRSC and TRSY were significantly increased within the pretreatment time of 3 h. The enzymatic hydrolysis effects of 3 h, 4 h and 5 h were similar, among which there were no significant difference.

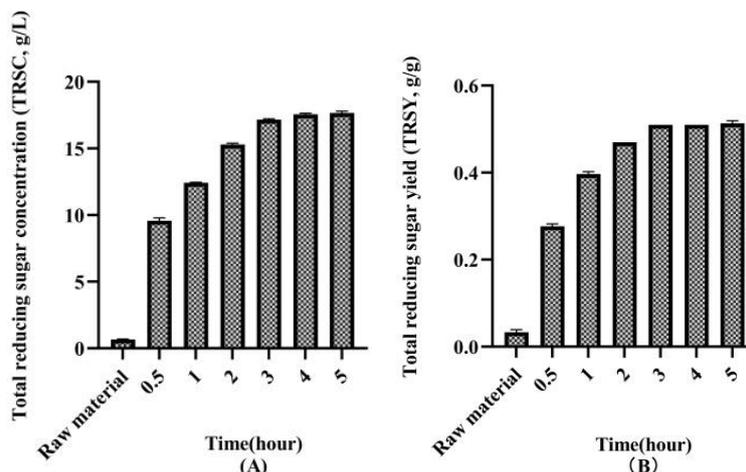


Figure 3. Enzymatic hydrolysis of SCB after pretreatment with different times.

Total reducing sugar content (A); Total reducing sugar yield (B). Pretreatment conditions: ChOH/G4, 170 °C, and the solid load of 10 %. All experiments and assays were performed in duplicate (n = 3).

3.4 Promotion of ChOH/G Pre-test with RSM

On the basis of primary data, the parameters as CHO/H/G (the quality ratio of ChOH to glycerol, pretreatment time and time) were considered furtherly through RSM by BBD for the aim of get the best reaction result especially in the aspects of TRSC and TRSY. These parameters of each factor after coding or actual condition on related response surface were shown in Table 1. In this paper, 17

groups of experiments were designed to acquire the response values of TRSC and TRSY according to BBD model with Design-Expert software, as shown in Table 3. Then BBD was used for further analysis of the experimental data to obtain two quadratic regression equations, in which TRSC and TRSY were functions and three factors were independent variables:

$$R1 = 17.5100 - 0.3300 * X1 + 0.2000 * X2 + 0.0730 * X3 + 1.0400 * X1 * X2 - 0.1900 * X1 * X3 - 0.0350 * X2 * X3 - 0.6400 * X1^2 - 1.3200 * X2^2 - 0.4500 * X3^2$$

$$R2 = 0.5100 + 0.0025 * X1 - 0.0100 * X2 + 0.0025 * X3 + 0.0300 * X1 * X2 - 0.0150 * X1 * X3 - 0.0100 * X2 * X3 - 0.0080 * X1^2 - 0.0330 * X2^2 + 0.0080 * X3^2$$

Table 3. Experimental design and the results of the Box-Behnken design.

Coding	Factors			Response values	
	Quality ratio	Pretreatment temperature	Pretreatment time	TRSC	TRSY
	(X ₁)	(X ₂ , °C)	(X ₃ , h)	(g/L)	(g/g)
1	1:2	150	4	16.72	0.51
2	1:6	150	4	13.99	0.44
3	1:2	190	4	15.02	0.43
4	1:6	190	4	16.46	0.48
5	1:2	170	3	16.37	0.48
6	1:6	170	3	16.08	0.50
7	1:2	170	5	17.14	0.50
8	1:6	170	5	16.07	0.48
9	1:4	150	3	15.53	0.46
10	1:4	190	3	16.03	0.46
11	1:4	150	5	15.51	0.49
12	1:4	190	5	15.87	0.45
13	1:4	170	4	17.64	0.52
14	1:4	170	4	17.53	0.51
15	1:4	170	4	17.32	0.51
16	1:4	170	4	17.56	0.50
17	1:4	170	4	17.48	0.49

R1 and R2 were respectively the predicted values of TRSC and TRSY, and X1 – X3 were coded values corresponding to the three factors in turn. As it can be seen in Table 4 and 5, the ANOVA results of the TRSC and TRSY models showed that the P-values were < 0.0001 and 0.0012 separately, with F-values of 76.19 and 13.46, which means these 2 ways were both of great significance. In details, in these 2 ways, the p-values of in the aspect of the lack-of-fit test were 0.1863 and 0.8537 separately, which were higher than 0.05, suggesting that the influence of the accidental parameters (like experimental incurrence operations) on final experiments outputs is not big. On the other side, the calculation method on the basis of RSM outputs were suitable considering there is just little error during test.

Table 4. The ANOVA results of the TRSC model.

Source	Sum of squares	df	Mean square	F-value	P-value (Pro > F)	
Model	16.54	9	1.84	76.19	< 0.0001	significant
X ₁	0.88	1	0.88	36.40	0.0005	
X ₂	0.33	1	0.33	13.77	0.0075	
X ₃	0.04	1	0.04	1.74	0.2282	
X ₁ X ₂	4.35	1	4.35	180.28	< 0.0001	
X ₁ X ₃	0.15	1	0.15	6.31	0.0403	
X ₂ X ₃	4.90E-03	1	4.90E-03	0.20	0.6658	
X ₁ ²	1.72	1	1.72	7.14E+01	< 0.0001	
X ₂ ²	7.33	1	7.33	303.90	< 0.0001	
X ₃ ²	0.86	1	0.86	35.63	0.0006	
Residual	0.17	7	0.02			
Lack of Fit	0.11	3	0.04	2.63	0.1863	not significant
Pure Error	0.06	4	0.01			
Cor Total	16.7	16				

Table 5. The ANOVA results of the TRSY model

Source	Sum of squares	df	Mean square	F-value	P-value (Pro > F)	
Model	0.011	9	1.19E-03	13.46	0.0012	significant
X ₁	5.00E-05	1	5.00E-05	0.56	0.4769	
X ₂	8.00E-04	1	8.00E-04	9.03	0.0198	
X ₃	5.00E-05	1	5.00E-05	0.56	0.4769	
X ₁ X ₂	3.60E-03	1	3.60E-03	40.65	0.0004	
X ₁ X ₃	4.00E-04	1	4.00E-04	4.52	0.0712	
X ₂ X ₃	4.00E-04	1	4.00E-04	4.52	0.0712	
X ₁ ²	2.70E-04	1	2.70E-04	3.04	0.1246	
X ₂ ²	4.59E-03	1	4.59E-03	51.77	0.0002	
X ₃ ²	2.70E-04	1	2.70E-04	3.04	0.1246	
Residual	6.20E-04	7	8.86E-05			
Lack of Fit	1.00E-04	3	3.33E-05	0.26	0.8537	not significant
Pure Error	5.20E-04	4	1.30E-04			
Cor Total	0.011	16				

For TRSC model, quality ratio brought about distinct effect on the pretreatment, with P = 0.0005, followed by pretreatment temperature (P = 0.0075). In the pair-factor interaction analysis, quality ratio and pretreatment temperature had significant interactions (P<0.0001), followed by quality ratio

and pretreatment time ($P = 0.0403$). However, in the quadratic term, the quality ratio, pretreatment temperature and pretreatment time caused obvious influence, with $P < 0.0001$, < 0.0001 and 0.0006 , respectively. While for TRSY model, the temperature can distinctly affect the pretreatment ($P = 0.0198$), and in the pair-factor interaction analysis, quality ratio and pretreatment temperature had significant interactions ($P = 0.0004$). But in the quadratic term, pretreatment temperature had obvious influence, with $P = 0.0002$.

From the forecast model, the condition can be conducted and get the highest TRSC as an incubation of SCB with ChOH/G3 at 170 °C for 4 h (Optimized-P1), while the maximum TRSY was achieved with ChOH/G2 at 150 °C for 5 h (Optimized-P2). Considering these promoted pre-test parameters, it is the forecasted that TRSC and TRSY's values could reached to 16.93 g/L and 0.52 g/g, separately. For the aim of confirming the best parameters, validation procedures were conducted. And it was found that both TRSC and TRSY were 17.36 g/L and 0.51 g/g, separately shown in Table 6. SCB's TRCS was increased after pretreated with Optimized-P1 ($P = 0.0310$), while the TRSY of SCB has no obvious gap within Initial and Optimized-P2 after recalling the primary experiment output than RSM promotion. Moreover, there is an obvious increase on efficacy of delignification starting at 88.19 % to 90.59 % ($P = 0.0119$). The above valued suggested that the introduce of ChOH/G to the procedure may provide a more effective way to improve the saccharification of enzyme with SCB.

Table 6. Main components and anzymatic hydrolysis of SCB before and after pretreatment

Pretreatment conditions	Solid recovery (%)	Glucan (%)	Xylan (%)	Lignin (%)		TRSC (g/L)	TRSY (g/g)
		Content	Content	Content	Delignification		
Untreated	100	40.16±0.40	23.07±0.84	25.23±1.50	—	0.66±0.03	0.03±0.01
Initial	59.40±0.25	56.30±0.28	29.64±0.27	5.02±0.25	88.19±0.59	17.16±0.06	0.51±0.00
Optimized ^a	59.66±0.06	53.75±2.34	28.59±1.11	4.03±0.05	90.32±0.11	17.36±0.04	0.52±0.01
Optimized ^b	59.75±0.11	57.18±0.42	28.37±0.86	4.08±0.07	90.59±0.22	17.10±0.02	0.51±0.01
Predicted ^a	—	—	—	—	—	16.93	—
Predicted ^b	—	—	—	—	—	—	0.52

Initial is components and enzymolysis effect of pretreated SCB before response surface optimization.

Initial pretreatment conditions: ChOH/G4, 170 °C, 3 h.

a Optimal pretreatment condition for the higher TRSC.

b Optimal pretreatment condition for the higher TRSY.

4. Conclusion

In this study, the ChOH/G method was established to pretreat SCB. Through the optimization experiment of RSM, the maximum values of TRSC and TRSY were 17.36 g/L and 0.51 g/g. At the same time the removal rate of lignin can exceed more than 90 %. It can provide a reference for further development of new solvents and conducive to the promotion of industrial application of sugarcane bagasse.

Acknowledgments

This study was supported by the the Program for New Century Excellent Talents in University (NCET-05-0745).

Highlights

An economical and green DES pretreatment process for SCB was established.

High concentration and yield of total reducing sugar were achieved.

The removal rate of lignin by DES pretreatment was up to 90%.

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