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Evaluation of steam pretreatment on sweet sorghum bagasse for enzymatic hydrolysis and bioethanol production

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ABSTRACT

Sweet sorghum bagasse (SSB) was steam-pretreated at various temperatures, residence times and SO_2 impregnation dosages. A 2^3 factorial experimental design was employed to investigate the effects of temperature, residence time and SO_2 impregnation and their interactions on enzymatic hydrolysis and ethanol production. The results showed that temperature, residence time, SO_2 impregnation and the interaction of residence time and SO_2 impregnation significantly affected enzymatic hydrolysis in an order of residence time > temperature > SO_2 impregnation > the interaction of residence time and SO_2 impregnation. Both of water-soluble and water-insoluble fraction derived from steam pretreatment were used for ethanol production, respectively. The results indicated that the effects of mentioned factors and their interactions on total ethanol yield were not significant. However, 200 °C, 7.5 min and 2.5% SO_2 impregnation could be determined as a group of optimal pretreatment conditions, in which the highest ethanol yield of 15.3 g/100 g SSB (dry basis) could be obtained without pentose fermentation.

1. Introduction

With world reserves of crude oil fast depleting and environmental degradation concerns growing, the development of alternatives to fossil fuels is receiving increased attention as it becomes an urgent global priority (Rubin, 2008). Ethanol has always been considered a better choice as it can reduces the dependence on reserves of crude oil and promises cleaner combustion leading to a healthier environment (Gupta, Sharma, & Kuhad, 2009). At present, researchers have transferred their attentions from food-based ethanol (the first generation) to non-food-based ethanol (the second generation), since the first-generation ethanol production has been limited by the resource competition between food and fuel production. Sweet sorghum [*Sorghum bicolor* (L.) *Moench*] has been regarded as one of the most promising crops for ethanol production (Gnansounou, Dauriat, & Wyman, 2005). Because it is a C_4 crop with higher photosynthetic efficiency and sugar and biomass yield. It also has a wide adaptability to harsh growth conditions, such as higher drought, water logging, salinity, and alkalinity tolerance (Corredor et al., 2009; Rubin, 2008). Based on these characteristics, the marginal lands could be utilized to grow sweet sorghum for ethanol production, which might give a potential solution to the completion between foods and fuels. Additionally, approximately 9.5% (wet basis) soluble carbohydrates (glucose, fructose and sucrose) and 10% (wet basis) insoluble carbohydrates (cellulose and hemicellulose) exist in sweet sorghum stalk (Sipos et al., 2009). These parts have both been regarded as the important sources for bioethanol production (Yu, Zhong, Zhang, & Tan, 2010). Recently, some applied technologies of ethanol production from sweet sorghum mainly have focused on the solid-state fermentation and juice fermentation of soluble carbohydrates in the stalk (Liu & Shen, 2008; Yu, Zhang, & Tan, 2008). However, regardless of whatever the mentioned technologies are employed, sweet sorghum bagasse (SSB) will be certainly left at large. Moreover, based on the recent economic analysis, the high cost is the bottleneck for the application in many cases of ethanol production, especially, a higher logistic cost, including the system for harvesting, collecting, preprocessing, transporting and handing of the raw materials (Corredor et al., 2009; Hess, Wright, & Kenney, 2007). Therefore, if the SBB (insoluble carbohydrates) could be effectively utilized for ethanol production, the total cost of "sorgnol"

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Table 1
The coded levels and actual values for the 2^3 factorial experimental design.

Run	Coded levels			Actual values			Log R_0
	Temperature	Time	SO_2	Temperature (°C)	Time (min)	SO_2 (%)	
1	−1	−1	−1	190	5	0	3.35
2	1	−1	−1	210	5	0	3.94
3	−1	1	−1	190	10	0	3.65
4	1	1	−1	210	10	0	4.24
5	−1	−1	1	190	5	5	3.35
6	1	−1	1	210	5	5	3.94
7	−1	1	1	190	10	5	3.65
8	1	1	1	210	10	5	4.24
9(a)	0	0	0	200	7.5	2.5	3.82
9(b)	0	0	0	200	7.5	2.5	3.82
9(c)	0	0	0	200	7.5	2.5	3.82

could be curtailed in some degree by sharing the co-logistic cost.

Enzymatic hydrolysis is a main method to obtain the liberated sugars from lignocellulosic material for ethanol fermentation. Prior to the enzymatic hydrolysis and fermentation, the lignocellulosic materials should be pretreated to break the lock of lignin and hemicellulose and expose cellulose for the attack by enzymes (Yang & Wyman, 2008). Currently, some similar raw materials, such as sugarcane bagasse and corn stover, were pretreated with various methods. For instance, SO_2-catalyzed steam pretreatment on sugarcane bagasse and corn stover (Öhgren, Galbe, & Zacchi, 2005; Sendelius, 2005; Varga, Réczey, & Zacchi, 2004), acid pretreatment on corn stover (Zhu, Lee, & Elander, 2005), ammonia fiber expansion (AFEX) pretreatment on sugarcane bagasse (Krishnan et al., 2010).Their optimal pretreatment conditions of corresponding methods were also investigated to improve the enzymatic hydrolysis and ethanol yield. Recently, various pretreatment on SSB for ethanol production, such as AFEX, lime and H_2SO_3-steam, have also been reported (Li, Balan, Yuan, & Dale, 2010; Umagiliyage, Choudhary, Liang, Siddaramu, & Haddock, 2010; Yu et al., 2010). In current pretreatment methods, SO_2-catalyzed steam pretreatment is one of the most thoroughly investigated methods for the bioconversion (Boussaid, Esteghlalian, Gregg, Lee, & Saddler, 2000; Grous, Converse, & Grethlein, 1986; Pan, Zhang, Gregg, & Saddler, 2004). It has been shown to be an inexpensive and effective process for the treatment of agricultural residues and hardwoods (Ballesteros, Oliva, Negro, Manzanares, & Ballesteros, 2004). However, as an optional method, few investigations of SO_2-catalyzed steam pretreatment on SSB have been carried out in recent. Furthermore, the optimized steam pretreatment conditions for the similar raw materials could not be directly used on SSB since their construction and components are not totally same. Therefore, the SO_2-catalyzed steam pretreatment was performed to investigate the effects of main factors on the enzymatic hydrolysis. The ethanol fermentation of water soluble fraction and water insoluble fraction from steam pretreatment was also carried out to estimate the effects of pretreatment conditions on biomass-ethanol yield from SSB and determine a group of optimal conditions for ethanol production.

2. Materials and methods

2.1. Raw material

Sweet sorghum variety, Liaotian 1, cultivated on the farm of Shanghai Jiao Tong University, China, was employed in this work. The bagasse was obtained after juice extraction by a three-roller mill (130, Debao Machinery Plant, Guangxi Province, China). The wet bagasse was air-dried and resulting samples had a moisture content of 8% (wet basis). The dried SSB was chopped by a small scale industry rubbing machine (9PR-1.6, Forestry and Agricultural Machinery Plant, Dandong, Liaoning Province, China) to less than 4 mm in length. The chopped bagasse was sealed in a plastic bag for the steam pretreatment.

2.2. Pretreatment

The steam pretreatment was carried out in a 2 L steam gun (StakeTech II, Stake Technology, Norval, Ontario, CA) at the Department of Wood Sciences, the University of British Columbia, Canada. 300 g (dry basis) SSB was impregnated overnight with different dosage of anhydrous SO_2 according to the experimental design (in Table 1). The SSB in each run was divided into 6 small bags (~50 g/bag), and each bag of SSB was introduced into the steam gun in batch for pretreatment. Once the desired retention time and temperature were reached, the steam pressure was discharged from the steam gun suddenly. The whole slurry, the mixture of water-soluble fraction (WSF) and water-insoluble fraction (WIF), was collected in a connected cyclone. After finishing pretreatment of 300 g SSB, the slurry was transferred from the cyclone and separated into WSF and WIF using vacuum filtration. Both of WIF and WSF were stored in a −20 °C freezer for further use.

2.3. Enzymatic hydrolysis

A commercial cellulase (Spezyme-CP, Gencencor, Palo Alto, CA) supplemented with β-glucosidase (Novzymes188, Bagsværd, Danmark) was used for the enzymatic hydrolysis. The cellulase activity of Spezyme-CP was 48.6 FPU/mL with the protein concentration of 133.9 mg/mL. The β-glucosidase activity of Novzymes188 was 458.4 CBU/mL with the protein concentration of 233.4 mg/mL. The WIF were enzymatically hydrolyzed in sodium acetate buffer (0.05 M, pH 4.8) with the substrate loading of 2% and 10%, respectively. The loading of 2% was used for the evaluation of pretreatment, and 10% was employed to obtain the hydrolysate for subsequent fermentation. The enzyme loading of Spezyme-CP and Novzymes188 was 20 FPU/g glucan and 40 CBU/g glucan. All runs of hydrolysis were performed in duplicate in the 150 mL screw flasks in the incubator with 150 rpm and 50 °C for 72 h. The hydrolysate afterwards was deactivated in the 100 °C oil bath for 15 min, and the deactivated hydrolysate was stored in the −20 °C freezer till for the subsequent fermentation.

2.4. Fermentation

2.4.1. WSF fermentation

The WSFs derived from steam pretreatment were directly employed for fermentation without detoxification and any external nutrients supplementation after the pH was adjusted to 6.0 using 50% NaOH solution. The yeast strain of *Sacharomyces cerevisia*, Tembec T1 (provided by Tembec Limited, Témiscaming, Québec,

Canada), was inoculated with a final concentration of 5 g/L (dry weight). The fermentation was carried out in 150 mL serum bottles for 48 h at 30 °C and 150 rpm. 250 μL samples were collected at the end of fermentation. The obtained samples were centrifuged at 13,000 rpm for 5 min and the supernatant was stored at −20 °C till for ethanol analysis.

2.4.2. WSF hydrolysate fermentation

Prior to the fermentation, the pH of hydrolysate was adjusted to 6.0 using 50% NaOH. Yeast strain of *Sacharomyces cerevisia* (Tembec T1) was inoculated into the hydrolysate at the final concentration of 3 g/L to carry out the fermentation with supplementation of 0.5 g/L $(NH_4)_2HPO_4$ and 1 g/L $MgSO_4{\cdot}7H_2O$ as nutrients. The fermentation conditions were totally same with that of WSF. After 48 h fermentation, 250 μL samples were collected and centrifuged at 13,000 rpm for 5 min. The supernatants were stored at −20 °C for further analysis.

2.5. Analysis

2.5.1. Monomeric sugars

The monomeric sugars, including arabinose, galactose, glucose, xylose, and mannose, were measured with a HPLC system (Dionex, ICS-3000, Sunyvale, CA), which was integrated with and an autosampler (Dionex, AS-50), a gradient pump (Dionex, GP50), an anion exchange column (Dionex, CarboPac PA1) and an electrochemical detector (Dionex, ED50). The mobile phase was the deionized water at a flow rate of 1.0 mL/min, and postcolumn addition of 0.2 M NaOH maintained optimization of baseline stability and detector sensitivity. The column needed to be reconditioned with 1 M NaOH after each sample. The samples had to be filtered with 0.45 μm syringe filter before they were prepared for running on the HPLC. The injection volume was 25 μL and the column temperature was maintained at 35 °C. The standard stock of the mentioned monomeric sugars above was prepared with a suitable concentration. Fucose (∼0.2 g/L) was used as an internal standard for sample analysis.

2.5.2. Oligomeric sugars

The oligomeric sugars in raw material, WIF and WSF needed to be acid-hydrolyzed before analysis. As for the raw material and WIS, the samples (∼0.2000 g) were triplicate prepared with the method of the modified Tappi T-222 om-88 as the reference described previously to obtain the monomeric sugars for analysis (Bura, Mansfield, Saddler, & Bothast, 2002). For the WSF, all the samples were hydrolyzed in duplicate to convert the oligomeric sugars into their corresponding monomers. Concretely, 0.697 mL of 72% H_2SO_4 was added into a certain volume of WSF sample and the total volume was made up to 20 mL with distilled water. The WSF samples were then autoclaved in 121 °C for 1 h to obtain the monomeric sugars. The obtained monomeric sugars from raw material, WIF and WSF were detected by HPLC as described above. The content of oligomeric sugars in raw material, WIF and WSF could be calculated according to their corresponding monomeric sugars content determined in this section.

2.5.3. Ethanol

Ethanol was analyzed by a Hewlett–Packard 5890 gas chromatograph equipped with a HP-Innowax column (15 m × 0.53 mm) with helium as the carrier gas (20 mL/min). The temperatures of the injection unit and flame ionization detector (FID) were set at 175 °C and 250 °C, respectively. The oven was heated to 45 °C for 2.5 min and the temperature was raised to 110 °C at a rate of 20 °C/min and later held at 110 °C for 2 min. The ethanol (chromatographic grade) and butanol (0.5 g/L) were used for the standard curve and internal standard substance separately. Samples of 0.1 mL were directly auto-injected into the column. All determinations were done by means of standard curves, and the final results were the average of two repetitions.

2.6. Experimental design

A 2^3 factorial experimental design was employed to justify the effects of main factors of SO_2-catalyzed steam pretreatment on the hydrolysis and ethanol yield (Chen, 2002). As shown in Table 1, the pretreatment temperature, residence time and dosage of SO_2 impregnation were chosen as the independent variables. The high and low level of these variables were coded as +1 and −1, respectively. Meanwhile, three center points (0) were also set to justify the repeatability of the experiment. In addition, the pretreatment severity can be defined as: $R_0 = t{\cdot}e^{(T-100)/14.75}$ (Overend & Chornet, 1987). Where, t is residence time (min), and T is the pretreatment temperature (°C). A third-order model as the following Eq. (1) is employed to estimate the response values.

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_1 X_2 + \beta_5 X_1 X_3 + \beta_6 X_2 X_3 + \beta_7 X_1 X_2 X_3 \quad (1)$$

where, Y is the predicted response, and X_1, X_2, X_3 stand for the variables of coded pretreatment temperature, residence time and SO_2 impregnation. The β terms are the parameters (regression coefficients) whose values are to be determined. β_1, β_2, β_3 represent the influence coefficient of the individual variable. β_4, β_5, β_6 represent the influence coefficient of interaction between each individual variable. β_7 stands for the influence coefficient of interaction of the three variables. The experiment was designed and analyzed by the software of Design Expert (Version 7.1, Stat-Ease, Inc., Minneapolis, MN).

3. Results and discussion

3.1. Composition of sweet sorghum bagasse

As shown in Table 2, the composition of the raw SSB indicated that both carbohydrate and lignin content were similar with the reported other typical herbaceous residues such as corn stover, and lower than those of typical hardwoods (poplar) and softwood (Douglas-fir) (Bura, Chandra, & Saddler, 2009; Yang, Boussaid, Mansfield, Gregg, & Saddler, 2002). In addition, xylan represented approximate 90% of the entire hemicellulose in SSB, which was also typical of other herbaceous feedstock and hardwoods compared to the mannose dominant hemicellulose in softwood. Approximate 60% of the total dry matter was polysaccharide, in which the largest fraction was glucan occupying 35.1% in total carbohydrate content. Thus, such high polysaccharide content could be potentially made SSB available for hydrolysis and subsequent ethanol fermentation. Moreover, the ash content accounted for 1.9% of the total dry matter of SSB, and it appeared to be very low compared with other agricultural residues, such as rice straw and corn stover. A lower ash content in lignocellulosic material was proved to be potentially beneficial for enzymatic hydrolysis (Yu & Chen, 2010). The extractives content in the SSB was 21.2%, which mainly included the residual soluble carbohydrates after juice extraction, proteins according to the preliminary analysis. Totally, the main composition of the employed SSB in present study was almost similar with the forage sorghum used in reference (Li et al., 2010).

3.2. Pretreatment

Based on the results of main composition of pretreated SSB in Run 9(a–c) (see Table 3), the RSDs (relative standard deviation) of glucan, xylan and lignin content in Run 9 were 3.0%, 7.7% and

Table 2
The composition of sweet sorghum bagasse.

Carbohydrates (%, wt/wt)					Lignin (%, wt/wt)		Ash (%, wt/wt)	Extractives (%, wt/wt)
Glucan	Xylan	Galactan	Arabinan	Mannan	AIL[a]	ASL[b]		
35.1 ± 1.0	19.4 ± 0.9	1.5 ± 0.1	1.4 ± 0.0	0.9 ± 0.0	18.4 ± 2.8	0.2 ± 0.1	1.9 ± 0.1	21.2 ± 2.0

[a] Acid insoluble lignin.
[b] Acid soluble lignin.

Table 3
The composition of pretreated SSB in different conditions.

Run	Glucan (%, wt/wt)	Xylan (%, wt/wt)	Mannan (%, wt/wt)	Arabinan (%, wt/wt)	Galactan (%, wt/wt)	AIL (%, wt/wt)	ASL (%, wt/wt)	Ash (%, wt/wt)
1	54.2 ± 1.8	14.9 ± 0.7	n.d.[a]	0.7 ± 0.0	n.d.	24.7 ± 0.9	1.9 ± 0.0	2.7 ± 0.0
2	61.8 ± 0.8	5.3 ± 0.1	n.d.	0.3 ± 0.0	n.d.	30.2 ± 0.6	1.7 ± 0.1	2.7 ± 0.1
3	60.8 ± 0.3	5.7 ± 0.1	n.d.	0.2 ± 0.0	n.d.	30.0 ± 0.1	1.6 ± 0.2	1.6 ± 0.0
4	62.7 ± 1.5	1.7 ± 0.0	n.d.	0.1 ± 0.0	n.d.	34.0 ± 0.3	1.7 ± 0.1	1.4 ± 0.0
5	59.6 ± 0.4	9.4 ± 0.26	n.d.	n.d.	n.d.	25.8 ± 0.9	1.9 ± 0.0	2.3 ± 0.0
6	59.3 ± 1.0	n.d.	n.d.	n.d.	n.d.	39.3 ± 0.6	1.6 ± 0.0	2.1 ± 0.1
7	64.5 ± 0.4	3.7 ± 0.3	n.d.	n.d.	n.d.	32.7 ± 1.4	1.7 ± 0.0	1.1 ± 0.0
8	63.6 ± 2.0	n.d.	n.d.	n.d.	n.d.	37.8 ± 2.7	1.5 ± 0.1	1.2 ± 0.0
9(a)	63.5 ± 0.5	4.9 ± 0.1	n.d.	0.3 ± 0.1	n.d.	27.7 ± 1.0	1.7 ± 0.1	1.9 ± 0.2
9(b)	59.9 ± 1.8	4.2 ± 0.4	n.d.	0.5 ± 0.0	n.d.	27.7 ± 2.8	1.9 ± 0.2	2.1 ± 0.2
9(c)	62.4 ± 0.5	4.5 ± 0.5	n.d.	0.2 ± 0.0	n.d.	30.8 ± 0.7	1.7 ± 0.0	2.0 ± 0.0

[a] "n.d." means "not detected".

Table 4
The solid recovery after pretreatment and the glucan–glucose conversion at the substrate loading of 2%.

Run	Temperature(°C) X_1	Time (min) X_2	SO_2 impregnation (%) X_3	Solid recovery[a] (%)	Glucan–glucose conversion (%)
1	190 (−1)	5 (−1)	0 (−1)	64.5	62.8
2	210 (1)	5 (−1)	0 (−1)	56.1	75.7
3	190 (−1)	10 (1)	0 (−1)	57.0	74.3
4	210 (1)	10 (1)	0 (−1)	55.1	87.3
5	190 (−1)	5 (−1)	5 (1)	56.5	71.7
6	210 (1)	5 (−1)	5 (1)	52.6	75.0
7	190 (−1)	10 (1)	5 (1)	52.4	87.2
8	210 (1)	10 (1)	5 (1)	48.6	100.0
9 (a)	200 (0)	7.5 (0)	2.5 (0)	51.6	87.8
9(b)	200 (0)	7.5 (0)	2.5 (0)	51.5	89.7
9 (c)	200 (0)	7.5 (0)	2.5 (0)	51.9	88.5

[a] Solid recovery is defined as the dry weight ratio after and before steam pretreatment.

5.7%, which were all in an acceptable range (<10%). It could be deduced that the steam pretreatment results could be repeatable in such pretreatment equipment. As expected, steam pretreatment preferentially attacked the hemicellulose fraction. The harsher the pretreatment conditions were, the higher amount of hemicellulose could be solubilized. In the harshest conditions (Runs 4 and 8), hemicellulose was almost totally removed from the solid fraction. Based on the results in Table 4, the solid recovery varied from 48.6% to 64.5% in a decrease trend, when residence time, temperature and SO_2 impregnation dosage increased. The decrease in solid recovery was due to the degradation of liberated sugars and/or extractives. In addition, a part of cellulose also could be solubilized under much severer conditions (higher temperature or longer residence time) (Varga et al., 2004).

3.3. Statistical analysis

In order to estimate the substrate reactivity to enzymes, the hydrolysis with the substrate loading of 2% was carried out using the WIF at various conditions. The hydrolysis results were presented in Table 4. The analysis of variance (ANOVA) was also performed and presented in Table 5, in which the sum of squares, mean sum of squares, *F*-values, and *p*-values of each term in the equation model (1) were estimated. According to the summarized ANOVA results, the *p*-value of the model was 0.0063, indicating that the model was reliable for the responses. Additionally, the three linear terms X_1 (temperature), X_2 (time) and X_3 (SO_2 impregnation) had significant effects on the glucan–glucose conversion responses (with the *p*-values of under $\alpha = 0.05$). The two-factor interaction terms X_2X_3 had *p*-value under 0.05, indicating that the interaction of residence time and SO_2 impregnation had a significant influence on the responses. The rest of two-factor interaction terms X_1X_2, X_1X_3 and the three-factor interaction $X_1X_2X_3$ had the *p*-value beyond 0.05, which indicated that the interactions of pretreatment temperature × SO_2 impregnation, pretreatment temperature × residence time and pretreatment temperature × residence time × SO_2 impregnation had no signif-

Table 5
The ANVOA results of the model.

Source	SS	df	MS	*F*	*p*
Model	940.8	7	134.4	159.2	0.0063
X_1	220.7	1	220.7	261.5	0.0038
X_2	504.8	1	504.8	598.0	0.0017
X_3	142.2	1	142.2	168.4	0.0059
X_1X_2	11.4	1	11.4	13.5	0.0666
X_1X_3	12.1	1	12.1	14.3	0.0634
X_2X_3	38.1	1	38.1	45.2	0.0214
$X_1X_2X_3$	11.5	1	11.5	13.7	0.0661
Curvature	194.0	1	194.0	229.8	0.0043
Pure error	1.7	2	0.8		
Total SS	1136.4	10			
Adjusted *R*-square	0.99				

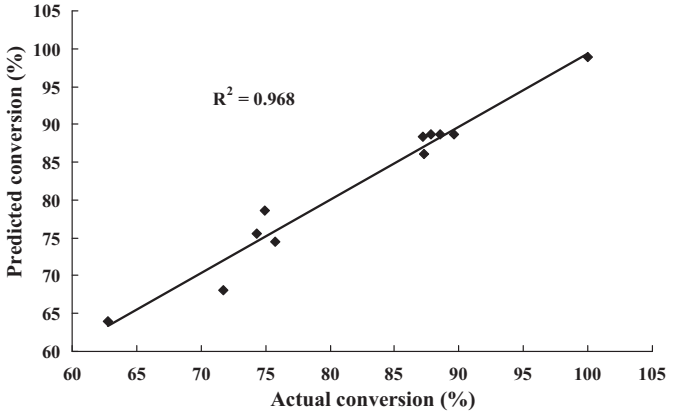

Fig. 1. The observed glucan–glucose conversion vs. the predicted conversion.

icant effects on the responses. The adjusted R^2-value was 0.99, suggesting that variations in the observations indeed account for most of the variation of the responses. The equation that related the glucan–glucose conversion as the dependent variable (Y, %) to other terms, as listed in Table 4, could be expressed in Eq. (2) as follows.

$$Y = 79.24 + 5.25X_1 + 7.94X_2 + 4.22X_3 + 1.19X_1X_2 - 1.23X_1X_3 + 2.18X_2X_3 + 1.20X_1X_2X_3 \quad (2)$$

where, Y is the glucan–glucose conversion (%); X_1 is the code of pretreatment temperature; X_2 is the residence time; X_3 is the SO_2 impregnation dosage.

The effects of individual variables and interactional effects could be estimated from Eq. (2). In this equation, it could be found that the influence coefficients of X_1, X_2, X_3 and X_2X_3 were 5.25, 7.94, 4.22, and 2.18, which meant that the influence sequence of these main factors on enzyme hydrolysis was residence time > temperature > SO_2 impregnation > the interaction of residence time × SO_2 impregnation. Based on Table 5, the insignificant term could be neglected, and a statistically significant model only with significant terms could be written as following Eq. (3):

$$Y = 79.24 + 5.25X_1 + 7.94X_2 + 4.22X_3 + 2.18X_2X_3 \quad (3)$$

And, the final model expression in terms of actual factors could be written as following Eq. (4):

$$\begin{aligned}\text{Glucanglucose conversion (\%)} &= -47.32 + 0.53 \ \text{temperature (°C)} + 2.30 \ \text{residence time (min)} \\ &\quad - 0.93\text{SO}_2 \text{ impregnation dosage (\%)} \\ &\quad + 0.35 \ \text{residence time (min)} \times \text{SO}_2 \text{ impregnation dosage (\%)}\end{aligned} \quad (4)$$

In order to check the validation of the final model, the comparison of experimental glucan–glucose conversion and the predicted values at various pretreatment conditions was carried out. Based on Eq. (4), the regression between the predicted glucan–glucose conversion and the actual values was plotted in Fig. 1. The correlation coefficient (R^2) of the predicted values and actual values was 0.968, which indicated Eq. (4) could be used as the prediction for potential glucan–glucose conversion according to the pretreatment conditions.

3.4. Effects of different pretreatment conditions on WIS hydrolysis

The crystallinity of cellulose, accessible surface area, protection of cellulose by lignin, the heterogeneous character of biomass particles, and cellulose sheathing by hemicellulose all contributed to the recalcitrance of lignocellulosic biomass to hydrolysis (Chang & Holtzapple, 2000). As we known, hemicellulose can have some degree of acetylation, for example, in the form of heteroxylan. The acetylation could be firstly broken and hydrolyzed into acetic acid in a critical starting temperature. The accumulation of resulting acetic acid could be increased with prolonging residence time. The hemicellulose hydrolysis could be well catalyzed in a certain high level acetic acid concentration. This was the autocatalyzed steam pretreatment process, which could be described by the Arrhenius Equation of thermo-chemical reaction (Eken-Sara olu, Mutlu, Dilma, & Avuolu, 1998; Mittal, 2007). According to the Arrhenius equation, the pretreatment temperature controlled the acetic acid formation rate rather than acetic acid accumulation in the steam pretreatment processes. In the autocatalyzed steam pretreatment process, the hemicellulose could be hydrolyzed more in a higher acetic acid concentration resulting from the acid accumulation by prolonging residence time. Thereby, temperature and residence time were both significant for the enzymatic hydrolysis of biomass. As reported, the optimal hemicellulose solubilization and enzymatic hydrolysis could be achieved by either high temperature with short residence time (270 °C, 1 min) or lower temperature with longer residence time (190 °C, 10 min) (Duff & Murray, 1996). In the present work, the similar results also appeared in Run 2 (210 °C, 5 min) and Run 3 (190 °C, 10 min), in which almost same glucan–glucose conversion could be obtained (see Table 4); When the SO_2 was applied for the catalyst by undergoing a combination of oxidation and disproportionation reactions that yield sulfuric acid within the raw materials (Shevchenko, Chang, Robinson, & Saddler, 2000). The resulting acid catalyst improved the partial hydrolysis and solubilization of the hemicellulose and hydrolytic reactions of lignin (Clark, Mackie, Dare, & McDonald, 1989). According to the results in Table 4, when the pretreatment temperature was fixed at 190 °C or 210 °C, the improvement of glucan–glucose conversion by individually increasing of residence time or SO_2 impregnation was both lower than that of increasing them simultaneously. For example, the conversion in Run 7 was higher than Runs 3 and 5. The ANOVA results in Table 5 again proved that the interaction of residence time and SO_2 impregnation could significantly affect the enzymatic hydrolysis. In addition, the referred studies indicated that higher pretreatment temperature could result in the formation of inhibitory materials for fermentation (Wright, 1988). though the enzymatic hydrolysis could be improve significantly. Moreover, the results in Table 4 showed that the a reasonable high conversion (87.2%) in Run 7 also could be achieved at the conditions of 190 °C, 10 min and 5% SO_2 impregnation. Therefore, prolonging residence time and increasing SO_2 impregnation instead of increasing temperature in steam pretreatment could be a potentially better choice for converting water insoluble fraction into bioethanol.

3.5. Estimation and optimization of pretreatment condition on total ethanol yield

In order to evaluate the effects of pretreatment conditions on total ethanol yield from the SSB, the fermentation using the WSF was performed. Meanwhile, the separate hydrolysis and fermentation (SHF) using WIF with the substrate loading of 10% was also carried out. The total ethanol yield was defined as the ethanol obtained from 100 g (D.W.) raw material. In this work, it included ethanol obtained from WSF and WIF. The results were presented in Fig. 2

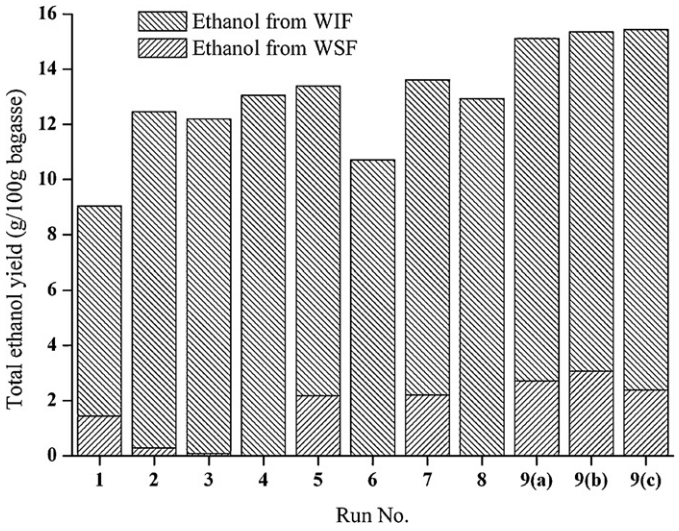

Fig. 2. The total ethanol yield in various conditions.

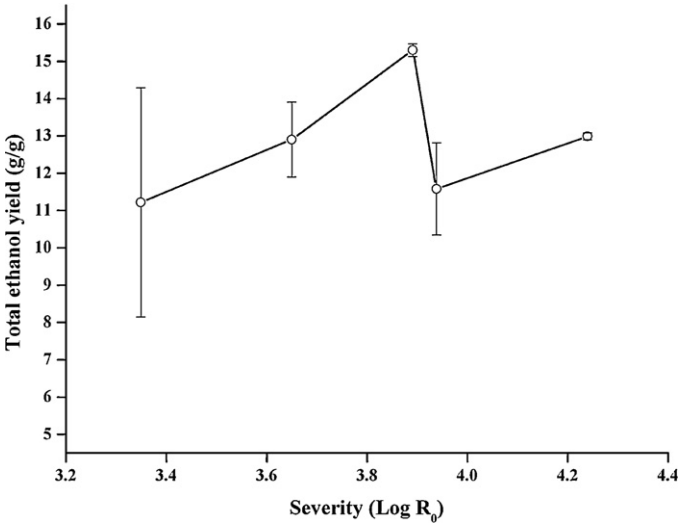

Fig. 3. The severity vs. the assorted total ethanol yield.

According to Fig. 2, the fermentation in WSF was almost totally inhibited when pretreatment temperature increased to 210 °C. Taking Runs 2, 4, 6 and 8 as examples, almost no ethanol was detected. In contrast to Run 1, WSF in Run 3 could not be fermented normally, when the residence time increased from 5 min to 10 min in pretreatment. Only 0.3 g/L ethanol could be detected in Run 3 at the end of fermentation. However, the presence of SO_2 in the pretreatment process at lower pretreatment severity seemed to have the function to protect the liberated pentose and hexose from the degradation. For instance, the fermentation in Run 7 (190 °C, 10 min and 5% SO_2 impregnation) went well compared with Run 3 (190 °C, 10 min and no SO_2 impregnation). The ethanol yield of WSF in Run 5 (190 °C, 5 min and 5% SO_2 impregnation) was also higher than that of Run 1 (190 °C, 5 min and no SO_2 impregnated). Additionally, the fermentation also could be well conducted in Run 9(a–c) with an increase of SO_2 impregnation in contrast to Run 1, although the pretreatment temperature and residence time were increased simultaneously. As reported in the referred studies, the presence of SO_2 in the steam pretreatment could improve the sugar recovery (Clark & Mackie, 1987), that was to mean, the sugar degradation and the production of inhibitory compounds could be decreased with the presence of SO_2 during the pretreatment (Morjanoff & Gray, 1987). Thus, the fermentation of WSF might go well with SO_2 participation in pretreatment when the severity was in a low level.

It could be obviously found that enhancing the severity or presence of SO_2 could improve the ethanol production in the SHF of WIF because the substrate accessibility to enzymes was increased and thereby more sugar could be liberated in the hydrolysis stream (Hendriks & Zeeman, 2009). Combining the results of WSF and WIF fermentation, it could be showed that the potential increasing of total ethanol yield by increasing the severity will be impaired by the fermentation inhibition in WSF (in Runs 4 and 8). However, when the low severity was employed, the potential ethanol production from WIF and WSF were both in lower levels, which also negatively affected the increase of total ethanol yield. The ANVOA results (data not shown) indicated that the influence of pretreatment temperature, residence time and SO_2 impregnation had no significant influences on the total ethanol yield, neither did their two-factor and the three-factor interactions. In order to determine a suitable pretreatment conditions for ethanol production, the total ethanol yield could be assorted according to their pretreatment severity to make a comparison. The assorted results were plotted in Fig. 3, and it indicated that the total ethanol yield could be enhanced by increasing the severity, and then decreased when the severity was in 4.24 (210 °C and 5 min). The highest total ethanol yield could be achieved at the severity of 3.82. Therefore, the pretreatment conditions of 200 °C, 7.5 min and 2.5% SO_2 impregnation could be selected as an optimal one for the ethanol production from SSB. In the conditions, the total ethanol yield could arrive at 15.3 g/100 g SSB (D.W.) with 72.7% hexosan to ethanol conversion. In contrast to the results in the reference, the ethanol yield of 17.1 g /100 g raw material (D.W.) from forage sorghum bagasse could be obtained by the ammonia fiber expansion (AFEX) pretreatment with the optimized conditions (30 min and 140 °C) (Li et al., 2010). The ethanol yield in the reference was higher than that of the current work, because the liberated glucose and xylose could be fermented by the employed yeast. However, only glucose could be fermented by yeast strain of Tembec T1 in this work. Therefore, if the ethanol from pentose fermentation were not considered, the optimized AFEX and SO_2-calalyzed steam pretreatment had a similar efficacy to covert sorghum bagasse to ethanol.

Additionally, as presented in Fig. 3, the temperature and the residence time were integrated as a variable (pretreatment severity). Thereby, the standard difference (SD) of the assorted ethanol yield could mainly reflect the influence of SO_2 impregnation on total ethanol yield except for the center points (Run 9). The SD was in the trend of decrease when the pretreatment became more severe. Results in Fig. 2 indicated that the total ethanol yield in Run 1 was 9.0 g/100 SSB (dry basis), whereas it could increase to 13.4 g/100 SSB (dry basis) in Run 5 when 5% SO_2 was impregnated. However, when the pretreatment severity was in highest, the impregnation of SO_2 had almost no influence on the total ethanol yield (see Runs 4 and 8 in Fig. 2). Therefore, it could be deduced that the SO_2 impregnation will be crucial to increase the total ethanol production in the low severity. While the severity was increased heavily, it was the temperature and the residence time that controlled the final ethanol production and the function of SO_2 will be weakened. As a result, it was suggested that the SO_2 had to be impregnated in SSB if the low severity was employed in steam pretreatment for ethanol production. On the contrary, when the high severity was employed, the acid impregnation seemed to be unnecessary.

4. Conclusions

Sweet sorghum bagasse was steam-pretreated according to the 2^3 factorial experimental design to evaluate the influences of three main factors and their interactions on substrate reactivity for hydrolysis. The results indicated pretreatment temperature, resi-

dence time, SO_2 impregnation and the interaction of residence time and SO_2 impregnation significantly affected enzymatic hydrolysis with a sequence of residence time > temperature > SO_2 impregnation > the interaction of residence time and SO_2 impregnation. The equation of glucan–glucose conversion (%) = −47.32 + 0.53 temperature (°C) + 2.30 residence time (min) − 0.93 SO_2 impregnation dosage (%) + 0.35 residence time (min) × SO_2 impregnation dosage (%) could be employed for predicting hydrolysis yield at the substrate loading of 2%. The mentioned factors and their interactions all had no significant influences on total ethanol yield. However, 200 °C, 7.5 min and 2.5% SO_2 impregnation could be the suitable pretreatment conditions, in which total ethanol yield of 15.3 g/100 g SSB (D.W.) with 72.7% hexosan to ethanol conversion could be achieved.

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