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SACCHARIFICATION OF ACID-PRETREATED SWEET SORGHUM STRAW BY CELLULASE FOR BIOETHANOL PRODUCTION

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Abstract. This study is focused on the yield of fermentative sugars liberated from the diluted sulfuric acid pretreatment of sweet sorghum straw followed by enzymatic saccharification of a commercial cellulase (Celluclast 1.5, Novozyme). The sweet sorghum straw was mixed with 3% dilute sulfuric acid with 10%w/v of solid loading and then, pretreated at various temperatures (120-190°C) for 10 min of pretreated times. The maximum yields of glucose and xylose from sweet sorghum straw were 0.234 g/g dry substrate and 0.208 g/g dry substrate, respectively, at the pretreatment condition: 120 ºC, 3%H_2SO_4 for 10 min. In this case, a total of 50.04% of glucan and 76.41% of xylan were converted to glucose and xylose, respectively. The saccharification conditions were 1.0-7.0% of the acid pretreated sweet sorghum straw, 15-35 FPU/g-substrate of cellulase, pH from 3 to 7 and temperature from 30 to 50 ^{o}C. A maximum saccharification yield of glucose was 0.366 g/g dry substrate at the optimal condition: 1.0-2.5% of the acid pretreated sweet sorghum straw, 30 FPU/g-substrate of cellulase, pH in the range 3-5, temperature in the range 30-50^{o}C and 96 h of incubation time. Furthermore, the saccharification conditions were optimized using a statistical analysis (Response surface methodology).

Keywords: Acid pretreatment, Bioethanol, Cellulase, Saccharification, Sweet sorghum straw

1. Introduction

Rapid price rising of petroleum-based fuels have recently increased the interest in alternative fuels. Lignocelluloses is one of potential choices due to sufficient abundance and generating very low net greenhouse emissions. Sweet sorghum *(Sorghum bicolor* (L.) Moench) is a tropical grass grown primarily in semiarid and arid parts of the world. It can adapt to a wide range of climates from the tropics to cool temperate areas. It is also drought and water logging, saline and alkaline tolerant. In addition, the straw or bagasse of sweet sorghum is an abundant and low-cost lignocellulosic material that can be synthetically used as a raw material for ethanol production with some byproduct with high additional value [1].

Sweet sorghum is mainly made up of celluloses, hemicelluloses and lignin. The natural structures of this biomass make it difficult for microorganism to utilize these component to produce ethanol. Generally, the lignin composition has been thought to be a critical factor to inhibit enzymatic hydrolysis by irreversible adsorption onto the cellulase, which is believed to reduce the amount of enzyme available for enzymatic hydrolysis [2]. Furthermore, the hemicelluloses–lignin matrix that surrounds the cellulose fraction in this biomass has been suggested to act as a physical barrier, which hinders the access of cellulase to the surface of the cellulose. It is believed that hemicelluloses removal may increase the pore volumes and surface areas of the solid residues and assist the access of cellulase to the cellulose structures [3].

The purpose of the pretreatment is to break the lignin seal, pre-hydrolyze the hemicellulose, and disrupt the crystalline structure of the cellulose. Efficient pretreatment method is required for the sequentially enzymatic hydrolysis to gives the maximal sugar productivity. Consequently, success of using renewable biomass for ethanol production depends upon the physical and chemical properties of the biomass,

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pretreatment methods, efficient microorganisms and optimization of the processing conditions. Pretreatment of lignocelluloses with acid gives a high recovery of hemicelluloses sugars in the liquid fraction with most of the cellulose remaining in the solid residue for sequential enzymatic hydrolysis. To date, the development of an integrated process combining optimized acid pretreatment, enzymatic saccharification and fermentation is thus of great interest for effective use of lignocellulosic materials for ethanol production [4].

The objective of this research is to evaluate the acid pretreatment method and the saccharification condition for converting sweet sorghum straw into fermentable sugars. The yields of combined monomeric sugar are determined. Furthermore, the optimum saccharification variables are also investigated using statistical analysis (RSM method).

2. Methods

2.1. Pretreatment of sweet sorghum straw

Sweet sorghum straw (SSS) in this study was obtained from The Suphanburi Field Crops Research Center. It consisted of 44.51% cellulose, 38.62% hemicellulose, 6.18% lignin and 10.69% ash. The chopped SSS was dried in oven at 70 ^{o}C to a constant weight. Thirty grams of chopped sweet sorghum were suspended in 300 ml of 3% aqueous solution of H_2SO_4 at 120, 150, 170 and 190 ^{o}C for 10 minutes. After pretreatment step, the hydrolyzates were neutralized with 40% NaOH, centrifuged and filtered through 0.45 μm filters before analysis total reducing sugars by DNS method and monomeric sugar (glucose, xylose, galactose, arabinose, and mannose) by HPLC. The solid residue was collected by filtration and washed extensively with distilled water until neutral pH. The acid pretreated sweet sorghum straw (SSS) was dried in the oven at 70 ^{o}C to a constant weight and used as the substrate for saccharification experiments.

2.2. Saccharification of the acid pretreated sweet sorghum straw

A typical hydrolysis mixture was consisted of 0.1 g of the acid pretreated SSS, 20 FPU/g substrate cellulase (Celluclast 1.5, Novozyme) and 2.0 ml of sodium phosphate buffer (pH 6.0). Microbial contamination was prevented by adding sodium azide (0.01 mg/ml). The mixture was incubated at 50 ^{o}C in a rotary shaker at 150 rpm for 7 days. Samples were obtained from the reaction mixture at different time intervals. Samples were cooled, then centrifuged for 10 min at 10,000 rpm and stored at -20 ^{o}C until analysis. The supernatant was used for analysis of total reducing sugars by DNS method and monomeric sugar by HPLC.

2.3. Analysis of monomeric sugar

High-performance liquid chromatography (HPLC) was used to determine the concentrations of monomeric sugar (xylose, glucose, galactose, arabinose, and mannose) using Aminex HPX-87P column (Bio-Rad, Richmond, USA) with a refractive index detector. The analysis condition was at 85°C using Milli-Q water as the eluent with a flow rate of 0.6 ml/min. Peaks area of samples were indentified and quantified by comparison with retention times of analytical standards (glucose, xylose, galactose, arabinose and mannose)

2.4. Statistical analysis

All experiments were performed in triplicate, and the related data were expressed as averages values. The experimental data from acid pretreatment were analyzed using the SPSS for Windows program followed by a factorial test. The experimental data from enzymatic saccharification were statistically analyzed using SPSS program by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Method Test to separate the means. Differences in means were judged significant when p values for the null hypothesis were 0.05 or less [5].

2.5. Response surface methodology

A factorial, central composite rotary design (CCRD) for four factors with replicates at the center point and star points were used in this investigation. The saccharification variables; a substrate concentration of 1–7%, cellulase concentration of 15–35 FPU/g substrate, a temperature of 30-70 ^{o}C and a pH of 3-7; were tested using a statistical analysis and RSM each at five coded levels (-α, -1, 0, +1, +α) as shown in Table 1. The actual levels of variables for CCRD experiments were selected based on the initial levels as the center points. A total of 30 experimental trials including 16 trials for factorial design, eight trials for axial points

(two for each variable) and six trials for replication of the central points were performed [6]. Statistical software package: Design-Expert (Stat-Ease, Inc., Minneapolis, USA) was used for regression analysis of experimental data and to plot response surface.

Table 1. Variables and their levels for the central composite experimental design.

Variable	Symbol	Code levels				
		-2	**-1**	**0**	**1**	**2**
Substrate (%w/v)	A	1%	2.5%	4%	5.5%	7%
Cellulase (FPU/g DS)	B	15	20	25	30	35
Temperature (^oC)	C	30	40	50	60	70
pH	D	3	4	5	6	7

3. Results and discussion

3.1. Acid pretreatment of sweet sorghum straw

Generally, glucan is the major component of SSS followed by xylan and acid-insoluble lignin. Arabinan, galactan and mannan accounted for only a small amount of the biomass composition. After pretreatment of SSS at different temperatures, the hydrolyzates were collected. Results for the pretreatment of SSS with 3%H_2SO_4 were shown in Table 2. The maximum yield of glucose was 0.234 g glucose/g DS at 120^{o}C for 10 min and the maximum yield of xylose was 0.208 g xylose/g DS at for the same condition. In this case, a total of 50.04% of glucan and 76.41% of xylan were converted to glucose and xylose, respectively. The experimental data indicated that glucose yields decreased at pretreated temperature above 120^{o}C. The xylose yields in the hydrolyzates gave similar results with increasing of pretreatment severity.

Table 2. Summary of average yields of monomeric sugar and %conversion using 3%H_2SO_4 with residence time for 10 min

Temp (°C)	**Yield $_{avg}$ (g monosugar /g dry substrate)**				**%Conversion[a]**	
	Glu	Xyl	Gal, Man, Ara	Total Sugars	Glu	Xyl
120	0.234±0.079	0.208±0.073	0.235±0.016	0.676±0.023	50.046 [b]	76.413 [c]
150	0.227±0.010	0.138±0.047	0.240±0.039	0.605±0.018	48.658	50.750
170	0.134±0.091	0.033±0.021	0.161±0.027	0.327±0.035	28.692	11.984
190	0.045±0.015	0.016±0.015	0.229±0.039	0.290±0.038	9.699	5.801

a: sweet sorghum straw consists of 42%glucan and 24%xylan
b: %conversion (g glucose produced/ g glucose theoretical)
c: %conversion (g xylose produced/ g xylose theoretical)

3.2. Enzymatic saccharification of the acid pretreated sweet sorghum straw

In this study, the SSS was pretreated with 3% H_2SO_4 at 120 ^{o}C for 10 min prior to hydrolysis. This treatment was effective in fractionating the hemicelluloses and lignin components. The pretreated SSS consisted of 69.50% cellulose, 0.44% hemicellulose, 19.53% lignin and 10.53% ash. Compared with the chemical components in the untreated materials, it was noted that dilute sulfuric acid pretreatment in SSS exhibited the increament of cellulose composition 56.14%, while the hemicellulose composition decreased by 96.86% [7]. The increment of cellulose content and reduction of hemicellulose content would allow for enhancement of enzymatic saccharification. A preliminary hydrolysis study showed that the cellulase concentration of 20 FPU/g-substrate could hydrolyze 4% the acid pretreated SSS and liberated glucose 0.344 g/g DS. The effect of saccharification time on total reducing sugars liberation was also investigated. A maximum yield of 0.344 g of reducing sugars/g DS was released after 96 h of hydrolysis and no significance change when extended saccharification time to 7 days due to the statistically analyzed using SPSS program (data not shown).

3.3. Optimization of saccharification conditions

The experimental results of saccharification conditions by the central composite design for four-factor-five level were of interest, the substrate concentration, the enzyme concentration, the temperature and the pH [8]. The responses were shown in Table 3.

Table 3 Experimental design and the results of the central composite design

Run	Factor A	Factor B	Factor C	Factor D	Response g glucose/ g DS	Run	Factor A	Factor B	Factor C	Factor D	Response g glucose/ g DS
1	1	-1	-1	1	0.203	16	-1	1	1	1	0.177

2	0	0	0	0	0.296	17	-1	1	-1	1	0.318
3	-1	1	1	-1	0.248	18	1	1	-1	1	0.265
4	1	1	1	1	0.101	19	-1	1	-1	-1	0.366
5	0	0	0	0	0.346	20	0	0	0	0	0.338
6	1	1	-1	-1	0.342	21	0	0	0	0	0.332
7	-1	-1	-1	1	0.312	22	0	-2	0	0	0.310
8	0	0	0	0	0.349	23	0	0	2	0	0.138
9	1	1	1	-1	0.229	24	0	0	0	2	0.026
10	-1	-1	1	-1	0.262	25	0	0	0	-2	0.330
11	-1	-1	-1	-1	0.337	26	-2	0	0	0	0.284
12	1	-1	1	1	0.083	27	0	0	-2	0	0.248
13	-1	-1	1	1	0.109	28	0	2	0	0	0.345
14	1	-1	-1	-1	0.280	29	2	0	0	0	0.266
15	1	-1	1	-1	0.227	30	0	0	0	0	0.286

The statistical treatment combinations of the test variables along with the measured response values, expressed as sugar yield corresponding to each combination, were summarized in Table 1. The application of RSM yielded the following a second-order polynomial equation for saccharification which could be written as follow:

$$Y = +0.323 - 0.018\times A + 0.013\times B - 0.0503\times C - 0.056\times D + 0.0034\times A\times B + 0.0055\times AC - 0.0080\times A\times D - 0.0054B\times C + 0.0046\times B\times D - 0.017\times C\times D - 0.013\times A^2 + 0.00046\times B^2 - 0.033\times C^2 - 0.037\times D^2 \quad \text{--------Eqution (1)}$$

Where Y is g-glucose/g the acid pretreated sweet sorghum straw

The accuracy of fit of the model was checked by plotting between the actual total reducing sugars and the predicted total reducing sugars. The normality assumption is satisfactory as normal residuals fall along a straight line as shown in Figure 1

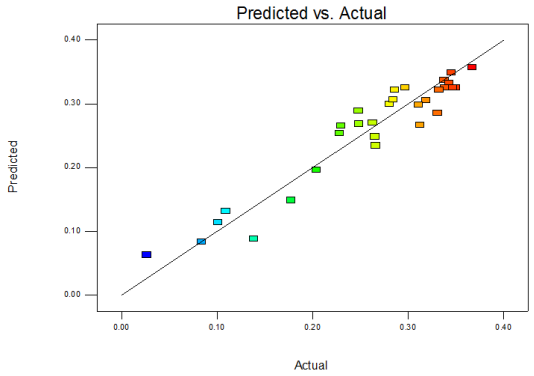

Figure 1 Correlation between actual and predicted the total reducing sugars liberated from the acid pretreated SSS.

The statistical significance of the above equation was checked by the *F* test, and the analysis of variance (ANOVA) for the response surface quadratic model (data not shown)[8]. The model *F* value of 10.89 and values of probability (*P*-values) >*F* or α< 0.0001 indicated that the model was statistically significant with confidence interval 95% and coefficient of determination (R^2) of the model was 0.9159.

From the above study, the optimum saccharification condition was 2.5% of the acid pretreated SSS, 30 FPU/g-substrate of cellulase, the pH of 4 and the temperature of 40 ^oC. Maximum yield of 0.366 g glucose/g DS (Run No.19) was obtained. Whereas several combinations of cellulase (15–35 FPU/g-substrate) and substrate concentrations (1.0-2.5%) gave no significant yields. The results from this study were consistent with Jeya *et al.*(2010) [8]. From an economic point of view, it is agreeable to choose the lowest possible enzyme concentration with the highest possible substrate concentration. An overall economic process must include the achievement of a high saccharification rate at a minimum enzyme concentration over short times [9]. Therefore, in the present study the cellulase concentration of 15 FPU/g-substrate and 2.5% of substrate concentration were chosen for further study.

Table 4. Combined yields of monosugar liberated from the acid pretreatment of the SSS (stage 1) and enzymatic saccharification of the pretreated of SSS (stage 2)

Conditions	Yield $_{avg}$ (g monosugar /g dry substrate)	%

	Glucose	Xyl	Gal, Man, Ara	Total sugars	Sacccharification
Stage 1 Acid pretreatment of SSS using 3% H_2SO_4 at 120°C,10 min	0.234±0.079	0.208±0.073	0.235±0.164	0.676±0.230	25.33
Stage 2 Enzymatic saccharification of the pretreated SSS using 30 FPU/g substrate, 2.5% SSS, 40°C, pH 4	0.366±0.001	0	0	0.366±0.001	47.09
Stage 1 + stage2	0.592±0.080	0.208±0.073	0.071±0.164	1.034±0.231	72.42

* Untreated SSS consists of 44.51% cellulose and 38.62% hemicellulose

** The acid pretreated SSS consists of 69.5% cellulose and 0.44% hemicelluloses

From Table 4 showed the partitioning of glucose, xylose and other monosaccharide released from the first pretreatment step (stage 1) while glucose was the majority monomeric sugar from the saccharification step (stage 2). Combined yields of glucose 0.592 g/g dry substrate and saccharification yield of 72.42% were obtained from these treatments.

4. Conclusions

From this study, sweet sorghum straw has a potential to be a source for the production of fermentative sugars that can be subsequently fermented to ethanol. The conversion of SSS into fermentative sugars consisted of two steps, the acid pretreatment followed by enzymatic saccharification.

5. Acknowledgments

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6. References

[1] S.Z. Li, and C. Halbrendt. Ethanol production in (the) People's Republic of China: Potential and technologies. *Applied Energy*. 2009, 86: 162–169.

[2] L. Zhu, J.P. O'Dwyer, V.S. Chang, and C.B. Granda. Structure features affecting biomass enzymatic digestibility. *Bioresour. Technol*. 2008, 99: 3817–3828.

[3] T.C. Hsu, G.L. Guo, W.H. Chen, W.S. Hwang. Effect of dilute acid pretreatment of rice straw on structural properties and enzymatic hydrolysis. *Bioresource Technology*. 2010, 101: 4907–4913.

[4] B. Buaban, H. Inoue, S. Yano, S. Tanapongpipat, V. Ruanglek, V. Champreda, R. Pichyangkura, S. Rengpipat and L. Eurwilaichitr. Bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting *Pichia stipitis*. *Journal of Bioscience and Bioengineering.* 2010, 110: 18–25.

[5] R.G.D. Steel and Torrie. J.H. Principles and procedures of statistics. *McGraw Hill Book* Co.Inc. New York USA. 1980.

[6] D.C. Montgomery. Response surface method other approaches to process optimization. *Design and Analysis of Experiment*. 2001, 427-461.

[7] A. Poonsrisawat and S. Teeradakorn. Conversion of sweet sorghum straw to sugars by dilute-acid saccharification. In : 3rd Technology and Innovation for Sustainable Development International Conference (TISD2010) Nongkhai, Thailand.

[8] M. Jeya, Y.W. Zhang, I.W. Kim, J.K. Lee. Enhanced saccharification of alkali-treated rice straw by cellulase from *Trametes hirsuta* and statistical optimization of hydrolysis conditions by RSM. *Bioresource Technology*. 2009, 100: 5155–516.

[9] B. Qi, X.Chen, F. Shen, Y. Su, and Y. Wan. Optimization of Enzymatic Hydrolysis of Wheat Straw Pretreated by Alkaline Peroxide Using Response Surface Methodology. *Ind. Eng. Chem. Res.* 2009, 48: 7346–7353.