Alkali Pretreatment on Chewing Cane Top and the Conversion of Fermentable Sugars

Zezheng Zhang [a], Yingxue Gong [b]

College of Life Science and Technology, Jinan University, Guangzhou 510632, China;

[a]245741783@qq.com, [b]tyxgong@jun.edu.cn

Abstract

In this study, the effects of dilute alkaline pretreatment conditions on the compositions of chewing cane top (CCT) were investigated using single factor and orthogonal experiments. The raw and pretreated CCT were further employed to enzymatic hydrolysis for the conversion of fermentable sugars. The results indicated that the optimal pretreatment conditions of CCT were 3% (w/v) NaOH, 121 ℃ pretreated for 60 min. Under the optimum conditions, the glucan, xylan and delignification of pretreated CCT reached the highest of 59.8%, 28.7% and 83.2%, respectively. After enzymatic hydrolysis of 48 h, the glucose concentration, total enzymatic efficiency, and glucose yield of pretreated CCT were 12.6 g/L, 88.7% and 632.0 g/kg substrate, respectively. These results indicated that the CCT is a valuable and promising feedstock for fermentable sugars production.

Keywords

Chewing cane top; Alkaline pretreatment; enzymatic hydrolysis; Sugars.

1. Introduction

Chewing cane (*Saccharum* var. Badila) is one of the most common harvest residues in southern China, which contains abundant glucan and xlycan. It is an attractive and promising lignocellulosic material for production of bio-ethanol. However, there were insufficient studies on the utilization of the top of chewing cane (CCT) for bio-ethanol production.

Ethanol production from lignocellulosic materials comprises two steps prior to fermentation: material pretreatment to break down the structure of the lignocellulosic matrix and enzymatic hydrolysis [1]. In our previous studies, several pretreatment methods were tried on sugarcane bagasse including dilute alkali [2], dilute acid [3], peroxide [3], concentrated alkali [4], alcoholates [5] and steam explosion [3,6]. Among these methods, dilute alkaline pretreatment was extensively used due to its ability to remove lignin and decrease the crystallinity of sugarcane bagasse, and thus, resulting in increased enzymatic efficiency.

In this study, CCT was used as a lignocellulosic material and the alkaline pretreatment conditions were optimized using single factors and orthogonal experiments. The enzymatic hydrolysis was further performed to convert CCT into fermentable sugars.

2. Materials and Methods

2.1 Preparation of CCT

The CCT (over 8 months) was cultivated in the local farm of Lanhe Town, Nansha District, Guangzhou, Guangdong Province, southern China. The material was squeezed, washed, air-dried, milled and sieved through an 80 mesh screen scale before use.

2.2 Single Factor Optimization of Alkali Pretreatment Condition

To investigate the effect of alkali concentration on pretreatment, the CCT was firstly pretreated with different concentration of NaOH (1%, 2%, 3%, 4%, and 5% w/v) with the solid loading of 1:10 (w/v) at 121 ℃ for 1 h. Then the effect of temperature on pretreatment was optimized by using different temperature (30 ℃, 90 ℃, 121 ℃) with 3% NaOH for 1 h. Finally, the pretreatment was carried out for different time (30, 60 and 90 min) with 3% NaOH at 121 ℃ to find the optimum time. After the pretreatment, the solid fraction was washed, air-dried, and stored in a hermetic bag at room temperature for composition analysis.

The solid recovery rate after pretreatment [5] were calculated as follows:

$$\text{Solid recovery rate (\%)} = \frac{\text{Rinsed solid (g)}}{\text{Raw solid (g)}} \times 100\% \quad (1)$$

where rinsed solid was the remaining SC after pretreatment and the raw solid was the untreated SC.

2.3 Enzymatic Hydrolysis of Raw and Pretreated CCT

Enzymatic hydrolysis of the raw and pretreated CCT was carried out at a substrate loading of 2% (w/v) in 25 mL of 0.1 M citric acid/citric sodium buffer (pH 4.8), containing 80 μg/mL tetracycline and 60 μg/mL nystatin to prevent microbial contamination. The adding of enzymes was Celluclast 1.5 L, Novozym 188 (Novozymes, Copenhagen, Denmark) and endo-1,4-β-xylanase of *Trichoderma longibrachiatum* (Sigma-Aldrich, St. Louis, MO, USA) with a loading of 5.97 FPU/g, 23.95 pNPGU/g and 3.34 U/g substrate, respectively. The mixtures were incubated at 45 ℃ in an orbital shaker at 160 rpm for 72 h. Samples of 1 mL were collected from the reaction mixtures at 12 h intervals. Each sample was centrifuged for 5 min at 10,000 g and the supernatants were through 0.22 μm filters (Sartorius, Gottingen, Germany) for analysis.

The glucan and xylan conversion rates, total enzymatic efficiency were calculated using the following equations [5,7]:

$$\text{Glucan conversion rate (\%)} = \frac{\text{Glucose concentration (g/L)}}{\text{Solid loading} \times \text{Glucan (\%)} \times 1.11 \text{ (g/L)}} \times 100\% \quad (2)$$

$$\text{Xylan conversion rate (\%)} = \frac{\text{Xylose concentration (g/L)}}{\text{Solid loading} \times \text{Xylan (\%)} \times 1.14 \text{ (g/L)}} \times 100\% \quad (3)$$

Total enzymatic efficiency (%)

$$= \frac{\text{Glucose concentration} + \text{Xylose concentration (g/L)}}{\text{Solid loading} \times (\text{Glucan (\%)} \times 1.11 + \text{Xylan (\%)} \times 1.14) \text{ (g/L)}} \times 100\% \quad (4)$$

where 1.11 is the coefficient of glucose converted from glucan, 1.14 is the coefficient of xylose converted from xylan.

2.4 Analytical Methods

The chemical compositions of the raw and pretreated CCT were determined following the standard procedure of National Renewable Energy Laboratory (NREL) [8], and the concentration of glucose, xylose, and arabinan were detected by High Performance Liquid Chromatography (HPLC). To ensure the accuracy and reliability, all the experiment were conducted in duplicate. The average value and the standard deviation of duplicate were analyzed.

The HPLC (LC-15C, Shimadzu, Japan) was equipped with a refractive index detector (RID-10A, Shimadzu) and an Aminex HPX-87H column (300×7.8 mm, Bio-Rad, USA). The column was operated at 55 ℃ with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 mL/min.

3. Results and Discussion

3.1 Effects of Alkaline Concentration on Chemical Compositions Of CCT

CCT was studied as a model material in this study mainly due to its large plantation in southern China and its potential use as lignocellulose feedstock. The glucan, xylan, arabinan, lignin (including acid insoluble lignin and acid soluble lignin) and ash content of raw CCT was 36.9%, 25.9%, 5.3%, 18.3% and 3.7%, respectively. Compared with other agricultural waste such as sugarcane bagasse [2]and elephant grass [9], the contents of glucan and xylan in CCT were higher, and lignin was lower.

Alkaline pretreatment is an effective method for low lignin content herbaceous [10]. As shown in Fig. 1, with the increase of NaOH concentrations form 1% to 3%, the content of glucan and xylcan increased gradually, while lignin, especially acid insoluble lignin, was removed significantly in the pretreated CCT. When the concentration of alkali was 3%, the glucan and xylan contents reached the highest of 59.8% and 28.7%, respectively and the lignin content was 2.6%. Compared with raw materials, the increment of glucan and xylan in pretreated CCT was 62.1% and 10.8%, respectively. Meanwhile, the decrement of acid soluble lignin, acid insoluble lignin and ash was 44.1%, 100% and 77.7% respectively. However, as the NaOH concentration was higher than 3%, the glucan and xlycan contents decreased slightly. This might be because the glucan and xylcan were partially removed by high concentration of NaOH. The change of arabinan amount in pretreated CCT was not obvious. This might be because the amount of arabinan was relatively low (less than 3.0%) and the NaOH pretreatment could not remove arabinan considerably. In addition, the ash content was decreased as the NaOH concentrations increased.

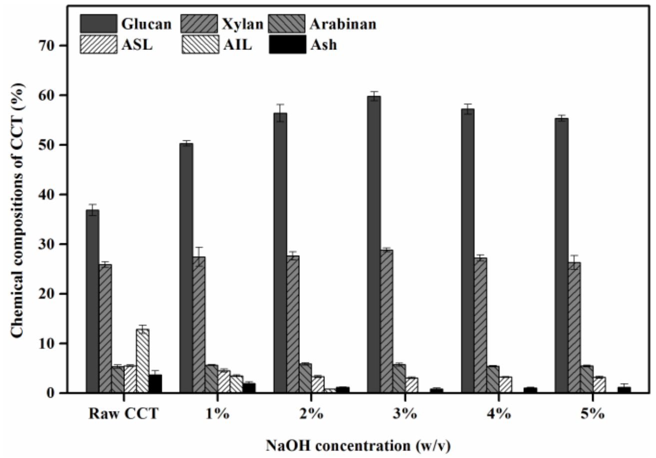

Fig. 1 Effects of different NaOH concentrations on the chemical compositions of CCT ASL, acid soluble lignin; AIL, acid insoluble lignin

3.2 Effects of Alkaline Temperature on Chemical Compositions Of CCT

To investigate the effect of pretreatment temperature on chemical composition, the CCT was pretreated with 3% NaOH at 60 ℃, 90 ℃ and 121 ℃ for 1 h (Fig. 2). With the increase of pretreatment temperatures, the glucan and xylan contents of pretreated CCT increased significantly, and the contents of lignin decreased gradually. The glucan and xylan contents of pretreated CCT achieved the highest at 121 ℃. At the temperature of 60 ℃ and 90 ℃, the acid-insoluble lignin of pretreated CCT decreased 81.1% and 89.7% compared with raw CCT. This indicated that the lignin could be moved effectively by NaOH solution even under mild temperature conditions.

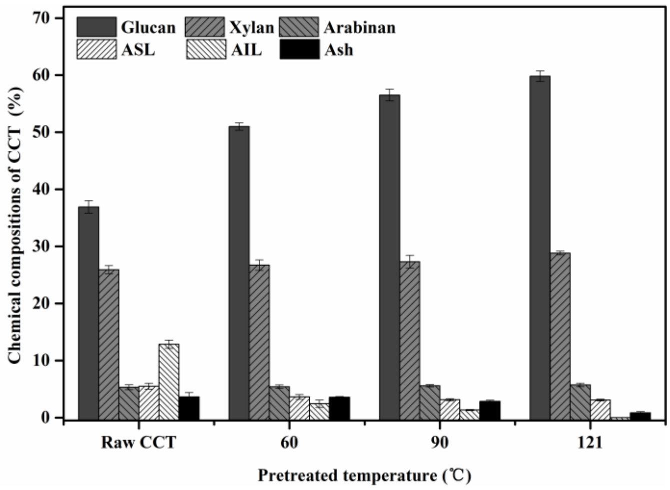

Fig. 2 Effects of different pretreated temperatures on the chemical compositions of CCT ASL, acid soluble lignin; AIL, acid insoluble lignin

3.3 Effects of Alkaline Time on Chemical Composition Of CCT

The effect of pretreatment time on the chemical compositions of CCT was shown in Fig. 3.With 3% NaOH at 121 ℃, the glucan and xylan contents of pretreated CCT achieved the highest when the pretreatment time was 60 min. When the time was extended to 90 min, the content of glucan and xylan decreased. This may be because parts of the glucan and xylan were degraded as the pretreated time was too long.

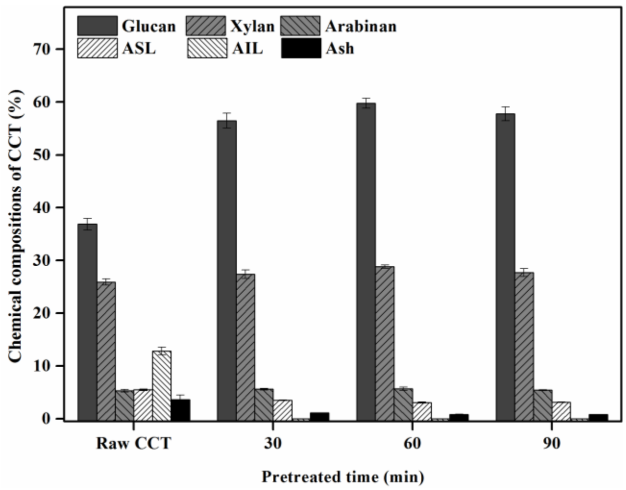

Fig. 3 Effects of different pretreated time on the chemical compositions of CCT ASL, acid soluble lignin; AIL, acid insoluble lignin

The solid recovery rate is a major mark reflecting the retention degree of carbohydrate after pretreatment. And the lignin removal rate was a key factor impacting the enzymatic efficiency of cellulose substrate in the following hydrolysis. With the increase of NaOH concentration in pretreatment, the solid recovery rates of CCT decreased gradually from 57.8% to 45.3%, while the lignin removal rates increased significantly from 56.3% to 82.6% (Table 1). Furthermore, with the increase of pretreatment temperature and the prolongation of pretreatment time, the solid recovery rates of CCT decreased and the lignin removal rates increased gradually. The solid recovery rates of CCT of this study were similar with some previous studies on sugarcane materials under similar alkaline pretreatment conditions, such as solid recovery rate of 52.8% in sugarcane bagasse under 1% NaOH and at 121 ℃ for 1 h [11], and 55% in sugarcane bagasse under 4% NaOH and at 121 ℃ for 0.5 h [12].

Table 1 the solid recovery rates and lignin removal rates of CCT under different NaOH concentrations, pretreated temperatures and time

		The solid recovery rates (%)	Lignin removal rates (%)
Alkali concentration (w/v)	1%	57.8	56.3
	2%	52.5	77.5
	3%	51.2	83.2
	4%	49.1	82.4
	5%	45.3	82.6
Temperature (℃)	60	55.2	67.0
	90	52.5	75.8
	121	51.2	83.2
Time (min)	30	51.4	80.9
	60	51.2	83.2
	90	49.1	82.8

3.4 Orthogonal Optimization Experiments of Pretreatment

Based on the results of single factors of alkali pretreatment, an orthogonal design assistant IIV3.1 was used and nine runs of orthogonal experiments were designed to optimize the pretreatment conditions. The total enzymatic efficiency after enzymatic hydrolysis 72 h was used as the test index.

Table 2 Analysis of orthogonal experimental results of CCT

Runs	A Concentration (w/v)	B Temperature (℃)	C Time (min)	Results
1	1%	60	50	63.7
2	1%	90	60	71.5
3	1%	121	70	76.9
4	3%	60	60	78.9
5	3%	90	70	82.2
6	3%	121	50	85.3
7	5%	60	70	71.8
8	5%	90	50	76.6
9	5%	121	60	81.1
Mean 1	70.700	71.467	75.200	
Mean 2	82.133	76.767	77.167	
Mean 3	76.500	81.100	76.967	
range	11.433	9.633	1.967	

According to the results obtained from range analysis in Table 2, among these three factors, the alkali concentration had the greatest effect on the total enzymatic efficiency, followed by the temperature, and the time had the least effect. The optimum pretreatment conditions were 3% NaOH, 121 ℃ and 60 min. Under these optimum conditions, the solid recovery rate and lignin removal rate of CCT were 51.2% and 83.2%, respectively (Table 1).

3.5 Enzymatic Hydrolysis

Some of our previous studies analyzed the different ratios of cellulases to xylanases and investigated the synergistic effect between them [3,5]. The cellulases and xylanases demonstrated the highest total efficiency when the ratio was 4:1. So in this study, the same enzyme proportion was applied in enzymatic hydrolysis.

The glucose and xylose concentrations during the enzymatic hydrolysis were shown in Fig. 4. The enzymatic digestibility of raw CCT was relatively low, and the glucose and xylose concentrations only reached of 1.8 g/L and 0.5 g/L respectively at 60 h. After the alkali pretreatment, the glucose and xylose concentrations of pretreated CCT reached 12.6 g/L and 5.0 g/L respectively at 48 h, which were 6.9 and 10.8-fold of the raw CCT. Thus, under the optimized pretreatment conditions, the pretreated CCT demonstrated high digestibility. That might be because most lignin of CCT was moved and the cellulose surface area increased [13]. After 72 h of enzymatic hydrolysis, the glucan conversion rate, xylan conversion rate and total enzymatic efficiency increased from 22.3%, 7.8% and 16.2% of raw CCT to 95.1%, 62.2% and 88.7% of pretreated CCT. Therefore, this optimized alkali pretreatment could significantly increase the enzymatic efficiency by breaking the lignin-carbohydrate binding and increasing the accessibility of enzymes to cellulose[10].

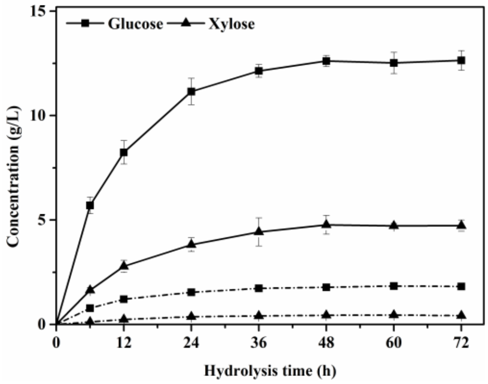

Fig.4 Glucose and xylose concentrations during the enzymatic hydrolysis of the raw (dash lines) and pretreated (solid lines) CCT

An ideal pretreatment will not only produce readily digestible substrates but also maximize the recovery of fermentable sugars [14]. After the enzymatic hydrolysis, pretreated CCT obtained approximate 632.0 g glucose and 248.5 g xylose from 1000 g material. Using other lignocellulosic materials like rice straw [15], corn cob [16] and sugarcane straw [17] as substrates, the glucose productions ranged from 102.0 to 576.0 g/kg substrate. So the pretreated CCT exhibited higher ability to be degraded and converted to fermentable sugars compared with the above lignocellulosic materials.

4. Conclusion

In this study, dilute alkaline was employed to pretreat CCT. The pretreatment conditions were optimized using single factors and orthogonal experiments. The optimal pretreatment conditions for CCT were using 3% NaOH at 121 ℃ for 1 h. Under these optimal conditions, the content of glucan and xylan of CCT reached the highest of 59.8% and 28.7%, respectively. After enzymatic hydrolysis of 48 h, the glucose concentration, total enzymatic efficiency, and glucose yield of pretreated CCT achieved the highest of 12.6 g/L, 88.7% and 632.0 g/kg substrate, respectively. These results indicated that dilute alkaline pretreatment could effectively remove the lignin, especially acid insoluble lignin, and the CCT had good prospects in fuel ethanol production.

Acknowledgements

This research was supported by the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry of China and the Science and Technology Planning Project of Guangdong Province (2015A010107007 and 2012B020311005). We appreciated Novozymes (China) for the generous gifts of Celluclast 1.5 L and Novozym 188.

References

[1] J.K. Saini, R. Saini, L. Tewari Lignocellulosic agriculture wastes as biomass feedstocks for second-generation bioethanol production: concepts and recent developments, 3 Biotech, vol. 5 (2015), 337-353.

[2] J. Li, J. Lin, P. Zhou, et al. One-pot simultaneous saccharification and fermentation: a preliminary study of a novel configuration for cellulosic ethanol production, Bioresource Technology, vol. 161 (2014), 171-178.

[3] J. Li, P. Zhou, H. Liu, et al. Synergism of cellulase, xylanase, and pectinase on hydrolyzing sugarcane bagasse resulting from different pretreatment technologies, Bioresource Technology, vol. 155 (2014), 258-265.

[4] J. Li, P. Zhou, H. Liu, et al. Ethanol production from xylan-removed sugarcane bagasse using low loading of commercial cellulase, Bioresource Technology, vol. 163 (2014), 390-394.

[5] Q. Huang, Q. Yan, J. Fu, et al. Comparative study of different alcoholate pretreatments for enhanced enzymatic hydrolysis of sugarcane bagasse, Bioresource Technology, vol. 211 (2016), 464-471.

[6] J. Li, K. Wu, W. Xiao, et al. Effect of antioxidant extraction on the enzymatic hydrolysis and bioethanol production of the extracted steam-exploded sugarcane bagasse, Biochemical Engineering Journal, vol. 82 (2014), 91-96.

[7] C. Huang, J. He, Y. Wang, et al. Associating cooking additives with sodium hydroxide to pretreat bamboo residues for improving the enzymatic saccharification and monosaccharides production, Bioresource Technology, vol. 193 (2015), 142-149.

[8] A. Sluiter, B. Hames, R. Ruiz, et al. Determination of structural carbohydrates and lignin in biomass, NREL, Golden, Co, vol. (2008).

[9] C. Eliana, R. Jorge, P. Juan, et al. Effects of the pretreatment method on enzymatic hydrolysis and ethanol fermentability of the cellulosic fraction from elephant grass, Fuel, vol. 118 (2014), 41-47.

[10] D. Cai, P. Li, Z. Luo, et al. Effect of dilute alkaline pretreatment on the conversion of different parts of corn stalk to fermentable sugars and its application in acetone-butanol-ethanol fermentation, Bioresource Technology, vol. 211 (2016), 117-124.

[11] L.D. Khuong, R. Kondo, R.D. Leon, et al. Bioethanol production from alkaline-pretreated sugarcane bagasse by consolidated bioprocessing using Phlebia sp. MG-60, International Biodeterioration and Biodegradation, vol. 88 (2014), 62-68.

[12] A.A. Guilherme, P.V.F. Dantas, E.S. Santos, et al. Evaluation of composition, characterization and enzymatic hydrolysis of pretreated sugar cane bagasse, Brazilian Journal of Chemical Engineering, vol. 32 (2015), 23-33.

[13] Y.Y. Liu, J.L. Xu, Y. Zhang, et al. Reinforced alkali-pretreatment for enhancing enzymatic hydrolysis of sugarcane bagasse, Fuel Processing Technology, vol. 143 (2016), 1-6.

[14] K. Li, J. Wan, X. Wang, et al. Comparison of dilute acid and alkali pretreatments in production of fermentable sugars from bamboo: Effect of Tween 80, Industrial Crops and Products, vol. 83 (2016), 414-422.

[15] H. Khaleghian, K. Karimi, T. Behzad Ethanol production from rice straw by sodium carbonate pretreatment and Mucor hiemalis fermentation, Industrial Crops and Products, vol. 76 (2015), 1079-1085.

[16] P. Li, D. Cai, Z. Luo, et al. Effect of acid pretreatment on different parts of corn stalk for second generation ethanol production, Bioresource Technology, vol. 206 (2016), 86-92.

[17] D.M.D. Carvalho, O. Sevastyanova, J.H.D. Queiroz, et al. Cold alkaline extraction as a pretreatment for bioethanol production from eucalyptus, sugarcane bagasse and sugarcane straw, Energy Conversion and Management, vol. 124 (2016), 315-324.