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Pretreatment of sweet sorghum bagasse by alkaline hydrogen peroxide for enhancing ethanol production

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Abstract−Effects of severe and mild alkaline hydrogen peroxide (AHP) pretreatment on ethanol production from sweet sorghum bagasse via pre-simultaneous saccharification fermentation, and the chemical structure changes of the substrates were investigated. The results showed that the bagasse pretreated by severe AHP could produce more ethanol than that of mild AHP. The maximum ethanol concentration of the bagasses from mild and severe AHP pretreatment with 8% bagasse loading was 7.642±0.140 g/L and 19.330±0.085 g/L, respectively. Moreover, the FTIR and NMR analysis illustrated that the molecule and surface structures of the pretreated bagasse were significantly changed compared with the control. The potential biomass energy production of the effluent from the pretreatment was also briefly discussed for future utilization of waste solution.The heat energy potentials of waste solution with severe and mild AHP pretreatment were 367.2 kJ/$L_{effluent}$ and 327.6 kJ/$L_{effluent}$, respectively.

Keywords: Sweet Sorghum Bagasse, Alkaline Hydrogen Peroxide Pretreatment, Ethanol Fermentation, Energy Potential, Biofuel

INTRODUCTION

To improve enzymatic hydrolysis for ethanol production with sweet sorghum bagasse, pretreatment is necessary. There are many well-known pretreatment methods for sweet sorghum bagasse or sugarcane bagasse: physical methods including milling [1] and steam explosion [2], chemical methods including acid, alkaline [3] and alkaline hydrogen peroxide (AHP) pretreatment [4], and biological pretreatment [5], etc. The main aim of these pretreatment methods is to improve accessibility of cellulose in the lignocellulose for enzyme attacking. Alkaline pretreatment has been considered as one of the most efficient pretreatment methods for sweet sorghum bagasse in these methods. It can dissolve the lignin and enhance the enzymatic hydrolysis of the bagasse [6,7]. Based on alkaline condition, hydrogen peroxide decomposition products can play the role of oxidization, leading to lignin further removal. There were many researches applying the AHP pretreatment for crop straws. Some researchers obtained the optimal pretreatment conditions at ambient temperature and pressure, namely, hydrogen peroxide concentration of 1%-5%, initial pH of 11.5-11.6 and pretreatment time of 24 h [5,6,8]. The hydrolysis yield efficiency and total sugar yield were more than 50% and 80%, respectively [9]. A modified pretreatment method associated with high temperature and pressure was obtained by our previous study, which was efficient in increasing the cellulose hydrolysis yield efficiency and total sugar yield up to 72% and 96%, respectively [7]. To be specific, the slurring sorghum bagasse was immersed with 5% (w/v) hydrogen peroxide before autoclaving at 121 °C for 60 min with 2% (w/w) sodium hydroxide solution; then the solution was cooled to ambient temperature and kept airtight in a dark place for 24 h. Generally, mild pretreatment conditions have low temperature, low concentration, and relatively short time. According to Yu et al., the temperature and chemical concentration of the mild alkaline pretreatment conditions were usually below 100 °C and 2%, respectively [8]. Cao et al. [7] pretreated sweet sorghum bagasse under 121 °C and high pressure through autoclaving. The results seemed better with relatively severe pretreatment conditions. Therefore, it is meaningful to compare these two methods for improving the enzymatic hydrolysis with the same raw materials. For convenience, the two methods are termed "mild" and "severe AHP" pretreatment in this paper. Besides, the concentration of bagasse in most researches was relatively low (about 2%), and evaluation was merely given from the aspect of pretreatment effect. Therefore, higher concentration of the bagasse was necessary to be investigated for the general ethanol fermentation from pretreated sweet sorghum bagasse. The pre-simultaneous saccharification fermentation (PSSF) had also been proposed to reduce the viscosity of the slurry at high substrate loadings [10].

Simultaneous saccharification fermentation (SSF) allows higher solids levels, higher final ethanol concentration, less energy and minimized production costs and thus was claimed as a promising technology [11]. A general option for the SSF is applying filtration after pretreatment, then followed by enzymatic hydrolysis and fermentation on the pretreated solids. For the PSSF strategy, hydroly-

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sis is performed previous to yeast inoculation, and then the sugar is converted to ethanol with inoculated microorganism in the same vessel. Subsequently, the hydrolysis and fermentation are performed simultaneously [12].

In this paper, to compare the impacts of two AHP pretreatment methods on enzymatic hydrolysis and ethanol fermentation from sweet sorghum bagasse, 8% (w/v) loading of the bagasse was used for enzymatic hydrolysis and ethanol production by employing PSSF strategy. Assessments of the better pretreatment method for sweet sorghum bagasse were conducted by contrastive analysis of PSSF, bagasse compositions and structure changes of the bagasse. In addition, the potential biomass energy production was briefly evaluated, which would provide a reference for making use of the effluent generated during the chemical pretreatment process in the future.

MATERIALS AND METHODS

1. Raw Materials

The dry sweet sorghum bagasse (Chongming No. 1) was obtained from the biomass energy engineering lab of Agricultural Biology and Ecology at Shanghai Jiao Tong University, China. Pretreatment chemicals including sodium hydroxide (NaOH) and hydrogen peroxide (H_2O_2, mass fraction of 30%) were bought from the Sinopharm Chemical Reagent Co., Ltd. The bagasse was milled and sieved to pass 40 mesh. The ground bagasse was washed by boiling water at ambient condition to substantially remove the major soluble sugars presented in the bagasse to avoid possible interference in the evaluation of the enzymatic hydrolysis of the bagasse [13]. Finally, the ground bagasse was dried to constant weight at 60 °C and sealed in plastic bag at room temperature.

2. Pretreatment Methods

Two kinds of AHP pretreatment methods were used in this study, labeled as A and B, respectively. Untreated bagasse was considered as the control in the following experiments. Each assay was duplicated and the results were averaged.

2-1. Method A: Mild AHP Pretreatment

About 5 g dry sweet sorghum bagasse was slurried with 60 mL 2% (w/v) hydrogen peroxide in a 100 mL serum bottle and adjusted with about 2 mL 10 mol/L NaOH until pH 11.5±0.2. The mixture was kept in a dark place at 35 °C for 24 h. The residues were centrifuged and washed with distilled water until neutral pH was achieved before being dried at 60 °C.

2-2. Method B: Severe AHP Pretreatment

About 5 g dry sweet sorghum bagasse was slurried with 50 mL 2% (w/v) sodium hydroxide solution for 5 min in a 100 mL serum flask, and then autoclaved at 121 °C for 60 min. The 10 mL 5% (w/v) hydrogen peroxide was mixed into slurry after the slurry cooled to ambient temperature. The mixture was kept in a dark place at 20 °C for 24 h. The residues were centrifuged and washed with distilled water until neutral pH was achieved before being dried at 60 °C.

3. Pre-simultaneous Saccharification Fermentation

Two commercial enzymes, including cellulase (Celluclast 1.5L, Sigma Aldrich) and β-glucosidase (Novzymes 188, Denmark), were applied for enzymatic hydrolysis of the sweet sorghum bagasse. The mixture with the 8% (w/v) loading of bagasse was hydrolyzed in sodium citrate buffer (50 mM, pH 4.8). The excessive enzyme loadings of Celluclast 1.5 L and β-glucosidase were about 60 FPU/g dry biomass and 80 IU/g dry biomass, respectively. The preliminary hydrolysis was conducted in a water bath shaker. Hydrolyzate was sampled in time intervals at 12, 24, 48, 72 and 96 h, respectively, and then centrifuged at 10,000 rpm for 10 min. The supernatants were kept at −20 °C until they were used for sugar and ethanol analysis.

Saccharomyces cerevisiae (CICC1308) was bought from China Center of Industrial Culture Collection (CICC) and used for ethanol fermentation. The medium was as follows: glucose 50 g/L, yeast 5 g/L, peptone 5 g/L, $MgSO_4 \cdot 7H_2O$ 1 g/L, K_2HPO_4 1 g/L. The pH was adjusted to 5.0 with 6 mol/L HCl solution. The medium was autoclaved at 121 °C for 20 min before being inoculated aseptically with *Saccharomyces cerevisiae*. Enzymatic hydrolysis was conducted at 50 °C for 12 h before the hydrolyzate cooled and then was inoculated with seed medium at the volume ratio of 1 : 10. Simultaneous saccharification fermentation assays were conducted at 36± 0.5 °C in a shaker at 100 rpm.

4. Analytical Methods

The pH value was determined directly by a pH meter (Mettler-Toledo International Inc.). Total solid (TS) and volatile solid (VS) of the bagasse were analyzed according to standard methods (24 h at 105 °C for TS, 4 h at 550 °C for VS). The total soluble sugar and total phenolic content (TPC) were determined by Phenol-Sulfuric method [14] and Folin-Ciocalteu method [15], respectively. The chemical oxygen demand (COD) of the pretreatment effluents was tested by standard method [16]. The cellulose and hemicellulose in sweet sorghum bagasse were measured by Van Soest's method [17]. The lignin content, including acid soluble lignin (ASL) and acid insoluble lignin (AIL), were determined by standard methods [18]. The reducing sugar in the hydrolyzate was determined by 3,5-dinitrosalicylic acid method [19]. The activities of the cellulase and β-glucosidase were tested according to the references [7].

Ethanol concentration in the hydrolyzate and volatile fatty acids (VFAs) concentrations in the effluents were analyzed by gas chromatography (Agilent 7890A GC system, USA) with a flame ionization detector and capillary column (Agilent, HP-Innowax, 30 m× 320 μm×0.25 μm, USA). Isopropanol was used as an internal standard for ethanol determination [7].

The microscopic structure of the sweet sorghum bagasse was represented by Fourier transform infrared raman spectroscopy (FTIR, EQUINOX 55, Bruker Company, Germany) [7]. The solid-state nuclear magnetic resonance (^{13}C-NMR) spectrum was obtained on Bruker Avance III spectrometer operating with cross polarization and magic angle spinning (^{13}C NMR CP/MAS) [20].

5. Statistical Analysis and Definitions

The results were expressed as mean±standard deviation. Statistical significance between means was tested by a one-way analysis of variance. Duncan's multiple range tests at the level of 5% were used to compare the means.

Recovery rate, ethanol yield in PSSF, remaining cellulose, remaining hemicellulose and remaining total lignin are defined as the following equations.

$$R_1 = \frac{W_1}{W_2} \times 100 \quad (1)$$

where R_1 is recovery rate, %; W_1 and W_2 is the dry sample mass after and before pretreatment, respectively, g.

$$R_2 = \frac{C_1}{C_2} \times R_1 \times 100 \quad (2)$$

where R_2 is remaining certain component content in biomass, %; C_1 and C_2 is the certain component content in biomass after and before pretreatment, respectively, %; R_1 is recovery rate, %.

$$Y = \frac{m_1}{m_2 \times 1.111 \times 0.511} \times 100 \quad (3)$$

where Y is ethanol yield in PSSF, %; m_1 is final produced ethanol, g; m_2 is glucan in dry sweet sorghum bagasse, g; 1.11 is the theoretical conversion rate from glucan to glucose; 0.511 is the theoretical conversion rate from glucose to ethanol.

RESULTS AND DISCUSSION

1. The Main Composition of Sweet Sorghum Bagasse after Pretreatment

As a perfect pretreatment method, the lignin should be mostly degraded while the cellulose and hemicellulose should be retained. Usually, the optimal pretreatment conditions mainly depend on the type of materials [3].

Table 1 shows the main composition of unpretreated sample (the Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B). The cellulose content of the bagasses was increased after being pretreated by method A or B. The cellulose content of bagasse pretreated by method A and B was 33.9% and 67.5% higher than the control, respectively. This meant that more cellulose could be hydrolyzed by cellulase for the pretreated samples compared with the control. It would provide more substrate for *Saccharomyces cerevisiae* to produce ethanol. The glucan content in sweet sorghum bagasse increased significantly after pretreatment ($p<0.05$). The sole hydrolyzate of glucan is glucose, which can be converted to ethanol directly. As a result, the glucan content can reflect the theoretical ethanol production of the bagasse. However, the actual enzymatic hydrolysis and ethanol production depend on the pretreatment effects. For hemicellulose content, it was decreased after being pretreated by method A or B. The hemicellulose content of bagasse pretreated by method A and B was 9.4% and 65.3% lower than the control, respectively. As we know, the lignin will restrict the hydrolysis of cellulose and hemicellulose by enzymes and prevent their activities from reaching to the maximum theoretical values. The removal of lignin increased enzymatic effectiveness by eliminating nonproductive binding and increasing accessibility to cellulose and hemicellulose [21]. Silverstein et al. [22] reported more than 60% lignin was removed by NaOH solution. In addition, the lignin in lignocelluloses includes ASL and AIL. The AIL in the lignocellulose will still prevent the enzyme hydrolysis step even if ASL can be dissolved through acid pretreatment. It can be seen from Table 1 that method A or B could remove plenty of AIL and ASL compared to the control. Furthermore, method B removed more AIL than method A from the bagasse. There was no difference between method A and method B about the ASL content removal capability ($p>0.05$).

The recovery rate of the bagasse was also applied to evaluate the pretreatment effect in this study. More potential fermentable substrate would be retained if high recovery rate was obtained [23]. Method A was about 1.3 times more than B concerning the recovery rate. In addition, the remaining fraction indicated the reaction yield of each component in the sample. More remaining cellulose and hemicellulose were also important for keeping more fermentable substrate in the following ethanol production [24]. Otherwise, quite a few fermentable materials would be lost. Fortunately, both method A and B could remain more than 90% cellulose. Besides, method A kept significantly more remaining hemicellulose than that of method B ($p<0.05$). That means much more hemicellulose was removed by method B during the pretreatment. Meantime, the lignin was also removed. Lignin removal was

Table 1. The main composition of unpretreated sample (the control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B)

	Control	A	B
Total solid (%)	91.995±0.110	88.828±0.111	89.195±0.127
Volatile solid (wet basis, %)	89.183±0.155	83.567±0.193	83.047±0.176
Acid insoluble ash (%)	0.767±0.062	0.648±0.147	0.682±0.069
Cellulose (%)	36.476±0.089^a	48.838±0.296^b	61.097±0.493^c
Hemicellulose (%)	21.927±0.196^a	19.861±0.836^a	7.619±0.889^b
Acid insoluble lignin (%)	7.373±0.050^a	6.940±0.132^a	1.962±0.200
Acid soluble lignin (%)	6.603±0.135^a	5.431±0.177^b	5.670±0.017^b
Total lignin (%)	13.98±0.19^a	12.37±0.31^b	7.63±0.18^c
Glucan	34.53±0.25^a	39±0.06^b	46.31±0.82^c
Recovery rate (%)	-	70.951±0.415^a	56.050±0.035^b
Remaining cellulose (%)	-	92.09±0.65^a	91.09±0.70^a
Remaining hemicellulose (%)	-	62.29±2.28^a	18.89±2.07^b
Remaining total lignin (%)	-	60.88±0.93^a	29.69±0.51^b

All the items are expressed on dry basis except volatile solid. Values with different letters ($^{a, b, c}$) at the same row mean significant difference at $p<0.05$

Table 2. Elements content of unpretreated sample (the control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B)

Elements	Control	A	B
C (%)	42.780±0.000	41.400±0.070	39.240±0.010
H (%)	6.137±0.020	6.084±0.010	6.136±0.040
N (%)	0.470±0.010	0.241±0.010	0.185±0.000
S (%)	0.210±0.050	0.030±0.000	0.120±0.020
O (%)	50.403	52.245	54.319
Ca (mg/g)	0.202±0.001	0.149±0.000	0.267±0.003
P (mg/g)	0.150±0.000	0.0470±0.001	0.098±0.001
K (mg/g)	0.881±0.006	0.195±0.001	0.212±0.002
Na (mg/g)	0.252±0.001	2.167±0.015	2.491±0.010

beneficial to the cellulose and hemicellulose hydrolysis.

Table 2 shows the element content of unpretreated sample (Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B). As can be seen, after pretreatment, the C, H, N and S content decreased. The N and S content reductions were beneficial to reduce the pollution during bagasse combustion. The delignification by AHP resulted in C and H content reduction in biomass. The C, H content decreased mainly caused by lignin removal. As we know, lignin mainly consists of phenolic compounds, which contain considerable amount of C and H. Other elements in the bagasse were also analyzed and shown as mg per g bagasse. Similarly, the P and K content decreased. The Na content increased maybe due to the NaOH inputs during pretreatment.

2. The Reducing Sugar Concentration in the Broth

Fig. 1 shows the reducing sugar concentration in the broth of unpretreated sample (Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B). The reducing sugar concentrations grew to a certain level after 12 h of hydrolysis. The reducing sugar in sample B was about 40.26 g/L, which was about twice and eight times more than A and the control, respectively. It implied that method B could improve the reducing sugar content in the enzymatic hydrolysis, which could provide enough substrate for the subsequent ethanol fermentation. The reducing sugar concentrations in the broth of sample A and the control decreased from the following 12 h after being inoculated the *saccharomyces cerevisiae*, and then the reducing sugar concentration decreased indistinctively ($p>0.05$), except for a slight decrease for sample A from 24 h to 48 h. Note that the reducing sugar concentration in sample B increased sharply in the first 12 h, and then decreased radically in the subsequent 24 h. It implies that there was more reducing sugar generated in the first 12 h for sample B. Reducing sugar in all samples seemed to keep constant after 48 h. It could be concluded that the reducing sugar generation rate by enzymatic hydrolysis was higher than the glucose consumption rate by *saccharomyces cerevisiae* for sample B, while a reverse deduction could be got for sample A and the control. For ordinary enzymatic hydrolysis of lignocellulose, the majority of the reducing sugars in the hydrolyzate are glucose and xylose. Besides, a small amount arabinose can be detected in the hydrolyzate [7]. The glucose could be converted to ethanol directly by *saccharomyces cerevisiae*, while the xylose and arabinose remaining in the hydrolyzate could be consumed by other microorganisms.

3. The Ethanol Concentration in the Broth

In the hydrolysis and fermentation process, the substrate loading was 8%, which would ensure producing sugar and ethanol as much as possible. The lower fluidity of the slurry would be an adverse factor for fermentation if the loading was higher than 8%.

Fig. 2 shows the ethanol concentration in the broth of unpretreated sample (Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B). Also from Fig. 2, for control and A the ethanol concentration increased remarkably from 12 h to 24 h, with no significant change after 48 h ($p>0.05$) because the glucose in the buffer solutions was used up and the residual sugar content was constant. Concerning the ethanol concentration of B, the ethanol concentration in the broth almost increased linearly during the first 48 h. Similarly, decrease of the reducing sugar concentration in the first 48 h can be seen from Fig. 1. A similar rea-

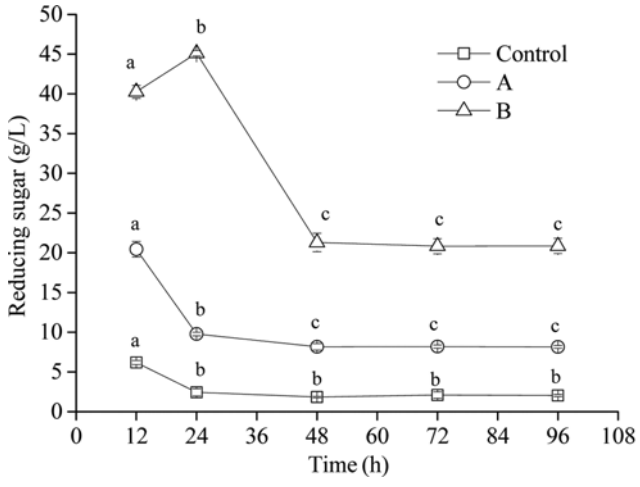

Fig. 1. The reducing sugar concentration in the broth of unpretreated sample (the control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B).

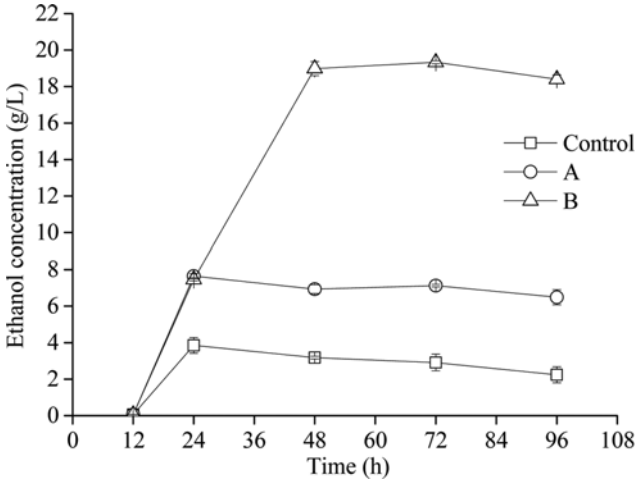

Fig. 2. The ethanol concentration in the broth unpretreated sample (the control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B).

son could be attributed to the sole fermentable sugar, glucose, was used up. Thus, the ethanol concentration from B was much more than A and the control because of the more cellulose in B. As a result, method B is a much better than method A if the assessment is based on the ethanol concentration.

The purpose of bagasse pretreatment is to improve ethanol yield from bagasse. Another evidence of the effects of pretreatments is the better ethanol yields in PSSF. According to Eq. (3), the ethanol yields in PSSF for unpretreated sample (Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B) were (19.83±0.06) %, (44.08±0.49) % and (88.57±0.53) %, respectively. Obviously, the ethanol yield of pretreated sweet sorghum bagasse was much higher than the control. Thus, the delignification by method A and B could significantly improve the final ethanol concentration by 122% and 347% ($p<0.05$) compared with the control. Note that the ethanol yield of bagasse pretreated by method B was about twice more than A. So, method B is much better than method A under current conditions.

4. The Structural Characterization Analysis for the Bagasse by FTIR

Fig. 3 shows the FTIR spectra of unpretreated sample (Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B). There are many different peaks shown in pretreated bagasses compared with the control. The absorption at about 3,342 cm^{-1} represents the stretching of hydroxyl and phenol in the sweet sorghum bagasse. The reduced transmittance of the pretreated bagasses indicates that some hydroxyl and phenol were removed. The absorption at 2,915 cm^{-1} represents the stretching of $-CH_3$ and $-CH_2$. The reduced transmittance of the bagasse pretreated by method B implies more fracture of carbon chains, while it seems that the bagasse pretreated by method A was not significantly changed compared with the control in the same position. The absorption at 1,700 cm^{-1} represents the bending mode stretching of the absorbed water and stretching of C=O in lignin [25]. It implies that there was less lignin content retained in bagasse pretreated by method B. The strong absorption at 1,160 cm^{-1} represents C-O-C stretching, which was assigned by cellulose and hemicellulose [26]. The stronger absorption at 1,160 cm^{-1} of the bagasse pretreated by method B means there is more cellulose or hemicellulose. This is corresponding to the results in section 1 about the main composition of sweet sorghum bagasse after pretreatment. The strong absorption at 1,049 cm^{-1} represents the stretching of C-O in cellulose and hemicellulose [5,26,27]. The transmittances at about 1,049 cm^{-1} of the bagasse pretreated by method A and B were higher than the control, which is a sign of the structural destruction of the bagasse. In short, method B was much more efficient in removing the C-O and C=O in the bagasse than method A. In contrast, the elemental analysis (Table 2) showed that the oxygen content in the pretreated samples was slightly increased. The increase of oxygen content probably was partly because O_2 evolved from H_2O_2 will combine with the lignin degradation products [28]. In sum, the structural destruction of the bagasse was beneficial to the enzymatic hydrolysis.

5. The Structural Characterization Analysis for the Bagasse by ^{13}C-NMR

The solid ^{13}C-NMR spectroscopy of unpretreated sample (Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B) are depicted in Fig. 4. All the observed signals were described according to the literature [29,30]. As can be seen from the spectra, the chemical shift at 105 ppm revealed the signal for C-1 of cellulose. The signals at 90 ppm and 85 ppm were corresponding to C_4, cellulose core chains and surface chains, respectively. The chemical shifts at 64 ppm and 58 ppm were assigned to C-6 of Cellulose and methoxy group of lignin, respectively. After pretreatment, the 58 ppm resonance from the samples pretreated by method B was much weaker than the others. This might be attributed to the lignin removal under the severe conditions of alkaline pretreatment [31].

6. The Bioenergy Potential Evaluation of the Effluent

The soluble sugar and VFAs generated and kept in the pretreatment solutions were due to the catalysis and dissolution function of NaOH. Although the soluble sugars such as xylose and arabinose were difficult to ferment by *Saccharomyces cerevisiae* directly, they could be converted to CH_4 and CO_2 by the methanogens, so as the VFAs [32]. Besides, yeast cells generated in the fermenta-

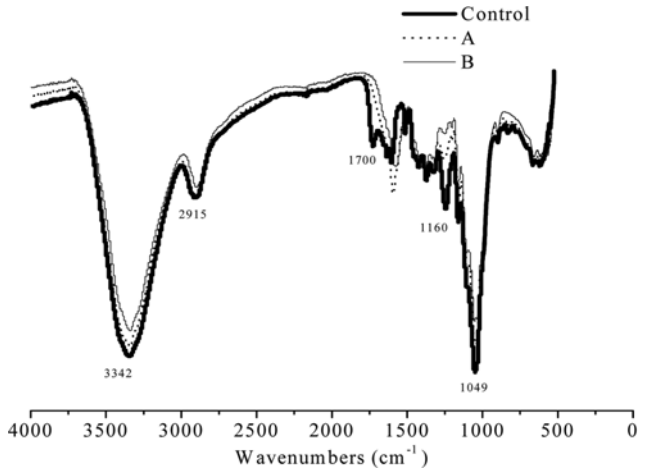

Fig. 3. FTIR spectra unpretreated sample (the control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B).

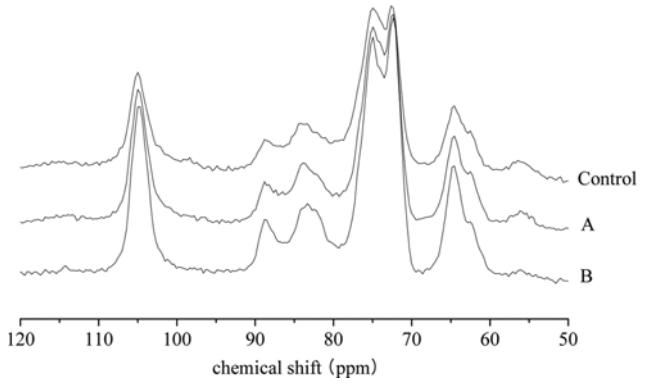

Fig. 4. The solid 13C-NMR spectroscopy of unpretreated sample (the control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B).

Table 3. The compositions of effluent from unpretreated sample (the control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B)

Test items	A	B
pH	10.01±0.10	8.51±0.18
Total soluble sugar (g/L)	108.955±0.348	94.183±6.847
COD (g/L)	25.882±2.003	29.098±0.781
TPC (g/L)	0.681±0.031	1.851±0.019
VFA (g/L)	72.688±3.324	99.748±0.365

tion broth were considered as one kind of organic nitrogen source for methane production. However, the salt generated during the neutralization of the effluent may cause potential inhabitation to the methanogens when applying anaerobic digestion. A possible method is to remove part of the salt by ion-exchange resin or to acclimate the microorganism to the high concentration of salt.

Table 3 shows the compositions of effluent from unpretreated sample (Control), mild AHP pretreated sample (A) and severe AHP pretreated sample (B). The pH values indicated that the effluents were alkaline because of the NaOH solution input. The pH value of B was less than A, which might be attributed to the neutralization of VFA generated during the pretreatment. The considerable amount of total soluble sugars in the effluent of the pretreated bagasse revealed the potential energy production if these soluble sugars were converted to methane. According to the theory of anaerobic digestion [33], each gram of methane production corresponds to the removal of 4 grams of COD. As a result, it could produce 9.1 L $CH_4/L_{effluent}$ (at standard temperature and pressure) and 10.2 L $CH_4/L_{effluent}$ theoretically for the effluent generated from method A and B, respectively. Energy potentials could be converted from m^3 of methane to kJ of heat energy using the factor of 36 kJ/L methane. Consequently, the heat energy potentials that could be produced from A and B were 327.6 $kJ/L_{effluent}$ and 367.2 $kJ/L_{effluent}$, respectively.

The VFA results showed that effluent from B generated much more VFA than A. This implies that more potential methane might be produced from B. The TPC in the effluent was mainly generated from the degradation of lignin, which might cause another sort of inhibition during the anaerobic digestion. The TPC of B was about 2.7 times more than A. It implies that the effluent from B would be harder to produce methane compared to A. In short, there was potential energy in the effluent after pretreatment. The actual performance of the anaerobic digestion from the effluent should be conducted in future.

CONCLUSIONS

The severe AHP pretreatment was much better to obtain more fermentable sugar and higher ethanol concentration than mild AHP pretreatment under the current substrate loading and experimental conditions. The maximum ethanol concentrations obtained from mild and severe AHP pretreatment were 7.642±0.140 g/L and 19.332±0.085 g/L, respectively, which were about twice and five times that of the control, respectively. The analysis of the effluent from the bagasse pretreatment implied that considerable energy potential could be obtained.

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