Dilute Alkali Pretreatment and Subsequent Enzymatic Hydrolysis of Amur Silvergrass for Ethanol Production

Fengqin Gao,[a,#,*] Fuyu Yang,[b,*] Ying De,[a] Ya Tao,[a] Na Ta,[a] Hui Wang,[a] and Qizhong Sun [a,#]

A dilute alkali pretreatment (NaOH) was used to remove lignin and some hemicelluloses, as well as to efficiently increase the accessibility of enzymes to the cellulose in Amur silvergrass. A single factor experiment was designed with 4 factors (1 to 5% w/w NaOH, 1/6 to 1/14 solid to liquid ratio, 15 to 90 min residence time, and 80 to 125 °C digestion temperature) with 3 duplicates of 5 levels for each factor. On the basis of the single factor test, an L_8 (2^4)-orthogonal experiment was conducted to identify the main influencing factor and the optimal factor combinations verified by an enzymatic hydrolysis and fermentation experiment. The main factors influencing ethanol production were NaOH concentration and digestion temperature, while residence time and solid to liquid ratio had a lesser effect. The enzymatic hydrolysis rate of cellulose reached 82.6%, and the highest conversion rate of ethanol was 78.3% with 4.0% (w/w) NaOH and a 1:6 solid to liquid ratio at 100 °C for 15 min. Scanning electron microscope (SEM) images of the lignocellulosic surface structure of non-pretreated and optimum pretreated Amur silvergrass displayed obvious differences. The lignin was the key recalcitrance-causing factor for ethanol production, which can be effectively removed by the NaOH.

Keywords: Amur silvergrass; Dilute alkali pretreatment; Lignocellulose; Enzymatic hydrolysis; Ethanol production

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INTRODUCTION

The demand and consumption of fossil fuels is expected to increase by 60% over the next 20 years as a function of both world economic development and population growth (David and Ragauskas 2010). Meanwhile, energy production from this finite resource emits SO_2 and CO_2, which both severely threaten humans and their living environment. In the face of this energy and ecosystem health crisis, saving energy, reducing consumption, and developing renewable and environmentally friendly energy sources are urgently important (Yang *et al.* 2015). Producing biomass energy from low-cost and abundant raw materials, which avoids issues surrounding "food or fuel," and has the potential for little environmental impact, is becoming a viable alternative (Sanchez and Cardona 2008; Gnansounou 2010). Cellulose ethanol is an alternative source of renewable energy that demonstrates great promise because it is renewable and clean. Lignocellulosic material includes agricultural and forest residues, other municipal wastes, waste paper, *etc.* (Noureddini and Byun 2010; Hee and Song 2011). Among the various types of lignocellulosic biomass, perennial grasses are considered an ideal source of

bioenergy and bioproducts (Efthymia 2018). The perennial Amur silvergrass is a potential lignocellulosic material for ethanol production because of the following characteristics: high yield and cellulose content, extensive adaptability, strong regeneration capacity, high dry matter caloric value, strong potential for carbon sequestration, and it is readily available in China (Li *et al*. 2016). However, there is a need for research on optimal techniques for the pretreatment of this forage for ethanol production. Among these methods, alkaline pretreatment is one of the most extensively investigated because it is relatively inexpensive, less energy intensive (Xu and Cheng 2011), and results in lesser sugar degradation (Janker-Obermeiera *et al*. 2012). This pretreatment involves the application of alkaline solutions like NaOH or KOH to remove lignin and some of the hemicelluloses, and it efficiently increases the accessibility of enzymes to the cellulose (Sun *et al*. 2005). Moreover, NaOH is reported to be the most effective alkali at low temperatures with extended pretreatment times because NaOH is a stronger base than lime or ammonia (Xu and Cheng 2011; Kim *et al*. 2016).

In this study, different combinations of dilute alkali pretreatment were evaluated for cellulose hydrolysis and delignification, and the efficiency of enzymatic saccharification and fermentation were evaluated under the best combinations. Through double evaluation of pretreatment and fermentation effects, it was possible to strengthen reliability and application value. At the same time, the approach used in this work provides reference data for further study of the ethanol production capacity of Amur silvergrass.

EXPERIMENTAL

Materials

Mature Amur silvergrass (*Triarrhena sacchariflora* [Maxim.] Nakai.) was harvested. The samples were air-dried in a field, and then they were cut into 2 to 4 cm lengths with a rubbing filament machine. Next, they were separated by the quadruple method, packed into sealed plastic bags, and stored at 4 °C.

Experimental Design and Pretreatment

A single factor experiment was designed with 4 factors (1 to 5% w/w NaOH, 1/6 to 1/14 solid to liquid ratio, 15 to 90 min residence time, and 80 to 125 °C digestion temperature) with 3 replicates of 5 levels for each factor (Table 1).

On the basis of the single factor test, an L_8 (2^4)-orthogonal experiment was conducted to identify the main factor or factor combinations influencing cellulose hydrolysis and delignification (Table 2). Finally, the optimal pretreatment combination was verified by an enzymatic hydrolysis and subsequent a fermentation experiment. Factor levels of the two most efficacious were selected using the data in Table 2.

The pretreatment steps were as follows: a 10 g, 2 to 4 cm sample of Amur silvergrass, along with the corresponding NaOH volume, was placed in a 500 mL glass container, and then it was sealed with aluminum foil. Each group pretreatment was performed according to a single factor and orthogonal experimental design in an autoclave. After pretreatment, the pretreated materials were separated through gauze, washed with water until it reached neutrality, dried at 40 °C in an oven, and then the 50 mL of filtrate solution was stored at 20 °C for a chemical composition analysis.

Table 1. Single Factor Experimental Design of Amur Silvergrass with a Dilute Alkali Pretreatment

Factors / Levels	NaOH Concentration (%)	Solid to Liquid Ratio (g/mL)	Residence Time (min)	Digestion Temperature (°C)
Levels of the single factor	1.0%	1:6	15	80
	2.0%	1:8	30	100
	3.0%	1:10	45	110
	4.0%	1:12	60	120
	5.0%	1:14	90	125
Fixed factors	1:8 g/mL, 30 min, 120 °C	2.0%, 30 min, 120 °C	2.0%, 1:8 g/mL, 120 °C	2.0%, 1:8 g/mL, 30 min

Table 2. Orthogonal Experimental Design of Amur Silvergrass with a Dilute Alkali Pretreatment

Factor / Experiment No.	NaOH Concentration (%)	Solid to Liquid Ratio (g/mL)	Residence Time (min)	Digestion Temperature (°C)
1	1 (2.0)	1 (1:6)	1 (15)	1 (100)
2	1	1	1	2 (110)
3	1	2 (1:8)	2 (45)	1
4	1	2	2	2
5	2 (4.0)	1	2	1
6	2	1	2	2
7	2	2	1	1
8	2	2	1	2

Enzymatic Hydrolysis and Fermentation

Enzymatic hydrolysis and fermentation experiments were simultaneously conducted. After pretreatment, 3 replicates of the 10 g samples of the pretreated grass were put into 100 mL serum glass bottles. A 3% peptone solution was added. When the solid to liquid ratio was 1:10, it was autoclaved for 20 min at 121 °C. The serum bottles were transferred to a superclean bench until the liquid sample temperature fell to 30 °C. Next, temperature-resistant active dry-Angel yeast at a concentration of 0.03% (w/v) and 20 U/g cellulase activated by a 2% sucrose solution were added to the solution. A simultaneous saccharification and fermentation (SSF) process was used for the enzymatic hydrolysis and the fermentation in a citric acid-sodium citrate buffer of pH 4.8 at 34 °C at 100 r/min for 96 h. During the fermentation, liquid samples were periodically taken for monitoring their glucose and ethanol.

Chemical Analysis

Amur silvergrass dry matter (DM) was measured using a drying determination method at 105 °C to ensure a constant weight. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed as described by previous research methodology (Van Soest *et al*. 1991). The concentrations of glucose and xylose were analyzed by high performance liquid chromatography (HPLC) (LC-20A, Shimadzu, Tokyo, Japan). The HPLC used an Agilent carbohydrate column at 30 °C with a methyl cyanide to water

ratio of 80:20, a refractive index detector at 25 °C with 40 MPa and a flow rate of 1 mL/min, and a sample size of 10 μL (Gao *et al*. 2014). The ethanol concentration was analyzed with the HP 6890 Series GC system (GC-2014, Shimadzu Co., Japan) and the PEG-20M column (PEG-20M, Aglient, American) under conditions per previous research (Bvochora *et al*. 2000). The images of lignocellulose surface structure were taken using a scanning electron microscope (SEM) (S-530, Hitachi Ltd., Tokyo, Japan).

Statistical Methods

An SPSS 16.0 one-way ANOVA was used in the single factor test at a significance level of 0.05. The range and comprehensive equilibrium method was used in the incomplete factorial experiments.

RESULTS AND DISCUSSION

Chemical Composition of Amur Silvergrass

The untreated Amur silvergrass consisted of 50.63 ± 3.66% cellulose, 28.49 ± 2.17% hemicellulose, and 9.41 ± 0.77% lignin. The crude protein and crude ash content were 8.03 ± 0.08% and 1.26 ± 0.02%, respectively.

Dry Matter Digestibility

Dry matter digestibility (DMD) means percentage of dry matter hydrolyzed by the raw sample after pretreatment, which exhibited an increasing trend with increasing levels of the four single factor conditions. The NaOH concentration pretreatment was more pronounced than the other factors.

$$\mathrm{DMD}(\%) = \left(1 - \frac{\text{remaining solid weight after pretreatment}}{\text{raw solid weight used for pretreatment}}\right) \times 100 \qquad (1)$$

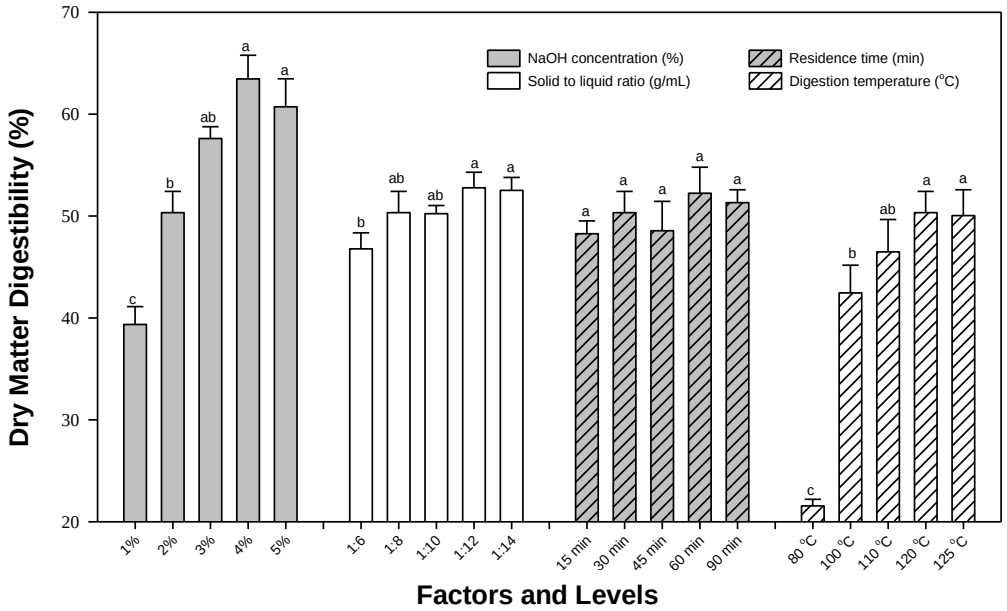

Fig. 1. Dry matter digestibility of Amur silvergrass under single factor pretreatment
Note: The different superscripts denote significant difference between different levels of the same treatment ($p < 0.05$). The same applies in subsequent figures and tables.

Dry matter digestibility increased significantly with NaOH concentration and peaked at 4% NaOH with a dry matter digestibility value of 63.45% (Fig. 1). The significant differences between 1%, 2%, and 3 to 5% NaOH suggested that dry matter digestibility could be promoted by dilute alkali pretreatment over a specific range of alkali concentration. The minor differences in the effects of the solid to liquid ratio, and no significant influence in digestion time ($p > 0.05$), indicated that extending the residence time and reducing the solid to liquid ratio had little effect on the improvement of dry matter hydrolysis efficiency. Therefore, selecting a shorter time and a higher solid to liquid ratio could be helpful for alkali pretreatment of Amur silvergrass. Dry matter digestibility varied significantly with temperature levels and had a substantial increase from 80 °C to 125 °C. Dry matter digestibility doubled from 80 °C to 100 °C, increased slightly to 120 °C, and then plateaued ($p > 0.05$). Because the temperature below 100 °C was not conducive to dry matter digestibility, orthogonal experiments were carried out at both 100 °C and 110 °C. In summary, pretreatment with change of NaOH concentration and digestion temperature had a greater efficacy on dry matter digestibility than solid to liquid ratio and residence time, but the increase of NaOH concentration and temperature would result in increasing rate of dry matter loss. So, combination optimal of NaOH concentration and digestion temperature was very important for reducing dry matter loss of Amur silvergrass.

Determination of Optimal Dilute Alkali Pretreatment Conditions

Lignocellulosic components

Lignocellulose is mainly composed of cellulose, hemicellulose, and lignin; as such, determination of degradation and removal of these components is essential for the process technology evaluation of Amur silvergrass. The cellulose degradation (CD), hemicelluloses removal (HR) and lignin removal (LR) of Amur silvergrass after pretreatment were calculated as following:

$$\mathrm{CD}(\%) = \left(1 - \frac{\text{remaining cellulose weight after pretreatment}}{\text{raw cellulose weight used for pretreatment}}\right) \times 100 \quad (2)$$

$$\mathrm{HR}(\%) = \left(1 - \frac{\text{remaining hemicellulose weight after pretreatment}}{\text{raw hemiellulose weight used for pretreatment}}\right) \times 100 \quad (3)$$

$$\mathrm{LR}(\%) = \left(1 - \frac{\text{remaining lignin weight after pretreatment}}{\text{raw lignin weight used for pretreatment}}\right) \times 100 \quad (4)$$

Cellulose degradation and hemicellulose and lignin removal tended to increase with an increase in alkali concentration (Table 3). At the same NaOH concentration, the hydrolysis efficiency of lignin was higher than that of hemicellulose and cellulose, with cellulose degradation being the weakest. At 4% NaOH concentration, cellulose degradation and hemicellulose and lignin removal reached their highest values at 45.2%, 74.4%, and 92.7%, respectively. When NaOH concentration increased from 4% to 5%, the hydrolysis efficiency of all three declined, which was probability due to increasing cellulose crystallinity of Amur silvergrass, similar to Chen (2011). Varying the solid to liquid ratio had no effect on lignocellulosic degradation ($p > 0.05$). Overall, cellulose degradation and hemicellulose removal did not change with increased residence time ($p > 0.05$); however, delignification increased slightly between 30 and 45 to 90 min ($p < 0.05$). Therefore, orthogonal experiments were carried out at 15 and 45 min, respectively, in the analysis of the three lignocellulosic components. After preliminary analysis, the

prolonging of residence time did not significantly improve the degradation of lignocellulosic components under the fixed concentration and temperature conditions. This indicated that time was not the main factor affecting the degradation of Amur silvergrass.

Table 3. Effect of Different Pretreatment Levels on the Lignocellulosic Components of Amur Silvergrass

Pretreatment Conditions		Cellulose Degradation (%)	Hemicellulose Removal (%)	Lignin Removal (%)
NaOH concentration (%)	1.0%	35.48 ± 1.90b	33.40 ± 2.03d	64.17 ± 1.72c
	2.0%	34.20 ± 2.69b	55.73 ± 1.28c	84.89 ± 3.05b
	3.0%	43.63 ± 1.15a	58.19 ± 2.16c	89.73 ± 1.67ab
	4.0%	45.17 ± 2.66a	74.40 ± 4.39a	92.70 ± 2.12a
	5.0%	43.12 ± 1.33a	66.57 ± 2.34b	90.63 ± 2.22ab
Solid to liquid ratio (g/mL)	1:6	31.74 ± 2.48a	53.40 ± 0.37b	79.31 ± 0.78b
	1:8	34.02 ± 2.69a	55.72 ± 1.27ab	84.89 ± 3.05ab
	1:10	31.50 ± 0.97a	55.69 ± 3.60ab	89.57 ± 2.33a
	1:12	34.69 ± 2.51a	60.28 ± 2.29a	86.51 ± 2.33a
	1:14	34.88 ± 1.59a	57.39 ± 0.15ab	87.55 ± 2.46a
Residence time (min)	15 min	30.62 ± 2.28a	55.49 ± 0.66a	84.46 ± 1.99b
	30 min	34.02 ± 2.69a	55.72 ± 1.27a	84.89 ± 3.05b
	45 min	31.49 ± 3.08a	50.75 ± 5.91a	87.68 ± 0.31a
	60 min	35.13 ± 3.39a	59.29 ± 3.34a	88.89 ± 2.06a
	90 min	33.01 ± 1.76a	57.40 ± 3.61a	90.60 ± 2.16a
Digestion temperature (°C)	80 °C	26.22 ± 1.72b	10.14 ± 1.59b	46.58 ± 2.15c
	100 °C	29.04 ± 1.82ab	45.24 ± 2.43a	72.30 ± 2.79b
	110 °C	31.87 ± 1.56ab	49.88 ± 3.77a	80.80 ± 3.76ab
	120 °C	34.02 ± 2.69a	55.72 ± 1.27a	84.89 ± 3.05a
	125 °C	38.13 ± 0.42a	54.65 ± 3.39a	84.66 ± 0.13a

Note: Different superscripts denote significant difference between different levels of the same treatment ($p < 0.05$).

Of all four pretreatment conditions, increasing the temperature had the greatest effect on the components of lignocellulose. Cellulose degradation and hemicellulose and lignin removal ranged from 26.2 to 38.1%, 10.1 to 54.6%, and 46.6 to 84.7%, respectively (Table 3). As the temperature rose from 80 °C to 100 °C, hemicellulose and lignin removal increased by 45% and 40%, respectively, whereas cellulose degradation only increased by 12%. In particular, hemicellulose hydrolysis increased by approximately 4-fold from 80 °C to 100 °C ($p < 0.05$). Lignocellulose hydrolysis increased slowly as the temperature increased to 100 °C. After that, there was no significant difference among other treatments ($p > 0.05$). The reason for this may be that generated steam pressure destroyed the structure of the lignocellulose and resulted in biomass porosity at 100 °C (Yang *et al*. 2015).

Therefore, lignin removal was greatly improved with increased NaOH concentration and digestion temperature, a finding shared with other studies on other agricultural residues (Kim and Han 2012). However, when experimental conditions become more severe, more solubilization of cellulose will be accompanied by higher delignification, which is not ideal for glucose yield. Consequently, appropriate combinations of pretreatment factors at the optimal levels, as found in this study, are very important for obtaining high yields of glucose and ethanol.

Concentration of glucose and xylose

For herbaceous plants, the main products of cellulose and hemicellulose hydrolysis, following dilute alkali pretreatment, are glucose and xylose. Also, their levels are a good reflection of the effect of pretreatment on cellulose and hemicellulose hydrolysis (Li *et al*. 2010). The hydrolysis reaction of dilute alkali pretreatment makes the lignocellulose matrix swell, decreases the degree of polymerization and crystallinity, increases the internal surface area, disrupts the lignin structure, and breaks the structural linkages between lignin and carbohydrates (Camesasca *et al*. 2015).

All of the treated groups indicated that xylose concentrations were much higher than glucose concentrations ($p < 0.01$).

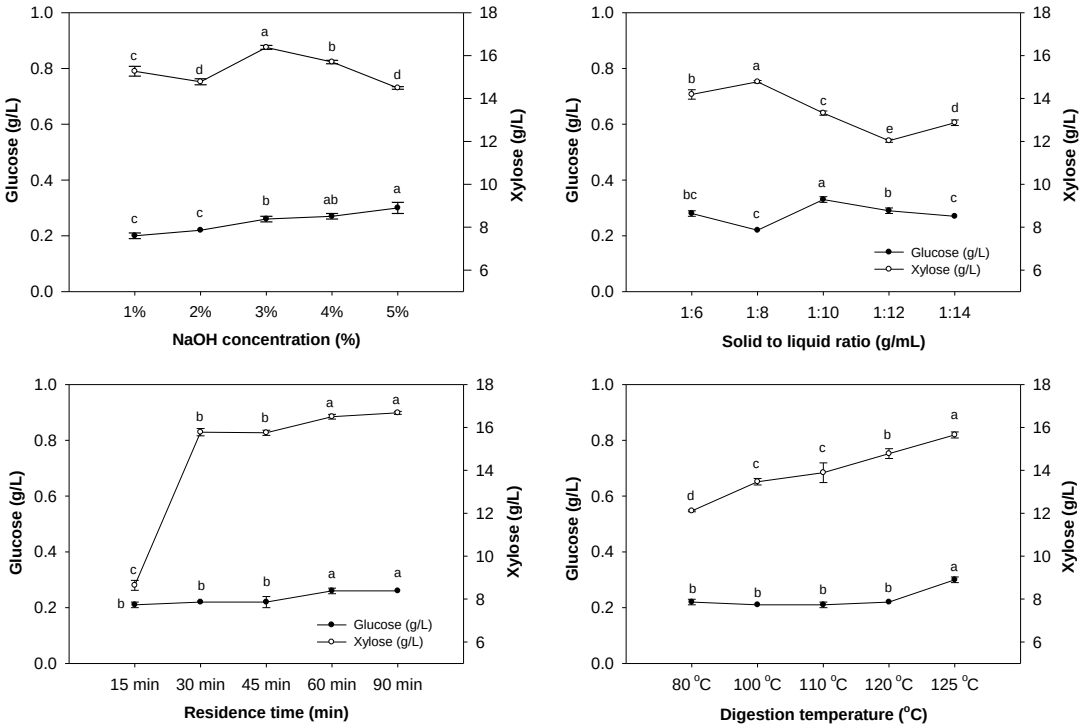

Fig. 2. The concentration of glucose and xylose of filtrate solution under factor pretreatment.

Generally, glucose concentrations were very low, but they significantly rose with increasing residence time, temperature, and alkali concentration (Fig. 2). This finding is consistent with others (Si 2015; Mikulski and Kłosowski 2018). It suggests that increasing the alkali concentration can also increase the degree of damage to the structure of the cell wall, making the sugar concentration increase continuously over a certain range. Xylose concentration continuously increased with increasing residence time and digestion temperature. However, it was more variable with increasing NaOH concentration and solid to liquid ratio (Fig. 2). Moreover, there was also a linear relationship between xylose concentration and digestion temperature. Xu *et al*. (2019) reported that alkaline dosage and delignification were positively correlated with reducing sugar yields. In this study it is suggested that a high alkali concentration treatment is not conducive to the preservation of xylose. The xylose concentration was approximately 40 to 80 times greater than the glucose concentration in the dilute alkali pretreatment. Consequently, dilute alkali pretreatment of Amur silvergrass appears to be more effective in the hydrolysis of hemicelluloses to produce xylose, rather than hydrolysis of cellulose to produce glucose. Furfural (0.10 to 0.22 g/L) and hydroxymethylfurfural (HMF) (0.05

to 0.18 g/L) were very low in NaOH pretreatment of Amur silvergrass, which may be due to detoxification with alkali removed inhibitors, and some studies have also shown in alkali pretreatment results (Negro *et al*. 2015; Camesasca *et al*. 2015; Jung and Kim 2017). Glucose production was much lower than cellulose hydrolysis yield, which perhaps indicates that cellulose hydrolysis produced more oligomers or that glucose was further degraded in the NaOH pretreatment. Thus, the optimized pretreatment conditions were essential for obtaining a maximum yield of glucose and xylose and further utilizing filtrate sugar.

Orthogonal Experiment

To determine which factors have the greatest influence on cellulose degradation, hemicellulose and lignin removal, an orthogonal experiment was conducted and the results were analyzed by extremum difference method. The greatest delignification and the conversion of hemicellulose to xylose occurred with a high NaOH concentration at a high temperature (Table 4). However, cellulose solubilization also increased, which caused a lower yield of glucose and ethanol for subsequent enzymatic hydrolysis and fermentation. To correct for this, a complete factorial experiment was arranged to determine the optimum conditions for enzymatic hydrolysis based on the results of the single factor analysis of Amur silvergrass with dilute alkali. The optimum combinations for cellulose were 2.0% (w/w) NaOH with a 1:6 solid to liquid ratio at 100 °C for 15 min. For maximum delignification, the optimum combination was 4.0% (w/w) NaOH with a 1:8 solid to liquid ratio at 110 °C for 30 min. Meanwhile, according to the comprehensive evaluation about low cost, saving energy and environmental protection, the optimum fermentation conditions were 4.0% NaOH, a solid to liquid ratio of 1:6, 15 min, and 110 °C (Table 4).

Table 4. Results of the Orthogonal Experiment Evaluating with Dilute Alkali Pretreatment on Amur Silvergrass

Experiment No.		Factors				Cellulose Degradation (%)	Lignin removal (%)
		NaOH Concentration (%)	Solid to liquid ratio (g/mL)	Residence time (min)	Digestion temperature (°C)		
1		1 (2.0%)	1 (1:6)	1 (15 min)	1 (100 °C)	28.03	72.66
2		1	1	1	2 (110 °C)	30.92	76.39
3		1	2 (1:8)	2 (45 min)	1	29.66	75.86
4		1	2	2	2	31.11	83.33
5		2 (4.0%)	1	2	1	30.26	83.01
6		2	1	2	2	32.34	87.22
7		2	2	1	1	30.77	80.89
8		2	2	1	2	33.56	88.34
Cellulose degradation	K1	119.72	121.55	123.28	118.72	∑ = 246.65	
	K2	126.93	125.10	123.37	127.93		
	$\overline{K1}$	29.93	30.39	30.82	29.68		
	$\overline{K2}$	31.73	31.28	30.84	31.98		
R		1.80	0.89	0.02	2.30		
Lignin removal	K1	308.24	319.28	318.28	312.42	∑ = 647.70	
	K2	339.46	328.42	329.42	335.28		
	$\overline{K1}$	77.06	79.82	79.57	78.11		
	$\overline{K2}$	84.87	82.11	82.36	83.82		
R		7.81	2.29	2.79	5.71		

Enzymatic Hydrolysis and Fermentation

The three optimal pretreatment combinations from the orthogonal experiment and untreated sample were tested, and the yield of glucose and ethanol was determined. The concentration of glucose was highest after 48 h of fermentation, and then it decreased with increasing fermentation time (Fig. 3).The results were similar to other studies for change trend of glucose concentration during the fermentation (Kuo *et al*. 2014; Hu *et al.* 2016). The concentration of ethanol was highest at 72 h, and it remained unchanged with increasing fermentation time. The concentration of glucose and ethanol in 4.0% NaOH/1:6 solid to liquid ratio/15 min/110 °C was the highest. This was slightly higher than that in the 4.0% (w/w) NaOH/1:8 solid to liquid ratio/110 °C /30 min pretreatment process ($p > 0.05$). Both of those were significantly greater than that in 2.0% (w/w) NaOH/1:6 solid to liquid ratio/100 °C/15 min process ($p < 0.05$), while untreated group was much lower than the three optimal pretreatment ones (Fig. 3). Under the optimized conditions, the ethanol concentration reached the maximum value at 60 hours, which shortened the fermentation time and saved costs compared with the other research (Camesasca *et al*. 2015). Under the pretreatment conditions of 4.0% NaOH/1:6 solid to liquid ratio/15 min/100 °C, the enzymatic hydrolysis rate (EHR) of residual cellulose was 82.6%, and the conversion rate (ECR) of ethanol was 78.3%. According to calculation of the conversion rate, 100 g of raw material could produce 29.8 g of ethanol under optimal process conditions.

$$\text{EHR}\ (\%) = \left(1 - \frac{\text{residual cellulose weight after fermentation}}{\text{residual cellulose weight after pretreatment}}\right) \times 100 \quad (5)$$

$$\text{ECR}\ (\%) = \left(1 - \frac{\text{residual glucose yield after fermentation}}{\text{total glucose yield used for fermentation}}\right) \times 100 \quad (6)$$

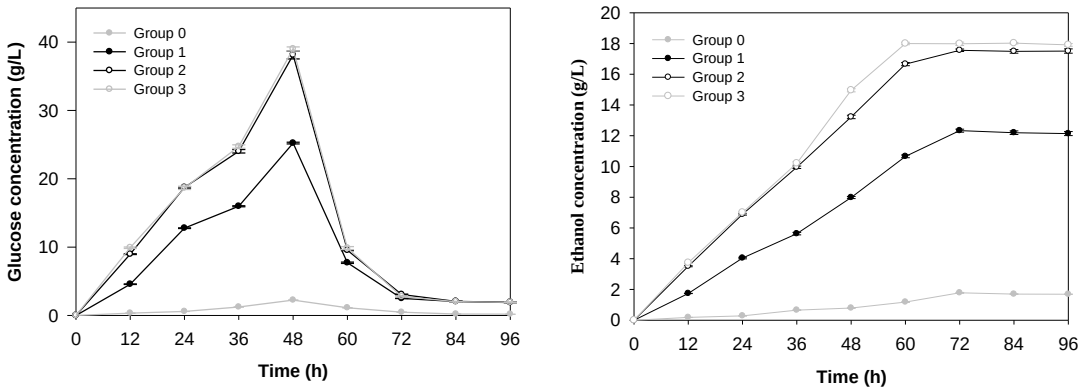

Fig. 3. Glucose and ethanol concentrations of Amur silvergrass after enzymatic hydrolysis and fermentation for 96 h, (group0) untreat; (group 1) 2.0%, 1:6, 15 min, 110 °C; (group 2) 4.0%, 1:8, 45 min, 110 °C; (group 3) 4.0%, 1:6, 15 min, 110 °C.

Images of the Lignocellulose Surface Structure

SEM analysis was used to observe the structure of Amur silvergrass before and after treatment with 4.0% NaOH. Untreated Amur silvergrass lignocellulose contains a large number of non-fibrous substances. The surface of the lignocellulose was rough and bonded together with obvious dense bundles of fibers (Fig. 4). After pretreatment with 4.0% NaOH, the texture of the cellulose in the cell wall was readily visible, the bundles were clear, the lignocellulose separation was good, and the interweaving structure was imperceptible. Lignocellulose pretreated with alkali was more easily enzymatically

hydrolyzed, while lignin could be dissolved well, and cellulose swelled in the alkali solution. Saponification under alkali pretreatment could break the unstable ester bonds among hemicellulose, cellulose, and lignin to remove lignin and some hemicellulose. This could also partially destroy the lignin structure. In lignin and hemicellulose, as the interstitial matrix material became dissolved, cellulose became exposed and partially degraded, and the lignocellulose structure collapsed. This change brought cellulase into contact with cellulose and decomposed it into monosaccharides. Therefore, the SEM images verified that dilute alkali pretreatment improved lignocellulose degradation.

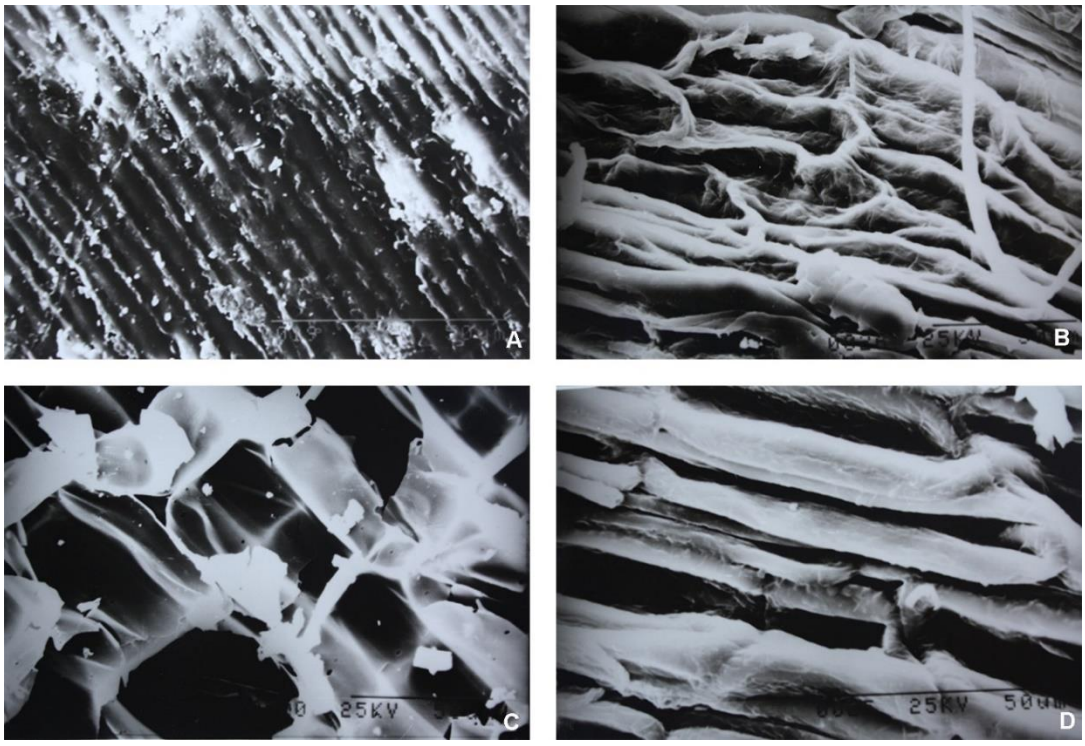

Fig. 4. The SEM images of lignocellulose surface structure before and after pretreatment on Amur silvergrass (A: External surface of lignocellulose before pretreatment (×1000); B: External surface of lignocellulose after pretreatment (×1000); C: Inner surface of lignocellulose before pretreatment (×600); D: Inner surface of lignocellulose after pretreatment (×600)

CONCLUSIONS

1. The appropriate concentration of NaOH pretreatment can remove more than 90% of lignin, degrade most hemicellulose, decompose a small part of cellulose, and improve the enzymatic hydrolysis rate of cellulose. The SEM images of the lignocellulose surface structure before and after the pretreatment verified the degradational change.
2. The main pretreatment factors driving degradation were NaOH concentration and temperature, followed by digestion time and then solid to liquid ratio.
3. The optimum pretreatment conditions were a 4.0% NaOH concentration, a 1:6 solid to liquid ratio, a 15 min digestion time and a 110 °C digestion temperature.
4. Under the optimum pretreatment conditions, the enzymatic hydrolysis rate of residual cellulose and the conversion rate to ethanol were 82.6% and 78.3%, respectively.

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