Pretreatment and enzymatic hydrolysis of bagasse by sodium metaaluminate

Meijie Chao [1, a], Jianghai Lin*.

Research Center for Molecular Biology, Institutes of Life and Health Engineering, College of Life Science and Technology, Jinan University, Guangzhou 510632, PR China

1571899839@qq.com; *Jianghai.lin@jnu.edu.cn

Abstract

In this study, sodium metaaluminate ($NaAlO_2$) was used to pretreat sugarcane bagasse to enhance the enzymatic efficiency. The results showed that when bagasse was pretreated with 3% NaAlO2, the content of cellulose and the removal rates of lignin were 48.18% and 63.25%, respectively. After enzymatic hydrolysis of bagasse pretreated with 5% NaAlO2, the total reducing sugar and total enzymatic hydrolysis efficiency were 3.89 g/L and 24.63%, respectively. This shows that $NaAlO_2$ can effectively remove lignin and improve the content of cellulose, but the effect of enzymatic hydrolysis is not obvious.

Keywords

$NaAlO_2$, pretreatment, enzymatic hydrolysis, bagasse.

1. Introduction

Lignocellulose is the most abundant renewable resource in the wolrd, and more than 50 billion tons of lignocellulose could be produced by terrestrial plants only every year. Lignocellulosic materials mainly come from agricultural and forestry wastes, such as wheat straw, corn straw, rice straw, bagasse, etc.[1]. However, these wastes have huge development prospects for recycling, such as to produce fuel ethanol[2] and high value-added chemicals[3], which can not only alleviate the energy crisis but also be environmentally friendly. Bagasse is a lignocellulose with higher cellulose content and less starch and soluble sugar content[4]. Moreover, bagasse is a good feedstock for ethanol production because of its high yield and low ash content.

Lignocellulose is coated with a tough layer of lignin that must be destroyed by certain chemically pretreatment method before it can be effectively enzymatic hydrolysis.. Alkali pretreatment is one of the most optimal treatment processes at present, and some strong bases such as NaOH[5] and KOH[6] are commonly used. The lignin of lignocellulose can be effectively removed under alkaline conditions to improve the utilization of cellulose[7]. However, the usage of strong base might increase the cost of pretreatment and the following post-pretreatment process of the waste water, and cause pollution to the environment It has also been reported that pretreatments with strong base and weak acid salts , such as Na_2CO_3[8], have obtained better results. $NaAlO_2$ is also a strong base and weak acid salt, and its pH is about 12 when dissolved in water. $NaAlO_2$ is widely used in desilication in papermaking industry[9], but has not been applied in pretreatment of lignocellulose so far.

In this study, we pretreated sugarcane bagasse (SCB) with $NaAlO_2$ to evaluate the effects of $NaAlO_2$ on the chemical components and the enzymatic efficiency of SCB. To our best knowledge, this is the first report that $NaAlO_2$ was involved in lignocellulose pretreatment.

2. Experiment datels

2.1 The effect of the concentration of NaAlO2 on SCB

In a 250 ml flask containing 5 g bagasse, 50 ml of different concentrations of $NaAlO_2$ (1%, 3% and 5%, w/v) were added and pretreated for 1 h at 121°C, then cooled down to room temperature. After pretreatment, the bagasse was washed with hot water until the pH was neutral and dried at 80℃ to constant weight.

2.2 Analysis of bagasse components

Cellulose, xylan, acid-soluble lignin (ASL) and acid-insoluble lignin (AIL) in pretreated and untreated bagasse were determined using methods provided by the National Renewable Energy Laboratory [10, 11].

2.3 Enzymatic hydrolysis

In a serum bottle, 0.5 g bagasse and 15 FPU of Cellic® CTec2 were added into 25 mL reaction system sodium citrate buffer (pH4.8) and bacteriostatic doxycycline and tetracycline were added to avoid microorganism contamination. Enzymatic hydrolyses were conducted in an orbital shaker incubator under 50℃ at 200 rpm . Samples were taken from every 12 h, boiled in water for 10 min to inactivate the enzymes, cooled down to room temperature, and centrifuged at 12,000 rpm for 5 min to collect supernatant. Thirty micro-liter of 10% H_2SO_4 were added to 570 μL the supernatants, and then filter with a 0.22 μm membrane filter.

2.4 Analysis method

Glucose and xylose concentrations were analyzed by HPLC (LC-15C, Shimadzu, Japan) equipped with a refractive index detector (RID-10A, Shimadzu, Japan) and an Aminex HPX-87H column (Bio-Rad, Richmond, CA, USA). Analyses were operated at 55°C with 5 mM H2SO4 as the mobile phase and a flow rate of 0.6 mL/min., and acid soluble lignin (ASL) was determined by a uv spectrophotometer at 240 nm and the acid insoluble lignin (AIL) was determined by the constant weight of an insoluble substance dried[12].

The following equations were used to calculate the lignocellulose compositions and recovery rate of pretreatment.:

$$\text{Cellulose composition (\%)} = \frac{\text{Glucose concentration (g/L)} \times \text{V (L)}}{\text{Solid mass(g)} \times 1.11 \times \text{a (\%)}} \times 100\%$$

$$\text{a (\%)} = \frac{\text{Glucose standard concentration (g/L)} \times \text{V(L)}}{\text{Glucose standard mass (g)}} \times 100\%$$

$$\text{Xylan composition (\%)} = \frac{\text{Xylose concentration (g/L)} \times \text{V (L)}}{\text{Solid mass(g)} \times 1.14 \times \text{b (\%)}} \times 100\%$$

$$\text{b (\%)} = \frac{\text{Xylose stangard concentration (g/L)} \times \text{V(L)}}{\text{Xylose stangard mass (g)}} \times 100\%$$

$$\text{Solid recovery (\%)} = \frac{\text{Solid mass after pretreatment (g)}}{\text{Solid mass before pretreatment (g)}} \times 100\%$$

a represented correction factor for glucose pyrolysis (%); b represented correction factor for xylan pyrolysis (%); V represented total volume of reaction hydrolysate (L).

3. Results and discussion

3.1 Compositions and solid recovery of bagasse

The component changes of SCB before and after pretreatment can be used as one of the parameters to evaluate the pretreatment effect. The main components of bagasse before and after being pretreated with $NaAlO_2$ at different concentrations are shown in Fig. 1. Obviously, with the increase of NaAlO2 concentration, the content of lignin, especially acid insoluble lignin in the pretreated bagasse decreased significantly compared with that in the unpretreated bagasse (total lignin 28%). When the concentration of $NaAlO_2$ reached 5%, the total lignin was removed by 72.7%. At the same time, the removal of lignin correspondingly increased the content of cellulose in the pretreated bagasse. However, when the $NaAlO_2$ concentration increased to 5%, the content of cellulose decreased slightly compared with 3% $NaAlO_2$, which might be caused by the destruction of cellulose structure by high alkali concentration. The content of xylan in $NaAlO_2$ was not different in different concentrations of $NaAlO_2$.

In addition, as shown in Table 1, with the increase of $NaAlO_2$ concentration, the bagasse recovered after pretreatment decreased gradually, from 78% to 65%. Although the loss of cellulose and xylan increased with the increase of $NaAlO_2$ concentration, the recovery rate of xylan was lower than that of cellulose. When $NaAlO_2$ concentration was 5%, the recovery rate of cellulose was 78.47%, while the recovery rate of xylan was only 60.1%. This indicated that more xylan and lignin dissolved in stronger base solution, and more raw materials were lost. Therefore, considering the removal rate of lignin and the recovery rate of raw materials, the pretreatment with 3% $NaAlO_2$ was considered to be the most effective. Being pretreated under this concentration, the cellulose and xylan contents of pretreated SCB were 48.18% and 25.04% respectively; the removal rate of lignin was 63.25%; and the content of total cellulose in the pretreated bagasse was the highest.

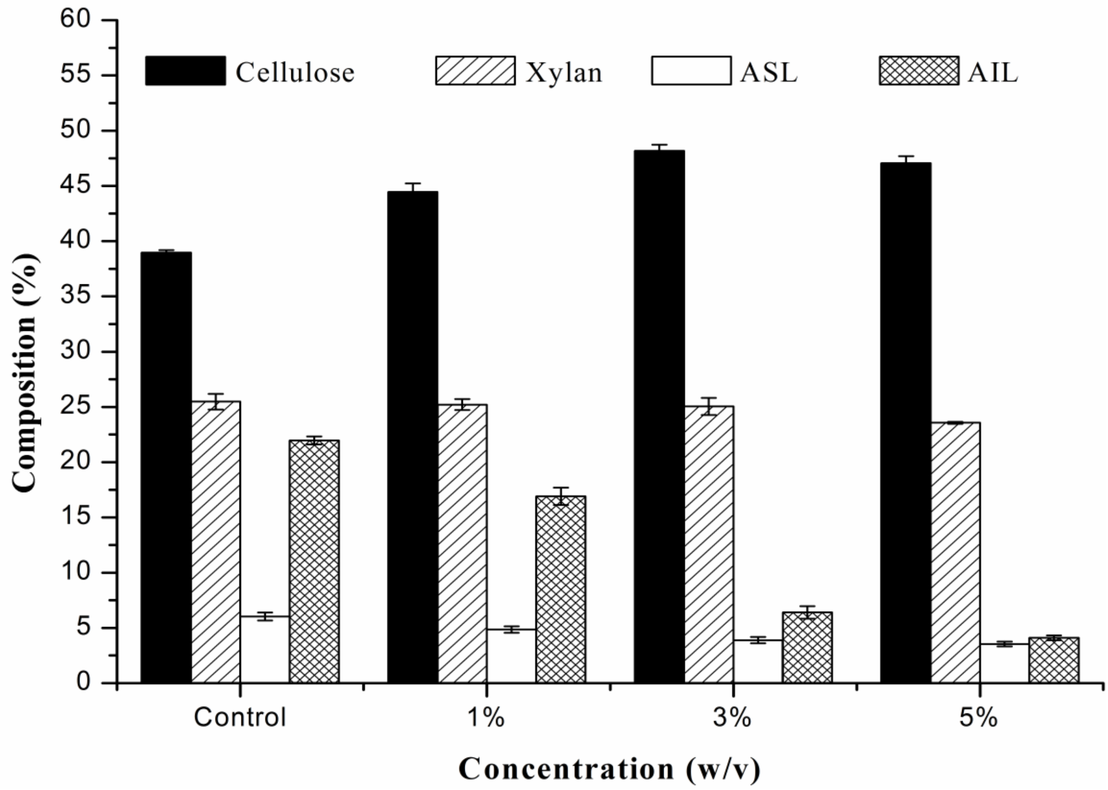

Fig 1 The main compositions were obtained unpretreated and pretreated bagasse with $NaAlO_2$

Table 1 The solid recovery were obtained unpretreated and pretreated bagasse with $NaAlO_2$

Concentration (w/v)	Solid recovery rate (%)	Cellulose recovery rate (%)	Xylan recovery rate (%)
Control	100	100	100
1%	78.60±0.21	89.63±0.28	77.76±0.19
3%	70.00±0.77	86.52±0.56	68.79±0.75
5%	65.00±0.56	78.47±0.36	60.10±0.20

3.2 Enzymatic hydrolysis

The effects of $NaAlO_2$ pretreatment at different concentrations on the enzymatic hydrolysis of SCB are shown in Fig. 2 and Fig. 3. As a whole, the total reducing sugar and total enzymatic hydrolysis efficiency of pretreated bagasse were obviously improved compared with that of untreated. When bagasse was pretreated with 5% $NaAlO_2$, the total reducing sugar and total enzymatic hydrolysis efficiency reached the maximum value of 3.89 g/L and 24.63%, respectively. This indicated that $NaAlO_2$ could be used as a pretreatment reagent for bagasse. However, the efficiency of enzymatic hydrolysis of total reducing sugars and total enzymatic hydrolysis after $NaAlO_2$ pretreatment was much lower than that of Na_2CO_3, [13].This might be due to the hydrolysis of $NaAlO_2$ at high temperature to form colloids attached to the surface of cellulose, thus hindering the hydrolysis of cellulose by cellulases[14].

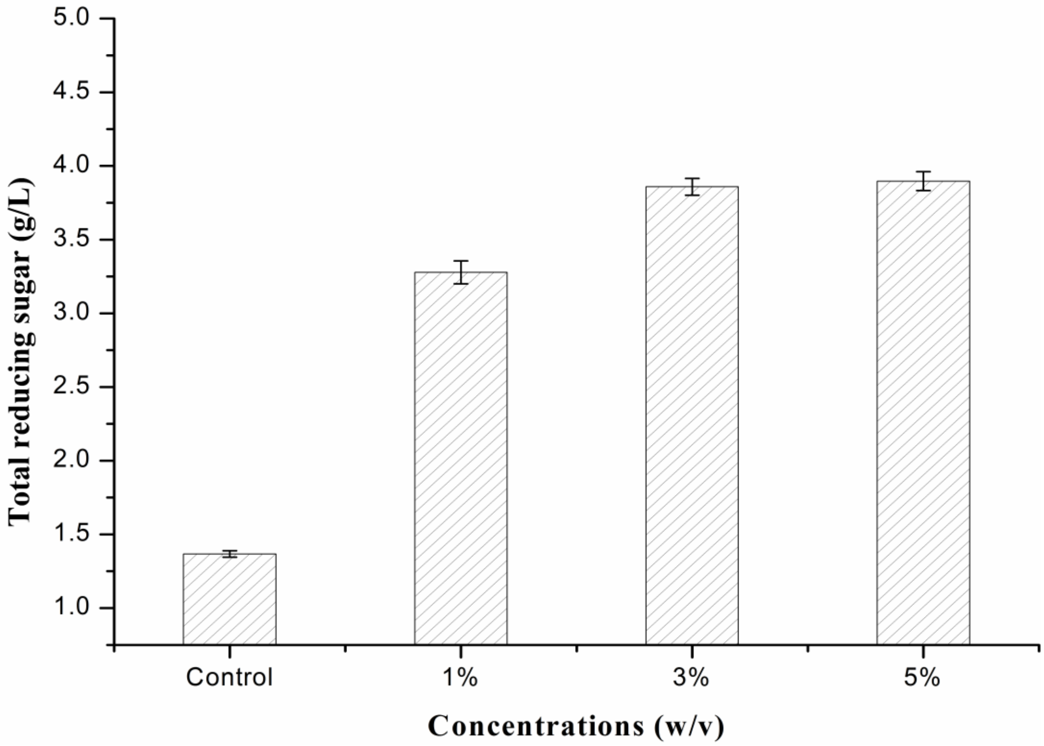

Fig 2 Total reducing sugars were obtained by enzymatic hydrolysis of bagasse pretreated with $NaAlO_2$ at different concentrations.

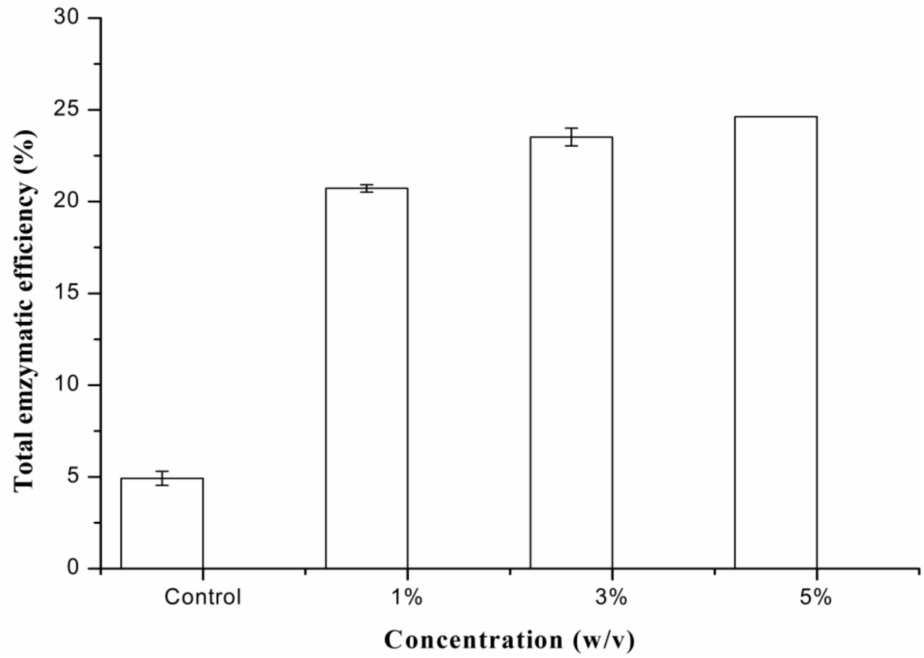

Fig 3 Total enzymatic efficiency were obtained by enzymatic hydrolysis of bagasse pretreated with $NaAlO_2$ at different concentrations.

4. Conclusion

It was found that $NaAlO_2$ pretreatment could effectively remove the lignin and improve the cellulose content of SCB. Our results indicated that the highest cellulose content was obtained when the $NaAlO_2$ concentration was 3%, and the cellulose and xylan contents of were 48.18% and 25.04% respectively. Enzymatic hydrolysis of pretreated SCB was studied, it was found that the total reducing sugars and total enzymatic hydrolysis efficiency of pretreated SCB were higher than those of unpretreated, but lower than those reported. In conclusion, this study introduced a novel potential pretreatment method for lignocellulose.

Acknowledgements

This work was supported by the Science and Technology Planning Project of Guangdong Province (2012B020311005 and 2015A010107007), the Program for New Century Excellent Talents in University (NCET-05-0745), and the Fundamental Research Funds for the Central Universities (21614333).

References

[1] Sanchez O J, Cardona C A. Trends in biotechnological production of fuel ethanol from different feedstocks, Bioresource Technology, Vol.99 (2008) No.13, p.5270-5295.

[2] Kanak R, Chandraraj K. Improved high solid loading enzymatic hydrolysis of low-temperature aqueous ammonia soaked sugarcane bagasse using laccase-mediator system and high concentration ethanol production, Industrial Crops & Products , Vol.131 (2019), p.32-40.

[3] Li F-H, Ding Z-T, Ke W-C, et al. Ferulic acid esterase-producing lactic acid bacteria and cellulase pretreatments of corn stalk silage at two different temperatures: ensiling characteristics, carbohydrates composition and enzymatic saccharificatio, Bioresource Technology, Vol.282 (2019), p.211-221.

[4] Wang Y-P, Li J-H, Liu Y-H, et al. Comprehensive utilization of bagasse: state of the art, Chinese Agricultural Science Bulletin, Vol.26 (2010) No.16, p.370-375.

[5] Yang H-Y, Shi Z-J, Xu J-F, et al. Bioethanol production from bamboo with alkali-catalyzed liquid hot water pretreatment, Bioresource Technology, Vol.274 (2019), p.261-266.

[6] Romero-Guiza M S, Wahid R, Hernandez V, et al. Improvement of wheat straw anaerobic digestion through alkali pre-treatment: carbohydrates bioavailability evaluation and economic feasibility, The Science of the total environment, Vol.595 (2017),651-659.

[7] Li X, Kim T H, Nghiem N P. Bioethanol production from corn stover using aqueous ammonia pretreatment and two-phase simultaneous saccharification and fermentation (TPSSF), Bioresource Technology, Vol.101 (2010) No.15, p.5910-5916.

[8] Nguyen T S, Zabeti M, Lefferts L, et al. Conversion of lignocellulosic biomass to green fuel oil over sodium based catalysts, Bioresource Technology, Vol.142 (2013), 353-360.

[9] Xu Y-J, Zhang W-P, Zhang D-J, et al. Bamboo cooking with simultaneous desilication of the black liquor by using calcium & aluminate salts, China Pulp & Paper, Vol.34 (2015) No 8, p.18-23.

[10] Sluiter A, Hames B, R. Ruiz C, et al. Determination of Structural Carbohydrates and Lignin in Biomass, NREL, Golden. 2008

[11] Z.F. Zhou, Z.A. Li : *Plant Physiology Experiment Instructio*, (www.bbioo.com, China 2008), p.82-86.

[12] Crocker D. NREL/TP-510-42618 NREL Laboratory Analytical Procedure, NREL, Golden. 2008

[13] Sewsynker-Sukai Y, Kana G E B. Microwave-assisted alkalic salt pretreatment of corn cob wastes: process optimization for improved sugar recovery, Industrial Crops & Products, Vol.125 (2018), p.284-292.

[14] Tang L-Y: *Technology and mechanism on simultaneous silicon-remain cooking in bamboo pulp based on extended deligeification* (MS., Shaanxi University of Science & Technology, China 2012), p.7.