Effects of Liquid-to-Solid Ratio and Reaction Temperature on NaOH Pretreatment of *Achnatherum splendens*

L. Tao[1,2], J. Ren[1,2,*], F.K. Yu[1,3] and T.R. Ni[1,3]

[1]School of Environmental and Municipal Engineering, Institute of Environmental Ecology, Lanzhou Jiaotong University, Lanzhou 730070, P.R. China

[2]Engineering Research Center for Cold and Arid Regions Water Resource Comprehensive Utilization, Ministry of Education, Lanzhou 730070, P.R. China

[3]School of Environmental Science and Engineering, Nankai University, Tianjin 300100, P.R. China

*Corresponding author: E-mail: renjun@mail.lzjtu.cn

In this study, the effects of several factors on NaOH pretreatment of *Achnatherum splendens* were investigated. It was found that NaOH loading, liquid-to-solid ratio and reaction temperature all had significant effects on delignification. These factors also had significant effects on the contents of lignin, hemicellulose and cellulose in the solids after NaOH pretreatment. The optimal pretreatment conditions were found to be 15 % of NaOH loading, 10:1 of liquid-to-solid ratio, 90 ºC and 90 min of reaction time. Under the optimal conditions, approximate 39 % of lignin was solubilized and the cellulose content was increased to 68.39 % while the lignin and hemicellulose contents were decreased to 9.28 %, 19.86 %, respectively. The lignin was effectively removed by NaOH pretreatment under mild conditions. The results could be considered as a step towards the optimization of NaOH pretreatment of *Achnatherum splendens*.

Key Words: Liquid-to-solid ratio, Reaction temperature, NaoH pretreatment.

INTRODUCTION

Due to increasing energy consumption and environmental concerns, there has been increased interest in using ethanol as an alternative to fossil fuels[1,2]. It is not sustainable to produce ethanol from grains such as corn and wheat in China and some other countries. Lignocellulosic materials are attractive feedstock for ethanol production because they are abundant, cheap and renewable. However, in lignocellulosic materials, cellulose, a liner polymer of glucose is associated with hemicellulose and surrounded by lignin seal. Lignin, a complex three-dimensional polyaromatic matrix prevents enzymes from accessing some regions of the cellulose polymers. Crystallinity of the cellulose further impedes enzymatic hydrolysis[3,4]. Therefore, pretreatment has been studied as a key step for the effective utilization of lignocellulosic biomass feedstock[5,6]. The objective of lignocellulosic biomass pretreatment is to alter the structure of the lignocellulosic matrix to increase cellulose digestibility using cellulase enzymes[7], which can be done by removing lignin, hemicelluloses, or a combination of the two. Lignin is a constituent that is known to inhibit saccharification enzymes and fermentative microorganisms[8-10]. Low-lignin substrates have improved microbial activity and enzyme efficiency, eventually lowering the enzyme requirement[11].

Several processes have been developed for pretreatment of lignocellulosic biomass, including steam explosion[12-15], liquid hot water pretreatment[16], acid pretreatment[17-21], alkali pretreatment[22-24], wet oxidation[25,26] and peracetic acid pretreatment[27,28]. Alkali has been recognized as one of the most effective agents for delignification and swelling of the fibers. The main effect of sodium hydroxide pretreatment on lignocellulosic biomass is delignification by breaking the ester bonds cross-linking lignin and xylan, thus increasing the porosity of the biomass[2,7,29]. The main purpose of chemical pretreatment under mild condition (<100 ºC) is delignification and separating cellulose fibers[24]. In this paper, *Achnatherum splendens* was pretreated by NaOH. *Achnatherum splendens*, which distributes widely in the north of China, is a tufty, perennial herbaceous plant of about 0.5-2.5 m in height. The plant grows rapidly and has a high tolerance for salt and drought. It is often found growing on infertile and poor land. Of special importance is the fact that the plant could be used for treatment of saline and alkali land and polluted soil. In this study, the factors that affect NaOH pretreatment of *Achnatherum splendens* were investigated. The factors of interest were NaOH loading, liquid-to-solid ratio and reaction temperature.

TABLE-1
EFFECT OF NaOH LOADING ON THE COMPOSITIONS IN NaOH TREATED *Achnatherum splendens*. VALUES ARE MEANS
OF TRIPLICATE MEASUREMENTS ± STANDARD DEVIATION. VALUES WITH THE SAME LETTERS ARE NOT SIGNIFICANTLY
DIFFERENT AMONG TREATMENTS AT $p < 0.05$ ACCORDING TO TUKEY HSD TEST

NaOH loading (%)	Lignin (%)	Delignification (%)	Hemicellulose (%)	Cellulose (%)
3	12.29 ± 0.06a	1.54 ± 0.46a	30.80 ± 0.39a	55.86 ± 0.42a
6	11.58 ± 0.14b	5.66 ± 1.15a	23.61 ± 0.89b	62.51 ± 0.03b
9	10.20 ± 0.20c	23.36 ± 1.54b	23.25 ± 0.11b	65.27 ± 0.51c
12	10.17 ± 0.43c	28.29 ± 3.05c	19.92 ± 0.59c	66.11 ± 0.58c
15	9.28 ± 0.34d	38.86 ± 2.21d	19.86 ± 0.62c	68.39 ± 0.57d
18	8.51 ± 0.11e	43.06 ± 0.73d	18.66 ± 0.85c	69.15 ± 0.52d
F-value	98.82***	278.67***	147.24***	313.67***

EXPERIMENTAL

Achnatherum splendens used in the study was obtained from Zhangye city in Gansu province of northwestern China. It was ground and screened to a nominal size of 20-60 mesh and then dried in oven at 105 ºC for 6 h. The oven-dried samples were stored in valve bags at room temperature until use for pretreatment. Cellulose, hemicellulose and lignin were determined by the methods described by Goering and Vansoest[30]. The initial composition of the *Achnatherum splendens* was determined to be 45.31 % cellulose, 31.46 % hemicellulose, 9.59 % lignin, 6.45 % moisture content, 1.96 % ash. Each sample was analyzed in duplicate. Statistical ANOVA analysis was determined by STATISTICA.

The pretreatment was carried out in a 1000 mL glass flask immersed in a water bath. The ground biomass was pretreated by 3-18 % of NaOH loading (based on initial dry materials) with 8:1-20:1 (v/w) of liquid-to-solid ratio at 60-100 ºC for 90 min. After NaOH pretreatment, the solids were washed with deionized water until neutrality and dried in oven at 105 ºC for 6 h. The oven-dried samples were stored in valve bags at room temperature for further analysis.

RESULTS AND DISCUSSION

Effects of NaOH loading (based on initial dry materials): The effects of NaOH loading in NaOH pretreatment were investigated. The results are summarized in Table-1. The pretreatment conditions were 3-18 % of NaOH loading (based on initial dry materials), 90 ºC, 10:1 (v/w) of liquid-to-solid ratio and 90 min of reaction time. The yield of lignin and hemicellulose in solids pretreated by NaOH were decreased from 12.29 %, 30.80 % to 8.51 %, 18.66 %, respectively when NaOH loading was increased from 3 % to 18 %, but the yield of cellulose was increased from 55.86 % to 69.15 %. Changes in NaOH loading caused significant decrease in lignin ($p < 0.001$) and hemicellulose ($p < 0.001$) and significant increase in cellulose ($p < 0.001$). Treatment with 3 % of NaOH loading achieved only 1.54 % of delignification. It indicated that 3 % NaOH is too low to affect delignification for treatment time up to 90 min and temperature up to 90 ºC. When NaOH loading was increased to 18 %, the solubilization of lignin was increased to 43.06 %. An increase in NaOH loading significantly improved delignification ($p < 0.001$).

As compared with the treatment of 15 % of NaOH loading, the lignin and cellulose contents of pretreated solids were significantly different at the treatments of 3, 6, 9 and 12 % of NaOH loading. There was significant difference between the treatments of 15 % and 18 % of NaOH loading for the content of lignin in pretreated solids. However, for cellulose content of pretreated solids, there was no significant difference between the treatments of 15 % and 18 % of NaOH loading. The content of cellulose in pretreated solids was significantly different at the treatments of 3, 6 and 9 % of NaOH loading as compared with the treatment of 15 % of NaOH loading. However, for hemicellulose content of pretreated solids, there was no significant difference among the treatments of 12, 15 and 18 % of NaOH loading (Table-1).

As compared with the treatment of 15 % of NaOH loading, delignification was significantly different at the treatments of 3, 6, 9 and 12 % of NaOH loading. However, for delignification, there was no significant difference between the treatments of 15 % and 18 % of NaOH loading (Table-1). It indicated that NaOH loading had no significant effects on delignification when higher than 15 %. Therefore, 15 % of NaOH loading was selected for NaOH pretreatment.

Effect of liquid-to-solid ratio: In order to study the effects of liquid-to-solid ratio, five different liquid-to-solid ratios (8:1, 10:1, 12:1, 15:1 and 20:1) were applied keeping the NaOH loading at 15 % (based on initial dry materials), the reaction temperature at 90 ºC and the reaction time for 90 min. The composition data after the treatment are summarized in Table-2. It was found that liquid-to-solid ratio had significant effects on yield of lignin ($p < 0.05$), hemicellulose ($p < 0.01$) and cellulose ($p < 0.05$). The lignin and hemicellulose contents of the NaOH pretreated solids were decreased from 10.29 %, 19.72 % to 9.32 %, 18.42 %, respectively when the liquid-to-solid ratio was increased from 8:1 to 10:1, but the cellulose content was increased from 66.94 to 68.34 %. The lignin and hemicellulose contents of solids pretreated by NaOH were increased to 9.76 and 19.78 %, respectively when the liquid-to-solid ratio was increased to 20:1, but the cellulose content was decreased to 66.78 %. Liquid-to-solid ratio had significant effects on delignification ($p < 0.05$). The delignification was increased from 31.70 to 38.71 % when the liquid-to-solid ratio was increased from 8:1 to 10:1. But when the liquid-to-solid ratio was increased to 20:1, delignification was decreased to 32.85 %. It became hard to keep the reaction system homogeneous because of less liquid being present when liquid-to-solid ratio was lower than 10:1. When NaOH loading was fixed, higher liquid-to-solid ratio led to lower NaOH concentration in the liquid phase, which further decreased the delignification.

For lignin, hemicellulose and cellulose contents of pretreated solids, there was significant difference between the

TABLE-2
EFFECT OF LIQUID-TO-SOLID RATIO ON THE COMPOSITIONS IN NaOH TREATED *Achnatherum splendens*. VALUES ARE
MEANS OF TRIPLICATE MEASUREMENTS ± STANDARD DEVIATION. VALUES WITH THE SAME LETTERS ARE
NOT SIGNIFICANTLY DIFFERENT AMONG TREATMENTS AT p < 0.05 ACCORDING TO TUKEY HSD TEST

Liquid-to-solid ratio (v/w)	Lignin (%)	Delignification (%)	Hemicellulose (%)	Cellulose (%)
8:1	10.29 ± 0.35a	31.70 ± 2.33a	19.72 ± 0.16a	66.94 ± 0.20a
10:1	9.32 ± 0.26b	38.71 ± 1.71b	18.42 ± 0.31b	68.34 ± 0.46b
12:1	9.61 ± 0.39ab	34.99 ± 2.65ab	18.50 ± 0.10b	67.62 ± 0.38ab
15:1	9.63 ± 0.29ab	34.96 ± 1.93ab	18.55 ± 0.39b	67.48 ± 0.10ab
20:1	9.76 ± 0.31ab	32.85 ± 2.15a	19.78 ± 0.44a	66.78 ± 0.52a
F-value	3.65*	4.52*	15.08**	6.23*

TABLE-3
EFFECT OF REACTION TEMPERATURE ON THE COMPOSITIONS IN NaOH TREATED *Achnatherum splendens*. VALUES ARE
MEANS OF TRIPLICATE MEASUREMENTS± STANDARD DEVIATION. VALUES WITH THE SAME LETTERS ARE
NOT SIGNIFICANTLY DIFFERENT AMONG TREATMENTS AT p < 0.05 ACCORDING TO TUKEY HSD TEST

Temperatures (ºC)	Lignin (%)	Delignification (%)	Hemicellulose (%)	Cellulose (%)
60	11.62 ± 0.14a	8.3 ± 1.07a	23.13 ± 0.43a	63.46 ± 0.24a
70	11.24 ± 0.49ab	13.09 ± 3.77a	21.84 ± 0.92ab	63.49 ± 0.47a
80	10.52 ± 0.36bc	25.39 ± 2.55b	21.63 ± 0.41bc	64.83 ± 0.29b
90	9.68 ± 0.45c	31.55 ± 3.15bc	18.97 ± 0.31d	66.53 ± 0.54c
100	9.58 ± 0.29c	34.48 ± 1.98c	18.65 ± 0.26d	67.69 ± 0.14d
F-value	18.53**	54.91***	41.65***	78.39***

treatments of 8:1 and 10:1 of liquid-to-solid ratio. However, there was no significant difference among the treatments of 10:1, 12:1, 15:1 and 20:1 of liquid-to-solid ratio for lignin content of pretreated solids. There was no significant difference among the treatments of 10:1, 12:1 and 15:1 of liquid-to-solid ratio for the contents of hemicellulose and cellulose in pretreated solids. However, for hemicellulose and cellulose contents of pretreated solids, there was significant difference between the treatments of 10:1 and 20:1 of liquid-to-solid ratio (Table-2).

As compared with the treatment of 8:1 of liquid-to-solid ratio, delignification was significantly different at the treatment of 10:1 of liquid-to-solid ratio. However, there was no significant difference among the treatments of 10:1, 12:1 and 15:1 of liquid-to-solid ratio for delignification. There was significant difference between the treatments of 10:1 and 20:1 of liquid-to-solid ratio for delignification. Treatment at 10:1 of liquid-to-solid ratio achieved 38.71 % of delignification which was the maximum delignification (Table-2). Therefore, 10:1 was the liquid-to-solid ratio selected for NaOH pretreatment.

Effect of reaction temperature: Table-3 summarizes the changes of composition with reaction temperature. The pretreatment conditions were 60-100 ºC, 12 % of NaOH loading (based on initial dry materials), 8:1 (v/w) of liquid-to-solid ratio and 90 min of reaction time. The amount of lignin and hemicellulose in solids after NaOH pretreatment were decreased from 11.62 %, 23.13 % to 9.58 %, 18.65 %, respectively when reaction temperature was increased from 60 to 100 ºC, but the amount of cellulose was increased from 63.46 to 67.69 %. Increasing of reaction temperature resulted in significant decrease in lignin ($p < 0.01$) and hemicellulose ($p < 0.001$) and significant increase in cellulose ($p < 0.001$). Delignification was in the range of 8.3-34.48 % with 60-100 ºC of reaction temperature. The reaction temperature had significant effects on delignification ($p < 0.001$). Treatment at 60 ºC achieved 8.3 % of delignification. It indicated that 60 ºC is too low to affect delignification. However, only 34.48 %

of lignin was dissolved when the temperature was increased to 100 ºC. Perhaps using higher temperatures could further remove lignin.

As compared with the treatment of 90 ºC, the content of lignin in pretreated solids was significantly different at the treatments of 60 ºC and 70 ºC. However, there was no significant difference among the treatments of 80, 90 and 100 ºC for the content of lignin in pretreated solids. As compared with the treatment of 90 ºC, hemicellulose and cellulose contents of pretreated solids were significantly different at the treatments of 60, 70 and 80 ºC. There was no significant difference between the treatments of 90 ºC and 100 ºC for hemicellulose contents of pretreated solids. However, there was significant difference between the treatments of 90 ºC and 100 ºC for the content of cellulose in pretreated solids (Table-3).

As compared with the treatment of 90 ºC, delignification was significantly different at the treatments of 60 and 70 ºC. However, there was no significant difference between the treatments of 80 ºC and 90 ºC for delignification. Similarly, there was no significant difference between the treatments of 90 and 100 ºC for delignification. However, there was significant difference between the treatments of 80 and 100 ºC for delignification (Table-3). It indicated that reaction temperature had no significant effects on delignification when over 90 ºC. Therefore, 90 ºC was selected for NaOH pretreatment.

On the basis of the collective experimental data of delignification was determined to the optimum operating condition of the NaOH pretreatment to be: 15 % of NaOH loading, 10:1 of liquid-to-solid ratio, 90 ºC and 90 min of reaction time. Under the optimal condition, approximately 39 % lignin was removed. Silverstein *et al.*[24] reported 65.63 % delignification as a result of pretreatment of cotton stalks with 2 % NaOH for 90 min at 121 ºC/15psi in the autoclave. The higher delignification level may be attributed to two reasons. One reason may be a higher reaction temperature of 121 ºC, which in this study was limited to 100 ºC and another reason may be related to the nature of *Achnatherum splenden*.

Conclusion

The lignin was effectively removed by NaOH pretreatment under mild conditions. It was showed that NaOH loading, liquid-to-solid ratio and reaction temperature all had significant effects on delignification. These factors also had signiifcant effects on the content of lignin, hemicellulose and cellulose in the solids after NaOH pretreatment. The optimum treatment conditions of NaOH pretreatment for *Achnatherum splendens* were found to be 15 % of NaOH loading (based on initial dry materials), 10:1 (v/w) of liquid-to-solid ratio, 90 ºC and 90 min of reaction time. Under these conditions, approximate 39 % of lignin was solubilized and the content of cellulose was increased to 68.39 % while the content of hemicellulose was decreased to 19.86 %. In addition, this study can serve as a step towards the optimization of NaOH pretreatment of *Achnatherum splendens*. Nevertheless, perhaps application of higher temperatures and pressures could be investigated. In addition, enzymatic hydrolysis using optimized pretreatment factors and ethanol fermentation need to be studied for bioethanol production since they could not be addressed in this study.

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