



## Pretreatment of *Achnatherum splendens* by Soaking in Aqueous Ammonia

REN JUN<sup>1,2,\*</sup>, TAO LING<sup>1,2</sup>, NI TIANRI<sup>1</sup>, HAO JIANXIU<sup>1</sup> and GAO YAMEI<sup>1</sup>

<sup>1</sup>School of Environmental and Municipal Engineering, Institute of Environmental Ecology, Lanzhou Jiaotong University, Lanzhou 730070, P.R. China

<sup>2</sup>Engineering Centre for Cold and Arid Regions Water Resource Comprehensive Utilization, Ministry of Education, Lanzhou 730070, P.R. China

\*Corresponding authors: Fax: +86 931 4956017; Tel: +86 931 4938177; E-mail: renjun@mail.lzjtu.cn

(Received: 6 November 2010;

Accepted: 22 June 2011)

AJC-10059

Soaking in aqueous ammonia was investigated as a pretreatment method for *Achnatherum splendens*. The method involves batch treatment of feedstock with aqueous ammonia (5-25 wt %) at 45-85 °C for 12-72 h. It was found that ammonia concentration, reaction temperature and reaction time all had significant effects on delignification. These factors also had significant effects on the lignin, hemicellulose and cellulose contents of soaking in aqueous ammonia-treated solids. Optimum conditions were obtained as follows: 10 wt % of ammonia, temperature 55 °C, 36 h of reaction time and 12:1 (based on wt) of liquid-to-solid ratio. Under these conditions, delignification reached to 26.58 % and the content of cellulose in pretreated solids increased to 64.20% while the content of hemicellulose decreased to 23.63 %.

**Key Words:** *Achnatherum splendens*, Aqueous ammonia, Delignification, Hemicellulose, Cellulose.

### INTRODUCTION

While the world's economy continues to grow, so does its dependency on energy resources. The most commonly used sources are oil, coal and natural gas, which are limited in nature and have caused serious environmental pollution. Fuel ethanol is a renewable energy resource that offers simultaneous environmental benefits. Grains such as corn and wheat are the primary materials for fuel ethanol production in the world. However, it is not sustainable to produce fuel ethanol from grains in China and some other countries. Lignocellulosic materials are attractive feedstock for fuel ethanol production because they are abundant, cheap and renewable. For fuel ethanol production, pretreatment has been studied as a key step for the effective utilization of lignocellulosic biomass feedstock, due to its recalcitrant nature. The objective of lignocellulosic biomass pretreatment is to alter the structure of the lignocellulosic matrix to increase cellulose digestibility using cellulose enzymes, 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<sup>1,2</sup>.

Several processes have been developed for pretreatment of lignocellulosic biomass, including steam explosion<sup>3,4</sup> acid pretreatment<sup>5,6</sup>, alkali pretreatment<sup>7,8</sup> and wet oxidation<sup>9,10</sup>. Using alkaline chemicals to remove lignin has been known to improve cellulose digestibility for years, but sodium hydroxide

and other bases are too expensive and too difficult to recover and recycle to make them viable for producing fuels and chemicals<sup>11</sup>. Pretreatment with ammonia has more recently been shown to be effective in improving cellulose digestion with the advantage of ammonia being recyclable due to its volatility. A process known as ammonia recycle percolation passes aqueous ammonia (10-15 wt %) through biomass at elevated temperatures (150-170 °C) and then recovers it for recycle<sup>12-15</sup>. A simpler process termed soaking in aqueous ammonia treats biomass with aqueous ammonia in a batch reactor at moderate temperatures under atmospheric pressure and achieves similar results to ammonia recycle percolation<sup>16-18</sup>. Kim and Lee<sup>17</sup> reported 62 % of lignin removal of corn stover treated by 15 wt % of ammonia with 1:6 (based on wt) of solid-to-liquid ratio, at 60 °C for 12 h. Xu *et al.*<sup>19</sup> showed that cellulose in straw pretreated by ammonia liquor (10 %) soaking for 24 h at room temperature increased 70.27 % compared with the untreated straw, whereas hemicellulose and lignin in pretreated straw decreased to 41.45 % and 30.16 %, respectively.

*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. This study was focused on evaluation of the

soaking in aqueous ammonia as a pretreatment method of *Achnatherum splendens*. The effects of reaction parameters on the composition were investigated. The reaction parameters of interest were ammonia concentration, reaction temperature and reaction time.

## EXPERIMENTAL

*Achnatherum splendens* used in the study was obtained from Zhangye city in Gansu province in Northwest China. It was ground and screened to a nominal size of 20-60 mesh and then dried in oven at 105 °C 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<sup>20</sup>. 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 analysis determined by ANOVA in STATISTICA.

**Pretreatment process:** The pretreatment was carried out in a 1000 mL glass flask immersed in a water bath. The ground biomass was treated with 5-25 wt % of aqueous ammonia at 45-85 °C for 12-72 h. Liquid-to-solid ratio was 12:1 (based on wt). After soaking, the solids were washed with deionized water until neutrality and dried in oven at 105 °C for 6 h. The oven-dried samples were stored in valve bags at room temperature for further analysis.

## RESULTS AND DISCUSSION

**Effect of ammonia concentration:** Table-1 summarizes the composition changes with the increasing of ammonia concentration. The pretreatment conditions were 5-25 wt % of ammonia, 75 °C, 48 h of reaction time and 12:1 (based on wt) of liquid-to-solid ratio. The lignin and hemicellulose contents of the soaking in aqueous ammonia-treated solids decreased from 11.78, 20.76 to 10.55, 19.40 %, respectively when ammonia concentration was increased from 5 to 25 wt % and the content of cellulose increased from 61.65 to 62.97 %. Increasing of ammonia concentration resulted in significant decrease in lignin ( $p < 0.001$ ) and hemicellulose ( $p < 0.01$ ) and significant increase in cellulose ( $p < 0.05$ ). Solubilization of lignin was in the range of 13.99-21.43 %, with 5-25 wt % of ammonia. The ammonia concentration had significant effects on delignification ( $p < 0.001$ ). Treatment at 25 wt % of aqueous ammonia achieved 21.43 % of delignification which was much lower than those observed from other treatment methods using aqueous ammonia<sup>17</sup>.

Compared with treatment of 5 wt % of ammonia, the lignin and hemicellulose contents of pretreated solids decreased significantly and the cellulose content increased significantly at treatment of 10 wt % of ammonia. For the lignin and hemicellulose contents of pretreated solids, there were no significant difference among the treatments of 10, 15, 20 and 25 wt % of ammonia. Compared with the treatment of 10 wt % of ammonia, there were significant increase in the content of cellulose in pretreated solids at the treatments of 15, 20 and 25 wt % of ammonia. However, for the content of cellulose in pretreated solids there was no significant difference among the treatments of 15, 20 and 25 wt % of ammonia (Table-1).

Delignification increased significantly at treatment of 10 wt % of ammonia compared with treatment of 5 wt % of ammonia. For the delignification, there was no significant difference among the treatments of 10, 15, 20 and 25 wt % of ammonia. It indicated that ammonia concentration had no significant effects on delignification when higher than 10 wt % (Table-1).

**Effect of reaction temperature:** The effects of reaction temperature on composition in soaking in aqueous ammonia were investigated. The results are summarized in Table-2. The pretreatment conditions were 45-85 °C, 15 wt % of ammonia, 48 h of reaction time and 12:1 (based on wt) of liquid-to-solid ratio. The reaction temperature had significant effects on the contents of lignin ( $p < 0.001$ ), hemicellulose ( $p < 0.001$ ) and cellulose ( $p < 0.001$ ) in soaking in aqueous ammonia-treated solids. When reaction temperature was increased from 45-85 °C, the hemicellulose content of soaking in aqueous ammonia-treated solids decreased from 20.41 to 18.15 %. The cellulose content of soaking in aqueous ammonia-treated solids was in the range of 60.08-63.70 %, with 45-85 °C of reaction temperature. The lignin content of soaking in aqueous ammonia-treated solids decreased from 10.67 to 9.66 % as reaction temperature was increased from 45 to 65 °C. However, when reaction temperature was increased from 65 to 85 °C, the lignin content of soaking in aqueous ammonia-treated solids increased from 9.66 to 11.67 %. Increase of reaction temperature showed significant effects on delignification ( $p < 0.001$ ). Delignification increased from 15.44 to 26.55 % as reaction temperature was increased from 45 to 65 °C. However, when reaction temperature was increased from 65 to 85 °C, delignification decreased from 26.55 to 10.56 %. The reason maybe that the higher reaction temperatures ( $> 65$  °C) led to higher volatility of ammonia in the liquid phase which led to lower ammonia concentration the liquid and further increased the content of lignin and decreased the delignification. Results from this study were opposite to those obtained by Kim and Lee<sup>17</sup>, who

TABLE-1  
EFFECT OF AQUEOUS AMMONIA CONCENTRATION ON THE COMPOSITIONS IN SAA-TREATED *ACHNATHERUM SPLENDENS*<sup>A</sup>

Ammonia concentration (%)	Lignin (%)	Delignification (%)	Hemicellulose (%)	Cellulose (%)
5	11.78±0.34a	13.99±2.45a	20.76±0.35a	61.65±0.24a
10	10.79±0.16b	20.89±1.22b	19.93±0.42b	62.21±0.25b
15	10.73±0.18b	20.99±1.32b	19.67±0.38b	62.81±0.06c
20	10.68±0.17b	21.08±1.26b	19.59±0.10b	62.83±0.09c
25	10.55±0.10b	21.43±0.71b	19.40±0.15b	62.97±0.26c
F-value	18.17***	13.44***	8.97**	5.45*

<sup>a</sup>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.

TABLE-2  
EFFECT OF REACTION TEMPERATURE ON THE COMPOSITIONS IN SAA-TREATED *ACHNATHERUM SPLENDENS*<sup>a</sup>.

Temperature (°C)	Lignin (%)	Delignification (%)	Hemicellulose (%)	Cellulose (%)
45	10.67±0.04a	15.44±0.28a	20.41±0.70a	61.69±0.1a
55	9.69±0.17b	26.31±1.32b	20.37±0.25a	63.70±0.24b
65	9.66±0.11b	26.55±0.87b	20.21±0.22a	62.84±0.15c
75	10.33±0.20a	20.85±1.54c	18.81±0.16b	62.57±0.26ac
85	11.67±0.07c	10.56±0.55d	18.15±0.12b	60.08±0.50d
F-value	102.18***	120.66***	25.12***	63.64***

<sup>a</sup>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.

TABLE-3  
EFFECT OF REACTION TIME ON THE COMPOSITIONS IN SAA-TREATED *ACHNATHERUM SPLENDENS*<sup>a</sup>.

Time (h)	Lignin (%)	Delignification (%)	Hemicellulose (%)	Cellulose (%)
12	10.47±0.36a	17.59±2.81a	26.50±0.28a	57.63±0.63a
24	10.07±0.06ab	21.09±0.64a	22.67±0.26b	60.96±0.24b
36	9.78±0.17b	26.84±1.31b	22.66±0.13b	61.90±0.12b
48	9.73±0.11b	26.85±0.81b	20.85±0.09c	63.57±0.49c
60	9.69±0.08b	26.90±0.61b	20.74±0.12c	66.20±0.25d
72	9.61±0.05b	27.12±0.37b	20.61±0.09c	66.41±0.13d
F-value	9.46***	26.62***	427.31***	255.79***

<sup>a</sup>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.

observed 50-70 % of delignification as reaction temperature was increased from 40 to 90 °C.

Compared with the treatment of 45 °C, the lignin content of pretreated solids decreased significantly and the cellulose content increased significantly at the treatment of 55 °C. However, for the content of hemicellulose in pretreated solids, there was no significant difference among the treatments of 45, 55 and 65 °C. There was no significant difference between the treatments of 55 and 65 °C for the lignin content of pretreated solids. However, the content of cellulose in pretreated solids decreased significantly at the treatment of 65 °C compared with the treatment of 55 °C. Compared with the treatment of 55 °C, the content of lignin in pretreated solids increased significantly and the contents of hemicellulose and cellulose decreased significantly at the treatments of 75 and 85 °C (Table-2).

There was a significant increase in delignification at the treatment of 55 °C compared with the treatment of 45 °C. There was no significant difference between the treatments of 55 and 65 °C for delignification. Delignification decreased significantly at the treatments of 75 and 85 °C compared with the treatment of 55 °C. Treatment at 55 °C achieved 26.31% of delignification which was insignificantly different from the maximum delignification of 26.55 % achieved in the treatment at a higher reaction temperature of 65 °C (Table-2).

**Effect of reaction time:** Table-3 summarizes the composition changes with the increasing of reaction time. The pretreatment conditions were 12-72 h of reaction time, 15 wt % of ammonia, 55 °C and 12:1 (based on wt) of liquid-to-solid ratio. Increasing of reaction time resulted in significant decrease in lignin (p < 0.001) and hemicellulose (p < 0.001) and significant increase in cellulose (p < 0.001). The amount of lignin and hemicellulose in soaking in aqueous ammonia-Treated solids decreased from 10.47, 26.50 to 9.61, 20.61 %, respectively when reaction time was increased from 12 to 72 h

and the amount of cellulose increased from 57.63 to 66.41 %. An increase in reaction time significantly improved delignification (p < 0.001). When the reaction time was increased from 12 to 72h, delignification increased from 17.59 to 27.12 %.

Compared with the treatment of 12 h of reaction time, the contents of lignin and hemicellulose in pretreated solids decreased significantly and the content of cellulose increased significantly at the treatment of 36 h of reaction time. There was no significant difference between the treatments of 24 h and 36 h of reaction time for the lignin, hemicellulose and cellulose contents of pretreated solids. For the lignin content of pretreated solids, there was no significant difference among the treatments of 36, 48, 60 and 72 h of reaction time. Compared with the treatment of 36 h of reaction time, the content of hemicellulose in pretreated solids decreased significantly and the content of cellulose increased significantly at the treatments of 48, 60 and 72 h of reaction time. For the hemicellulose content of pretreated solids, there was no significant difference among the treatments of 48, 60 and 72 h of reaction time. For the content of cellulose in pretreated solids, there was no significant difference between the treatments of 60 and 72 h of reaction time (Table-3).

For the delignification, there was no significant difference between the treatments of 12 and 24 h of reaction time. Compared with the treatment of 36 h of reaction time, there were significant decreases in delignification at the treatments of 12 and 24 h of reaction time. However, there was no significant difference among the treatments of 36, 48, 60 and 72 h of reaction time for the delignification (Table-3). It indicated that, the reaction time had no significant effects on the delignification when longer than 36 h in aqueous ammonia pretreatment (Table-3).

On the basis of the collective experimental data of delignification, we determined the optimum operating conditions for

the soaking in aqueous ammonia to be: 10 wt % of ammonia, 55 °C, 36 h of reaction time and 12:1 (based on wt) of liquid-to-solid ratio. We did a complementary experiment using the combination of the optimum operating condition and found delignification was 26.58 %. The delignification in the soaking in aqueous ammonia under the optimal conditions is much lower than that of NaOH pretreatment (39 at 15 % of NaOH loading, 10:1 of liquid-to-solid ratio, 90 °C and 90 min). The delignification in the soaking in aqueous ammonia under the optimal condition was also much lower than those obtained by Kim *et al.*<sup>16</sup>, who observed 66 % of delignification at 75 °C for 48 h of reaction time with 15 wt % of ammonia. The lower delignification level of the aqueous ammonia pretreatment may be attributed to the nature of *Achnatherum splendens*.

### Conclusion

It was found that ammonia concentration, reaction temperature and reaction time all had significant effects on delignification. These factors also had significant effects on the lignin, hemicellulose and cellulose contents of soaking in aqueous ammonia-treated solids. Optimum conditions were obtained as follows: 10 wt % of ammonia, 55 °C, 36 h of reaction time and 12:1 (based on wt) of liquid-to-solid ratio. Under these conditions, delignification reached to 26.58 % and the content of cellulose in pretreated solids increased to 64.20 % while the content of hemicellulose decreased to 23.63 %. In addition, this study can serve as a step towards the optimization of pretreatment of *Achnatherum splendens*. Nevertheless, 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.

### ACKNOWLEDGEMENTS

This research was supported by Program for Chongjiang Scholars and Innovative Research Team in University (IRT0966) and by the National Natural Science Foundation (No. 30970490).

### REFERENCES

1. V.S. Chang and M.T. Holtzapfle, *Appl. Biochem. Biotechnol.* **84**, 37 (2000).
2. C.A. Mooney, S.D. Mansfield, M.G. Touhy and J.N. Saddler, *Bioresour. Technol.* **64**, 113 (1998).
3. C. Martin, M. Galbe, N.O. Nilvebrant and L.J. Jonsson, *Appl. Biochem. Biotechnol.*, **98**, 716 (2002).
4. B. Palmarola-Adrados, M. Galbe and G. Zacchi, *J. Chem. Technol. Biotechnol.*, **80**, 85 (2005).
5. L.S. Yan, H.M. Zhang, J.W. Chen, Z.X. Lin, Q. Jin and H.H. Jia, *Bioresour. Technol.*, **100**, 1803 (2009).
6. G.L. Guo, W.H. Chen, W.H. Chen, L.C. Men and W.S. Hwang, *Bioresour. Technol.*, **99**, 6046 (2008).
7. X.B. Zhao, F. Peng, K.K. Cheng and D.H. Liu, *Enzym. Microb. Technol.*, **44**, 17 (2009).
8. R.A. Silverstein, Y. Chen, R.R. Sharma-Shivappa, M. Boyette and J.A. Osborne, *Bioresour. Technol.*, **98**, 3000 (2007).
9. C. Martín, H.B. Klinké and A.B. Thomsen, *Enzym. Microb. Technol.*, **40**, 426 (2007).
10. C. Martin and A.B. Thomsen, *J. Chem. Technol. Biotechnol.*, **82**, 174 (2007).
11. T.A. Hsu, in ed.: C.E. Wyman, Pretreatment of Biomass, Handbook on Bioethanol Production and Utilization, Applied Energy Technology Series, Taylor & Francis, Washington DC, p. 179 (1996).
12. T.H. Kim and Y.Y. Lee, *Bioresour. Technol.*, **96**, 2007 (2005).
13. T.H. Kim, J.S. Kim, C. Sunwoo and Y.Y. Lee, *Bioresour. Technol.*, **90**, 39 (2003).
14. P.V. Iyer, Z. Wu, S.B. Kim and Y.Y. Lee, *Appl. Biochem. Biotechnol.*, **57**, 121 (1996).
15. S.B. Kim and Y.Y. Lee, *Appl. Biochem. Biotechnol.*, **57**, 47 (1996).
16. T.H. Kim, F. Taylor and K.B. Hicks, *Bioresour. Technol.*, **99**, 5694 (2008).
17. T.H. Kim and Y.Y. Lee, *Appl. Biochem. Biotechnol.*, **136**, 81(2007).
18. T.H. Kim and Y.Y. Lee, *Appl. Biochem. Biotechnol.*, **124**, 1119 (2005).
19. Z. Xu, Q.H. Wang, Z.H. Jiang, X.X. Yang and Y.Z. Ji, *Biomass Bioener.*, **31**, 162 (2007).
20. H.K. Goering and P.J. Vansoest, Forage Fiber Analysis, Agriculture Handbook. Agricultural Research Services, United States Department of Agriculture, No. 379 (1970).