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Effects of Liquid-to-Solid Ratio and Reaction Time on Dilute Sulfuric Acid Pretreatment of *Achnatherum splendens*

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In sulfuric acid pretreatment of *Achnatherum splendens*, the ground biomass was pretreated by 0.5-3 % (w/v) sulfuric acid with 8:1-20:1 (v/w) of liquid-to-solid ratio at 100 °C for 1-4 h. Increasing sulfuric acid concentration, liquid-to-solid ratio and reaction time had significant effects on hemicellulose solubilization. Increasing the sulfuric acid concentration, liquid-to-solid ratio and reaction time caused significant decrease in hemicellulose and significant increase in formation of cellulose and lignin. The optimum treatment conditions of sulfuric acid pretreatment for *Achnatherum splendens* were 2 % (w/v) sulfuric acid, 15:1(v/w) of liquid-to-solid ratio, 3 h of reaction time and temperature 100 °C. Under these conditions, 92.57 % hemicellulose was solubilized and the content of cellulose in pretreated solids increased to 66.79 %. This study can serve as a step towards the optimization of pretreatment of *Achnatherum splendens*.

Keywords: Sulfuric acid pretreatment, Hemicellulose solubilization, Hemicellulose, Cellulose.

INTRODUCTION

Lignocellulosic materials are attractive feedstock for fuel ethanol production because they are abundant, low-cost and renewable. Pretreatment has been studied as a key step for the effective utilization of lignocellulosic biomass feedstock, due to its recalcitrant nature. Several processes have been developed for pretreatment of lignocellulosic biomass, including steam explosion[1], acid pretreatment[2-6], alkali pretreatment[7,8] and wet oxidation[9,10]. Acid pretreatment involves the use of sulfuric acid[11,12], nitric acid, hydrochloric acid[13] and phosphoric acid[14] to remove hemicellulose components and expose cellulose for enzymatic digestion[15]. The main reaction that occurs during acid pretreatment is the hydrolysis of hemicellulose, especially xylan as glucomannan is relatively stable. Agricultural residues such as corncobs and stovers have been found to be particularly well suited to dilute acid pretreatment[16]. Dilute acid pretreatment of lignocellulosic biomass is one of the most effective pretreatment methods[17,18]. When crofton weed stem was treated by 1 % (w/v) of sulfuric acid concentration with 8:1 (v/w) of liquid-to-solid ratio at 120 °C for 120 min, approximately 46 % of the raw material was dissolved in the pretreatment; YRSAH was 32.89 %; and the cellulose content was increased to 61.14 %[19]. Silverstein *et al.*[8] showed that sulfuric acid pretreatment resulted in a xylan reduction of 95.23 % for 2 % acid, 90 min, 121 °C/15 psi.

Achnatherum splendens 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 sulfuric acid pretreatment of *Achnatherum splendens*. The effects of reaction parameters on the composition were investigated. The reaction parameters of interest were sulfuric acid concentration, liquid-to-solid ratio and reaction time.

EXPERIMENTAL

Achnatherum splendens used in the study was obtained from Zhangye city in Gansu province in Northwestern 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[20].

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 was determined by ANOVA in STATISTICA.

Sulfuric acid at concentrations of 0.5-3 % (w/v) were used to pretreat 20 g ground biomass at liquid-to-solid ratio of 8:1-20:1 (v/w). Treatments were performed in triplicate at 100 °C for residence times of 1-4 h in a 1000 mL glass flask immersed in a water bath. After sulfuric acid pretreatment, the solids were separated by filtering, 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 sulfuric acid concentration: The effects of sulfuric acid concentration in sulfuric acid pretreatment were investigated. The results are summarized in Table-1. The pretreatment conditions were 0.5-3 % (w/v) sulfuric acid, 10:1 (v/w) of liquid-to-solid ratio, 2 h of reaction time and 100 °C. The major compositional changes occurred in hemicellulose. The hemicellulose content of the sulfuric acid pretreated samples decreased from 25.37 to 6.46 % when sulfuric acid concentration was increased from 0.5 to 3 % and the yield of cellulose and lignin increased from 52.40, 11.45 to 64.53, 16.91 %, respectively. Changes in sulfuric acid concentration caused significant decrease in hemicellulose ($p < 0.001$) and significant increase in cellulose ($p < 0.001$) and lignin ($p < 0.001$). Hemicellulose solubilization was significantly affected by sulfuric acid concentration ($p < 0.001$). Treatment with 0.5 % sulfuric acid achieved only 31.82 % of hemicellulose solubilization. It indicated that 0.5 % sulfuric acid was too low to affect hemicellulose solubilization for treatment time up to 2 h and temperature up to 100 °C. When sulfuric acid concentration was increased to 3 %, the hemicellulose solubilization increased to 85.89 %.

Compared with the treatment of 2 % sulfuric acid, the hemicellulose content of pretreated solids increased significantly and the cellulose and lignin contents decreased significantly at the treatments of 0.5, 1 and 1.5 % sulfuric acid. There was no significant difference between the treatments of 2 % and 2.5 % sulfuric acid for the content of hemicellulose in pretreated solids. There was no significant difference among the treatments of 2, 2.5 and 3 % sulfuric acid for cellulose content of pretreated solids. There was a significant increase in the content of lignin in pretreated solids at the treatments of 2.5 and 3 % sulfuric acid compared with treatment of 2 % sulfuric acid. However, there was no significant difference between the treatments of 2.5 and 3 % sulfuric acid for lignin content of pretreated solids (Table-1).

Compared with the treatment of 2 % sulfuric acid, there were significant decreases in hemicellulose solubilization at the treatments of 0.5, 1 and 1.5 % sulfuric acid. There was no significant difference between the treatments of 2 and 2.5 % sulfuric acid for hemicellulose solubilization. However, there was a significant increase in hemicellulose solubilization at the treatment of 3 % sulfuric acid compared with the treatment of 2 % sulfuric acid (Table-1). The overall view was that 2 % sulfuric acid is near the optimum level as it represented the relative low sulfuric acid concentration that can give a satisfactory pretreatment effect (78.87 % of hemicellulose solubilization).

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 sulfuric acid concentration at 2 %, the reaction temperature at 100 °C and the reaction time for 2 h. The composition data after the treatment are summarized in Table-2. The yield of hemicellulose ($p < 0.001$), cellulose ($p < 0.001$) and lignin ($p < 0.001$) in sulfuric acid pretreated samples was significantly influenced by liquid-to-solid ratio. The hemicellulose content of the sulfuric acid pretreated samples decreased from 10.67 to 6.67 % when the liquid-to-solid ratio was increased from 8:1 to 20:1 and the cellulose and lignin contents increased from 60.71, 14.68 to 64.18, 16.30 %, respectively. The effect of liquid-to-solid ratio on hemicellulose solubilization was significant ($p < 0.001$). The hemicellulose solubilization increased from 75.36 to 84.99 % when the liquid-to-solid ratio was increased from 8:1 to 20:1. The reason maybe that higher liquid-to-solid ratio led to higher sulfuric acid loading when sulfuric acid concentration was fixed at 2 % which further increased the hemicellulose solubilization.

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

TABEL-1
EFFECT OF SULFURIC ACID CONCENTRATION ON THE COMPOSITIONS IN SULFURIC ACID TREATED *Achnatherum splendens*

Sulfuric acid concentration (%)	Hemicellulose (%)	Hemicellulose solubilization (%)	Cellulose (%)	Lignin (%)
0.5	25.37 ± 0.41a	31.82 ± 1.09a	52.40 ± 0.56a	11.45 ± 0.19a
1.0	19.09 ± 0.56b	51.71 ± 1.42b	56.12 ± 0.44b	12.72 ± 0.08b
1.5	16.41 ± 0.48c	59.97 ± 1.17c	58.26 ± 0.62c	13.60 ± 0.14c
2.0	9.36 ± 0.08d	78.87 ± 0.19d	63.58 ± 0.38d	15.48 ± 0.31d
2.5	8.65 ± 0.31d	80.64 ± 0.07d	64.49 ± 0.15d	16.37 ± 0.15e
3.0	6.46 ± 0.31e	85.89 ± 0.67e	64.53 ± 0.37d	16.91 ± 0.40e
F-value	1058.27***	1409.22***	388.93***	248.69***

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 LIQUID-TO-SOLID RATIO ON THE COMPOSITIONS IN SULFURIC ACID TREATED *Achnatherum splendens*

Liquid-to-solid (v/w)	Hemicellulose (%)	Hemicellulose solubilization (%)	Cellulose (%)	Lignin (%)
8:1	10.67 ± 0.18a	75.36 ± 0.42a	60.71 ± 0.35a	14.68 ± 0.52a
10:1	9.87 ± 0.11b	77.00 ± 0.26b	63.31 ± 0.15b	15.32 ± 0.18ab
12:1	9.53 ± 0.37b	77.80 ± 0.86b	63.64 ± 0.15bc	15.34 ± 0.11ab
15:1	7.10 ± 0.23c	84.11 ± 0.51c	63.73 ± 0.23bc	15.71 ± 0.05bc
20:1	6.67 ± 0.17c	84.99 ± 0.38c	64.18 ± 0.21c	16.30 ± 0.36c
F-value	179.98***	208.28***	109.35***	11.97***

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

Compared with the treatment of 15:1 of liquid-to-solid ratio, hemicellulose solubilization decreased significantly at the treatments of 8:1, 10:1 and 12:1 of liquid-to-solid ratio. However, there was no significant difference between the treatments of 15:1 and 20:1 of liquid-to-solid ratio for hemicellulose solubilization (Table-2). It indicated that the liquid-to-solid ratio had no significant effects on hemicellulose solubilization when higher than 15:1 (v/w).

Effect of reaction time: Table-3 summarized the composition changes with the increasing reaction time. The pretreatment conditions were 1-4 h of reaction time, 2 % sulfuric acid, 15:1 (v/w) of liquid-to-solid and temperature 100 °C. Increasing of reaction time resulted in significant decrease in hemicellulose ($p < 0.001$) and significant increase in cellulose ($p < 0.001$) and lignin ($p < 0.001$). The hemicellulose content of the sulfuric acid pretreated samples decreased from15.52 to 3.01 % when reaction time was increased from 1 to 4 h and the contents of cellulose and lignin increased from 59.63, 12.76 to, 67.65, 17.61 %, respectively. Hemicellulose solubilization was in the range of 63.17-93.87 % with 1 to 4 h of reaction time. An increase in reaction time significantly improved hemicellulose solubilization ($p < 0.001$). Treatment with 1 h of the reaction time achieved only 63.17 % of hemicellulose solubilization. It indicated that 1h was too short to affect hemicellulose solubilization for sulfuric acid concentration up to 2 % and temperature up to 100 °C.

Compared with the treatment of 1 h of reaction time there was a significant decrease in hemicellulose content of pretreated solids at the treatment of 2 h of reaction time. There was no significant difference among the treatments of 2, 3 and 4 h of reaction time for the content of hemicellulose in pretreated solids. Compared with the treatment of 3 h of reaction time, the cellulose and lignin contents of pretreated solids decreased significantly at the treatments of 1 and 2 h of reaction time. There was a significant increase in cellulose and lignin contents of pretreated solids at the treatment of 4 h of reaction time compared with the treatment of 3 h of reaction time (Table-3).

Compared with the treatment of 3 h of reaction time, there were significant decreases in hemicellulose solubilization at the treatments of 1 and 2 h of reaction time. However, there was no significant difference between the treatments of 3 and 4 h of reaction time for hemicellulose solubilization (Table-3). It indicated that reaction time had no significant effects on hemicellulose solubilization over 3 h.

On the basis of the collective experimental data of hemicellulose solubilization, the optimum operating condition of the sulfuric acid pretreatment was determined to be: 2 % (w/v) sulfuric acid, 15:1 (v/w) of liquid-to-solid, 3 h of reaction time and 100 °C. Under the optimal conditions, 92.57 % hemicellulose was removed. Results from this study are comparable to those obtained by Silverstein *et al.*[8]. They observed 95.2 % solubilization of xylan in cotton stalks at 121 °C/15 psi for 90 min with 2 % (w/v) H_2SO_4.

Conclusion

The major effect of sulfuric acid pretreatment was hemicellulose solubilization. It was showed that sulfuric acid concentration, liquid-to-solid ratio and reaction time all had significant effects on hemicellulose solubilization. These factors also had significant effects on the contents of lignin, hemicellulose and cellulose in the solids after sulfuric acid pretreatment. The optimum treatment conditions of sulfuric acid pretreatment for *Achnatherum splendens* were 2 % (w/v) sulfuric acid, 15:1 (v/w) of liquid-to-solid ratio, 3 h of reaction time and temperautre 100 °C. Under these conditions, 92.57 % hemicellulose was solubilized and the content of cellulose increased to 66.79 %.

Sulfuric acid pretreatment was a pretreatment method suitable for *Achnatherum splendens* which could give a satisfactory pretreatment effect (92.57 % of hemicellulose solubilization) under optimum conditions. 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.

TABLE-3
EFFECT OF REACTION TIME ON THE COMPOSITIONS IN SULFURIC ACID TREATED *Achnatherum splendens*

Time (h)	Hemicellulose (%)	Hemicellulose solubilization (%)	Cellulose (%)	Lignin (%)
1	15.52 ± 0.29a	63.17 ± 0.68a	59.63 ± 0.29a	12.76 ± 0.11a
2	7.17 ± 0.29c	84.50 ± 0.63b	63.95 ± 0.19b	15.66 ± 0.24b
3	3.58 ± 0.11c	92.57 ± 0.22c	66.79 ± 0.31c	16.69 ± 0.40c
4	3.01 ± 0.34c	93.87 ± 0.70c	67.65 ± 0.28d	17.61 ± 0.10d
F-value	1338.77***	1716.87***	531.39***	151.88***

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

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