Dilute Sulfuric Acid Hydrolysis of *Pennisetum* (sp.) Hemicellulose

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Dilute sulfuric acid hydrolysis of *Pennisetum* (sp.) hemicellulose was investigated in this work. The hemicellulose was obtained by ethanol precipitation of hydrolysate produced *via* the microwave-assisted H_2O_2-NaOH extraction from the *Pennisetum* (sp.). Acid hydrolysis was performed by varying the process parameters, including the sulfuric acid concentration, hydrolysis temperature, solid to liquor ratio, and the reaction time. The xylose yield was selected as the target of process optimization and the orthogonal experiment of L9 (3^4) was designed to optimize the process conditions. The highest xylose yield of 86.5% could be obtained under the conditions of an acid concentration of 1%, the hydrolysis temperature of 105 °C, a solid to liquor ratio of 1:15, and a reaction time of 4 h. Fourier transform infrared spectroscopy (FTIR) analysis confirmed that most of the hemicellulose had been depolymerized into xylose.

Keywords: Hemicellulose; Dilute sulfuric acid; Hydrolysis; Xylose

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INTRODUCTION

Hemicelluloses, also termed as heteropolysaccharides, are non-cellulosic polysaccharides consisting of D-xylose, D-mannose, L-arabinose, D-glucose, D-galactose, and 4-O-methyl-D-glucuronic acid residues, which are arranged in different proportions and with different substituents (Sun and Tomkinson 2003). Converting hemicellulose into xylose is essential for value-added product production, such as xylitol. Many methods have been developed for this purpose, such as steam explosion, dilute acid hydrolysis, alkaline treatment, enzymatic hydrolysis, and γ-valerolactone (GVL) (Yang *et al.* 2013; Vivekanand *et al.* 2013; Luterbacher *et al.* 2014). Among these, dilute acid hydrolysis showed promising application because of its low cost and high efficiency. Hu *et al*. (2010) employed the dilute sulfuric acid at atmospheric pressure to hydrolyze wood and produce maple sugar. Zu *et al*. (2014) used dilute hydrochloric acid to maximize the xylose yield from corn stover for sugar production. Tang *et al*. (2011) used dilute acid to hydrolyze hemicellulose from *Eulaliopsis binata*. Fractionation is the prerequisite process for the holistic utilization of biomass as lignin. The hemicellulose and cellulose of biomass have different properties, and thus alter the end-use applications. In the past, technologies have been developed and evaluated to separate hemicellulose from biomass, such as steam/hot water treatments, organosolv processes, as well as chemical treatments (Sindhu *et al.*

2011; Koo *et al*. 2011; Martin-Sampedro *et al*. 2014; Chandra *et al*. 2016). Alkaline peroxide has shown promising results to separate the hemicellulose, lignin, and cellulose into three different streams (Sun *et al*. 2001). Additionally, peroxide, as an environmentally friendly chemical, has been used in the pulp bleaching stage since the 1940s (Sun and Tomkinson 2003). In contrast, microwave irradiation would favor the composition fractionation of biomass due to the high-speed oscillation. Therefore, the proposed method is effective in the fractionation of hemicellulose from biomass. In fact, microwave-assisted H_2O_2-NaOH extraction has been established as a technique to separate hemicellulose from biomass (Jiang *et al.* 2016).

Because of the depletion of fossil fuels, biomass has attracted a great amount of attention for the production of value-added products (Dodds and Gross 2007). In this regard, the utilization of biomass to produce a broad range of value-added products, such as bio- materials, bio-fuels, and bio-chemicals, has been regarded as a promising strategy for its renewable and abundant (Zhang *et al.* 2014) availability.

Pennisetum (sp.) belongs to the classification of angiosperms monocotyledoneae grasses, and is a perennial plant with an average height of 3 to 5 m, and a maximum height of 8 m. The grasses are drought and cold resistant and can grow in poor climate conditions, like the desert. This biomass has carbohydrate contents that would make it a suitable candidate for bio-material production. To make the sustainable plantation of this species, the exploration of value-added product production from *Pennisetum* (sp.) is needed.

In the past, the production of hemicellulose from the *Pennisetum* species has not been studied in detail. In this study, hemicelluloses were extracted from this species *via* a microwave-assisted H_2O_2-NaOH process. The objective of this study is to investigate the influence of the process parameters, such as the sulfuric acid concentration, hydrolysis temperature, solid to liquor ratio, and reaction time on the dilute acid hydrolysis of the extracted hemicelluloses. The xylose yield was selected as the target. The orthogonal experiment was designed for the parameter optimization. Fourier transform infrared spectroscopy (FTIR) was used to analyze the composition changes.

EXPERIMENTAL

Materials

Pennisetum (sp.) was collected from Xintai City, of the Shandong province in China. The chemical compositions (on mass basis) of the raw materials were analyzed for cellulose according to TAPPI T429 cm-84 (1984), hemicellulose according to TAPPI T223 cm-84 (1984), Klason lignin according to TAPPI T222 om-88 (1988), and for ash according to TAPPI T211 om-93 (1993). Sulfuric acid was purchased from a chemical company (Calvin, Jinan, China). Deionized water was used for all the experiments.

Methods

Hemicellulose production and extraction

Hemicellulose was extracted from the raw material *via* a microwave-assisted H_2O_2-NaOH process and dissolved in spent liquor. The extraction process was conducted in a water bath using a three-neck boiling flask with a condenser and electrical stirrer. First, 10 g of screened grass (40- to 60-mesh) was added to the flask. Then, 250 mL of H_2O_2-NaOH solution (0.5 wt.% of H_2O_2 and 4 wt.% of NaOH) by dissolving chemicals into deionized water was added into the flask and mixed well with the grass. After that, the flask was heated in a water bath heater at 50 °C for 30 min. Next, the flask was treated with microwaves in a microwave generator (YUHUA Instrument MCR-3, Gongyi City, China) for 4 h. Once the reaction was complete, the hydrolysis liquor was recovered through vacuum filtration and then concentrated. Finally, the hydrolysis liquor was mixed with ethanol (3-fold) to precipitate the dissolved hemicellulose *via* centrifuging at 8000 rpm for 10 min and was then vacuum dried. Based on the raw material, a hemicellulose yield of 84.7% was obtained.

Dilute sulfuric acid hydrolysis

The extracted hemicellulose was treated with varied concentrations of dilute sulfuric acid in a 200-mL high-pressure stainless steel vessel with a Teflon liner. The vessel was maintained in an oil bath for temperature control. The process conditions of these reactions were listed in Table 1.

Table 1. Process Conditions

Parameters	Process Conditions				
Sulfuric acid Concentration (%)	0.5	1	2	3	4
Temperature (°C)	90	100	110	120	125
Solid to Liquor ratio	1:5	1:10	1:15	1:20	1:25
Time (h)	1	2	3	4	5

In this set of experiments, 5 g of hemicellulose was placed in high-pressure stainless steel vessels, and then the required amounts of acid and water were added to the system. Next, the vessel was sealed and immersed in a pre-heated oil bath to treatment under the desired conditions. Once the reaction was completed, the vessel was removed from the oil bath and cooled with cold tap water. Lastly, the mono-sugars were separated from the hydrolysate *via* vacuum filtration.

Determination of xylose yield

The concentration of xylose in the hydrolysate of the acid hydrolysis treatment was determined using an ion chromatography (IC) unit (Thermo ICS-5000, Waltham, MA, USA) equipped with a CarboPacTMPA1 column (Dionex-300, Dionex Corporation, Canada) and a pulsed amperometric detector (PAD). The xylose yield was calculated according to Eq. 1,

$$Y = (C \times V \times 100) / m \qquad (1)$$

where Y is the xylose yield (%), C is the xylose concentration (g/L), V is the volume of the hydrolysate (L), and m is the weight of the hemicellulose (g).

Additionally, the other mono-sugars were also determined by IC unit.

High Performance Liquid Chromatography (HPLC) analysis

The degraded byproducts of hemicellulose such as formic acid, acetic acid, furfural, and hydroxymethylfurfural (HMF) were analyzed by HPLC equipped with ZORBAXSB-C18 (4.6×150 mm) column at 25 °C with 0.01 mol/L H_3PO_4 (v/v) as eluent at 1.0 mL/min. The injection volume was 20 µL.

FTIR analysis

The FTIR analysis of the hemicellulose and hydrolyzed residue was performed in a Vertex 70 (Bruker, Ettlingen, Germany) spectrophotometer. Each spectrum was recorded in a frequency range of 500 to 4000 cm^{-1} using a potassium bromide (KBr) disc. The KBr was previously oven-dried to reduce the interference of water.

RESULTS AND DISCUSSION

The biomass had a composition of 44.0 wt.% cellulose, 25.8 wt.% hemicellulose, 15.8 wt.% Klason lignin, and 4.3 wt.% ash. In the hydrolysate, produced from the dilute sulfuric acid hydrolysis of hemicellulose, xylose was the most abundant monomeric sugar. Therefore, the xylose yield was selected as the target of process optimization.

Effect of Process Parameters on Xylose Yield

The effect of the process parameters on the xylose yield are shown in Fig. 1.

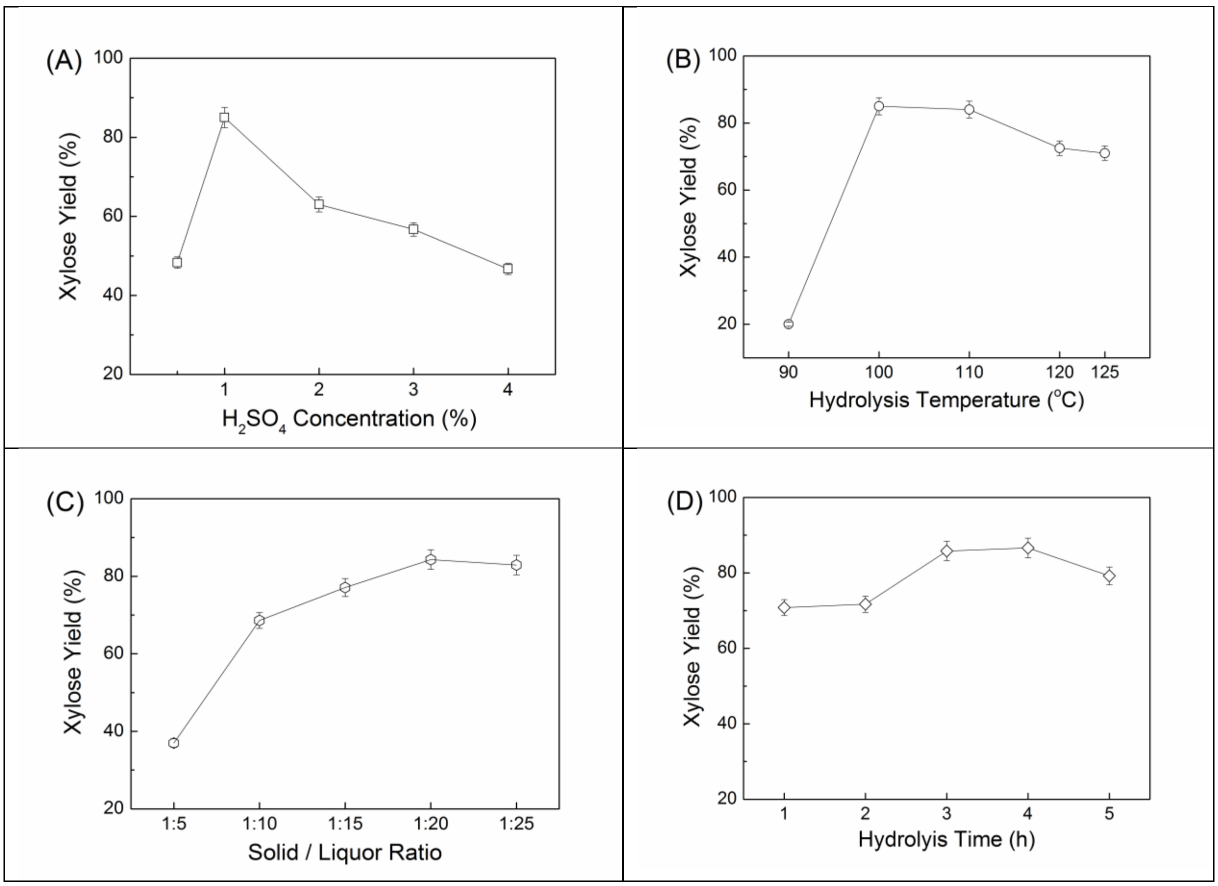

Fig. 1. Effect of process parameters on xylose yield in the dilute sulfuric acid hydrolysis; (A) effect of H_2SO_4 concentration, other conditions as solid to liquor ratio 1:20, temperature 110 °C, and 3 h time, (B) effect of hydrolysis temperature, other conditions as H_2SO_4 concentration 1%, solid to liquor ratio 1:20, and 3 h time, (C) effect of solid to liquor ratio, other conditions as H_2SO_4 concentration 1%, temperature 110 °C, and 3 h time, and (D) effect of hydrolysis time length, other conditions as H_2SO_4 concentration 1%, solid to liquor ratio 1:20, and temperature 110 °C

As can be seen from Fig. 1(A), the xylose yield in the hydrolysate liquor increased at low sulfuric acid concentrations, and then it decreased sharply when the acid concentration increased to 2%. This phenomenon could be attributed to the xylose's degradation to furfural at high sulfuric acid concentrations (Hu *et al.* 2010).

Additionally, the hydrolysis temperature had a noticeable effect on the xylose yield, as shown in Fig. 1(B). The xylose yield was only 20% at 90 °C; however, it greatly increased to approximately 4 times the original yield when the temperature was increased to 100 °C. In the past, the xylose yield was also increased as a function of the temperature (Hu *et al.* 2010). This could be explained by the energy barrier effect. When the temperature was increased beyond 120 °C, the xylose yield decreased minimally, which was due to the degradation of xylose (Liu *et al*. 2008).

Figure 1(C) shows the effect of the solid to liquor ratio on the xylose yield. The xylose yield increased gradually as the ratio increased, and then it leveled off beyond 1:20. This is explained by the mass transfer effect, *i.e.*, a higher solid to liquor ratio would favor the reaction.

Figure 1(D) shows the effect of the hydrolysis time on the xylose yield. The figure shows that a longer time of 3 h to 4 h was favorable for the xylose yield. Shorter or longer times decreased the xylose yield to a certain extent.

These observations were in agreement with other reports. Xu and Hanna (2010) also observed that the lower hydrolysis temperature and the longer duration of time was beneficial for xylose production when studying the dilute acid hydrolysis of corn dried distillers grains (Xu and Hanna 2010). Hu *et al.* (2010) concluded that 105 °C and 180 min were suitable conditions for the xylose liberation when studying the dilute sulfuric acid hydrolysis of sugar maple wood extract (Hu *et al*. 2010).

Orthogonal Experiment

Table 2. L9 (3^4) Orthogonal Experiment

Test ID	Process Parameters				Xylose Yield (%)
	H_2SO_4 (%)	Temp. (°C)	S/L	Time (h)	
1	0.5	95	1:15	2	18.5 ± 0.4
2	0.5	100	1:20	3	42.0 ± 0.3
3	0.5	105	1:25	4	68.1 ± 0.3
4	1	95	1:25	3	30.2 ± 0.8
5	1	100	1:15	4	84.9 ± 0.2
6	1	105	1:15	3	85.5 ± 0.2
7	1.5	95	1:20	4	49.5 ± 0.2
8	1.5	100	1:25	2	51.8 ± 0.2
9	1.5	105	1:20	2	69.3 ± 0.7
Average 1	43.2	31.9	62.3	45.8	
Average 2	61.8	59.9	51.3	53.3	
Average 3	59.7	72.9	51.1	65.6	
Deviation between Averages	18.6	41.0	11.2	19.8	
Best Combination	A2	B3	C1	D3	

The xylose yield was selected as the target of process optimization, and an L9 (3^4) orthogonal experiment was designed to evaluate and optimize the process parameters. As can be seen in Table 2, the process parameters had a substantial effect on the xylose yield, which varied from 18.5% to 85.5%. Through analyzing the deviation between the average values, it was found that the temperature had the highest amount of influence on the xylose yield, which increased sharply when the hydrolysis temperature was elevated. The hydrolysis time and sulfuric acid concentration similarly affected the yield. When the other conditions were held constant, increasing the sulfuric acid concentration would decrease the xylose yield, whereas, prolonging the hydrolysis time led to an increased xylose yield. The influence of the solid to liquor ratio was smallest in the selected experiment scopes. Therefore, a rather low solid to liquor ratio was suggested from both the economic and effectiveness of point.

Based on the optimized conditions the sulfuric acid hydrolysis of hemicellulose was reproduced, and the highest xylose yield of 86.5% was obtained.

Composition of Hydrolyzed Hemicellulose Liquor

The composition of dilute sulfuric acid hydrolyzed hemicellulose liquor was comprehensively analyzed by IC and HPLC, and results are listed in Table 3. As shown, xylose was the major mono-sugar in the liquor, followed by arabinose and glucose. The overall mono-sugar was 96.4% based on the hemicellulose weight. On the other hand, the sum of degraded byproducts such as formic acid, acetic acid, furfural, and HMF was only 1.5%, which indicated that degradation reaction was negligible under the optimized hydrolysis conditions.

Table 3. Composition of Sulfuric Acid Hydrolyzed Hemicellulose Liquor under Optimized Conditions (acid concentration = 1%, hydrolysis temperature = 105 °C, solid to liquor ratio = 1:15, and reaction time = 4 h)

Mono-sugars	Composition	Xylose	Arabinose	Glucose	Rhamnose	Galactose
	Content (%)	86.5	7.48	1.35	0.89	0.14
Degraded byproducts	Composition	Formic acid	Acetic acid	Furfural	HMF	
	Content (%)	0.74	0.71	0.05	0.03	

FTIR Analysis

The FTIR spectra of hemicellulose after the ethanol precipitation of hemicellulose derivatives (*e.g.*, mainly xylose) and dilute acid hydrolysis are shown in Fig. 2. As is apparent in spectrum (a), the absorptions at 3241, 2923, 1424, 1044, and 897 cm^{-1} are indicative of the native hemicelluloses (Sun and Tomkinson 2005; Peng *et al*. 2011). The broadband at 3241 cm^{-1} was assigned to the hydroxyl group (O-H), and the peak at 2923 cm^{-1} indicated C-H stretching in the CH_2 and CH_3 groups. The adsorption at 1636 cm^{-1} was due to the bending of the absorbed water. A sharp band at 897 cm^{-1} was assigned to the β-glucosidic linkages between the sugar units. In spectrum (b), the typical hemicellulose adsorption bands at 1424, 1044, and 897 cm^{-1} had disappeared or weakened, which indicated that the hemicellulose depolymerisation reaction happened thoroughly.

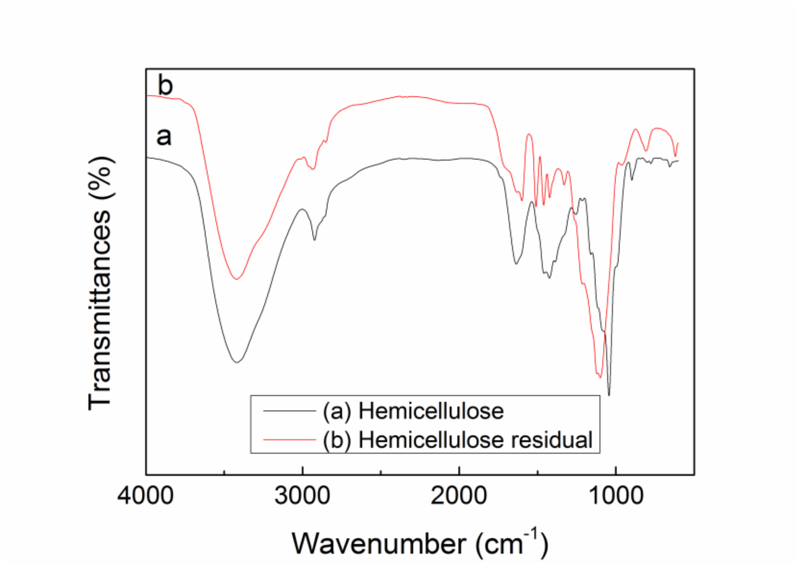

Fig. 2. FTIR spectra of hemicellulose and hemicellulose derivatives after dilute sulfuric acid hydrolysis; (a) hemicellulose, (b) hemicellulose residue hydrolyzed under condition of acid concentration of 1%, hydrolysis temperature of 105 °C, solid to liquor ratio of 1:15, and reaction time of 4 h

CONCLUSIONS

1. Dilute sulfuric acid hydrolysis is a promising method to convert *Pennisetum* (sp.) hemicellulose to xylose. The higher temperature in the studied scopes contributed to a higher xylose yield. The optimized conditions for the hydrolysis was an acid concentration of 1%, hydrolysis temperature of 105 °C, solid to liquor ratio of 1:15, and a reaction time of 4 h. The highest xylose yield obtained was 86.5%.
2. The FTIR analysis confirmed the structure of hemicellulose that was produced *via* microwave-assisted H_2O_2-NaOH extraction and ethanol precipitation. The FTIR analysis also confirmed the depolymerisation of hemicelluloses with the dilute sulfuric acid hydrolysis process.

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