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Hydrolysis of hemicellulose to produce fermentable monosaccharides by plasma acid

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ABSTRACT

In this paper, plasma acid was obtained by treating distilled water with dielectric barrier discharge to hydrolyze hemicellulose. The orthogonal experiment $L_{25}(5^6)$ was used to optimize such hydrolysis conditions. The total reducing sugar (TRS) was measured by the DNS method. To determine whether the oligosaccharide existed in the hydrolysis products, it was hydrolyzed by sulfuric acid for a second time following the same procedure as reported earlier. The monosaccharide compositions of the hydrolyzed sample were analyzed by high-performance liquid chromatography (HPLC) and Fourier transformed infrared spectroscopy (FTIR). The results showed that pH 2.81 of plasma acid, 100 °C and 50 min were assigned as an optimal hydrolysis condition by plasma acid. Under this condition, the hemicellulose was hydrolyzed completely to produce monosaccharides including xylose, glucose, and galactose with the mole ratio being 17:3:1. The yields of xylose, glucose, and galactose were 38.67%, 9.28% and 3.09%, respectively. Compared with the hemicellulose hydrolysis results by sulfuric acid, it is concluded that plasma acid is an environmental-friendly and efficient method to explore and hydrolyze the hemicellulose existed in biomass.

1. Introduction

Plant biomass is easily accessible from various industries including agriculture, forestry and livestock. They mainly consist of cellulose, hemicellulose and lignin. The corn stalk, for instance, contains 35% hemicellulose. Hemicellulose differs from cellulose. They comprise a group of polysaccharides composed of a combination of 5- and 6-carbon ring sugars. Though the ratio of hemicellulose varies depending on the type of plant biomass, in many cases, xylan accounts for the main ingredient of hemicellulose in poaceae plants and hard woods.

Xylan can be converted to xylose and further to xylitol (Nigam & Singh, 1995; Roberto, Felipe, & Lacis, 1991) which can be applied in food industry, teeth protecting and medical fields (Roberto, Mussatto, & Rodrigues, 2003). To hydrolyze the xylan, a few methods are used currently. The principal hydrolysis methods are mainly focused on steam explosion method, dilute acid method and alkali method etc. Due to the lower cost and simple process, the dilute acid method is the most popular way to hydrolyze xylan (Mosier, Ladisch, & Ladisch, 2002). However, a large amount of byproduct would be produced and prohibit xylan fermentation further (Walther, Hensirisak, & Aghlevor, 2001). Additionally, the residual inorganic acid in the hydrolyzed sample could not be separated easily. Knowing the above disadvantage of the inorganic acid method, the development of a new, green, economical process for the conversion of hemicellulose into xylose is essential.

Dielectric barrier discharge of water vapor will produce a great number of protons. These protons make plasma with a preliminary acidic. The concept of plasma acid was initially presented by Changjun Liu and Jijun Zou in hydrolysis of starch catalyzed by dielectric barrier discharge plasma (Liu & Zou, 2004). Obviously, the plasma acid has a great advantage over sulfuric acid in cost and it can be obtained and separated more easily for it coming from water.

So far, the plasma acid has been seldom investigated. The relation between the discharge conditions and the pH of the plasma has not been further explored. Therefore, in this work, the tentative relationship between discharge duration and the pH of the plasma was investigated. The hemicellulose was hydrolyzed by plasma acid under various discharge conditions and the DNS method was applied to measure the total reducing sugar (TRS) in order to optimize the hydrolysis condition. To get the solid reducing sugar, the sample after hydrolysis was evaporated and cooled. Moreover, it was hydrolysis by sulfuric acid for a second time to determine whether the oligosaccharide existed in the hydrolysis products. High-performance liquid chromatography (HPLC) and Fourier transformed infrared spectroscopy (FTIR) were carried out

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to investigate the monosaccharide compositions of the hydrolysis sample.

2. Experiment

2.1. Materials

The feedstock hemicellulose was purchased from Shanghai Xitang biotechnology Co., Ltd (purity ≥ 85%). Its main ingredient is xylan with a molecular formula of $(C_5H_8O_4)_n$. The average degree of polymerization of xylan is about 150–200.

3,5-Dinitrosalicylic acid (DNS) reagent, H_2SO_4 and NaOH were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. Potassium sodium tratrate, phenol and sodium sulfite were purchased from Dalian Beijiang Chemical Industry Co., Ltd.

2.2. Atmospheric pressure plasma treatment

The plasma treatment equipment, CTP1200, was made by Coronalab (China). Before the plasma discharge, 5 ml of distilled water was placed on the surface of the quartz electrode. Then, it was treated by plasma at discharge power of 80 W and discharge gap of 8 mm for 10 s, 20 s, 30 s, 60 s, 90 s, 120 s and 180 s, respectively. The treated water was diluted to 50 ml in order to test the pH value conveniently by pH detector, pH-3C, made by Aohua Instruments Co., Ltd (Changzhou, China).

2.3. Drawing of the standard curve with DNS method

In the carbohydrate, monosaccharide and disaccharide with aldehyde group or ketone group are reducing sugars. They could be oxidized to sugar acid when it was heated in alkaline condition, while the DNS is converted into brownish red 3-amino-5-nitro-salicylic acid. The color of the tested sample is due to the mole number of the aldehyde group or ketone group existed in the reducing sugars. It could be characterized by the integrated optical density (IOD) value in the UV spectrophotometer. Thus, the DNS method is applied to measure the mole numbers of TRS obtained from hemicellulose hydrolysis.

By the DNS method, drawing a standard curve is necessary. Ten mixture solutions were obtained by blending the distilled water (1.9 ml), the DNS mixture solvents (1.5 ml) and glucose solutions (0.1 ml) with different concentrations for 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml and 10 mg/ml, respectively. Each of the mixture solutions was heated at 100 °C for 15 min, and cooled down and diluted into 25 ml. The IOD value of each sample was measured at 540 nm through UV5000 spectrophotometer, made by Nanjing Haohai Instrument Co., Ltd.

A DNS mixture solvent mentioned above was obtained as follows. The DNS (6.3 g) and 2 mol/l sodium hydroxide (262 ml) were added to the 500 ml hydrothermal solution of potassium sodium tartrate (185 g). Then, the crystallization phenol (5 g) and sodium sulfite (5 g) were put into the mixture solution. It was cooled down and diluted to 1000 ml and stored in the brown bottle for later use.

2.4. Measurement of the TRS of hydrolysis sample by DNS method

2 ml DNS mixture solvent and 1 ml hydrolysis sample were heated for 15 min in a boiling water bath, cooled down and diluted into 25 ml. The IOD value of the mixture was measured in spectrophotometer at 540 nm. Then the molarity of TRS was obtained based on a standard curve and the total mole numbers of the TRS could be calculated.

Table 1
Factors and levels of orthogonal experiment design.

Level	A (pH)	B (temperature/°C)	C (t/min)
1	3.94	20	10
2	2.81	40	20
3	1.66	60	30
4	1.42	80	40
5	1.35	100	50

2.5. Hemicellulose hydrolysis by plasma acid

Hemicellulose (0.1 g) was mixed with the plasma acid (10 ml) with different pH value in the conical flask. Heated the conical flask at different temperature for various time periods, applied by an orthogonal experiment design method $L_{25}(5^6)$. To test the IOD value, the hydrolysis sample was diluted to 50 ml and then 1 ml was taken to test the IOD. Table 1 listed the schedule of the orthogonal test in which the parameters, pH value (A), hydrolysis temperature (B), and hydrolysis time (C) were selected as the three key factors. Each factor had five levels. Twenty-five experiments with different combinations of the factors and levels would be conducted. The IOD of hydrolysis sample was obtained and taken as an index point to evaluate the hydrolysis results under different factors and levels. The higher the IOD value was, the more the TRS was.

2.6. Hydrolysis by sulfuric acid

The controlled experiment was made with sulfuric acid to hydrolyze hemicellulose (0.1 g). The test was carried out at 4% sulfuric acid at 121 °C for 1 h in an oil bath, following the same procedure as reported earlier (Liu, Fatehi, Jahan, & Ni, 2011). Under this condition, hemicellulose was completely converted to monosaccharides (Saeed et al., 2012).

Therefore, an additional sulfuric acid hydrolysis at above condition was carried out on the hydrolyze products of plasma acids. If there was oligosaccharide in the products, it could be converted to monosaccharides (Shi, Fatehi, Xiao, & Ni, 2011) and promote the IOD value. By comparing the IOD value before and after second hydrolysis, whether the oligosaccharide existed in the hydrolyze products was determined.

2.7. HPLC analysis

HPLC, 6890N, was made by Agilent Technologies Co., Ltd. (America). The monosaccharide compositions of the hydrolyzed sample were investigated by HPLC with a HP-5 column (Agilent Technologies Co., Ltd) using N_2 at a flow rate of 1 ml/min and an FID detector.

2.8. FTIR analysis

FTIR, Spectrum one-B, was made by PerkinElmer Instruments Co., Ltd (America). The sample was pressed into a pellet to investigate the chemical structure.

3. Results and discussion

3.1. Standard curve

As shown in Fig. 1, the standard glucose curve was drawn with the obtained IOD value as vertical coordinate y and the glucose molarity as horizontal coordinate x (mol/ml). Obviously, it was a straight line whose regression equation was $y = 100231x - 0.0036$. With the measured IOD value y, the unknown molarity of TRS x (mol/ml) was obtained.

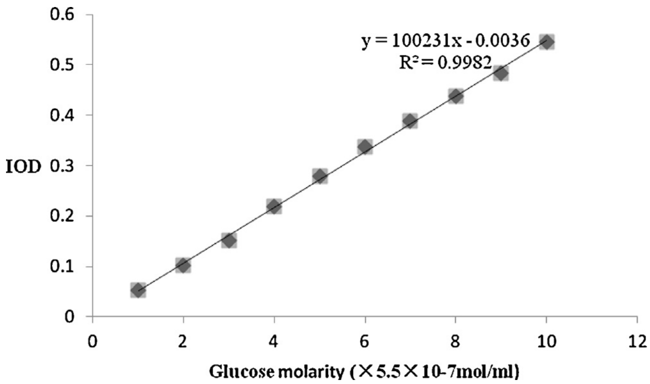

Fig. 1. Standard glucose curve.

3.2. Plasma treatment results

To concisely illustrate the relations between the discharge time and the pH value of plasma acid, a curve was drawn where the discharge time was horizontal coordinate and the pH value was vertical coordinate. The curve was shown in Fig. 2.

The acidity of the plasma acid increased dramatically from 6.83 to 1.66 when the discharge time varied from 10 s to 90 s from point A to point E. It could be concluded that the acidity of plasma acid was related to discharge time positively and it was affected significantly by the discharge time. As the time increased from 90 s to 180 s, the acidity varied slightly from 1.66 to 1.35 which was presented with the curve tending to a horizontal line. Therefore, it could be seen that the influence of the discharge time weakened with the time extension.

A large number of electronics with high energy were produced during the process of discharging. With the presence of electric field, a variety of electric particles moved along the direction of the field. Due to the light mass, the electronics possessed an excellent migrating rate and reached the surface of discharge medium prior to any other particles. Therefore, an electronic layer formed at the surface of the medium which was called plasma sheath with negative charge. This negative plasma electronic layer will attract H^+ protons, produced by the discharge of water, forming a layer of protons beyond the surface of medium. This made the plasma treated water in this experiment contain a great number of H^+ protons and form plasma acid (Liu & Zou, 2004).

3.3. Hemicellulose hydrolysis by plasma acid

Table 2 shows the results of the orthogonal experiment of by plasma acid hydrolysis. The K value for each level of a factor was

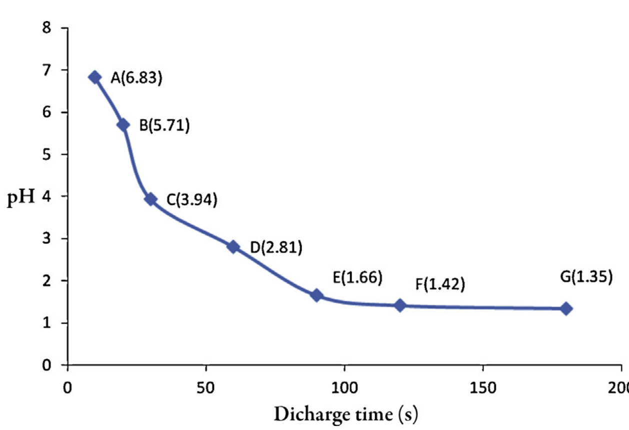

Fig. 2. Relations between the discharge time and the pH value of plasma acid.

Table 2
Results of orthogonal experiment.

No.	A	B	C	IOD
1	1(A_1)	1(B_1)	1(C_1)	0.213
2	1	2(B_2)	2(C_2)	0.506
3	1	3(B_3)	3(C_3)	0.612
4	1	4(B_4)	4(C_4)	0.478
5	1	5(B_5)	5(C_5)	0.695
6	2(A_2)	1	2	0.417
7	2	2	3	0.519
8	2	3	4	0.612
9	2	4	5	0.662
10	2	5	1	0.233
11	3(A_3)	1	2	0.136
12	3	2	3	0.163
13	3	3	4	0.166
14	3	4	5	0.190
15	3	5	1	0.192
16	4(A_4)	1	2	0.140
17	4	2	3	0.137
18	4	3	4	0.166
19	4	4	5	0.120
20	4	5	1	0.175
21	5(A_5)	1	2	0.134
22	5	2	3	0.175
23	5	3	4	0.177
24	5	4	5	0.174
25	5	5	1	0.152
K_1	0.503	0.208	0.251	
K_2	0.543	0.3	0.267	
K_3	0.169	0.346	0.321	
K_4	0.148	0.327	0.322	
K_5	0.162	0.347	0.368	
R	0.395	0.139	0.117	
Optimal level	A_2	B_5	C_5	

the average of three experimental values. The range value (R) for each factor was the difference between the maximal and minimal K value of the five levels. The larger the value of R, the more influence the factor had on the content of total reducing sugar. Table 2 shows the R for A (pH value), B (hydrolysis temperature), and C (hydrolysis time) factors were 0.395, 0.139, and 0.117, respectively. Therefore, it could be learnt that the pH value had the most significant influence on the results. And the hydrolysis temperature was next important factor compared with the time. In this study, the order of significance was pH value, hydrolysis temperature, and hydrolysis time.

From the results of the experiment it was learnt that hemicellulose could be hydrolyze by plasma acid with certain temperature and time. The analysis results showed that the maximal K was achieved at K_2 level for A factor, K_5 level for B factor, and K_5 level for C factor. Thus, the optimal level was A_2 B_5 C_5, which could be expressed by that the pH value was 2.81, hydrolysis temperature was 100 °C and hydrolysis time was 50 min. To confirm the optimized results, parallel tests were made on A_2 B_5 C_5 for three times. The results showed that the average IOD were 0.721 for the optimal method. With the IOD value of 0.721, the molarity of TRS was calculated to be 7.229×10^{-6} mol/ml according the equation $y = 100231x - 0.0036$, respectively. Multiplied by total volume 50 ml, the final results were 3.61×10^{-4} mol.

3.4. Hydrolysis by sulfuric acid

The IOD of the hemicellulose hydrolyzed by sulfuric acid was 0.614. The TRS were 3.08×10^{-4} mol, lower than 3.61×10^{-4} mol products hydrolyzed by plasma acid.

An additional sulfuric acid hydrolysis was carried out on the hydrolyze products obtained under the optimal condition by plasma acids. The result showed that the IOD of the second hydrolyze products by sulfuric acid was 0.729, close to 0.721

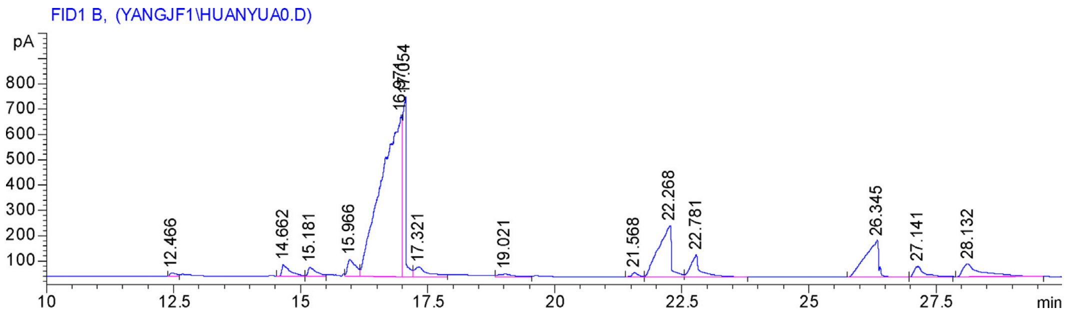

Fig. 3. HPLC profile of the hydrolysis sample by plasma acid.

obtained by plasma acid. Therefore, it was shown that the hemicellulose was hydrolyzed completely to produce monosaccharides. Also, it proved that plasma acid was more efficient than sulfuric acid in hemicellulose hydrolysis. The monosaccharide yield was higher than traditional inorganic acid.

3.5. HPLC analysis

To further investigate the monosaccharide compositions, the solid sample was analyzed through HPLC analysis. The HPLC results of solid sample were shown in Fig. 3.

Fig. 3 shows that there were three main elution peaks at 17.054 min, 22.268 min and 22.781 min. According to the standard HPLC profile of monosaccharides sample, the three elution peaks were due to the presence of xylose, glucose and galactose, respectively. Thus, it can be concluded that the hydrolyzed sample mainly consisted of a few kinds of monosaccharides, including xylose, glucose, and galactose. Furthermore, the mole ratio of xylose, glucose, and galactose can be calculated to be 17:3:1 according the area proportion from the HPLC profile in Fig. 3. With the TRS, the mole ratio and the molecular weight of each monosaccharide, the mass of xylose, glucose, and galactose could be counted easily to be 38.67 mg, 9.28 mg and 3.09 mg respectively. Therefore, the yield of xylose, glucose, and galactose were 38.67%, 9.28% and 3.09% via this equation $c = a/b \times 100\%$ °C in which the a (mg) was monosaccharide mass and the b (mg) was the original mass of the hemicellulose (100 mg) used in this experiment. The monosaccharides were 51.04 mg and the yield was 51.04%.

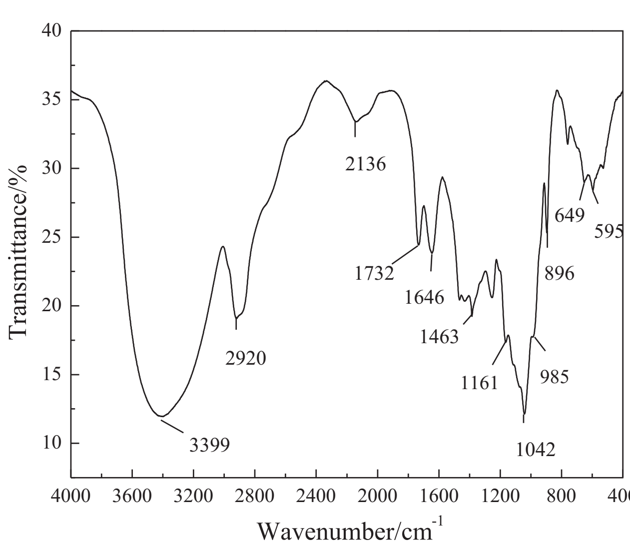

Fig. 4. FTIR spectrum of hydrolysis sample by plasma acid.

3.6. FTIR analysis

The FTIR spectrum of hydrolysis sample was shown in Fig. 4. The hydrolysis sample showed intense characteristic peaks at about 3399 cm^{-1}, 2920 cm^{-1}, 1732 cm^{-1}, 1646 cm^{-1}. The absorption peak at 3399 cm^{-1} was due to stretching vibration of —OH and the and 2920 cm^{-1} was attributed to the C—H stretching vibration. The peaks at 1732 cm^{-1} and 1646 cm^{-1} were caused by the C=O stretching vibration and O—H deforming vibration, respectively. The absorption bands among 1400–1200 cm^{-1} were attributed to the C—H deforming vibration. These function groups were related closely to the obtained xylose, glucose, and galactose because these monosaccharides were all aldose. Combined with HPLC analysis, it can be inferred that the hydrolyzed sample contained monosaccharides and it definitely proved that the hemicellulose can be hydrolyzed effectively by plasma acid.

4. Conclusion

The plasma acid was achieved by treating distilled water with dielectric barrier discharge plasma. The acidity of the plasma acid was adjusted to the discharge conditions. The hemicellulose could be hydrolyzed by plasma acid effectively and the optimal hydrolysis conditions were as follows: plasma acid pH of 2.81, 100 °C and 50 min. Under this optimal condition, the hydrolyzed TRS was 3.61×10^{-4} mol in 100 mg hemicellulose sample, higher than that of 3.08×10^{-4} mol by sulfuric acid hydrolysis. The hemicellulose was hydrolyzed completely to produce monosaccharides including xylose, glucose, and galactose with the mole ratio being 17:3:1. The yield of xylose, glucose, and galactose were 38.67%, 9.28% and 3.09%, respectively. The yield of monosaccharide was 51.04%.

Compared with hydrolysis results by sulfuric acid, undoubtedly, the plasma acid has proven to be an effective and environmentally friendly method to hydrolyze hemicelluloses. It could be obtain by plasma discharge of water in a short time. Additionally, it will definitely have a promising future to investigate plasma acid and apply it to other fields, such as hydrolysis cellulose and other plant based biomass.

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