HYDROLYSIS OF WHEAT STRAW HEMICELLULOSE AND DETOXIFICATION OF THE HYDROLYSATE FOR XYLITOL PRODUCTION

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Xylitol can be obtained from wheat straw hemicellulose containing a high content of xylan. This study describes a new system of hydrolysis, utilizing a mixed solution of formic acid and hydrochloric acid in which xylan can be hydrolyzed effectively. The hydrolysate contains a high content of formic acid, which markedly inhibits the fermentation. One of the most efficient methods for removing inhibiting compounds is treatment of the hydrolysate with ion-exchange resins. Formate can be removed by a factor of 77.78%, and furfural, acetic acid, phenolic compounds can be removed by 90.36%, 96.29%, and 77.44%, respectively after the hydrolysate has been treated with excess $Ca(OH)_2$ and D311 ion-exchange resin. The xylose from the hydrolysis process can be fermented by *Candida tropicalis* strain (AS2.1776) to produce xylitol with a yield of 41.88 % (xylitol/xylose).

Keywords: Straw hydrolysate; Formic acid; Xylose; Xylitol; Fermentation

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INTRODUCTION

Xylitol, a natural five-carbon sugar alcohol with sweetness comparable with that of sucrose has found increasing use in the food and medical–pharmaceutical industries (Mitchell 2006). It has anticariogenic properties, it is appropriate to replace glucose in diets of diabetics, and it has been recommended for parenteral nutrition and to prevent otitis, osteoporosis, and lung infections (Choi et al. 2000; Villarreal et al. 2006). Xylitol is currently manufactured by the chemical reduction of the five-carbon sugar xylose, which is one of the major components present in hemicellulose hydrolysates. As the hemicellulose hydrolysate fraction also contains other sugars, the chemical process includes expensive separation and purification steps to remove these by-products from xylose or xylitol. Xylitol can also be produced by microbiological methods with xylose-utilizing yeasts, especially species of the genus Candida (Oh and Kim 1998).

In order to obtain a better bioconversion yield of fermentation from xylose to xylitol, benign pretreatment methods of lignocelluloses for production of higher quality of xylose have been investigated, including acid, alkali, ray, steam explosion, and combinations of different methods. Among these pretreatments, formic acid is a novel agent utilized for hydrolysis of lignocellulose into fermentable sugars and exhibits the potential prospects of industrial application because the reaction conditions employed are mild, such as temperatures below 70^{o}C (Sun et al. 2007). Moreover, the corrosion of formic acid to the reaction apparatus is much less than that by the concentrated inorganic acid, such as higher concentration of sulphuric acid. Formic acid together with the lower concentration of hydrochloric acid can be effectively recovered and reused. However, it was found that formic acid hydrolysate of lignocellulosics such as wheat straw cannot be directly applied as a fermentation substrate owing to the presence of various fermentation inhibitors in it.

Acidic hydrolysate of hemicellulose comprises a considerably complex mixture of components. These compounds can be divided into three major groups: organic acids (acetic, formic and levulinic acids), furan derivatives, for example, furfural and 5-hydroxymethylfurfural (5-HMF), and phenolic compounds derived from sugar degradation or released from degradation of lignin polymer (López et al. 2004). Among these fermentation inhibitors, especially residual formic acid during pretreatment and in reaction system is inhibitory to the fermenting microorganism when formic acid concentrations are higher and exceed the tolerance threshold of the microorganism used (Zimbardi et al. 2007; Rao et al. 2006). However, these problems can be overcome by detoxification of the hydrolysate prior to fermentation (Nilverbrant et al. 2001; Palmqvist et al. 2000). Various approaches are being considered to remove fermentation inhibitors or minimize their formation, CaO or $Ca(OH)_2$, activated charcoal, ion-exchange resins, solvent extraction, intracellular acidification, yeast strain variation, decomposition of polymerization catalyzed by laccase enzymes, recombinant strains, and adaptation of the microbial strains have been reported (Rao et al. 2006).

Assays with $Ca(OH)_2$, solvent extraction (ether and ethyl acetate) and D311 ion-exchange resin for hydrolysate of formic acid hydrolysis of wheat straw was explored in this study. Effects of treatment time, temperature, content of hydrochloric acid in the mixed solution, and ratio of liquid/solid (g/g) on the yield of xylose were evaluated to detoxify the formic acid hydrolysate of wheat straw. Then, the resulting hydrolysate was fermented by *Candida tropicalis* strain (AS2.1776) to produce xylitol.

EXPERIMENTAL

Materials

The wheat straw was obtained from a local farmer in the suburb of Zhengzhou, Henan Province of China during July, 2007. The straw was air-dried, milled, screened to select the fraction of particles with a size lower than 0.5 mm, and washed with distilled water. D311 ion-exchange resin was purchased from the Anhui Sanxing resin Company. Formic acid was purchased from the Shanghai Lingfeng Chemical Company. Hydrochloric acid was purchased from the Guangdong Donghong Chemical Company. The pure water was provided by the Wetsons company in Guangzhou. All chemicals are analytical grade.

Methods

Determination of main components of wheat straw

Contents of moisture, ash, klason lignin, and pentosan in wheat straw were determined by the Chinese Standard of GB/T 2677.2-1993, GB/T 2677.3-1993, GB/T 2677.8-1994 and GB/T 2677.9-1994 respectively. Cellulose content was determined by the nitric acid–ethanol method (Wang. 1995).

Influence of temperature on the hydrolysis

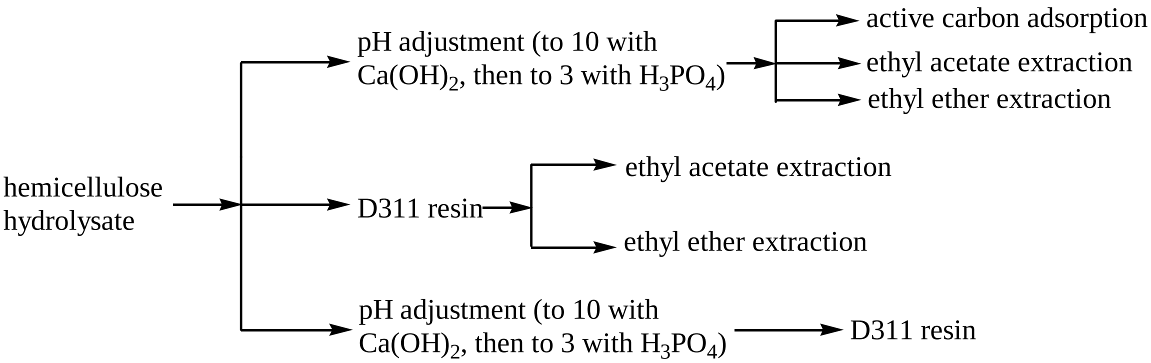
The hydrolysis of wheat straw hemicellulose was carried out by placing 1 g of wheat straw material in a flask that contained 24 g of a formic acid solution (78.22 wt % formic acid, 17.78 wt % water, and 4 wt % hydrochloric acid). The reaction was carried out at different temperatures (60, 65, 70, and 75 ^{o}C) and residence times (0.5~9 h) at atmospheric pressure. After reaction, formic acid and hydrochloric acid were extracted by a depressurization procedure. The final products were obtained for further analysis after washing, filtration, and desiccation.

Hydrolysis of wheat straw with formic acid

Saturated formic acid solution that contained 86.24 wt% formic acid and 17.78 wt% water solution with hydrochloric acid as the catalyst at different ratios of 2%, 4%, 7% and 10 wt% was employed for hydrolysis of wheat straw. The hydrolysis of wheat straw hemicellulose was carried out by placing 1 g of wheat straw material in three flasks that contained 49 g, 24 g, and 16 g of a saturated formic acid solution (78.22 wt % formic acid, 17.78 wt % water, and 4 wt % hydrochloric acid), respectively. The reaction was carried out at different temperatures (60, 65, 70, 75 ^{o}C) and residence times (0.5~9 h) at atmospheric pressure. After reaction, formic acid and hydrochloric acid were extracted by a depressurization procedure. The final products were obtained for further analysis after washing, filtration and desiccation.

Removal of residual formic acid and detoxification of hydrolysates of wheat straw

An orthogonal test design $L9(3^4)$ was used to optimize the suitable hydrolysate detoxification procedure with 20 g D311 ion-exchange resin, several methods of detoxification was designed as follows:



The condition of active carbon adsorption was as follows: Dosage of active carbon 2 wt % of hydrolysate, then placed in a incubator, 120 rpm, 80 ^oC for 45min. The condition of solvent extraction was as follows: The ratio of solvent/hydrolysate was 3:1(V/V), and the time of extraction was 5 min, then delamination occurred.

Fermentation of hydrolysates for production of xylitol

Fermentation medium (25 mL) was prepared and added into 250 mL Erlenmeyer flasks with wheat straw hydrolysate after detoxification by the above procedure. The following nutrients were supplemented (g/L): yeast extract 2, KH_2PO_4 2, ammonium tartrate 7, and $MgSO_4$ 2. All the materials were sterilized in an autoclave for 20 min at a temperature of 121 ^oC. Adapted cells (*Candida tropicalis* strain AS2.1776, from China General Microbiological Culture Collection Center, CGMCC, 1%) were used for inoculation. During fermentation they were incubated for 72 h at 30 ^oC in a rotary shaker at 150 rpm of oscillating speed.

HPLC Analysis and GC-MS analysis

During the hydrolysis procedure, after the reaction formic acid and hydrochloric acid were extracted by a depressurization procedure, then 10 ml of deionizated water were added into the Erlenmeyer flask, the resulting solution was filtered firstly through quantitative filter paper, and HPLC and GC-MS analyses were carried out.

The high-performance liquid chromatography (HPLC) system consisted of a Waters 600E system controller, a Waters 717 automatic sampler, a Waters 410 differential refractometer, and a Waters Sugar pak I column. The mobile phase was pure water and ran at a flow rate of 1.1 mL/min. The HPLC system was operated at 90 ^oC. The sample volume injection was 10 µL. Standard samples and hydrolyzate samples were filtrated by a 0.45µm filter and analyzed in duplicate.

Gas chromatography (GC) in combination with mass spectrometry (MS) was performed using an HP-INNOWAX column (produced by Agilent) with helium as the carrier gas. The initial temperature was 80 ^oC for 2 min and then the temperature was increased at 10 ^oC/min to 250 ^oC, with the temperature held at that level for 10 min. Mass spectra were obtained at 70 eV. Absolute alcohol was used for solvent.

RESULTS AND DISCUSSION

Main Components of Raw Material

Table 1 lists the main chemical properties of the wheat straw. The results show that pentosan (the main component of hemicellulose) content was 23.96%. As xylose is the dominant sugar in the hydrolyzates from the pentosan, therefore we think that wheat straw is a low cost, attractive feedstock for production of higher value-added production of xylitol.

Table 1. Main Components of Wheat Straw

Component	Moisture	Ash	Klason lignin	Cellulose	Pentosan	Other
Content (%)	8.02	8.71	17.89	28.52	23.96	12.74

Influence of Different Factors on the Hydrolysis

During hydrolysis of wheat straw with form acid, it was found that the residence time and temperature were important factors influencing the hydrolysis process. In order to explore optimal reaction conditions, the effects of reaction temperature (55, 60, 65, 70 °C) and residence time (from 0.5 to 9 h) on the xylose yield were examined (Fig. 1). The figure indicates that the hydrolysis of hemicellulose was strongly affected by the temperature. The optimal hydrolysis conditions of wheat straw in this study were found to be 65 °C for 0.5 h. Under optimal conditions, the yield of xylose was 65.53 % (xylose/pentosan). The effects of content of HCl (2, 4, 7, and 10 %) and residence time (from 0.5 to 9 h) on the xylose yield were examined (Fig. 2).

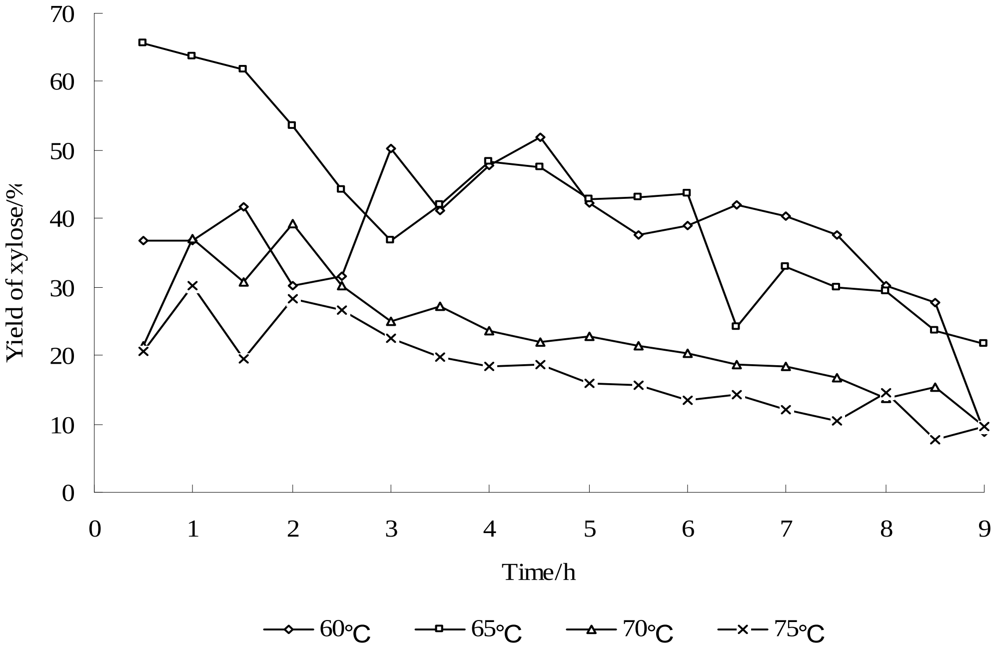


Figure 1. Effect of temperature on the yield of xylose

The results in Fig. 2 show that a lower content of HCl wasn't beneficial to the hydrolysis of hemicellulose because it demanded a long time to release the hemicellulose. A high content of HCl accelerated the hydrolysis of hemicellulose strongly but also enhanced the degradation of xylose in the hydrolysate. The optimal hydrolysis conditions were found to be 10 wt% of HCl for 0.5 h. Under optimal conditions, the yield of xylose was 89.61 % (xylose/pentosan). The effects of ratio of liquid/solid (49:1, 24:1, 16:1) and residence time (from 0.5 to 9 h) on the xylose yield were examined (Fig. 3). The optimal hydrolysis conditions were found to be 24:1 for 0.5 h. Under optimal conditions, the yield of xylose was 65.53 % (xylose/pentosan). The optimal conditions of hydrolyzing the wheat straw using formic acid and hydrochloric acid (10% hydrochloric acid) were 24:1 ratio, 65 °C for 0.5 h with a yield of xylose of 89.61 % (xylose/pentosan).

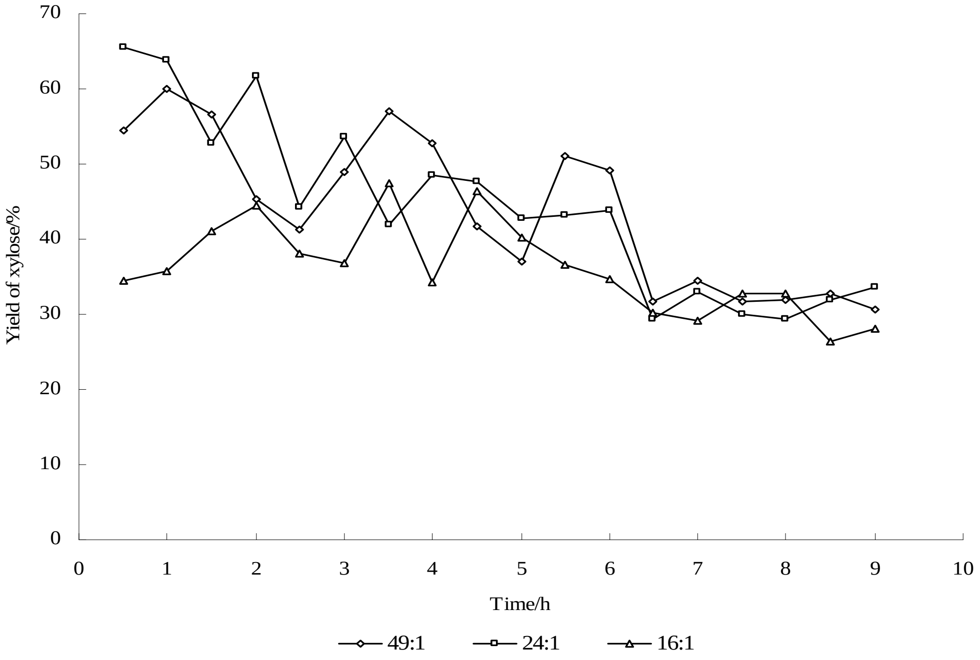


Figure 2. Effect of concentration of HCl on the yield of xylose

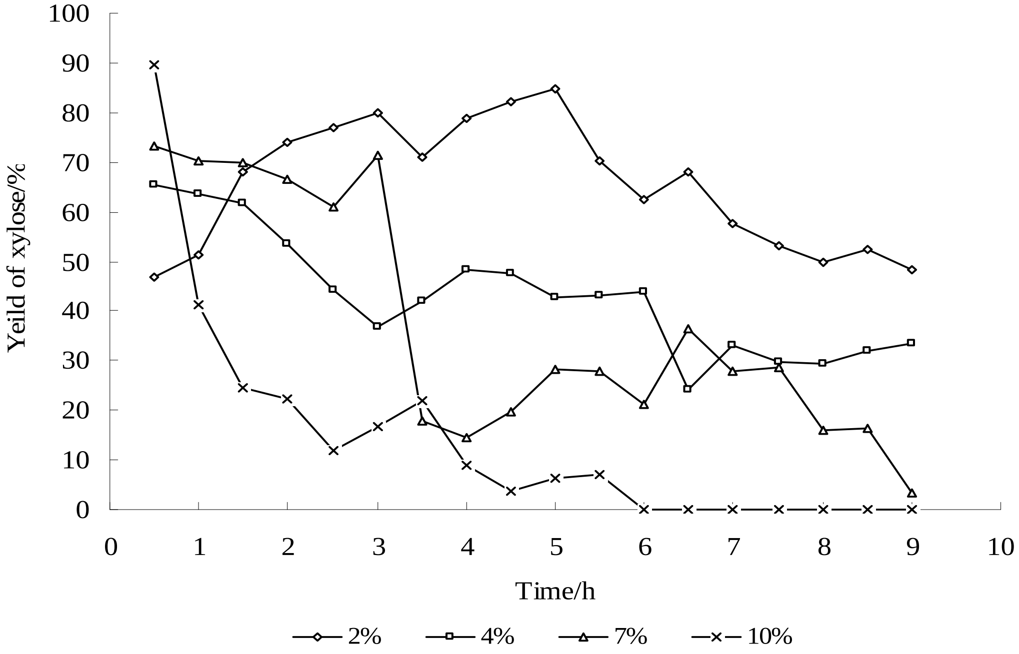


Figure 3. Effect of ratio of L/S on the yield of xylose

Figure 4 shows the components in the wheat straw hydrolysate when hydrolyzed under the optimal conditions. In the HPLC spectrum the peak of 5.906' was the retained time of formic acid with a concentration of 16.5127 g/L. The highest peak represents the retained time of xylose (11.439') and 10.551' and 12.904' for glucose and arabinose, respectively. The yield of reducing sugars was measured by the DNS method. The concentration of xylose, glucose, arabinose, and total reducing sugars was 2.9839 g/L, 0.1409 g/L, 0.4877 g/L, and 3.6125 g/L, respectively. The content of xylose, glucose, and arabinose in reducing sugars was 89.74 %, 3.74 %, and 6.52 %, respectively.

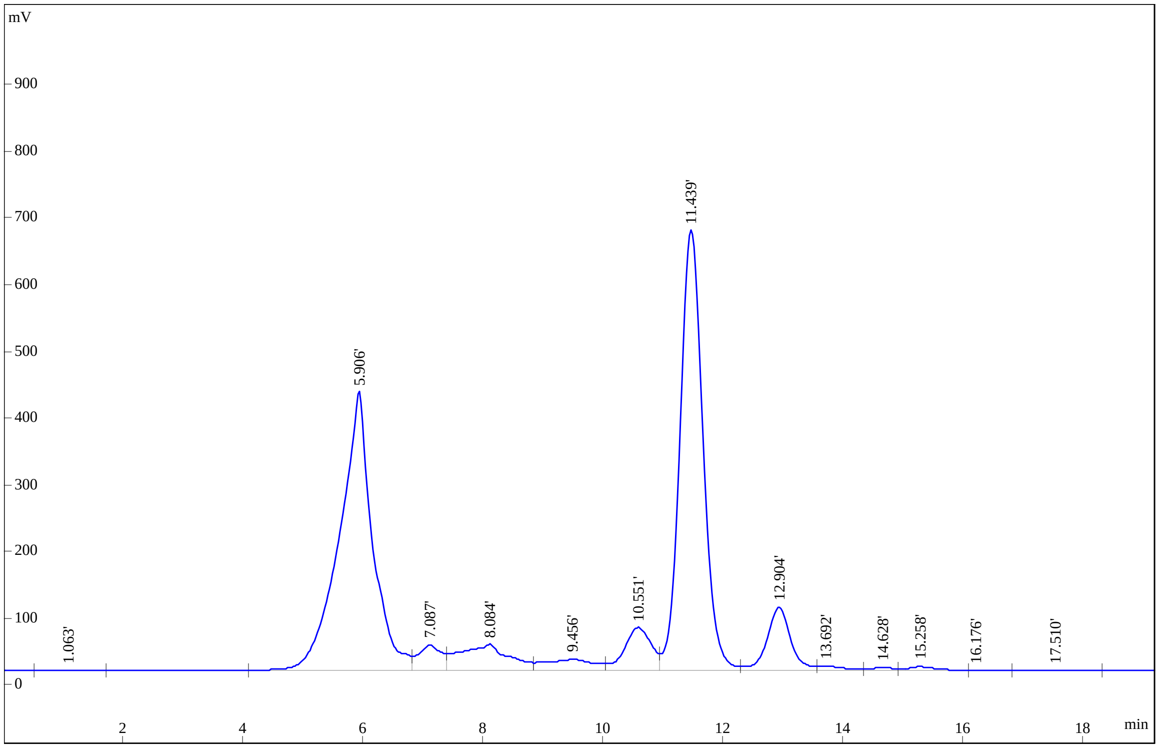


Figure 4. The HPLC spectrum of hydrolysate of wheat straw in formic acid solution

Effect of Formic Acid on Fermentation of Xylose

The effects of concentration of formic acid (0.01, 0.1, 0.5, 1, 2, 5, and 10 g/L) on fermentation of xylose were examined (Fig. 5). The initial concentration of xylose was 50 g/L. It could be found that a high concentration of formic acid (>5 g/L) restrained the fermentation of xylose strongly to produce xylitol. However, to our surprise, it was found that a low concentration of formic acid (<5 g/L) was beneficial to the fermentation, and the highest xylitol yield was achieved at a 52.88 % level when there was 2 g/L formic acid in the hydrolysates. The reason for the results could be ascribed to possible physiological adaptation of the *Candida tropicalis* strain (AS2.1776) to low concentration of formic acid; however, in-depth exploration of its mechanism will be expected in further studies.

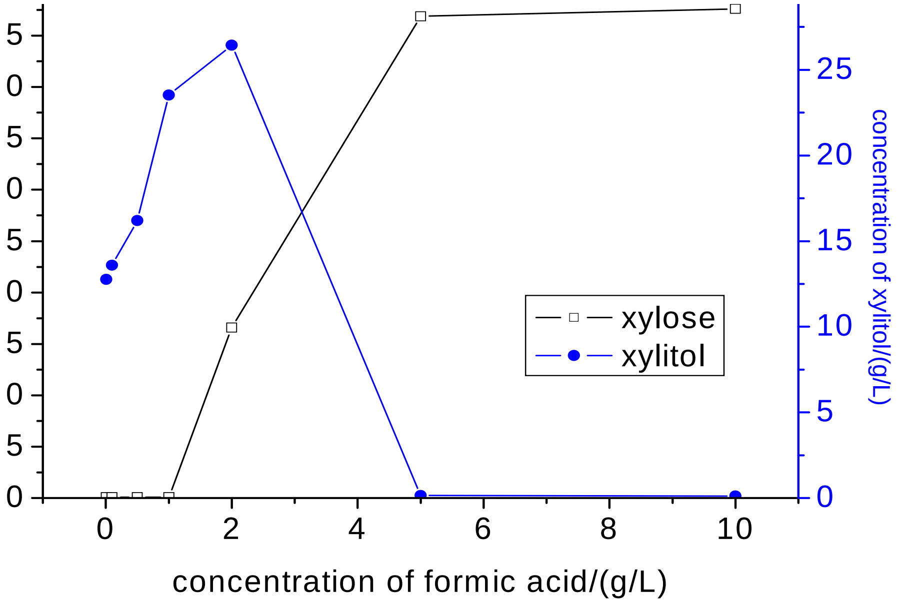


Figure. 5. Effect of formic acid on fermentation of xylose to produce xylitol

Removal of residual formic acid in hydrolysates with D311 ion-exchange resin

An orthogonal test was designed to remove formic acid by 20 g D311 ion-exchange resin (Table 2.). We found that the use of D311 ion-exchange resin was an efficient method for removal of formic acid. The effect of dosage of formic acid on removal was more significant than that of the concentration of formic acid or the velocity of flow. The highest yield achieved 99.99 % removal of formic acid under the following conditions: concentration of formic acid: 50 g/L; dosage of formic acid: 22.84 m mol; velocity of flow: 6 mL/min.

Table 2. Orthogonal Test Results of Formic Acid Removal by D311 Resin

Serial number	Concentration of formic acid/(g/L)	Dosage of formic acid/(m mol)	Velocity of flow/(mL/min)	Yield of formic aid removing/%
1	10	22.84	2	99.87
2	10	57.10	4	99.88
3	10	91.36	6	81.89
4	30	22.84	4	99.96
5	30	57.10	6	99.97
6	30	91.36	2	97.31
7	50	22.84	6	99.99
8	50	57.10	2	99.98
9	50	91.36	4	99.94
K1	281.64	299.82	297.16	
K2	297.24	299.83	299.78	
K3	299.91	279.14	281.85	
k1	93.88	99.94	99.05	
k2	99.08	99.94	99.93	
k3	99.97	93.05	93.95	
Range	6.09	6.89	5.98	

The mechanism of the removal process of formic acid in the hydolysates of wheat straw by ion-exchange resin is proposed as follows:

$$\text{R£- OH} + \text{HCOOH} \rightleftharpoons \text{R£- HCOO} + \text{H}_2\text{O} \qquad (1)$$

$$\text{R£- HCOO} + \text{NaOH} \rightleftharpoons \text{R£- OH} + \text{HCOONa} \qquad (2)$$

Results of Detoxification and Fermentation

Furfural, acetic acid and phenolic compounds produced in the process of hydrolysis are toxic to fermentation of hydrolysate. During the removal process of residual formic acid in wheat straw hydrolysate, the effect of D311 resin on detoxification was also examined (Figs. 6 and 7) with GC-MS measurement. Figure 6 indicates that in the hydrolysate varieties of toxic compounds such as acetic acid, furfural, and hydroxymethylfurfural, and others resulting from hydrolysis of the structural components, for example, cellulose, hemicellulose and lignin, were very complicated. Figure 7 indicates that D311 resin was an efficient agent for removal of toxic compounds in the hydrolysate when residual formic acid was removed. Except for the residual formic acid (at 5.37 min) in the hydrolysate, toxic compounds such as furfural (at 4.88 min), acetic acid (15.62 min) and phenolic compounds had been removed by 70.74 %, 64.08 % and 57.73 %, respectively, after the treatment with D311 resin.

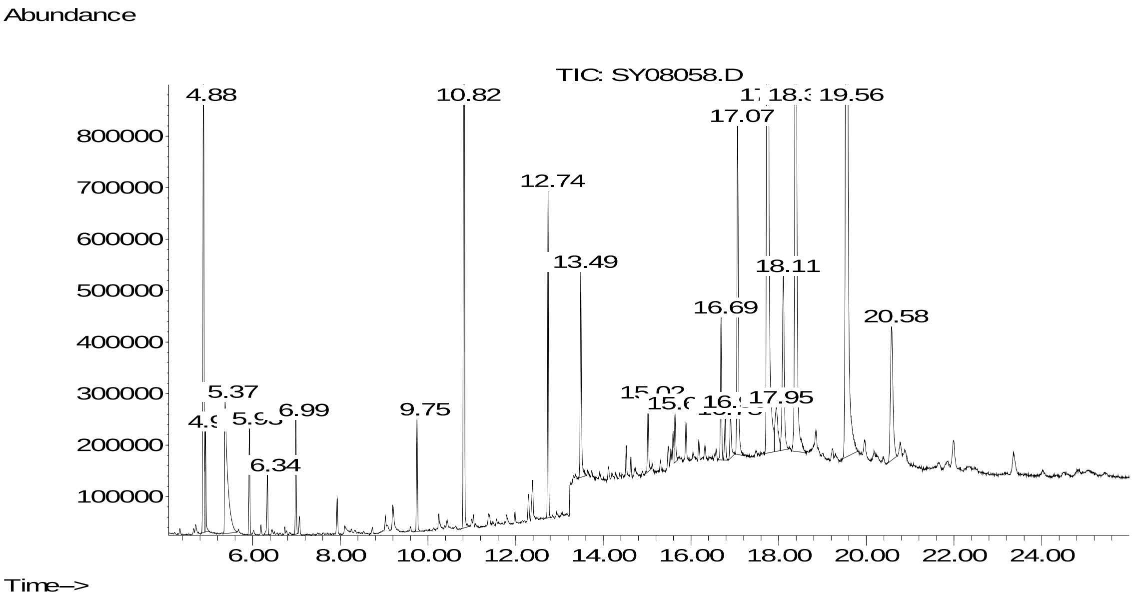


Figure 6. GC-MS chromatogram of hydrolysate of wheat straw in formic acid (10 % HCl)

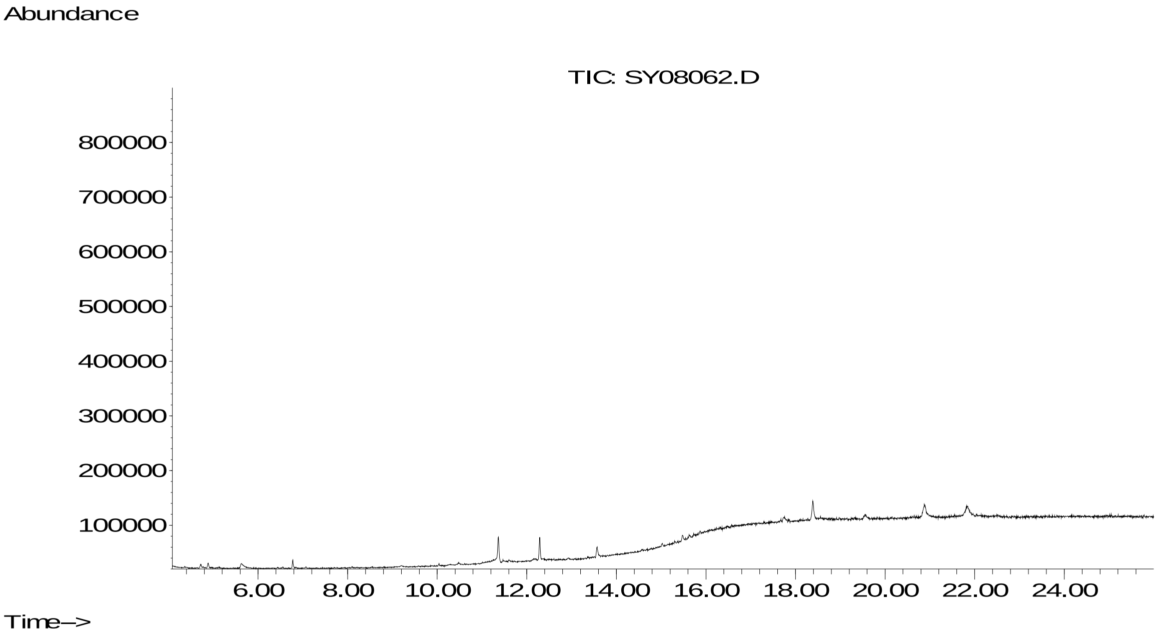


Figure 7. GC-MS chromatogram of hydrolysate of wheat straw detoxified by D311 resin

The results for the removal of xylose and inhibitors in formic acid hydrolysate are shown in Table 3. All treatments were considered efficient for the inhibitor removal, with some differences between their effects on hydrolysate composition. The treatment of $Ca(OH)_2$ with active carbon adsorption removed furfural, acetic acid, and phenolic compounds by 83.64%, 96.37%, and 99.57% respectively, but xylose was adsorbed strongly. Furfural and acetic acid were almost totally removed by $Ca(OH)_2$ with D311 resin treatments. The treatment of $Ca(OH)_2$ with D311 resin removed formate, furfural, acetic acid, and phenolic compounds by 77.78 %, 90.36 %, 96.29 %, and 77.44 %, respectively. The highest xylitol yield was achieved at 41.88 % after the $Ca(OH)_2$ and D311 resin treatments with a volumetric productivity of 0.24 g/(L·h).

Table 3. The Results of the Removal of Xylose and Inhibitors in Formic Acid Hydrolysate

Measures	Yield of removal / %				
	formic acid	furfural	acetic acid	phenolic	xylose
$Ca(OH)_2$+ active carbon	/	83.64	96.37	99.57	61.07
$Ca(OH)_2$+ ethyl acetate	/	59.76	92.19	48.23	/
$Ca(OH)_2$+ ether	/	63.51	89.37	37.13	/
D311 resin + ethyl acetate	97.63	74.25	63.54	62.83	23.34
D311resin + ether	98.99	81.14	59.96	64.75	19.18
$Ca(OH)_2$+D311 resin	77.78	90.36	96.29	77.44	21.64
D311 resin	96.94	70.74	64.08	57.73	18.67

The bioconversion of lignocellulose such as agricultural residues to xylitol contains two key steps: hydrolysis of hemicellulose to xylose and the following fermentation by yeast or bacteria to convert fermentable xylose to xylitol. The hydrolysis process currently used is either concentrated acid hydrolysis or enzymatic hydrolysis. In this study, it was demonstrated that hydrolysis of lignocellulose with formic acid is a novel pretreatment pathway for conversion of hemicellulose into fermentable xylose. In the hydrolysis system of formic acid, the reaction temperature is lower (below 70 ^{o}C), and reaction speed is faster (about 0.5 h) than that with dilute sulfuric acid (1 h 120 ^{o}C) (Cheng et al. 2009). Moreover, the corrosion of organic acid to reaction apparatus is lower than that of concentrated inorganic acid. Formic acid together with hydrochloric acid can be effectively recovered and reused, so it could be asserted that formic acid hydrolysis is a prospective pathway for conversion of lignocellulose in to fermentable xylose and exhibits the potential industrial application because of its simple procedure and fast process. However, formic acid hydrolysates of wheat straw cannot be directly used as fermentation substrates, owing to various fermentation inhibitors in it, so an efficient detoxification process is indispensable before it is subjected to fermentation. In this experimentation, treatment of D311 resin and $Ca(OH)_2$ was shown to remove most parts of the fermentation inhibitors, and the highest xylitol yield was achieved to 41.88 % from fermentation of the hydrolysate after detoxification, thus providing a new procedure for conversion of lignocellulose to high-value chemicals such as xylitol.

CONCLUSIONS

1. Hydrolysis of wheat straw hemicellulose was strongly affected by time, temperature, content of hydrochloric acid in the mixed solution, and ratio of liquid/solid. The optimal conditions for the hydrolysis of hemicellulose were found to be 10 wt % of HCl, 24:1 ratio, and 65 °C for 0.5 h. The yield of xylose was 89.61 % (xylose/ pentosan). The yield of reducing sugars was measured by the DNS method, and the content of xylose, glucose, and arabinose was 89.74%, 6.52%, and 3.74%, respectively.
2. An efficient method for removing inhibiting compounds was to treat the hydrolysate with D311 resin. The treatment by $Ca(OH)_2$ with D311 resin removed formate, furfural, acetic acid, and phenolic compounds by 77.78%, 90.36%, 96.29%, and 77.44%, respectively. The hydrolysate could be fermented by *Candida tropicalis* strain (AS2.1776) to produce xylitol with a yield of 41.88 % (xylitol/xylose).

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