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Pretreatment of Sugar Cane Bagasse Hemicellulose Hydrolysate for Xylitol Production by Yeast

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ABSTRACT

A total of six known xylitol-producing yeast strains were screened for production of xylitol from xylose. *Candida* sp. 11-2 proved to be the best producer. It was chosen to study its ability to produce xylitol from hemicellulose hydrolysate derived from sugar cane bagasse. The hydrolysate was prepared by dilute sulfuric acid (2–3% [w/v]) hydrolysis, with a high-solid, low-liquid ratio followed by leaching. Owing to the inhibitors present in the hydrolysate, different treatments were studied to overcome its effect. In order to reach higher xylitol productivity, treated hydrolysates were concentrated by vacuum evaporation in rotavapor to provide a higher initial xylose concentration. After treatment, *Candida* sp. 11-2 was able to ferment xylose in hemicellulose hydrolysate to produce xylitol.

Index Entries: Xylose; xylitol; sugar cane bagasse hemicellulose hydrolysate; *Candida* sp.

INTRODUCTION

In the past years, the number of works related to obtaining high sweetening power products has increased. One of such product is xylitol, a naturally occurring five-carbon polyalcohol. This alcohol has other important properties in addition to its sweetening effect that make it desirable for sugar-free confections: It is completely safe for the teeth because of it anticaries properties, it is tolerated by diabetics, and it has a high negative heat of solution *(1,2)*. Xylitol is present in certain fruits and vegetables, but the small amounts render its extraction uneconomical. Producing xylitol by microbiological means can overcome this problem. Many yeast species, especially those belonging to *Candida* species, are good xylitol producers *(3)*. Xylitol is formed as a metabolic intermediate product of D-xylose fermentation: D-xylose can be converted to xylitol by NADPH-dependent aldehyde reductase, or can be isomerized to D-xylose by D-xylose isomerase, and then reduced to xylitol by NADH-dependent xylitol dexhydrogenase *(4)*.

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A good source of D-xylose is sugar cane bagasse, the fibrous residue obtained after the extraction of sugar from sugar cane. This waste has been used as raw material for hydroxymethyl furfural production, paper pulp, acoustical boards, press woods, and agriculture mulch. Sugar cane bagasse can be hydrolysed using dilute acid with high-solid, low-liquid ratio to obtain a mixture of fermentable sugars with xylose as the major component *(5)*.

In the hydrolysate, some byproducts generated in the hydrolysis, such as acetic acid, furfural, phenolic compounds, or lignin-degradation products can be present. These are potential inhibitors of the microbial metabolism. In order to provide hydrolysates suitable as fermentation substrate, different treatments, such as acidified activated charcoal or cation-exchange resins, can be carried out to remove these toxic substances *(3)*.

The initial xylose concentration is an important factor to obtain a high xylitol production. This initial xylose concentration can be increased by concentrating the hydrolysate using vacuum evaporation. During the concentrating of hydrolysate, there is also an increase in toxic substances that can act as inhibitors. Therefore, it is important to pretreat concentrated hydrolysate to remove these toxic substances. In this article, we investigate the effect of different pretreatments of sugar cane bagasse hydrolysate on the production of xylitol from hydrolysate by *Candida* sp. 11-2.

MATERIALS AND METHODS

Microorganisms

The yeast cultures were purchased from American Type Culture Collection (ATCC; Rockville, MD). The yeast cultures chosen were *Candida parapsilosis* ATCC 28474, *Candida guilliermondii* ATCC 20118, *C. guilliermondii* ATCC 42050, *Pachysolen tannophilus* ATCC 32691, *Candida* sp. 11-2 (laboratory strain), and the yeast *Debaryomyces hansenii* NRRL Y-7426 (obtained from the Northern Regional Research Laboratory, USDA, Peoria, IL).

Growth of Yeast Cells

The yeast cells were incubated for 3 d at 32°C in an incubator shaker at 200 rpm (New Brunswick). The liquid medium contains 1% glucose, 1% xylose, 3 g/L Bacto-yeast extract, 3 g/L Bacto-malt extract, and S g/L Bacto-peptone.

Fermentation

Fermentation with Pure Xylose

The fermentation batches to compare different yeasts was performed at 28°C in 50-mL Erlenmeyer flasks (containing 10 mL of culture media) placed in a gyratory shaker at 180 rpm for 4 d. The culture media was prepared with pure xylose (15% [w/v]) and supplemented with nutrients with the following composition per liter: 3 g Bacto-yeast extract, 3 g Bacto-malt extract, and 5 g Bacto-Peptone. Yeast cells from growth medium were harvested by centrifugation and transferred into fermentation medium.

Fermentation with Hydrolysates

The fermentation of hydrolysates was carried out under the same conditions as described for with pure xylose. The same amounts of nutrients were added in

the hydrolysate to eliminate the effect of nutrients that might affect the fermentation of different concentrations of substrate.

Acid Hydrolysate

Sugar cane bagasse hemicellulose hydrolysate was obtained by hydrolysis of ground bagasse in dilute sulfuric acid g 2–3% [w/v]) at 100°C followed by downflow leaching using water according to the method described by Ladisch *(5)*. Hydrolysate has a low pH value and was kept at room temperature.

Treatments

1. Neutralization: The pH of the hydrolysate was increased to 4.5–6 by the addition of calcium carbonate. Calcium sulfate of approx 15 g/L formed during neutralization was removed from liquid by filtration.
2. Activated charcoal and neutralization: Activated charcoal (Savannah Foods & Industrials, Inc., Savannah, GA) was washed and equilibrated with HCl 0.4*N*. After being packed into a column (1.5 × 25 cm), charcoal was washed with water, and the acid hydrolysate was applied to the column. The hydrolysate collected was neutralized by addition of calcium oxide slurry and calcium carbonate to pH 4.5–6.0. The calcium sulfate was removed by filtration.
3. Cation–exchange resins and neutralization: Acid cation-exchange resins (Dowex 50 WX4, 200–400 mesh) were washed with NaOH (0.1*N*) followed by water. The resin was packed into a column (1.5 × 25 cm) and after washing with water, the acid hydrolysate was applied to the column. The hydrolysate collected was treated with calcium oxide slurry and calcium carbonate to increase the pH to 4.5–6.0. Calcium sulfate formed was removed by filtration.

Analytical Methods

Sugar and alcohol were analyzed using a Hitachi high-performance liquid chromatographic system consisting of an AS-4000 Intelligent Auto Sampler, a Hitachi L3350 refractive index monitor, a Hitachi L-6000 pump, and a Hitachi D-2500 chromatointegrator. Separation was achieved using an organic acid column (Aminex HPX-87 H Ion Exclusion Column 300 × 7.8 mm, Bio-Rad, Hercules, CA) at 60°C with 0.008*N* sulfuric acid as eluant at 0.8 mL/min over a 18-min period. Figure 1 shows the liquid chromatography of acid hydrolysate without treatment.

RESULTS AND DISCUSSION

Comparison of Yeast

Table 1 shows the fermentation batches carried out with pure xylose under microaerobic conditions for the different yeast chosen. Only *C. guilliermondii* 20118, yeast mutant 11-2, *D. hansenii* NRRL Y-7426 showed substantial xylose consumption and xylitol production (0.72, 0.99, and 0.90g/L/h, respectively). The other strains were poor D-xylose utilizers under these fermentative conditions. The yeast mutant, *Candida* sp. 11-2, was chosen to perform the further experimentation.

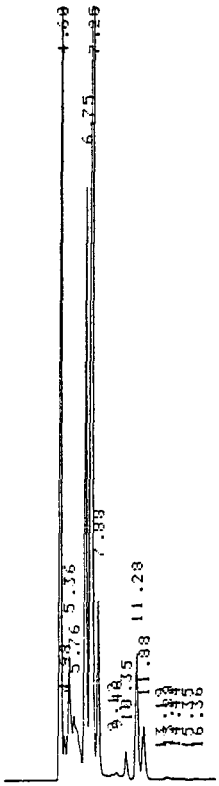

Fig. 1. Liquid chromatogram of acid hydrolysate. 4.60, phytic acid + sulfuric acid; 6.75, glucose; 7.25, xylose; 7.88, arabinose; 9.48, lactic acid; 10.35, glycrol; 11.28, acetic acid.

Comparison of Different Treatments and Different Dilutions

Table 2 shows the typical chemical composition of bagasse acid hydrolysate. Glucose and xylose are the fermentable sugars. Xylose (6.69%) can be fermented to xylitol by yeast, and glucose (2.39%) can be fermented to ethanol. Phytic acid present (4.87%) can be removed from hydrolysate by an alkaline treatment. Acetic acid (1.39%) can become an inhibitor when it is present in significant amounts. In order to increase the xylitol production, the simple neutralization of this hydrolysate was compared to other treatments, like activated charcoal and cation-exchange resins. The effect of the initial xylose concentration on yeast fermentation after each treatment was also studied. The results after 48 h of fermentation are shown in Table 3.

The increase in initial xylose concentration resulted in the decrease in the amount of xylose utilized in the "only neutralization" sample, indicating the presence of an inhibitor(s) in the hydrolysate that inhibits the utilization of xylose. The inhibitory effect can be removed by activated charcoal treatment. From our experience, approx 1 g of charcoal can treat about 20 mL of original sugar cane bagasse hemicellulose hydrolysate. In all the cases, xylitol yield from xylose is low when hydrolysates with different treatment were used as substrate. The reason for low xylitol yield is not known. We believe the extraneous chemicals present in the hydrolysate could inhibit the release of xylitol into the medium. This possibility is currently under investigation.

Table 1
Xylitol Production from Pure Xylose by Different Yeasts

Yeast	48 h				96 h		
	Initial xylose, g/L	Xylose used, %	Xylitol, g/L	Qp, g/Lh	Xylose used, %	Xylitol, g/L	Qp, g/Lh
C. parapsilosis 28474	152.72	26.94	6.72	0.14	88.32	17.84	0.19
C. guillierrnondii 20118	155.97	40.11	33.28	0.69	80.33	68.96	0.72
C. guilliermondii 42050	148.98	8.11	5.91	0.12	25.24	13.79	0.14
P. tannophilus 32691	152.78	14.42	0.91	0.02	45.91	0.88	0.01
Mutant of *Candida* sp. 11-2	152.47	54.77	56.35	1.17	97.33	95.35	0.99
D. hansenii NRRL Y-7426	149.50	93.89	105.82	2.21	100	86.00	0.90

Table 2
Typical Chemical Composition of Acid Hydrolysate

Constituent	Wt%
Moisture	83.64
Xylose	6.69
Glucose	2.39
Arabinose	0.79
Acetic acid	1.39
Phytic acid	4.87

Table 3
Xylitol Production after 48 h of Fermentation
for the Different Treatments and with Different Initial Xylose Concentrations

Treatment	Initial xylose, g/L	Xylose used, %	Xylitol, g/L	Qp, g/Lh	Ethanol, g/L)
Only neutralization	58.38	5.07	2.55	0.053	0
	43.25	8.38	4.19	0.073	1.38
	27.99	95.94	4.32	0.066	9.06
Activated charcoal	42.96	94.75	10.54	0.205	12.18
and neutralization	34.66	96.77	7.32	0.137	6.13
	22.70	96.31	4.62	0.071	1.83
Cation-exchange resins	45.80	83.94	10.10	0.195	7.185
and neutralization	32.58	90.31	8.32	0.158	2.011
	24.33	89.49	5.96	0.066	0.878

The lowest xylose consumption corresponded to the hydrolysate only neutralized. This is owing to the fact that there are substances in the fermentation media that make the fermentation processes difficult. Consequently, the xylitol production was very poor, especially when the media was not diluted, making the

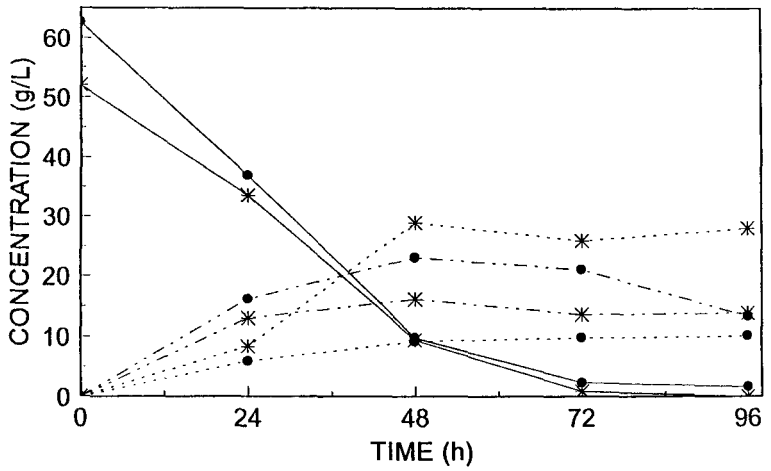

Fig. 2. Comparation of hydrolysates treated with activate charcoal. Without passing through a cation-exchange resin: (—●—) xylose concentration, (- -●- -) xylitol concentration, (—●- -) ethanol concentration. Passing through a cation-exchange resin: (—✳—) xylose concentration, (-✳-) xylitol concentration, (—✳- -)ethanol concentration.

effect of these substances stronger. However, the results show that the hydrolysates treated with activated charcoal or cation-exchange resins produced xylitol readily. This confirms that the samples without a pretreatment have significant inhibition. During the activated charcoal treatment, the acetic acid is removed. Some toxic byproducts formed during acid hydrolysis of cellulosic materials, such as 5-hydroxymethyl furfural and furfural (with significant fermentation inhibition *[6]*) and other chemicals toxic to microorganisms (like phenolic compounds, especially in its oxidized form *[7]*) are removed during these treatments. The highest xylitol productivity (0.205 g/L/h), corresponding to 10.54 g/L, was obtained from hydrolysate treated with activated charcoal. For this reason, it was selected to be the best treatment. Another reason is its low cost as compared to the cation-exchange resins.

Glucose and other sugars that can be present in the hydrolysate in insignificant amounts, like mannose and galactose, were utilized by the yeast for growth and ethanol production, but not for their corresponding polyol production. The amounts of ethanol found in some experiments were even higher than the xylitol concentration. This is because *Candida* sp. 11-2 is able to produce both ethanol and xylitol from xylose. The highest ethanol concentration (12.183 g/L) appeared under the same conditions that led to the highest xylitol concentration. Another sugar present, arabinose (0.79%), was hardly consumed. A similar behavior was observed by Meyrial et al. *(8)*.

Concentration of the Hydrolysate Treated with Activated Charcoal

The hydrolysate treated with activated charcoal was concentrated by vacuum evaporation in a rotavapor. An additional treatment with cation-exchange resins and neutralization was conducted. The xylitol production from that treated with cation-exchange resins was compared with that without the resin treatment. Figure 2 shows the results after 96 h of incubation. After 48 h, the highest xylitol production

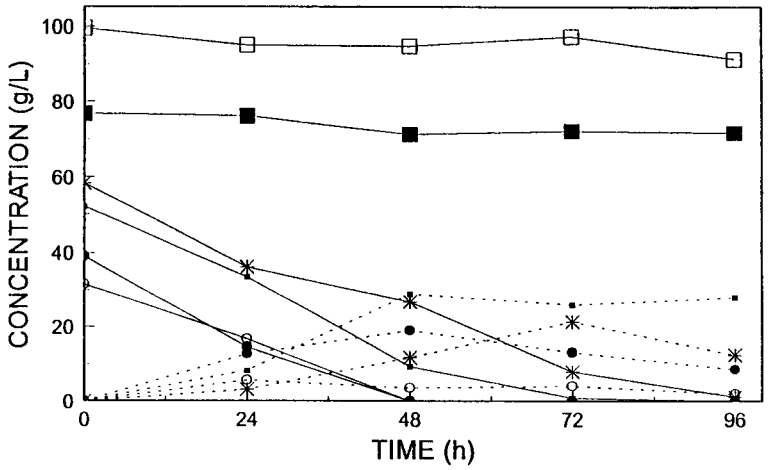

Fig. 3. Effect of the initial xylose concentration. Concentration of xylose with the following initial xylose concentrations: (—○—) 30 g/L, (—●—) 40 g/L, (—■—) 50 g/L, (—✳—) 60 g/L, (—■—)75 g/L, (—□—) 100 g/L. Concentration of xylitol with the following initial xylose concentration: (- -○- -) 30 g/L, (- -●- -) 40 g/L, (- -■- -) 50 g/L, (-✳-) 60 g/L.

was reached. When the hydrolysate was not treated with cation-exchange resins, the xylitol productivity and the final xylitol concentration after 48 h were 0.192 g/L/h and 9.22 g/L, respectively, whereas when it was treated, the xylitol productivity and the final xylitol concentration increased to 0.602 g/L/h and 28.90 g/L, respectively. When the hydrolysate was treated with resins, the ethanol concentration was lower (16.10 g/L) than the xylitol production (28.90 g/L), but when the hydrolysate was not treated with resins, the ethanol production was higher (23.03 g/L) than the xylitol production (9.22 g/L).

Effect of the Initial Xylose Concentration

The hydrolysate treated with activated charcoal was concentrated to obtain different concentrations of initial xylose. After being applied to a column with cation-exchange resins, the hydrolysates were neutralized and supplemented with nutrients. Figure 3 shows the fermentation results after 96 h with different initial xylose concentrations. The rate of xylitol production increased with increasing initial xylose concentration from 30 to 50 g/L, reaching a maximum of 28.90 g/L after 48 h of fermentation. The decrease in xylitol production is dramatic with a further increase in the initial xylose concentration. With an initial xylose concentration of 60 g/L the xylose was mostly utilized and reached the maximum xylitol production of 21.35 g/L after 72 h. With initial xylose concentration higher than 75 g/L, xylose was not consumed at all.

Figure 4 shows the ethanol production from different initial xylose concentrations. Ethanol increased with increasing initial xylose concentration to 60 g/L, reaching a maximum of 26.87 g/L after 72 h of fermentation. Ethanol production was very low when the initial xylose concentration in the hydrolysate was 75 g/L or higher.

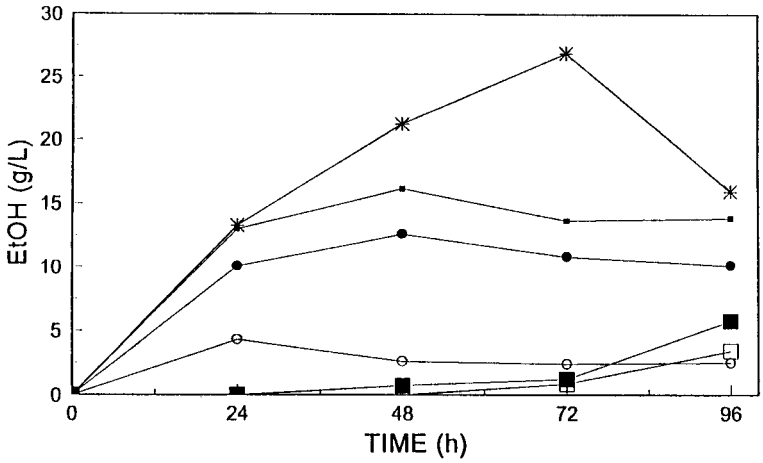

Fig. 4. Ethanol production with the following initial xylose concentrations: (—○—) 30 g/L, (—●—) 40 g/L, (—▪—) 50 g/L, (—✳—) 60 g/L, (—■—)75 g/L, (—□—) 100 g/L.

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