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Pretreatment of Sugar Cane Bagasse Hemicellulose Hydrolyzate for Ethanol Production by Yeast

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ABSTRACT

Sugar cane bagasse hemicellulose hydrolyzate was prepared by dilute sulfuric acid (3% w/v) hydrolysis with a high-solid, low-liquid ratio followed by leaching. The hydrolyzate contains 11% (w/v) of fermentable sugars with xylose as the major component, which comprises up to 75% of the total reducing sugars. The neutralized hydrolyzate exhibited strong inhibition toward cell growth and ethanol production by yeasts. The inhibitory effect of hydrolyzate can be alleviated by treating hydrolyzate either with ion-exchange resins or with acidified activated charcoal.

Index Entries: Sugar cane bagasse; hemicellulose hydrolysis; xylose; ethanol; activated charcoal.

INTRODUCTION

Sugar cane bagasse is the fibrous residue obtained after the extraction of sugar from sugar cane. Traditionally, it has been used as fuel in sugar mills and raw materials for hydroxymethyl furfural production. Bagasse has also been used as raw materials for paper pulp, acoustical boards, press woods, and agricultural mulch. Similar to other agriculatural residues, bagasse contains a significant portion of hemicellulose materials that can be hydrolyzed to its constituent carbohydrates easily. The hydrolysis product contains a mixture of sugars with xylose as the major component (*1*). These sugars as well as cellulose-derived carbohydrates are

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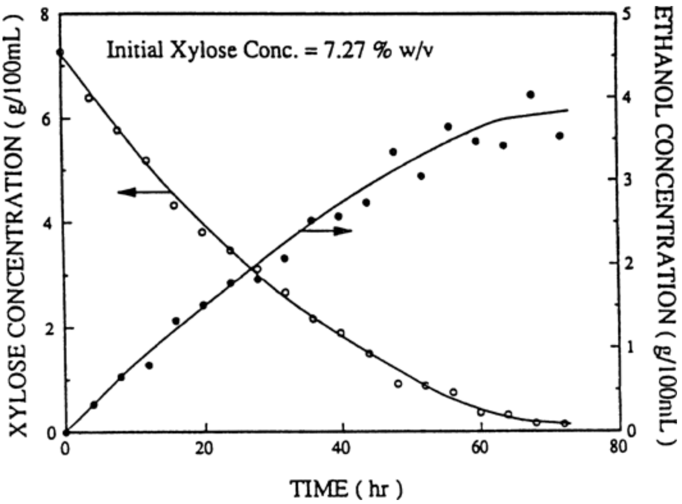

Fig. 1. Production of ethanol by *Candida* yeast from D-xylose. ○ Xylose; ● ethanol.

potential substrates for fermentation to useful products. Previously, obtaining hemicellulose carbohydrates from bagasse using dilute acid hydrolysis with a high-solid, low-liquid ratio has been reported. The hydrolyzate contains over 11% (w/v) of fermentable sugars with xylose comprising up to 75% of the total reducing sugars. It has also been shown that ethanol and xylitol could be produced from acid hydrolyzate by yeasts if the hydrolyzate was treated with an cation-exchange resin (2,3). In order to prepare hydrolyzate suitable as fermentation substrate economically, experiments have been undertaken to remove extraneous materials that would otherwise interfere with fermentation. This article reports the treatment of hydrolyzate by acidified activated charcoal as compared to the simple neutralization and the more costly ion-exchange resin treatment.

MATERIALS AND METHODS

Microorganisms

Candida sp. was selected with respect to its ability to convert xylose to ethanol (*see* Fig. 1). Cultures were maintained on Bacto-yeast extract, Bacto-malt extract, Bacto-peptone, xylose agar slants (YMA-Difco).

Acid Hydrolyzate

Sugar cane bagasse hemicellulose hydrolyzate was obtained by hydrolysis in dilute sulfuric acid (3% w/v) at 100°C followed by downflow leaching using water according to the method described by Ladisch (4). Hydrolyzate has a low pH value and was kept at room temperature.

Alkaline Treatment and Neutralization

The pH of the hydrolyzate was adjusted to 4.5 by addition of calcium oxide slurry. The mixture was filtered to remove calcium sulfate. After filtration, additional CaO slurry was added to a pH of 8.5. The precipitates formed were removed by centrifugation. Concentrated phosphoric acid was then added to bring pH down to 7.

Activated Charcoal Treatment

Activated charcoal (Savannah Foods & Industrials, Inc., Savannah, GA) was washed and equilibrated with dilute sulfuric acid (0.05*M*) after being packed into a column (1.5×25 cm). Acid hydrolyzate was applied to the column at a volume ratio of 250 mL hydrolyzate to 20 mL of void volume. Samples were collected after void volume, and column was eluted with water to ensure the recovery of sugars. Activated charcoal-treated samples were concentrated by vacuum evaporation to their original concentrations.

Cation-Exchange Resins Treatment

Acid hydrolyzate was applied to a column with strong acid cation-exchange resins (Dowex 50W-X4, 200–400 mesh) that was previously washed and equilibrated with dilute sulfuric acid (0.05*M*). Samples were collected after void volume and concentrated to their original concentrations with vacuum evaporation at 40°C.

Fermentation of Hydrolyzates

Pretreated neutralized hydrolyzates used in fermentation experiments were supplemented with nutrients with the following composition per liter: Bacto-yeast extract, 1.5 g; Bacto-Peptone, 2.0 g; and Bacto-malt extract, 1.5 g. Flask cultures used for fermentation were capped with rubber stoppers; carbon dioxide produced was allowed to escape through a hypodermic needle.

Analytical Methods

Sugars and organic acids were analyzed using a Waters Associates high-performance liquid chromatographic system consisting of a model 660 solvent programmer, a refractive index detector, a 712 WISP unit, a Dionex gradient pump, and a Hewlett-Packard 3390A report integrator. Separation was achieved using an organic acid column (ORH-801, Interaction Chem. Inc., Mountain Views, CA) at room temperature with 0.01*N* sulfuric acid as eluant as 0.3 mL/min over a 30-min period. Figure 2 shows the liquid chromatogram of acid hydrolyzate. Ethanol was quantified using

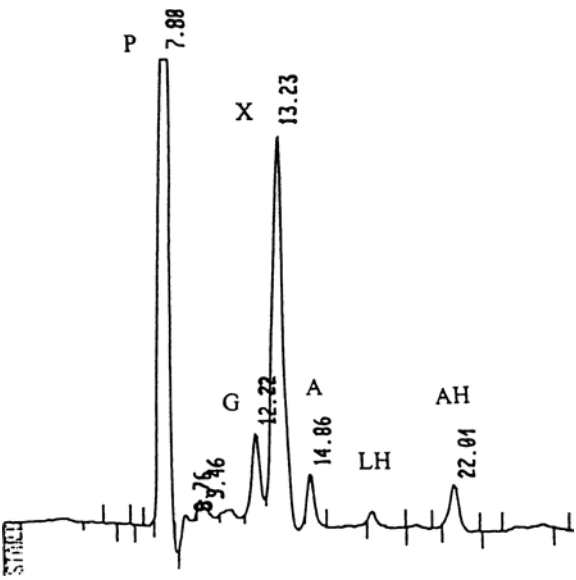

Fig. 2. Liquid chromatogram of acid hydrolyzate. P = phytic acid + sulfuric acid, G = glucose, X = xylose, A = arabinose, LH = lactic acid, and AH = acetic acid.

gas chromatography, and phytic acid was estimated according to the procedures of Reddy et al. (*5*) following alkaline precipitation of phytic acid from hydrolyzate.

RESULTS AND DISCUSSION

The chemical composition of bagasse acid hydrolyzate is shown in Table 1. Total fermentable sugars (xylose + glucose) is about 11% with small amounts of arabinose (1.1%), acetic acid, (1.67%), and lactic acid (0.7%). Phytic acid content is about 2% and can be removed from hydrolyzate on alkaline treatment. Hydrolyzate has a pH value of below 1 from the presence of about 3% of sulfuric acid. Because of its low pH value, the hydrolyzate can be kept at room temperature indefinitely without loss of its sugar content.

Table 2 summarizes the results of ethanol production from treated hydrolyzates by yeasts. Results show ethanol was produced readily from ion-exchange-resins-treated sample. The amounts of ethanol produced reached about 4% after 96 h of incubation. Comparable results, although at a slower rate, were obtained from activated charcoal-treated sample. Alkaline-treated sample showed significant inhibition as compared to ion-exchange-resins-treated sample.

It has been reported that toxic byproducts from carbohydrates, such as 5-hydroxymethyl furfural and furfural can be formed during acid hydrolysis of cellulosic materials, and these chemical have been shown to

Table 1
Chemical Composition of Acid Hydrolyzate

Constituent	Wt %
Moisture	83.7
Xylose	9.06
Glucose	1.88
Arabinose	1.10
Acetic acid	1.67
Lactic acid	0.70
Phytic acid	2.10

Table 2
Effect of Treatment of Hydrolyzates on Ethanol Production by *Candida sp.*

	Ethanol produced, % w/v			
Treatment	24 h	48 h	72 h	96 h
Alkaline	0.65	0.93	1.82	2.40
Activated charcoal	0.80	1.83	2.21	3.65
Cation exchange	1.10	2.10	2.90	4.20

inhibit fermentation (*6*). Other chemicals, such as phenolic compounds, especially in its oxidized form, are toxic to microorganisms (*7*). Results of fermentation experiments indicate those toxic materials could have been removed by ion-exchange resins.

Activated charcoal has been demonstrated to be a standard methodology for removal of dissolved organic matters in waste water treatment and for the removal of pigments in sugar industries. When acidified activated charcoal was used to treat hydrolyzate, the adsorption of pigments was effective, and the recovery of sugars was almost 100%. Although some inhibition of rate of fermentation was observed, the growth of cells was not affected.

In summary, this study has demonstrated the importance of the pretreatment of hemicellulose hydrolyzate to be used as fermentation substrate. The economics dictate the extent of pretreatment. For the production of ethanol by yeast from acid hydrolyzate, the use of activated charcoal is preferred because of its low cost and low capital investment as compared to the use of ion-exchange resins.

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