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The Effect of Cell Density on the Production of Xylitol from D-Xylose by Yeast

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ABSTRACT

The rate of xylitol production from D-xylose increased with increasing yeast cell density. The optimal temperature for xylitol production is 36°C, and the optimal pH range is from 4.0 to 6.0. At high initial yeast cell concentration of 26 mg/mL, 210 g/L of xylitol was produced from 260 g/L of D-xylose after 96 h of incubation with an indicated yield of 81% of the theoretical value.

Index Entries: D-xylose; xylitol; *candida sp.*; fermentation; yeast.

INTRODUCTION

Xylitol, a naturally occurring five-carbon sugar alcohol, is a minor constituent of many fruits and berries (*1*). It is also a normal metabolic intermediate in mammalian cardohydrate metabolism. For example, the human body produces 5–15 g of xylitol/d under normal metabolism.

Xylitol is a sweetner with sweetness Eq of 1 that can replace sucrose on a weight-for-weight basis. Xylitol has a substantially lower viscosity than sucrose and has a negative heat effect when dissolved in a solution. With these properties, xylitol has found its increasing usage in the food industry, especially in confectionery products (*2*).

Production of xylitol from its natural source is impractical because of the relatively small quantities in which it occurs. Traditionally, xylitol is

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produced through chemical reduction of purified xylose or xylose-containing materials, such as birch hemicellulose hydrolyzates (3). However, the purification of xylose and the separation of xylitol from other sugar alcohols are difficult, making xylitol an expensive product, thus limiting its utilization. To expand its utilization, a cheaper source of xylitol will be helpful.

Xylitol can be produced from xylose through biological hydrogenation. Many yeast species, especially those belonging to *Candida* species, are good xylitol producers (4,5). These yeast species possess an active reductive enzyme, xylose reductase, which catalyzes the reduction of xylose as the initial step in xylose metabolism, often resulting in the secretion of xylitol extracellularly as a metabolic byproduct.

Previously, we have shown that high concentrations of xylitol can be produced from xylose by a *Candida* yeast (6). We have also shown that xylitol can be readily produced from sugar cane hemicellulose hydrolyzates (7,8). In this study, we investigate the effect of different initial yeast cell concentrations on the rate of xylitol production from xylose.

MATERIALS AND METHODS

Microorganisms

Candida sp. B-22 was selected with respect to its ability to produce high concentrations of xylitol from xylose. Cultures were maintained on Bacto-yeast extract, Bacto-malt extract, Bacto-peptone, and xylose agar slants (YMA-Difco).

Substrates

D-Xylose, D-glucose, L-arabinose, xylitol, and L-arabitol were purchased from Sigma Chemical Company (St. Louis, MO).

Media

Yeast extract-malt extract-peptone (YMP) medium was used for growth and fermentation. It contained the following composition per liter: Bacto-yeast extract, 3 g: Bacto-malt extract, 3 g; and Bacto-peptone, 5 g. Concentration of the carbon source in growth media was 10 g/L.

Fermentation

Shake-flask experiments were conducted in 125-mL Erlenmeyer flask (in duplicate), each containing 20 mL YMP with appropriate amounts of substrates. Sugar solution was autoclaved separately. Inocula were prepared by growing yeast cells in flasks in the same media with 2% xylose for 48 h. Yeast cells were harvested aseptically by centrifugation. After washing the yeast pellets with sterile water, yeast cells were transferred

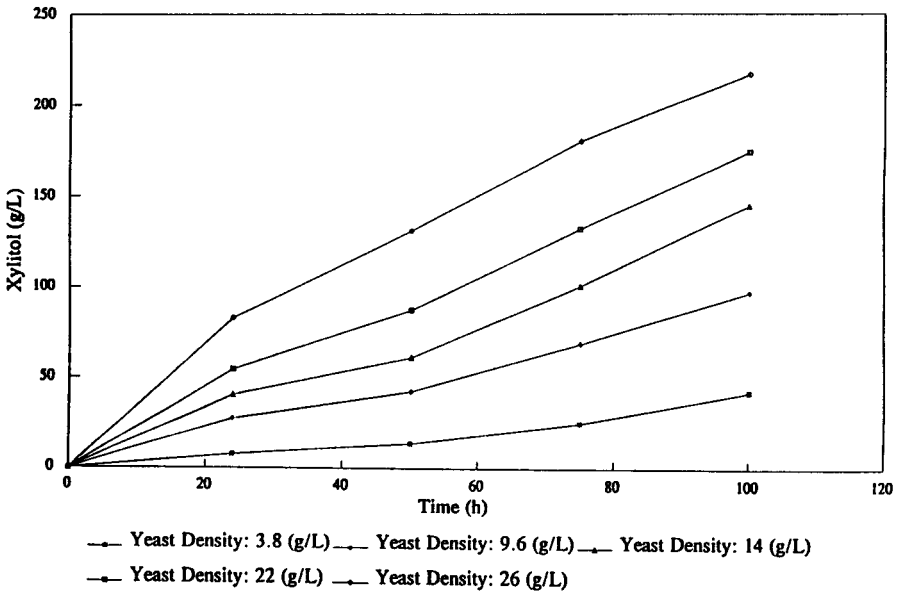

Fig. 1. Effect of yeast concentration on the production of xylitol from xylose. The temperature was 35°C, the pH was 5.4, and xylose concentration was 260 g/L.

to fermentation substrates. Fermentations were carried out aerobically at 35°C and 200 rpm in a incubator shaker. After fermentation, samples were taken in duplicate and subjected to analysis.

Analytical Methods

Carbohydrates were analyzed using a Water Associates high-performance liquid chromatographic system consisting of a model 660 solvent programmer, a refrective 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 ambient temperature with 0.01*N* sulfric acid as eluant at 0.3 mL/min over 30-min period. Ethanol was quantified using gas chromatography.

Determination of Cell Dry Weight

Samples were collected by centrifugation. The cell pellets were washed in distilled water, pelleted again, collected, and dried at 85°C for 48 h.

RESULTS AND DISCUSSION

The time-course for the production of xylitol from xylose at a high initial concentration of xylose (26%, w/v) vs initial cell concentration is shown in Fig. 1. The increase in the rate of xylitol production was linear

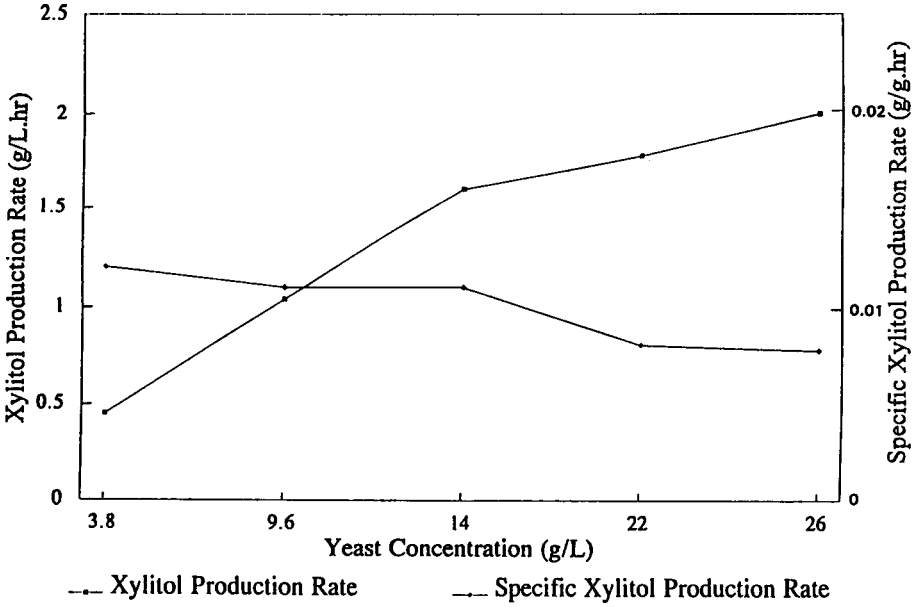

Fig. 2. Effect of yeast concentration on xylitol production rate and specific xylitol production rate.

over an initial yeast concentration range of 3.8–26 mg/mL (Fig. 2). It is apparent that a high concentration of xylose can be rapidly and efficiently converted to xylitol at relatively high cell densities. The decrease in fermentation time is dramatic with high-cell-density fermentation as compared to low-cell-density fermentation. With high-cell-density fermentation, the completion of biological conversion was obtained after 100 h of incubation. At a lower cell concentration, the fermentation time increased significantly resulting in a difference of 80% in xylitol yield between cell density of 3.8 and 26 mg/mL after 100 h of incubation.

Some reductions in specific xylitol production rates were observed at high initial cell densities (Fig. 2). This is similar to the typical ethanol fermentation in that high cell densities often resulted in a sharp decrease in specific ethanol production rate. The reduction in specific xylitol production rate is not as profound as in ethanol fermentation and could be in part the result of oxygen limitations.

The initial fermentation rate of xylose to xylitol was relatively constant over a temperature range of 35–40°C (Fig. 3). At an incubation temperature of 45°C or higher, the fermentation rate was sharply reduced. Whereas pH has no significant effect on xylitol production over a range of 4–6. Fermentation byproducts include glycerol (<1%) and ethanol (0.2–0.5%) in all the fermentations.

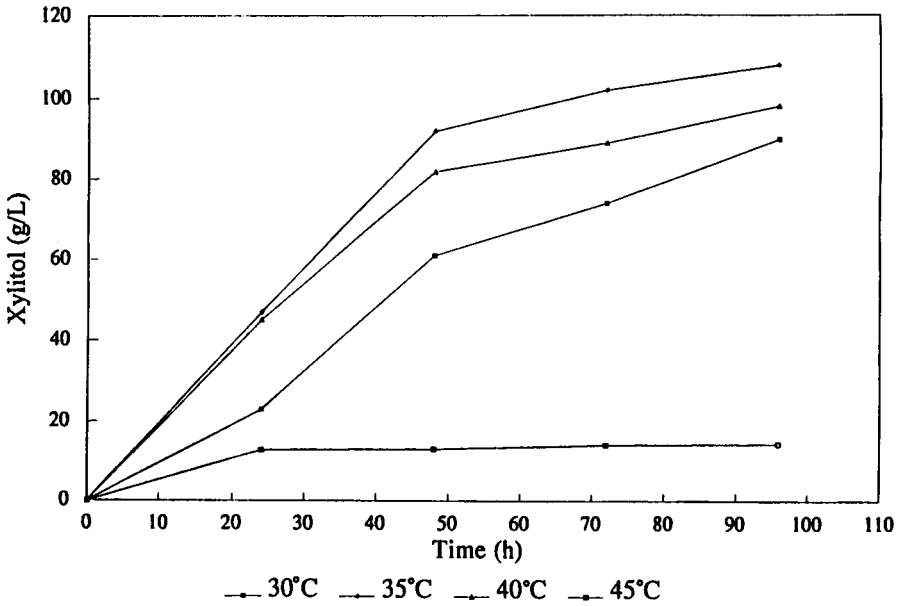

Fig. 3. Effect of temperature on the production of xylitol from xylose by yeast. The pH was 5.4, the cell concentration was 14 g/L, and xylose concentration was 150 g/L.

In summary, high initial yeast cell concentration is beneficial for the biological conversion of xylose, producing high concentrations of xylitol as end product. Since high concentration of xylitol can be produced with only small amounts of byproduct, the recovery of xylitol can be achieved without elaborate purification process.

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