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Production of Xylitol from D-Xylose by *Debaryomyces hansenii*

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ABSTRACT

Xylitol, a naturally occurring five-carbon sugar alcohol, can be produced from D-xylose through microbial hydrogenation. Xylitol has found increasing use in the food industries, especially in confectionary. It is the only so-called "second-generation polyol sweeteners" that is allowed to have the specific health claims in some world markets. In this study, the effect of cell density on the xylitol production by the yeast *Debaryomyces hansenii NRRL Y-7426* from D-xylose under microaerobic conditions was examined. The rate of xylitol production increased with increasing yeast cell density to 3 g/L. Beyond this amount there was no increase in the xylitol production with increasing cell density. The optimal pH range for xylitol production was between 4.5 and 5.5. The optimal temperature was between 28 and 37°C, and the optimal shaking speed was 300 rpm. The rate of xylitol production increased linearly with increasing initial xylose concentration. A high concentration of xylose (279 g/L) was converted rapidly and efficiently to produce xylitol with a product concentration of 221 g/L was reached after 48 h of incubation under optimum conditions.

Index Entries: *Debaryomyces hansenii*; D-xylose; xylitol; biological hydrogenation; yeast.

INTRODUCTION

Sucrose is one of the most important ingredients of confectionary products, as it provides body, texture, and preservative properties, besides its sweetening effect. However, it is well known that consumption of

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sucrose and fermentable carbohydrates facilitates the development of plaque, dental caries, and periodontal disease. To avoid these problems, noncariogenic polyol sweeteners are increasingly used. One of these, xylitol, may be considered as the best of all nutritive sweeteners because it has anticaries properties *(1)*. Besides, it is tolerated by diabetics and it has a high negative heat of solution. For all these properties, xylitol is desirable for sugar-free confections *(2)*. Unfortunately, it is one of the most expensive polyol sweeteners *(3)*. Availability and cost of production are the obstacles impeding the increased use of xylitol.

Xylitol is a normal metabolic intermediate in animals. The human body produces 5–15 g of xylitol per day during normal metabolism *(2)*. Xylitol occurs naturally in many fruits and vegetables (such as lettuce, cauliflower, strawberries) and constitutes part of the human diet. However, fruits and vegetables contain a small amount, usually less than 900 mg/100 g, rendering its extraction uneconomical. In industrial scale, it can be produced through chemical reduction of xylose derived from hemicellulosic hydrolyzate. This process includes extensive purification and separation steps to remove polymers of other sugars and other by-products present in the raw materials *(4)*.

Xylitol can be formed too, as a metabolic intermediary product of D-xylose fermentation: D-xylose can be converted to xylitol by NADPH-dependent aldehyde reductase, or can be isomerized to D-xylulose by D-xylose isomerase, and then reduced to xylitol by NADH-dependent xylitol dehydrogenase *(5)*. Many yeast strains have the ability to produce xylitol from xylose extracellularly as a normal metabolic activity *(6)*. The prominent strains that produce xylitol include *Candida sp. (7)*, *C. guillermondi (8–10)*, *C. boidinii (11)*, *C. tropicalis (12)*, *C. parapsilosis (13)*, and *D. hansenii (14,15)*. However, D-xylose is an expensive substrate for xylitol production. Recent developments in obtaining xylose-rich hemicellulose hydrolyzates from lignocellulosic materials have identified economic source of xylose availability *(16)*. As a result, xylitol can be produced from such materials as an option for effective utilization of lignocellulosic biomass *(17)*.

In this report, we studied some characteristics of *D. hansenii* NRRL Y-7426 for xylitol production from xylose.

MATERIALS AND METHODS

Micro-organism

The yeast strain used in this study, *D. hansenii NRRL Y-7426*, was obtained from the Northern Regional Research Laboratory, (Peoria, IL). The yeast was grown for 3 d at 32°C in an incubator shaker at 200 rpm (New Brunswick), in a liquid media with 1% of glucose, 1% of xylose, 3 g/L of Bacto-yeast extract, 3 g/L of Bacto-malt extract, and 5 g/L of Bacto-peptone.

Fermentation

Shake flask fermentation experiments were carried out at 24°C, in 50-mL Erlenmeyer flasks (containing 10 mL of culture media) placed in a gyratory shaker at 180 rpm for 2 to 4 d. The sterile culture medium was prepared with pure xylose (12%) and supplemented with nutrients with the following composition per liter (pH 5.7): Bacto-yeast extract, 3 g; Bacto-malt extract, 3 g; and Bacto-peptone, 5 g.

Analytical Methods

Xylose and xylitol were analyzed using a Hitachi high-performance liquid chromatographic system consisting of an AS-4000 Intelligent Auto Sampler, a Hitachi L-3350 refractive index monitor, a Hitachi L-6000 pump, and a Hitachi D-2500 chromato-integrator. Separation was achieved using an organic acid column (Aminex HPX-87 H Ion Exclusion Column 300 × 7.8 mm, Bio-Rad, Hercules, CA) at 0.81 bar and 60°C with 0.01 N sulfuric acid as eluant at 0.8 mL/min over a 18-min period.

Determination of Cell Dry Weight

The cells were collected by centrifugation and dry weight was determined.

RESULTS AND DISCUSSION

Effect of the Cell Density

Figure 1 shows the effect of cell density on the xylitol production from D-xylose by *D. hansenii NRRL Y-7426*. When the initial yeast concentration increased gradually from 0.3 g/L to 3 g/L, the xylitol production increased from 60.1 g/L to 105.8 g/L after 72 h of fermentation. However, when the initial yeast concentration increased to 7.5 and 15 g/L, there was a little decrease in xylitol production. With 3 g/L of initial yeast concentration, xylose was rapidly converted to xylitol. In contrast, the rate of xylose utilization was slow and xylitol assimilation was lower at initial yeast concentration of 0.3 g/L. The highest xylitol productivity, 1.47 g/L/h, was obtained at 3 g/L of initial yeast concentration after 72 h of fermentation where 105.8 g/L of xylitol was produced. Similar results were reported by Cao et al. *(18)*, with *Candida* sp. B-22, the rate of xylitol production increased with increasing initial yeast concentration. After 72 h of fermentation, xylitol concentration decreased slowly as a result of lower residual xylose concentration and the assimilation of xylitol itself.

Effect of the Initial pH

The effect of the pH was studied using the same amount of yeast cells that led to the highest xylitol productions. Figure 2 shows the results after

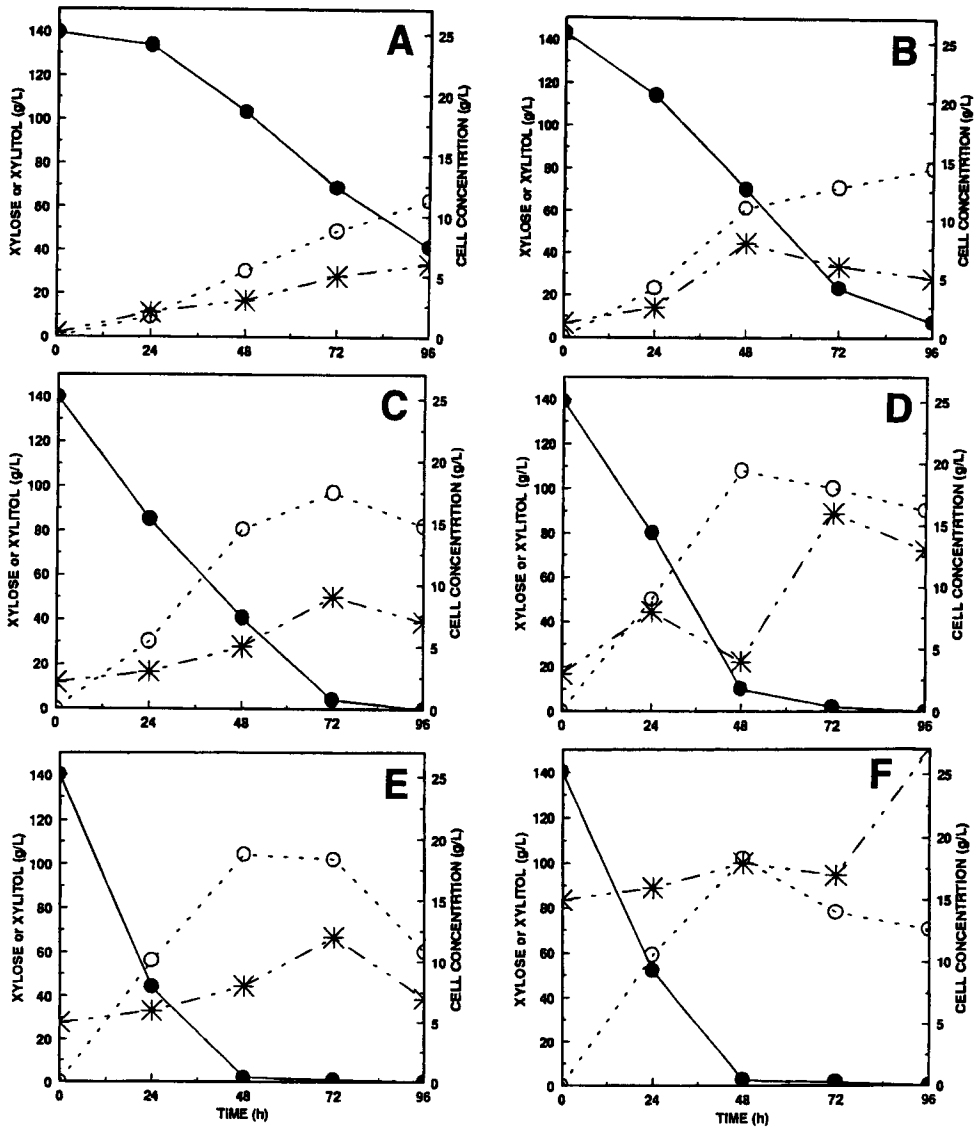

Fig. 1. Effect of the cell density on the production of xylitol from xylose by *D. hansenii*. Symbols: ●—●, xylose; ○- - -○, xylitol; ✳— · ·✳, cell concentration. **(A)** 0.3 g/L, **(B)** 1.2 g/L, **(C)** 2.1 g/L, **(D)** 3 g/L, **(E)** 7.5 g/L, **(F)** 15 g/L.

48 h of fermentation under different initial pH. The optimal pH range for xylitol production was between 4.5 and 5.5. The xylitol concentration and the xylitol productivity were 86.29 g/L and 1.80 g/L/h at initial pH of 4.5. Whereas at initial pH of 5.5, xylitol concentration was 91.91 g/L and the productivity was 1.91 g/L/h. The xylitol concentrations at pH 3 and 6.5 were 69.91 g/L and 66.53 g/L, respectively. The xylitol concentration was lowest at both pH 8.0 (49.02 g/L) and pH 2.0 (53.32 g/L). The results indi-

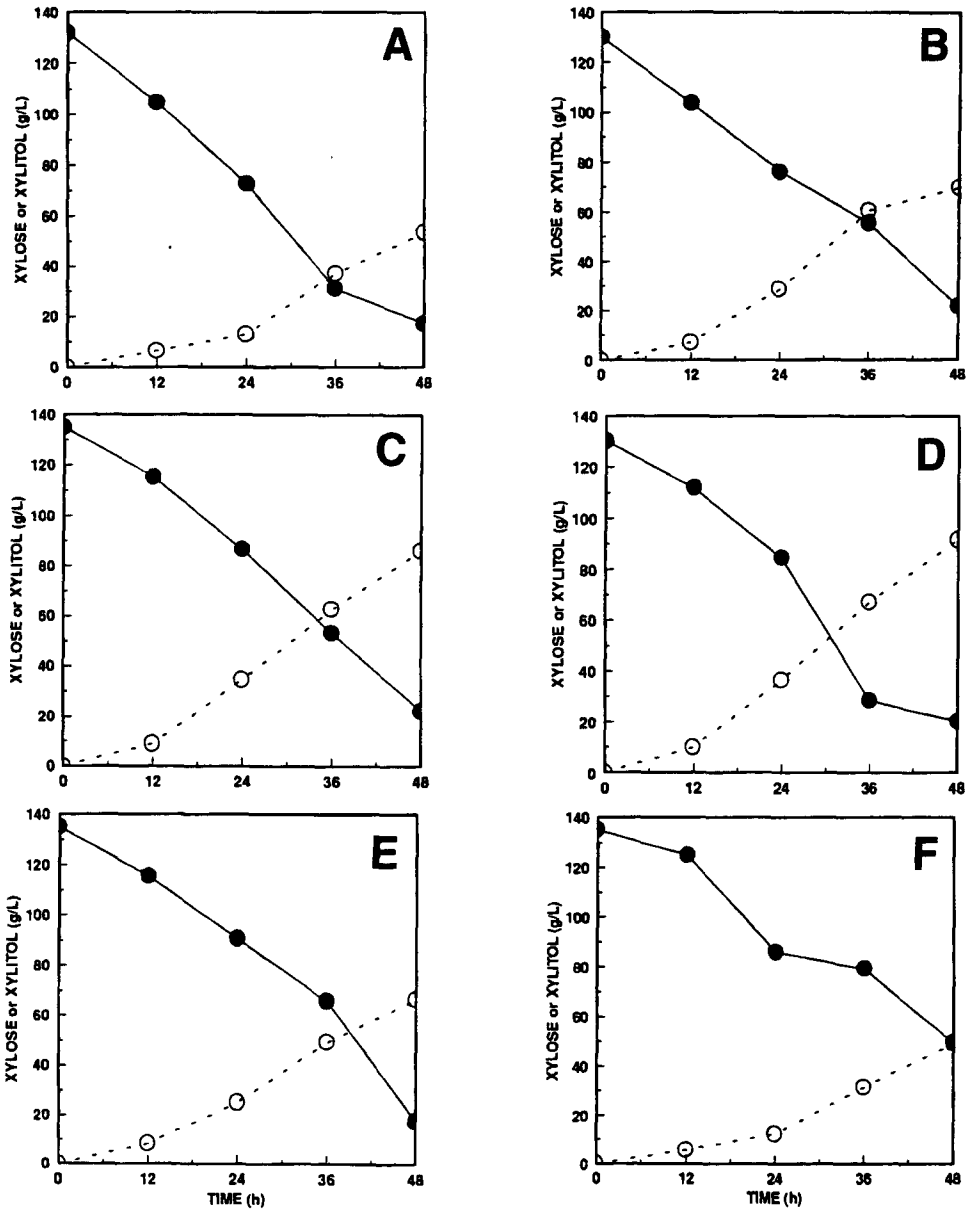

Fig. 2. Effect of the initial pH on the production of xylitol from xylose by *D. hansenii*. Symbols: ●—●, xylose; O- - -O, xylitol. **(A)** pH = 2, **(B)** pH = 3, **(C)** pH = 4.5, **(D)** pH = 5.5, **(E)** pH = 6.5, **(F)** pH = 8.

cated that both the xylitol productivity and xylitol yields were influenced by initial pH. However, yeast cell growth is relatively resistant to pH change. This is indicated by the ability of *D. hansenii* to utilize xylose to produce xylitol at a relatively low pH environment.

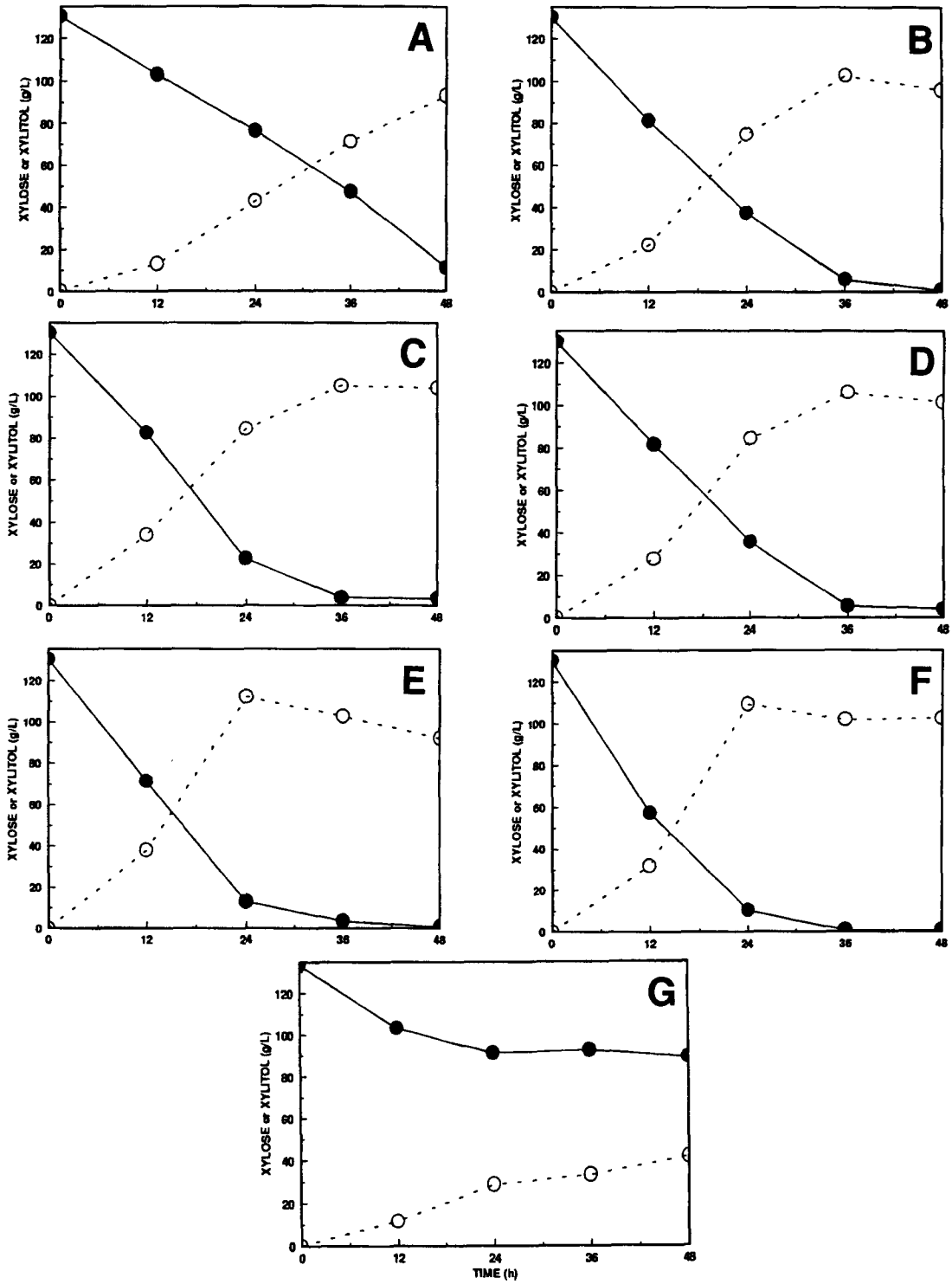

Fig. 3. Effect of the temperature on the production of xylitol from xylose by *D. hansenii*. Symbols: ●——●, xylose; ○- - -○, xylitol.**(A)** 24°C, **(B)** 28°C, **(C)** 30°C, **(D)** 32°C, **(E)** 35°C, **(F)** 37°C, **(G)** 44°C.

Effect of the Temperature

The effect of temperature was studied at optimal pH 5.5 with 3 g/L initial yeast concentration for xylitol production from pure xylose. Figure 3 shows the fermentation carried out with the different temperatures chosen. The fermentation rate of xylos e to xylitol was relatively constant over a temperature range of 28 to 37°C, and in this interval of temperatures, xylose was consumed rapidly and xylitol was produced efficiently (around 100 g/L). At 24°C, fermentation of xylose to xylitol was carried out slowly, although it reached a high xylitol concentration of 93.04 g/L. The decrease in fermentation rate was dramatic with a high temperature of 44°C as xylose utilization was slow, and xylitol production was lower (41.88 g/L). Cao et al. *(18),* found that the initial fermentation rate of xylose to xylitol with *Candida* sp. B-22 was relatively constant over a temperature range of 35–40°C, however at incubation temperature of 45°C or higher, the fermentation rate was sharply reduced.

Effect of the Shaker Speed

The shaker speed is related to the concentration of dissolved oxygen that plays an important role in the fermentation of xylose into xylitol. With high aeration, the xylose fermentation shifted to growth phase, whereas under low speeds (anaerobic conditions) xylose could not be assimilated by yeast without NADH-linked xylose reductase. To improve the xylitol production, microaerobic conditions are required. Figure 4 shows the fermentation carried out at 32°C with three different shaker speeds during 48 h. When the shaker speed was 100 rpm, xylose was consumed slowly and the xylitol production was only 40.55 g/L. This is because of the low speed required to dissolve enough oxygen in the media. Working with 300 rpm, xylose was consumed rapidly to produce xylitol (99.67 g/L). Whereas at 200 rpm, xylose was consumed slowly, but reached a xylitol concentration of 113.54 g/L. Both 300 and 200 rpm can be considered microaerobic conditions. Parajó et al. *(15)* found aerobic condition in 300 rpm working with bigger flasks, this is because of the concentration of dissolved oxygen that was higher under these conditions. Figure 4 shows the yeast concentration under the different shaker speeds. At 100 rpm there was a little increase in yeast concentration. Whereas at 200 and 300 rpm, the increase in cell concentration was much higher.

Effect of the Initial Xylose Concentration

The effect of the initial xylose concentration was studied at shaker speed of 300 rpm. Figure 5 shows the xylose consumption and the xylitol production after 48 h of fermentation. Under these microaerobic conditions, all xylose was consumed after 48 h of incubation and xylitol production started without lag period. With low initial xylose concentrations,

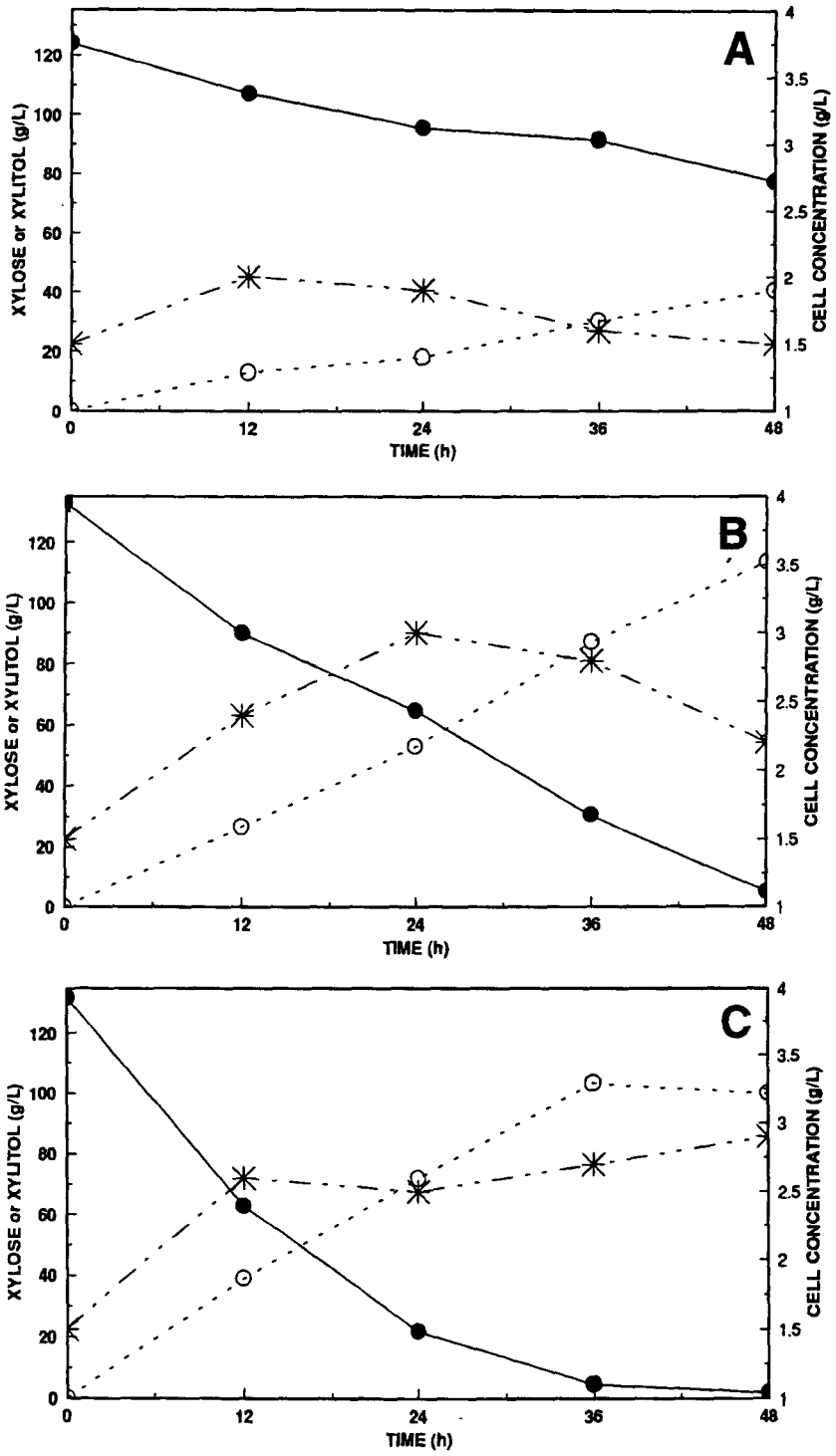

Fig. 4. Effect of the shaker speed on the production of xylitol from xylose by *D. hansenii*. Symbols: ●——●, xylose; O- - -O, xylitol; ✳— ··✳, cell concentration. **(A)** Shaker Speed = 100 rpm; **(B)** Shaker Speed = 200 rpm; **(C)** Shaker Speed = 300 rpm.

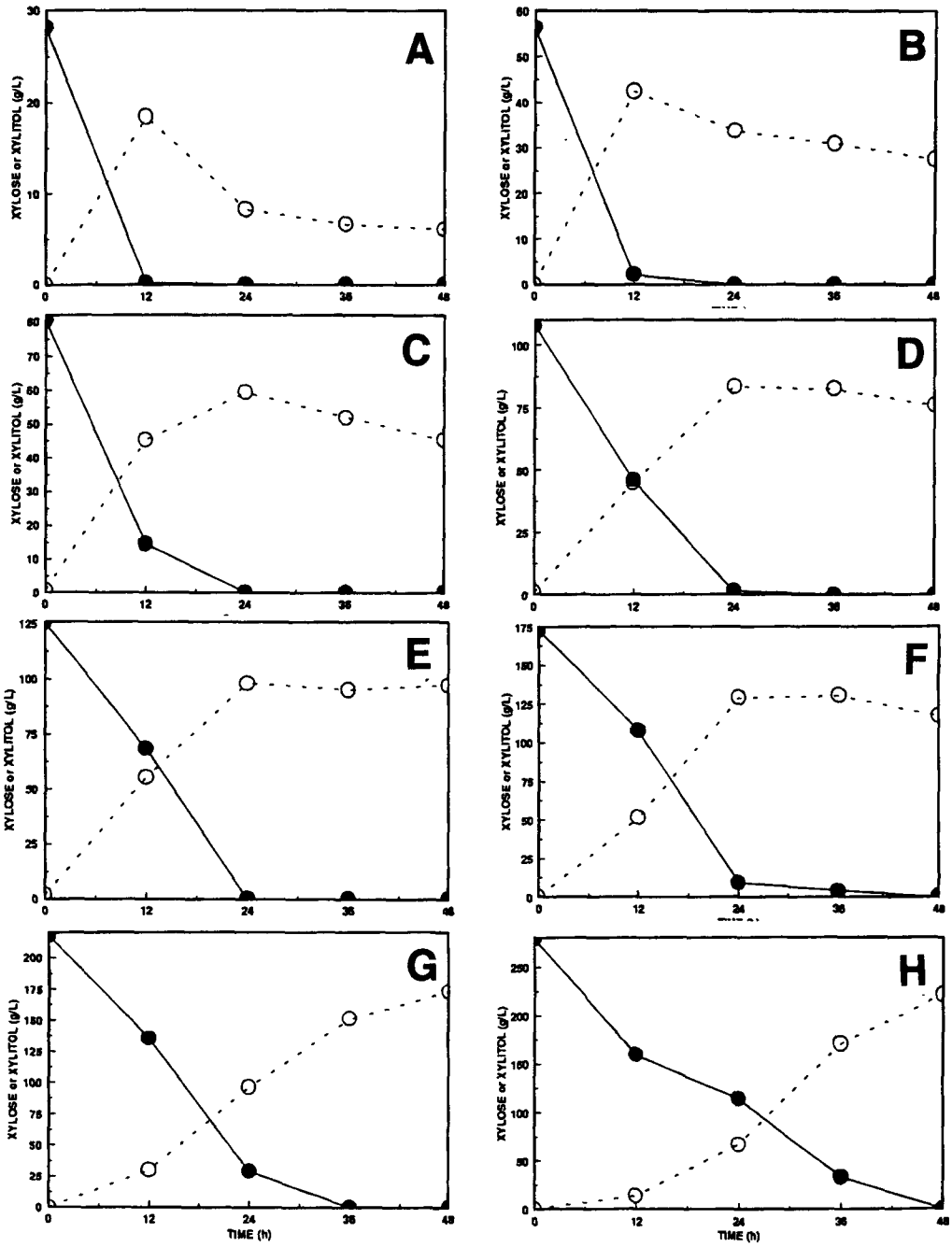

Fig. 5. Effect of the initial xylose concentration on the production of xylitol from xylose by *D. hansenii*. Symbols:●——●, xylose; ○- - -○, xylitol. **(A)** X = 30 g/L; **(B)** X = 60 g/L; **(C)** X = 80 g/L; **(D)** X = 110 g/L; **(E)** X = 125 g/L; **(F)** X = 175 g/L; **(G)** X = 220 g/L; **(H)** X = 280 g/L.

xylose was converted rapidly and efficiently into xylitol. When the initial xylose concentration was very high at 217 and 279.24 g/L, xylose was consumed slowly at first, a result of the high osmotic pressure. After 24 h, when xylose decreased, xylose was consumed rapidly and reached a xylitol concentration of 173.08 g/L with a xylitol productivity of 3.6 g/L/h when initial xylose concentration was 217 g/L. At an initial xylose concentration of 279.24 g/L, final xylitol concentration was 221.12 g/L after 48 h of incubation with a productivity of 4.6 g/L/h. The xylitol weight yields were 79.76 and 79.19% for xylose concentration of 217 and 279.4 g/L, respectively. Similar effect of high initial xylose concentration on yeast productivity was observed by Meyrial et al. (*10*) with *C. guillermondii* at an initial xylose concentration of 300 g/L.

CONCLUSIONS

Xylose, the feedstock for xylitol, is abundant in nature in the form of hemicellulose as the major component of lignocellulose materials. It can be easily obtained by hydrolysis of hemicellulose. The use of this yeast or other high-xylitol producing yeast strains for xylitol production from biomass-derived xylose can provide a firm basis for expansion of supply of xylitol in food arena.

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