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Fermentation of Xylose to Ethanol by *Pichia stipitis*

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*Pichia stipitis*에 의한 Xylose의 Ethanol 발효

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ABSTRACT

Batch fermentation runs were made with initial xylose concentrations of 2%, 4%, 8%, and 10%. The maximum yields were 0.46, 0.45, 0.43, and 0.42g ethanol/g xylose for 2%, 4%, 8%, and 10% xylose respectively. Xylitol formation was insignificant over a wide range of specific oxygen supply rates and xylose concentrations. The maximum specific productivities were 0.110, 0.110, 0.241, and 0.0961g ethanol/hr-g DCW for 2% through 10% xylose concentration.

INTRODUCTION

Efficient utilization of xylose should be integral part of the process for converting hardwood residues to ethanol. Xylose comprises a significant fraction of these materials and it is much more readily recovered from hemicellulose than is glucose from cellulose(1).

The fairly recent discovery that *Pachysolen tannophilus* can ferment xylose sparked interest in the route for xylose fermentation and subsequently several other yeast species were discovered to possess this ability as well (2-5). Whereas most of these yeasts were characterized by a low ethanol yield and production rate on xylose, *Pichia stipitis* appeared promising for xylose fermentation(6).

Temperature, pH, and xylose effects on ethanol production by *Pichia stipitis* have been investigated by many researchers(7-9). The majority of previous studies on xylose fermentation by *Pichia stipitis* have not concentrated on the effects of oxygen. This study was undertaken to evaluate such parameters in xylose fermentation by *Pichia stipitis*.

MATERIALS AND METHODS

Microorganism

The microorganism used throughout this study was *Pichia stipitis* CBS 5776. The stock culture was kept on agar slants. The media was as follows: 20g / l xylose, 3g / l yeast extract, 3g / l malt extract, 5g / l peptone, and 15g / l agar.

Inocula Preparation

A loop of the stock culture was transferred into each of two test tubes containing 10ml of the following: 20g / l xylose, 3g / l yeast extract, 3g / l malt extract and 5g / ℓ peptone. These two test tubes were inoculated for 24 hours at 32℃ before being added to the fermentor. The initial dry cell weight(DCW) was ca. 0.2g / l as determined indirectly by turbidity measurements.

Preparation Fermentation of Media

The fermentation media used throughout this study was 6.7g / l yeast nitrogen base (DIFCO). The carbon source was D-xylose in concentration of 2%, 4%, 8%, and 10%(W / V). Since xylose caramelizes and forms furfural in the presence of yeast nitrogen base at high temperatures, the xylose and yeast nitrogen base solutions were autoclaved separately for 15 minutes at 15 psig and 121℃. The media was inoculated after the pH was adjusted to 4.5 and the temperature was controlled at 32℃.

Batch Fermentation Reactor

A bench-top fermentor was kept at 32℃ and a pH of 4.5. Air was sparged through the media after passing through a filter while a rotameter measured the flow rate. The agitation rate was kept 200 rpm. The working volume of the fermentor was 500ml. An antiform agent (Antifoam C, Sigma Chemical Company) was used at 200 ppm.

Analytical Methods

The dry cell weight(DCW) was determined turbidometrically. Xylose and xylitol measurements were made by liquid chromatography. The column was packed with an ion-exchange resin(Bio-Rad Aminex Q-15S, calcium form). Ethanol concentrations were determined by gas chromatograpy equipped with a flame ionization detector. An internal standard of 1% n-propanol was used.

RESULTS AND DISCUSSION

The specific productivity was determined using a time-averaged dry cell weight during exponential growth. The cell mass at inoculation was approximately 0.2 g / l. The ethanol concentration after the lag phase was used as the initial point in specific productivity evaluation. In this way the effect of the nutrient media on the lag time was minimized. The volumetric productivity and substrate consumption rates were also evaluated after the lag phase.

Each set of fermentations (initial xylose concentrations 2%, 4%, 8%, and 10%) included one controlled run in which there was no oxygen supply. This microaerobic condition was not truly anaerobic since the media was not flushed with nitrogen. Oxygen in the fermentor head space would be dissolved in the media. This condition was used as an approximation to evaluate the parameters in near anaerobic conditions. *Pichia stipitis* requires oxygen to grow and strict anaerobic conditions are not possible for ethanol production with growing cells. Even non-growing cells would require a small amount of oxygen supply for cell maintenance.

2% Xylose Fermentations

The ethanol yield reached maximum at 0 specific oxygen supply rate (SOSR) in 2% initial xylose runs(Fig. 1). The highest yield was 0.46 which is 90% of the theoretical yield. Because of the small amount of xylitol

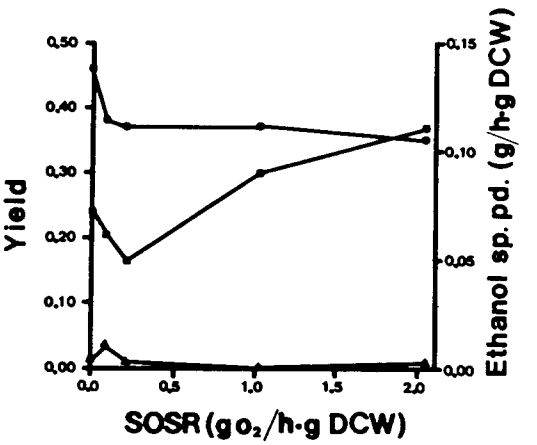

Fig. 1. Graph of 2% Initial Xylose Batch Fermentations (●, ethanol yield; ▲, xylitol yield; ■, ethanol specific productivity)

produced at this concentration, the ethanol yield was larger overall than any other sets. The highest xylitol yield was 0.03 and it occurred at 0 SOSR. As mentioned earlier, xylitol yields reached a maximum under limited oxygen supply rates. The yields at higher specific oxygen supply rates were 0.01. The xylitol formation of this level seems to be the lowest ever reported in xylose fermentation.

The specific productivity with 2% xylose increased with the specific oxygen supply rate. The yield decreased from 0.46 at 0 SOSR to 0.35 at 2.045 g O_2 / hr-g DCW SOSR. For the most part, the yield remained fairly constant over the oxygen supply rate span specified in Figure 1. Higher oxygen supply rates would be a preferable operating condition since there is little decrease in the ethanol yield.

4% Xylose Fermentations

Fermentations with 4% initial xylose have shown a similar trend observed with the 2% data(Fig. 2). The ethanol yield is highest at 0 SOSR and decreases slightly with the oxygen supply rate. Even at this high oxygen supply rate, the yield did not decrease noticeably. The lower ethanol yield may be due to ethanol loss by evaporation since the fermentation time was nearly twice as long as that in the 2% run. The xylitol yield was as low as in the 2% xylose runs. The highest xylitol yield was 0.018 and it occurred at microaerobic conditions. It

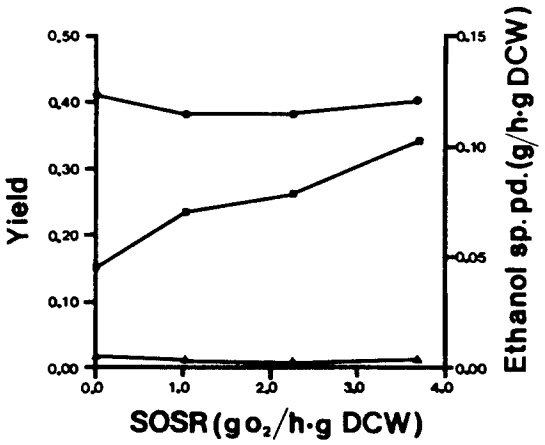

Fig. 2. Graph of 4% Initial Xylose Batch Fermentations (●, ethanol yield; ▲, xylitol yield; ■, ethanol specific productivity)

further decreased to 0.012 at 3.693 g O_2 / hr-g DCW.

There was not an optimum operating point with regard to aeration for ethanol yield in the 2% and 4% runs even though the xylitol yield decreased with aeration. At the microaerobic condition more xylose is channeled into ethanol production than cell growth. The average dry cell weight under high aeration(2.045g O_2 / hr-g DCW) was larger than that at the microaerobic condition by 0.64g / l. The ethanol yield was less at this high aeration than that of microaerobic condition by 0.1g ethanol / g xylose. The low yield of 0.35 could be explained by the higher cell growth occurring under increased aeration. An optimum condition for ethanol yield did not exist because the decrease in xylose conversion to ethanol was dependent to a much greater extent on cell growth than xylitol yield. The same reasoning applies to the 4% fermentation.

8% Xylose Fermentations

The fermentation with 8% initial xylose is characterized by a somewhat high xylitol yield and the existence of an optimum operating point in terms of ethanol yield-(Fig. 3). The 0.08 xylitol yield observed under the microaerobic condition runs was the highest of all fermentation runs made in this study. The high xylitol yield caused reduction in the ethanol yield at microaerobic conditions. The ethanol yield was much lower at this point (0.35g ethanol / g xylose). It increased at higher aeration rates as the xylitol yield decreased. At 0.712 g O_2 / hr-g DCW the xylitol yield was reduced to 0.004 and the ethanol yield peaked to 0.43. At the highest aeration the cells consumed a high level of energy generated through respiration. The ethanol yield therefore dropped considerably. High aeration did not seem to affect the pathway from xylose to xylulose. The xylitol yield was constant after 0.7 g O_2 / hr-g DCW and was not affected by the oxygen supply beyond this point. The decrease in ethanol yield does not appear to be related with the conversion of xylose to xylulose, rather it is believed to be the result of increased reliance on respiration.

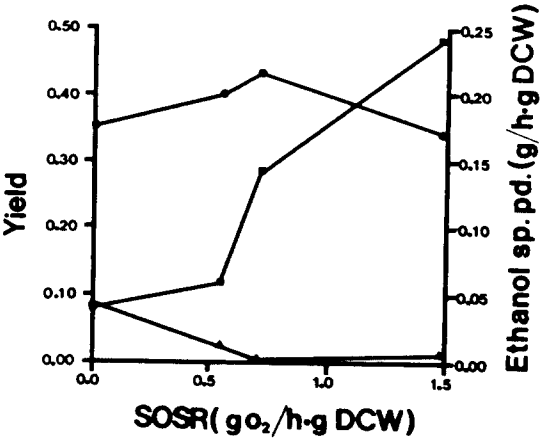

Fig. 3. Graph of 8% Initial Xylose Batch Fermentations (●, ethanol yield; ▲, xylitol yield; ■, ethanol specific productivity)

10% Xylose Fermentations.

Ethanol yields in the 10% initial xylose runs gradually decreased from 0.42 at microaerobic conditions to 0.29 with an specific oxygen supply rate of 1.310 g O_2 / hr-g DCW(Figure 4). The xylitol yield was surprisingly low at the microaerobic condition. It increased to 0.027 at 0.369 g O_2 / hr-g DCW then decreased to 0.020 at 1.310 g O_2 / hr-g DCW.

Specific and Volumetric Productivity

The specific productivity for 8% initial xylose was much higher than the rest(Fig. 1,2,3, and 4). It reached a maximum of 0.241 g / hr-g DCW or 2.1 times higher than the largest in any of the 2%, 4% or 10% runs.

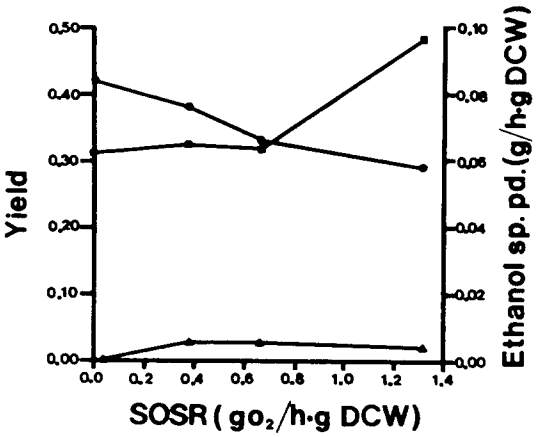

Fig. 4. Graph of 10% Initial Xylose Batch Fermentations
(●, ethanol yield; ■, xylitol yield; ■, ethanol specific productivity)

Xylose concentration appears to have a profound effect on the specific productivity. It has been reported that the specific productivity at 5% initial xylose is 2 times higher than that at 2%(7). Excluding the 8% results, the remaining specific productivity data were essentially identical. The 2% and 10% runs had very similar results while the 4% runs resulted in slightly higher productivity. All four runs, however, gave the same range of specific productivity(ca. 0.06 g / hr-g DCW) at microaerobic conditions. At this low oxygen supply rate the ethanol production is independent of xylose concentration.

At microaerobic conditions the effect of xylose concentration on volumetric ethanol production is also insignificant(Fig.5). As the oxygen supply increased, the difference in the ethanol volumetric productivity became more pronounced. Volumetric productivity for 10% was higher than that of any other runs including 8% even though the specific productivity was higher at 8%. This could be explained by the high cell growth rate observed at 10% (Fig. 6). The cells in 10% xylose grew faster so that more cells were present to ferment xylose. The 2% and 4% runs showed similar volumetric productivity data as did the 8% and 10% runs. In the lower xylose concentration range the ethanol volumetric productivity was substantially lower than those observed at 8% and 10% xylose concentrations because the average dry cell weight in these runs was 2-3 times less. The xylose volumetric consumption rate shows a trend similar to the ethanol volumetric productivity(Fig.7). The effect of specific oxygen supply rate on the growth rate. μ, is presented in Figure 6. Oxygen increases μ in a Monod-like relationship. At 4% initial xylose μ leveled off above 1.04 g O_2/hr-g DCW.

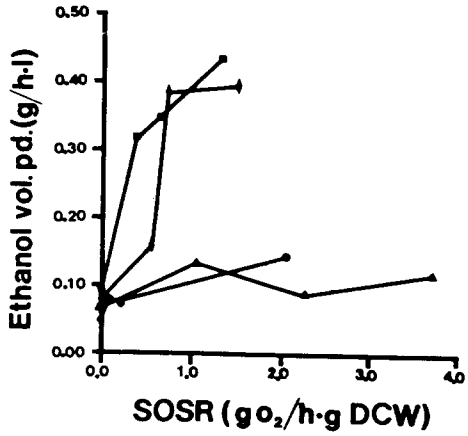

Fig. 5. Effect of Specific Oxygen Supply Rate on Ethanol Volumetric Productivity
(●, 2%; ▲, 4%; ◆, 8%; ■, 10%)

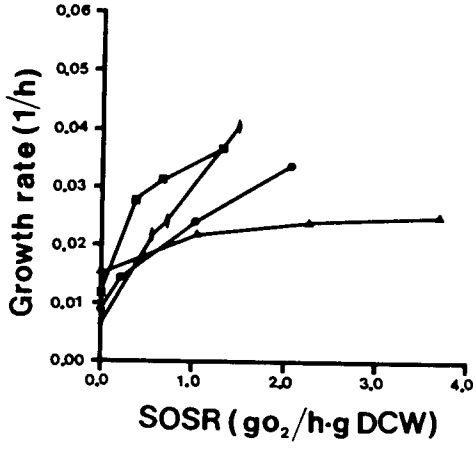

Fig. 6. Effect of Specific Oxygen Supply Rate on the Cell Growth Rate
(●, 2%; ▲, 4%; ◆, 8%; ■, 10%)

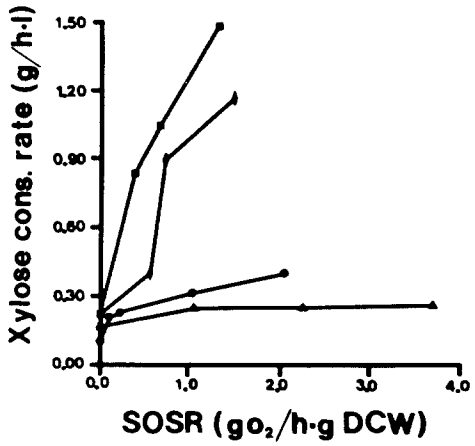

Fig. 7. Effect of Specific Oxygen Supply Rate on Xylose Volumetric Consumption Rate
(●, 2%; ▲, 4%; ◆, 8%; ■, 10%)

요 약

Xylose의 초기 농도가 2%, 4%, 8% 그리고 10%의 경우에 대하여 회분식 발효 실험을 하였다. 최대 ethanol 수율은 2%, 4%, 8%과 10%의 xylose의 농도에 대하여 각각 0.46, 0.45, 0.43 그리고 0.42로 나타났다. Xylitol은 넓은 범위의 specific oxygen supply rate와 xylose농도에서 거의 생성되지 않았다. 최대 specific productivity는 2−10%의 농도에 대하여 0.11, 0.11, 0.241, 0.0961 g ethanol / hr·g DCW의 값을 보여 주었다.

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