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Ethanol Production from Xylose by *Pichia stipitis* Using Cell-recycled Bioreactor

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Pichia stipitis 세포의 재순환 생물반응기를 이용한 Xylose로부터 Ethanol 생산

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ABSTRACT

To increase the volumetric productivity a continuous cell-recycled system was implemented. Cell concentrations between 9.2 and 15.0 g / l were obtained in the continuous fermentor study. At a 4% xylose feed and a specific oxygen supply rate(SOSR) of 1.04 g O_2 / hr-g DCW the ethanol yield was 0.36 at dilution rate of 0.0349 hr^{-1}. The ethanol volumetric productivity was increased to 0.244 g / hr-ℓ at this same dilution rate. This represented a 260% increase over that of the batch fermentation.

INTRODUCTION

Continuous cell-recycled fermentation with *Saccharomyces cerevisiae* has been studied by several researchers to improve the ethanol volumetric productivity(1-4). Fermentation of xylose by *Pachysolen tannophilus* has also been studied in a continuous cell-recycled fermentor(5-6). Immobilized cells of *Pichia stipitis* have been used in batch and packed bed systems for the fermentation of xylose to ethanol. The reported ethanol volumetric productivity was 0.18 g / hr-ℓ in a 5% xylose batch system while the packed bed system obtained 0.56 g / hr-ℓ (7).

In order to achieve high productivity, it is important that cells used as a biocatalyst be kept at a high concentration. High cell concentrations will also enable stable operating conditions in the fermentation.

To operate at high cell conditions in a continuous system, cells must be separated from the exit stream to avoid washing out with the product. Devices used to separate cells have included cell settlers and hollow fiber membranes (1, 4, 8). Cell settlers require a flocculating yeast and often do not separate cells as efficiently as hollow fiber membranes. Centrifugation may also be used but it is too cumbersome for laboratory scale operation. Hollow fiber membranes offer the most simple and efficient cell separation and thus were used in this study.

In this study, a continuous cell-recycled system was constructed and employed to ferment xylose to ethanol by *Pichia stipitis*. Of particular interest was the evaluation of the ethanol volumetric productivity, yield, and specific productivity

MATERIALS AND METHODS

Inoculum

Two one-liter fermentors were employed to harvest cells to be used for cell-recycled fermentation. The conditions in these vessels were 32℃, pH 4.5, and 200 rpm agitation.

Pichia stipitis CBS 5776 was grown on 15%(W / V) and 0.67% yeast nitrogen base (DIFCO) under high aeration. The cells were centrifuged at 6000 rpm for 20 minutes. The one liter fermentor used in the continuous system was inoculated with the cells harvested from the seed fermentors.

Analytical Methods

The dry cell weight (DCW) was determined gravimetrically as described elsewhere(5). Xylose and xylitol measurements were made by liquid chromatography. Ethanol concentrations were determined by gas chromatography.

Continuous Fermentor System

A one liter fermentor with a 500ml working volume was connected to a hollow fiber membrane (Diaflo Model H1MPO 1-43, 0.1 μm cutoff). The cell filter system was designed to avoid protein accumulation in the fermentor using membrane with pore size large enough to pass proteins but small enough to retain cells(0.1 μm cutoff). It has been reported that the accumulation of non-volatile toxic substances including certain proteins inhibits cell growth(9). The feed to the fermentor was 4% xylose, 0.67% yeast nitrogen base (DIFCO) and 200 ppm antifoam(Antifoam C, Sigma). The temperature was controlled at 32℃ and the pH at 4.5 using a controller (Horizon Model 5997-20) connected to a peristaltic pump(Masterflex Model 7520-10) delivering 0.1M NaOH. Air was sparged through the fermentor at 1.04 g O_2 / hr-g DCW.

Fouling of the membrane occurred in several hours of operation due to cell deposit on the membrane wall. This was avoided by periodically backflushing permeate liquid from the collector through the filter. A computer control was incorporated into the fermentation system for automatic backflushing. During normal operation a peristaltic pump drew the permeate into a collector connected to the fermentor(Fig. 1). The backflush and permeate pumps were connected to an analog / digital convertor and a microcomputer. The operation proceeded as follows: the permeate pump was run for 15 minutes until the computer turned it off, at which point the backflush pump was turned on for three minutes. This procedure was repeated for the duration of fermentation run. In this way the membrane sustained well over extended period allowing the fermentor system to run continuously for several weeks.

RESULTS AND DISCUSSION

Operating condition Rationale

The continuous cell recycle system was operated at a xylose feed of 4% and 1.04 g O_2/hr-g DCW for several reasons:

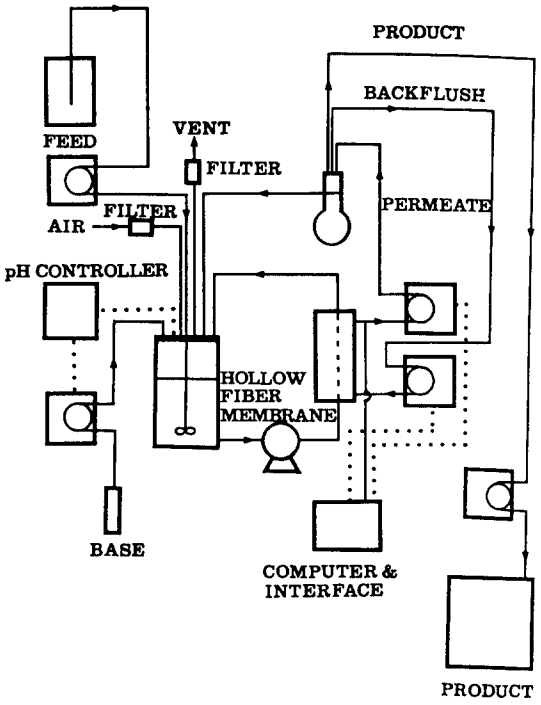

Fig. 1. Schematic Diagram for Continuous Fermentation with Cell Recycle

1. The xylose concentration in hemicellulose hydrolyzate would not exceed 4%.

2. The ethanol yield was much higher at limited aeration in the 4% batch fermentations. Considering the high cell concentrations, the actual oxygen uptake by the cells would be lower than expected. The oxygen mass transfer coefficient, K_{La}, was not linear with the aeration rate(5). A ten fold increase in the oxygen supply rate would not bring about a ten fold increase in the oxygen available to the cells; therefore, a lower amount of oxygen would be available to the cells than suggested by the specific oxygen supply rate(SOSR).

3. The mean residence time would be shorter for a 4% feed. It was much easy to work with dilution rates between 0.0349 hr^{-1}- and 0.015 hr^{-1} than half that magnitude.

4. Oxygen was supplied at 1.04 g O_2 / hr-g DCW to keep the cells viable. Comparison between batch and continuous cell-recycled systems was facilitated by operating at identical specific oxygen supply rates.

Continuous Fermentation with Cell Recycle

The dilution rate was varied from 0.0349 to 0.0150 hr^{-1} (mean residence time 28.7-66.7 hr). The dry cell weight remained relatively constant throughout each individual run. Cell concentrations stayed between 9.2 and 15 g / l (Table 1).

Growing cells in a nitrogen present media (0.67% yeast nitrogen base) were employed instead of non-growing cells even though non-growing yeasts exhibit higher ethanol yields. Production of ethanol is severely depressed under nitrogen deficient conditions : moreover, essential nutrients and oxygen must be supplied to keep the cells metabolically active(5).

It was found that the fermentation system did not decrease the viability of the cells. The shear stress encountered by the cells in the permeate and backflush peristaltic pumps or the heat transfer from the filter pump did not have any noticeable effects.

The highest ethanol yield obtained was 0.36 and it occurred at the dilution rate of 0.0349 hr^{-1}. The ethanol yield did not show a noticeable relationship with the dilution rate as it stayed relatively constant throughout the dilution rate applied in the test(Fig. 2). The ethanol yields in the continuous fermentation runs were lower than those obtained in batch fermention by about 25%. The lower yield in cell-recycled systems may be due to the greater requirements for cell maintenance. In high cell density cultures, the benefit of increased productivity was somewhat offset by reduction of the ethanol yield.

The highest ethanol yield occurred at the point where the lowest xylitol yield was obtained. There was negligible xylitol formation at the dilution rate of 0.0349 hr^{-1} (Fig. 2). Xylitol formation in general decreased with the dilution rate.

The specific productivities in continuous cell-recycled runs were much lower than those in batch fermentations. The specific productivity was only 0.0172 g / hr-g DCW at a dilution rate of 0.0349 hr^{-1} as compared to 0.0778 g / hr-g DCW obtained in the batch fermentation. This may be explained by the non-linear relation between the aeration rate and the oxygen mass transfer coefficient, K_{La}(5). It was observed during the 4% batch fermentations that the specific productivity increased with the specific oxygen supply rate. By increasing the cell con-

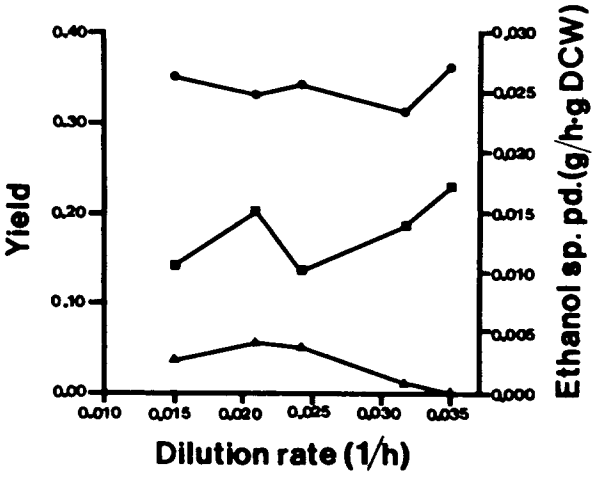

Fig. 2. Effect of SOSR on Yield and Specific Productivity
(●, ethanol yield; ■, xylitol yield; ■, specific productivity)

Table 1. Continuous Cell-recycled Fermentation with 4% Xylose Feed.

Dilution Rate (h^{-1})	Yield		Qp (g/h·l)	Q_S (g/h·l)	Ethanol Specific Productivity (g/h · DCW)	DCW (g/l)
	Ethanol	Xylitol				
0.0349	0.36	0	0.244	0.681	0.0172	14.8
0.0316	0.31	0.010	0.173	0.566	0.0138	12.5
0.0241	0.34	0.049	0.161	0.423	0.0101	15.0
0.0208	0.33	0.055	0.198	0.601	0.0151	9.2
0.0150	0.35	0.037	0.104	0.296	0.0106	9.8
Batch Fermentation						
	0.45	0.011	0.093	0.253	0.0778	1.2

centration ten-fold, the aeration rate was increased by ten to operate at a SOSR of 1.04 g O_2 / hr-g DCW ; however, K_{La} did not increase linearly with aeration. The cells did not assimilate as much oxygen as in the batch fermentations because of the lower K_{La} in the cell-recycled system. The oxygen supply rate was not increased beyond the level of 1.04 g O_2 / hr-h DCW to preserve the ethanol yield which was already at a low level(64% of theoretical). Any increase in aeration may bring about an increase in respiration and a further decline in ethanol yield.

The volumetric ethanol productivity, Q_p, and the volumetric xylose consumption rate, Q_s, showed a similar trend with regard to the specific productivity(Fig. 3). The Q_p varied from 0.104 g / hr^{-1} to 0.244 g / hr^{-1} which was only 1.11 to 2.63 times higher than that obtained in the 4% batch fermentation. This is lower than expected considering that the cell concentration was about 10 times that in the batch fermentor. The specific productivity was approximately 7 times lower in the continuous fermentation with cell recycle than in the batch fermentation. Among the probable causes for low productivity in the continous runs are : a small oxygen uptake rate because of low K_{La} and the lack of exposure to oxygen and nutrients while being circulated through the fiber system.

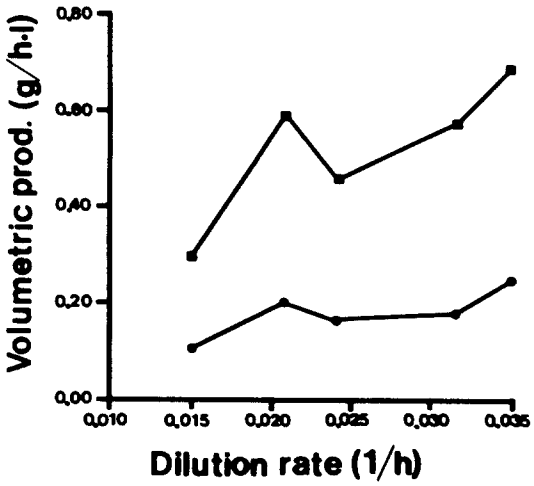

Fig. 3. Effect of SOSR on Ethanol Volumetric Productivity and Xylose Consumption Rate
(●, ethanol volumetric productivity; ■, xylose consumption rate)

요 약

Volumetric productivity를 향상시키기 위하여 미생물 재순환에 의한 연속 시스템을 이용하였다. 연속발효조의 연구에서 9.2g / l와 15.0g / l의 범위의 세포 농도를 유지할 수 있었다. 4%의 xylose의 feed와 1.04g O_2 / hr-g DCW의 specific oxygen supply rate의 조건에서 희석율이 0.0349 h^{-1}일 때 ethanol 수율은 0.36이었다. Ethanol volumetric productivity는 같은 희석율에서 0.244 g / hr-ℓ로 증가하였으며 이것은 회분배양값의 260%이었다.

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