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CONTINUOUS ETHANOL PRODUCTION FROM WOOD HYDROLYSATE BY CHEMOSTAT AND TOTAL CELL RETENTION CULTURE

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Abstract - Chemostat and total cell retention cultures with internal filter system of *Saccharomyces cerevisiae* H1-7 were carried out to produce ethanol from wood hydrolysate. Maximum ethanol productivity obtained in a chemostat with the aeration rate of 1 vvm was 3.79 g/(L·h). This was 20% higher than that in a chemostat without aeration. However, the substrate was not completely consumed at the dilution rate with the maximum productivity. The realistic productivity, which has higher than 99% conversion rate of substrate, was 2.95 g/(L·h). The maximum productivity in the total cell retention culture was 6.65 g/(L·h) at the dilution rate of 0.19 h^{-1} and the residual glucose concentration was negligible.

Key words: *Wood Hydrolysate, Toxic Materials, Continuous Ethanol Fermentation, Realistic Productivity, Internal Filter System*

INTRODUCTION

Recently fuel ethanol production from biomass, especially cellulosic materials, has been drawing increasing attention due to low cost. However, little progress has been made on ethanol fermentation using cellulosic materials because of the presence of various toxic substances liberated from lignocellulose pretreatment and hydrolysis processes [Chung and Lee, 1985; Spindler et al., 1991; Castro et al., 1994]. It has been reported that the toxicity of the hydrolysates could be overcome using high cell density [Chung and Lee, 1985].

Achieving a high productivity in a bioreactor plays a crucial role in determining the economics of bulk biochemical products such as ethanol. High cell density culture coupled with a continuous operation would yield the theoretical productivity of the bioreactor, but in practice this has seldom been realized in the bioreactor operation. During continuous operation, cells will be washed out unless the dilution rate is kept lower than the cell growth rate. Cell recycle using membrane has been a very popular method in achieving a high bioreactor productivity [Lee and Chang, 1987; Chang et al., 1994]. The membrane filter modules for cell recycle located outside the bioreactor have several drawbacks such as difficulties in sterilization and cell recycling operation. To overcome these problems, we have employed an internal filter system, which allowed microbial separation to be carried out inside the fermentor [Chang et al., 1993; Lee et al., 1994].

We report here primarily the result of the continuous ethanol production from wood hydrolysate by a chemostat and a total cell retention culture for high cell density with the internal filter system.

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MATERIALS AND METHODS

1. Microorganism

The yeast strain used in this study was *Saccharomyces cerevisiae* H1-7 obtained from Suwon University in Korea. This strain was maintained on agar slant containing 0.3% yeast extract, 0.5% Bacto peptone, 2% glucose, and 2% agar at 4°C.

2. Preparation of Wood Hydrolysate

Chip of oak wood, the most abundant species in Korea, was made by a chipper designed and built in our laboratory as an initial feed material. Steam explosion of the chip materials was conducted in a 8 L exploder designed in our laboratory for 3 minutes at 215°C. Five kg (dry weight) of residue after explosion and washing was enzymatically hydrolysed with Celluclast (Novo Co., Denmark) and Novozym (Novo Co., Denmark) in a reactor with 30 L working volume (Korea Fermentor Co., Korea) for 3 days at 50°C. The enzyme loading was 20 IU and 30 IU/g residue for filter paper and cellobiase activities, respectively. The hydrolysate was centrifuged and the supernatant contained 39 g/L of glucose. By doubling the enzyme loading, the supernatant containing 81 g/L of glucose was obtained from wood hydrolysate.

3. Culture Medium

The seed culture medium consisted of 2% glucose, 0.3% yeast extract, and 0.5% Bacto-peptone. The fermentation medium consisted of the supernatant of the wood hydrolysate (the glucose concentrations were specified in the experimental results), 3 g/L yeast extract, and 5 g/L peptone.

4. Chemostat

The seed culture was prepared by inoculating a loopful of cells from a stock plate into 100 mL of seed culture medium. The seed culture was grown in a shaking incubator overnight at 30°C before transfer to the fermentor. A 2 L fermentor with 1 L working volume (LSL BIOLAFITTE SA, France) was used

in this study. The inoculum volume was 10%(v/v) of the fermentor working volume. For a chemostat culture, the fermentor was operated batchwise for 24 hr in the first place. The level of the fermentor was controlled at a fixed level so that the inlet flow rate was exactly equal to the outlet flow rate. Medium feeding rate and the flow rate out of the reactor were adjusted by a peristaltic pump (Cole Parmer Co., USA). Steady state conditions were observed after more than 3 working volume changes in the fermentor. Temperature and agitation speed were controlled at 30°C and 250 rpm, respectively. Initial pH was adjusted at 4.7-4.9. Aeration rates were adjusted between 0.3 and 1 vvm by operation conditions.

5. Total Cell Retention Culture

Details of the experimental set-up and the operation protocol for a total cell retention culture with the internal filter system were reported previously [Chang et al., 1993; Lee et al., 1994]. Total cell retention culture was carried out with a working volume of 1.7 L in a 2.5 L fermentor (Korea Fermentor Co., Korea) incorporated with a filter module. Temperature, agitation speed, and aeration were controlled at 30°C, 350 rpm, and 0.3 vvm, respectively. The filter was made of porous stainless steel and was sterilizable (Motto Co., USA). The pore size of the filter was 2 μm. The filter module consisted of 9 vertical cylindrical filter tubes with a diameter and a height of 9 and 90 mm, respectively. The lower frame of the filter was made of stainless steel. The total surface area of the filter module was approximately 229 cm^2.

6. Assays

Ethanol was measured by gas chromatography equipped with a flame ionization detector (HP5890A, Hewlett-Packard Inc., USA). Glucose was measured by an enzymatic glucose oxidase-peroxidase method (Glucose-E Kit, Yeoungdong Pharm. Inc., Korea). Cell number was counted by a hemacytometer (American Opticals Inc., USA). Cell dry weight was determined after two rounds of centrifugation and washing with distilled water, and drying at 105°C for 1 day.

RESULTS AND DISCUSSION

1. Chemostat

Chemostat culture with *Saccharomyces cerevisiae* H1-7 was carried out with wood hydrolysate as the substrate. Fig. 1 shows the results of steady state kinetics of nonaerated chemostat culture. The yeast concentration was 2.2×10^7/mL at the dilution rate of 0.033 h^{-1} and then gradually decreased to 0.55×10^7/mL as the dilution rate increased to 0.14 h^{-1}. The maximum ethanol concentration was 27.7 g/L at the dilution rate of 0.067 h^{-1} and decreased to 22.53 g/L as the dilution rate increased to 0.14 h^{-1}. Ethanol yield was in the range of 0.376-0.462 g ethanol/g glucose at dilution rates from 0.033 to 0.14 h^{-1}. The low ethanol concentration and the yield were due to the maintenance and the growth of yeast. Maximum productivity in this run was 3.15 g/(L·h) at the dilution rate of 0.14 h^{-1}. But the cost of the substrate, which is the single largest expense in ethanol fermentation, is estimated to represent at least 60-70% of the total production cost. It is necessary to run at the dilution rate that gives near complete conversion of the substrate. In that sense the realistic ethanol productivity having higher than 99% of substrate conversion was 1.86 g/(L h) at the dilution rate of 0.067 h^{-1}. The low productivity was from the presence of toxic chemicals in wood hydrolysate [Clark and Mackie, 1984]. Unlike starch materials, wood contains lignin which is degraded into various phenolic components like vanillin and *p*-coumaric acid during pretreatment like steam explosion [Marchal et al., 1992]. The phenolic components produced were known to have the strong inhibitory effect on the cell activity and growth [Clark and Mackie, 1984; Marchal et al., 1992].

Aerobic chemostat was also carried out to increase the ethanol productivity and yeast concentration (Fig. 2). Yeast concentration obtained in this run was about 3 times higher than that obtained in the anaerobic chemostat. Maximum productivity and the realistic productivity were 3.79 g/(L·h) and 2.95 g/(L·h), respectively, which were 20.3% and 58.6% higher than the values obtained in the anaerobically operated chemostat. The productivities and yields under the various operating conditions were summarized at Table 1

2. Total Cell Retention Culture

Total cell retention culture with the internal filter system was carried out with wood hydrolysate of 39 g/L glucose at the dilution rate of 0.39 h^{-1} (Fig. 3). The initial cell concentration was 5.3×10^7/mL and the initial glucose concentration was

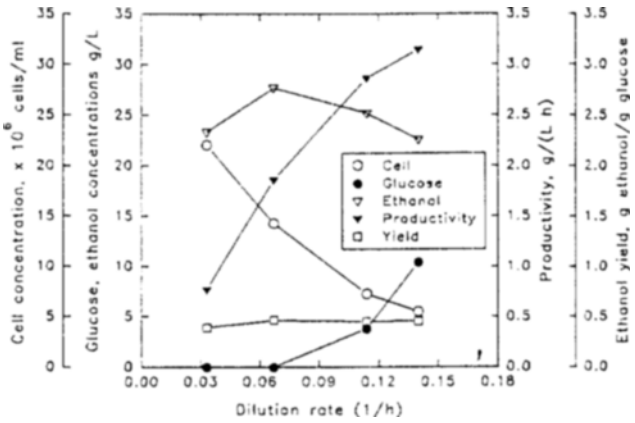

Fig. 1. Nonaerated chemostat culture, (S_0=60 g/*l*).

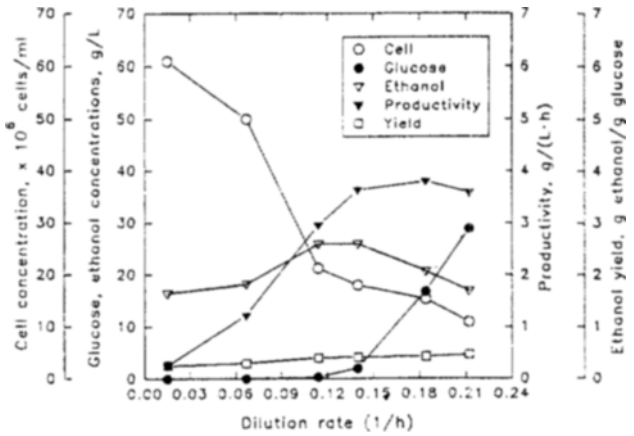

Fig. 2. Aerated chemostat culture, (S_0=65 g/*l*, aeration rate=1 vvm).

Table 1. Summary of results on the continuous fermentations

	Chemostat		Total cell retention culture	
	No aeration	Aeration rate =1 vvm	Aeration rate =0.3 vvm	
Feeding glucose concentration, g/L	60	65	39	81
Cell concentration, $\times 10^7$ cell/ml	0.55	1.65	20.7	8.2
Maximum productivity, g/(L·h)	3.15	3.79	3.42	6.65
Realistic productivity, g/(L·h)*	1.86	2.95	3.42	6.65
Yield	0.383	0.408	0.462	0.433

*: The ethanol productivity at the dilution rate to achieve 99% substrate consumption

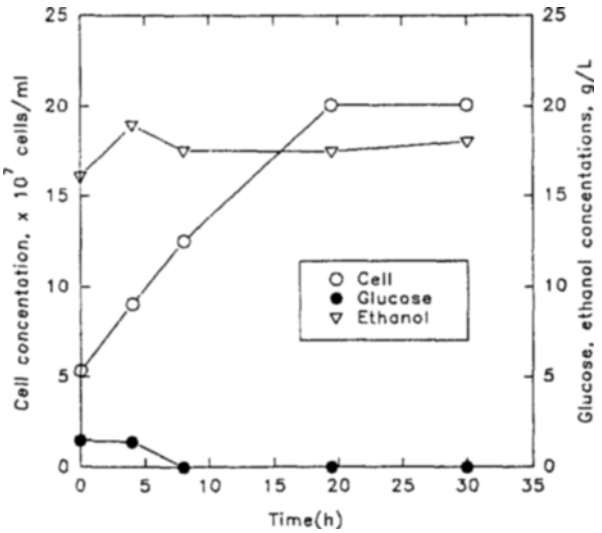

Fig. 3. Total cell retention culture with the internal filter system, (S_0=39 g/*l*, D=0.19 h^{-1}, aeration rate=0.3 vvm).

about 1.5 g/L. The glucose concentration was completely depleted at 8 hr due to the low feeding glucose concentration. The cell concentration increased slowly to 2.07×10^8/mL at 30 hr which was about 13 times higher than that by aerobic chemostat at the similar dilution rate. But cell concentration no longer increased because of the toxic materials existed in wood hydrolysate. The average ethanol concentration and ethanol yield of the filtrate during substrate limitation period was 18.01 g/L and 0.462 g ethanol/g glucose, respectively.

Considering ethanol separation cost, it is necessary to have a high ethanol concentration in the fermentation broth and thus the wood hydrolysate supernatant containing 81 g/L glucose concentration was used (Fig. 4). In the beginning of the fermentation until 3 hr, the glucose concentration increased rapidly to 44.85 g/L due to low concentration of yeast cells. Then the glucose concentration gradually decreased and became completely depleted at 20 hr. Yeast cell concentration obtained in this run was 8.2×10^7/mL and was lower because of low initial cell concentration and toxic materials. But the ethanol concentration and ethanol yield were 35.07 g/L and 0.433 g

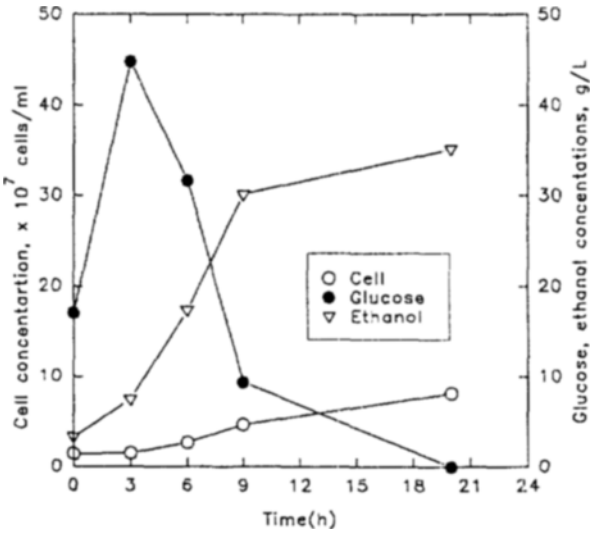

Fig. 4. Total cell retention culture with the internal filter system, (S_0=81 g/*l*, D=0.19 h^{-1}, aeration rate=0.3 vvm).

ethanol/g glucose, respectively. Both the maximum and the realistic ethanol productivities were 6.65 g/(L·h) which were 75 and 125% higher than those by aerobic chemostat. Both productivities were also 36% and 202% higher than those with an external cell recycle by natural settling [Chung and Lee, 1985]. The internal filter system has also significant advantages over the traditional external cell recycle systems, because of the reduced maintenance and the operation requirements.

From these results, we found that the total cell retention culture with the internal filter system has advantages over anaerobic and aerobic chemostats in terms of productivity, cell concentration, substrate utilization and ethanol yield. The total cell retention culture also had two times higher realistic productivity than that of the ethanol production system with external recycling. Toxic compounds contained in wood hydrolysate were likely responsible for the lower cell concentration obtained in total cell retention culture with high glucose concentration hydrolysate. In our laboratory, some works are being done to find some economic ways to remove toxic compounds from the hydrolysate to enhance the fermentation efficiency.

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