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세포재순환 에탄올 발효에서 세포농도와 희석률의 최적화

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Optimization of Cell Concentration and Dilution Rate in Cell Recycled Ethanol Fermentation

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ABSTRACT

The rheological characteristics of the ethanol fermentation broth were pseudoplastic when the yeast concentration was above 150g/L. From the viewpoint of rheological properties, the cell concentration below 150 g/L was recommended for ethanol fermentation. Since the cell floc was formed at the cell concentration of 100 g/L, yeast cells were not much plugged in the pores of the membrane. The cell concentration above 100 g/L was desirable when considering the permeability of the membrane. Since ethanol productivity was the highest when the cell concentration was 130 g/L in cell recycled ethanol fermentation. The optimal dilution rate was determined at 1.3 h^{-1} at constant cell mass of 130g/L. At this dilution rate, the ethanol productivity and glucose conversion ratio ware 80 g/L · h and 0.94, respectively.

INTRODUCTION

With the development of biotechnology, high density culture of microorganism has become very important to enhance product concentration and productivity. To maintain the high cell density, it is necessary to feed the nutrients continuously and to reduce substrate and product inhibitions. Therefore, fed-batch culture and cell recycled continuous culture have been studied[1-22]. Especially, cell recycled continuous culture has many advantages. Since the dilution rate can be maintained much higher than the maximum specific growth rate, productivity can be maintained high. Inhibitory substances can be removed and in situ product separation is also possible, which makes down-stream processing easi-er and more economical. In wastewater treatment, activated sludge processes employing cell recycle have been used successfully. The growing prospects of ethanol as a fuel and future chemical feedstock have prompted considerable efforts to increase the efficiency of production in cell recycled continuous bioreactor[3-19].

Cysewski and Wilke[3, 4, 5] and Ghose and Tyagi[6, 7] used a settling tank to recycle yeast cells, where productivity was increased about four times compared to the results obtained from

a conventional continuous bioreactor. The major constraint of this system was in the requirement of a large settler to allow enough residence time for the cells to be settled. Margaritis and Wilke [8, 9] designed a rotor fermentor which combined the function of fermentor and filter in one unit. They reported that the ethanol productivity could be increased by ten times compared with an ordinary continuous bioreactor. Since another constraint in conventional fermentation processes was end-product inhibition, fermentation productivity can also be increased by decreasing the product inhibition effect. Cysewski and Wilke[7, 10] demonstrated that alcohol can be removed continuously during fermentation by operating under vacuum condition. The main disadvantage in vacuum fermentation was high power consumption for maintaining vacuum conditions[5]. In the 1980's, with the development of membrane technology, the shorter residence times of cell and the higher efficiency of seperation have become available[11]. Rogers *et al.*[12] and Hoffman *et al.* [13] employed flat plate membrane and Nishizawa *et al.*[14], Cheryan and Mahaia[15], Lee and Chang[16] used hollow fiber membrane and Lafforgue *et al.* [17] and Jarzebski *et al.* [18] chose tubular type membrane. Product inhibition can be reduced and cell density can be maintained high by employing bioreactor combined with a membrane unit. In ethanol fermentation employing membrane bioreactor, fermem-tation broth has characteristics of pseuoplastics[23], carbon dioxide is entrapped in the bioreactor and the permeability of the membrane is deterioated as the cell grows to high density. But when the cell concentration is low, the ethanol productivity becomes low. Determination of the optimal cell concentration for the ethanol production is therefore very important in cell recycled ethanol fermentation system.

In this article, the optimal cell concentration was determined considering the rheological properities of fermentation broth and the permeability of the membrane. The optimal dilution rate was determined for the maximum ethanol productivity and maximum glucose conversion at the optimal cell concentration.

MATERIALS AND METHODS

Microorganism and medium

The microorganism used in the ethanol fermentation studies was *Saccahromyces cerevisiae* ATCC 24858. The growth medium was 20g/L glucose, 5g/L bactopeptone, 3g/L malt extract, and 3g/L yeast extract. The production medium consisted of 100g/L glucose, 5g/L yeast extract, 5g/L KH_2PO_4, 5g/L $(NH_4)_2SO_4$ and 1g/L $MgSO_4 \cdot 7H_2O$. When glucose concentration was varied, all other components were also varied in proportion to the glucose concentration.

Apparatus

The experimental setup is shown in Figure 1. To a 2-L jar fermentor(Bioflo model C30, New Brunswick Scientific Co.) a flat-plate membrane module (Millipore, Lab Cassete XX42 YLC Ko) was attached. The effective area of a membrane sheet was 60cm² with pore size of 0.2μm. The material of the membrane was nitrocellulose. Operation condi-

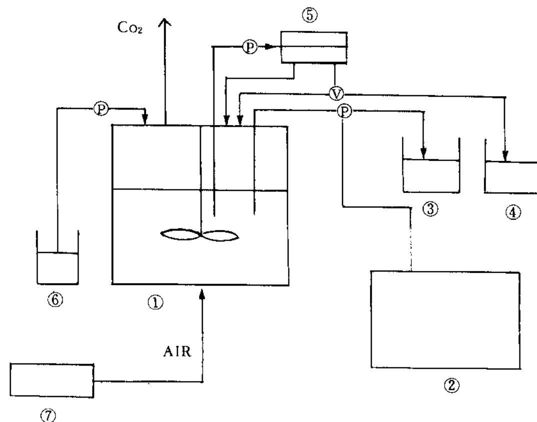

Fig. 1. Schematic diagram of experimental setup.
① fermentor ⑥ medium reservoir
② computer ⑦ compressor
③ cell bleeding reservoir Ⓟ peristaltic pump
④ permeate reservoir Ⓥ valve
⑤ membrane module

tions were chosen to keep the membrane fouling to a minimum, i. e. the flow rate of the broth at the entrance of the membrane module was 200ml /min, and the pressure across the filtration unit was below 1.5 atm. Part of the permeate stream was removed from the system at a desired rate to balance with the medium stream and the rest of the permeate was recirculated to the fermentor. Cell bleeding rate was manipulated by using a peristaltic pump interfaced to the personal computer(IBM AT).

Yeast cells for the innoculum were grown in a 250ml flask containing 50ml growth medium in a rotary shaking incubator for 16 h at 30℃ and transferred to the 2-L jar fermentor. The culture was grown batchwise followed by the feeding of the production medium for continuous operation. The batch culture time was 9 h for initial glucose concentration 30g/L. The working volume was 600ml including the amount of hold up in the filter. The initial pH was 4.5 and the agitation speed of the stirrer was 400 rpm. The temperature was maintained at 30℃ and the air flow rate was 0.5vvm.

Assay

Cell mass was estimated after diluting the sample and measuring its absorbance at 525nm using spectrophotometer(Kontron, model UVIKON 930) and comparing with the calibration curve. Dry cell weight was determined by centrifuging the cell suspension, resuspending in distilled water and drying at 90℃ for 24 h. Glucose concentration was determined by DNS(dinitrosalicyclic acid) method. 1ml of the supernatant of the centrifugated broth was reacted with 1ml of the DNS color reagent at 100 ℃ for 5 min. 10ml of distilled water was added after the reaction and optical density was measured at 546 nm. Ethanol was measured by gas chromatography(Yanaco G-1800) with a thermal conductivity detector(column material=porapak Q, carrier flow rate=30 cc/min. oven temperature =140℃). The viscosity of the fermentation broth was measured by Haake viscometer. The cell configuration was photographed using optical microscope(Olympus AH-6).

RESULTS AND DISCUSSION

The optimal cell concentration in cell recycled ethanol fermentation was determined considering rheological properties of the fermentation broth and separation performance of the membrane and ethanol productivity in cell recycled ethanol fermentation. The apparent viscosity of the fermentation broth is shown in Figure 2. Non-Newtonian behavior began to occur when the cell concentration was about 77g/L. At this concentration, the fermentation broth was almost Newtonian because apparent viscosity was not changed much for various shear rates. When the cell concentration was above 150g/L, the fermentation broth was clearly non-Newtonian. Non-Newtonian fluid is usually described by power law equation. The power law equation is,

$$\tau = k(\dot{\gamma})\ n \quad \cdots\cdots(1)$$

where $\dot{\gamma}$ is shear rate, n is the flow behavior index and k is the consistency index. As shown in Figure 3, as the cell concentration was increased, the flow behavior index was decreased. Therefore, yeast suspension could be classified as pseudoplastic in range of high concentration. Malinowski *et al.* [23] also reported that yeast suspensions were psuedopasic when the cell concentration was above 75g/L. As shown in Figure 4, the viscosity of the fermentation broth was increased suddenly when the cell concentration was around 150g/L. From the viewpoint of the power requirement by aeration and agitation, it is better to avoid the high cell concentration as far as the ethanol productivity is not much influenced.

The time courses of the membrane flux and the cell concentration in cell recycled ethanol fermentation are represented in Figure 5. The membrane was cleansed with 0.1 N NaOH for 3 min to overcome membrane fouling. The peak of the flux was shown after the cleansing of the membrane. At low cell concentration the flux was decayed rapidly, while the cell concentration was

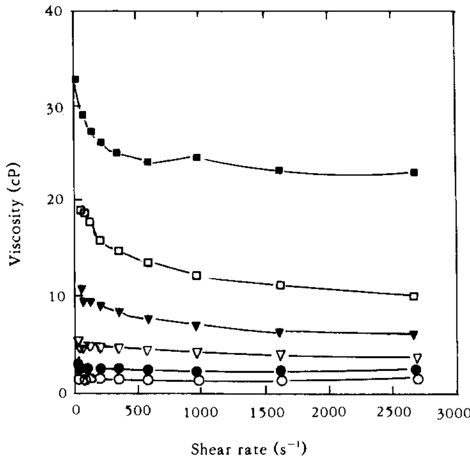

Fig. 2. Apparent viscosity of the fermentation broth vs. shear rate for various cell concentrations in cell recycled ethanol fermentation.
■:X=238 g/L, □:X=162 g/L,
▼:X=157 g/L, ▽:X=110 g/L
●:X= 77 g/L, ○:X=16 g/L

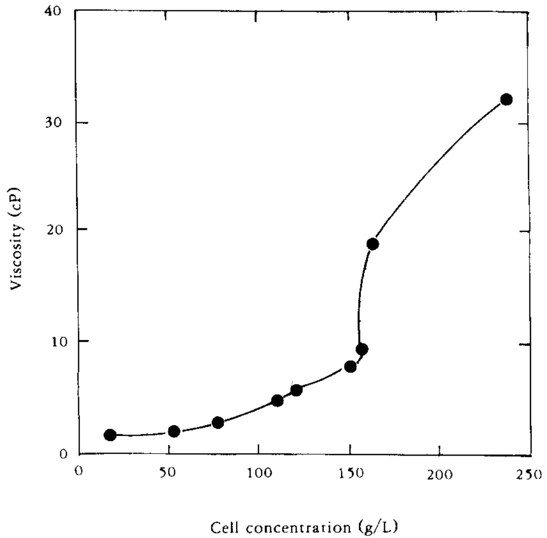

Fig. 4. Change of apparent viscosity of fermentation broth at the constant shear rate of 75 sec^{-1}

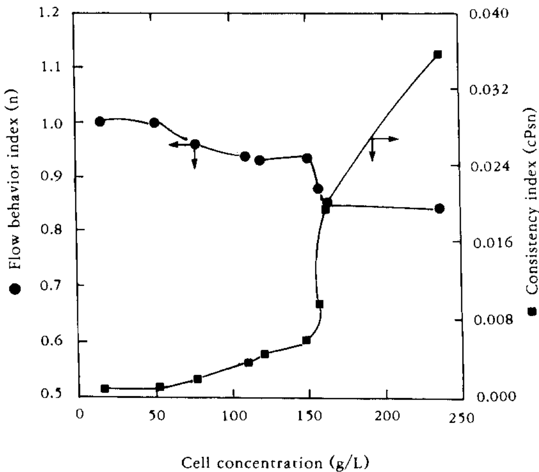

Fig. 3. Flow behavior index and consistency index of the fermentation broth for various cell concentrations in cell recycled ethanol fermentation.

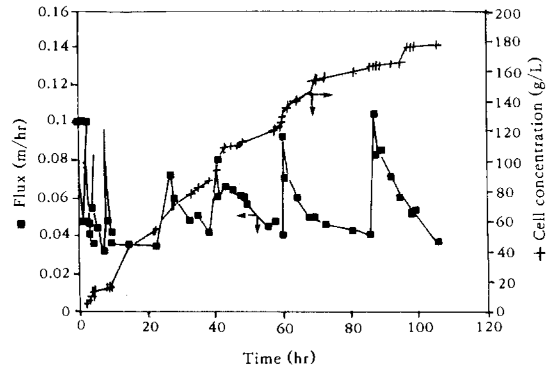

Fig. 5. Time courses of flux and cell concentration in cell recylcled ethanol fermentaiton.

above 100g/L the flux decay was delayed for approximately 25h. At high cell concentration, the membrane flux was higher compared to the result obtained at low cell concentration. As the cell concentration increased, the cell population was dense as shown in Figure 6 and cell Floc seemed to be formed. The cell floc was observed to be larger than the pore size. Therefore, the pore of the membrane was not much plugged and the deposited materials were swept by the influx of yeast slurry. The membrane flux was thus higher than the flux at low cell concentration where the cells were much plugged in the internal pores of the membrane as reported by Cheryan and Mehaia[15]. Form the viewpoint of the membrane flux, it is better to maintain the Cell concentration above 100g/L to get high flux through

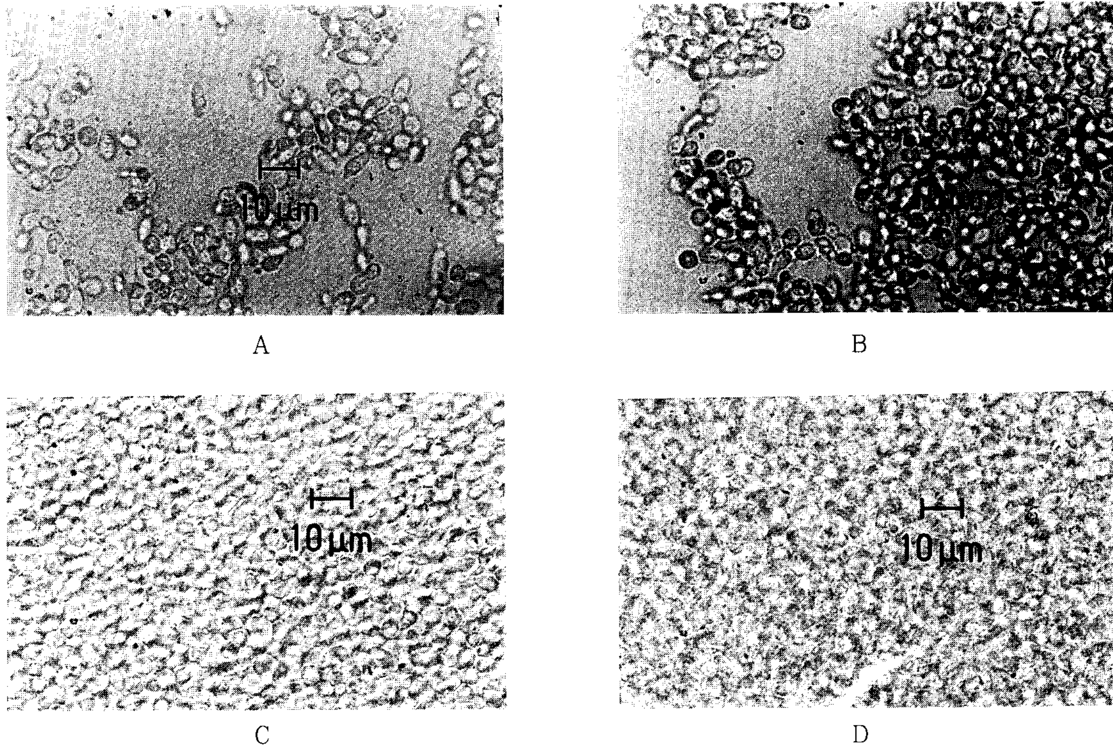

Fig. 6. Cell configurations for various cell concentrations. A: 16 g/L, B: 77 g/L, C: 157 g/L, D: 238g/L

the membrane.

Figure 7 shows the time courses of the ethanol fermentation in the cell recycled reactor. It shows that the ethanol concentration was the hightest at 66 g/L when the cell concentration was approximately 130g/L and decreased as the cell concentration was increased further. At the cell concentration of 130g/L, the apparent viscosity of the fermentation broth was still low(Figure 4), the yeast suspensions were slightly pseudoplasitc(Figure 2) and the permeability of the membrane was good(Figure 5). The optimum cell concentration was thus determined as 130g/L.

At the constant cell concentration, the ethanol productivity was increased with the increase in the dilution rate. The productivity was decreased subsequently after reaching the maximum value. The dilution rate at the maximum ethanol productivity was not optimal beyond this dilution rate because substrate utilization was less than 60% [15, 19]. The optimal dilution rate could be selected as $1.3h^{-1}$ as shown in Figure 8 when the cell concentration was constant as 130g/L by bleeding the cells manually. At this dilution rate, the ethanol productivity was 80g/L/h and the glucose conversion was 93.9%. When the dilution rate was 1.5 h^{-1}, the ethanol productivity had the maximum value of 82.5 g/L/h, but the value of the glucose conversion was lowered by 15% than that of the case in which the dilution rate was $1.3h^{-1}$. Therefore, the dilution rate recommended in cell recycled ethanol fermentation was determined as $1.3h^{-1}$ under the experimental conditions used in this study.

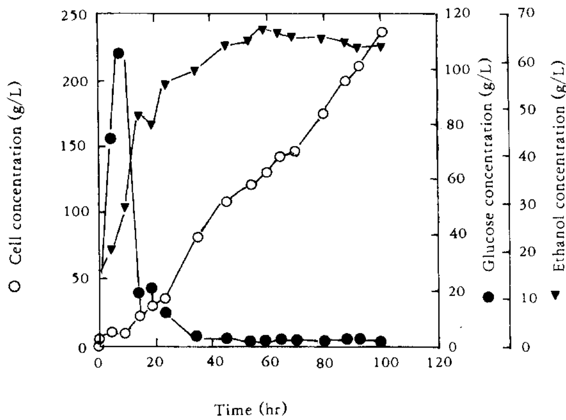

Fig. 7. Time courses of cell recycled ethanol fermentation at constant dilution rate of 0.3 h^{-1} (inlet glucose concentration = 150g/L).

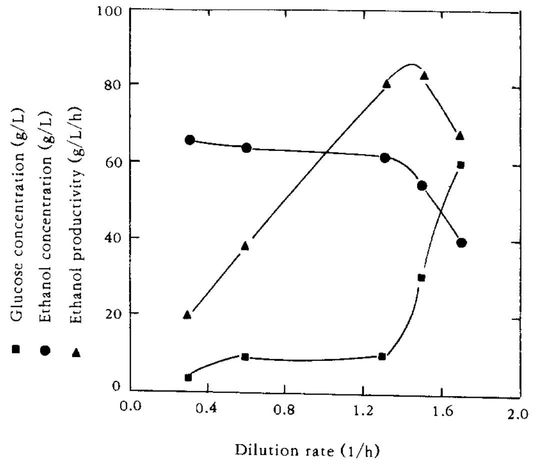

Fig. 8. The determination of the optimal dilution rate at the cell concentration of 130 g/L(inlet glucose concentration = 150g/L)

요 약

효모를 이용한 에탄올 발효에서 세포 농도가 130g/L일 때 배양액은 약간의 가소성 유체의 성질을 보이며 세포 농도가 증가할수록 cell floc의 형성으로 인하여 세포 분리를 위한 막의 투과 성능은 향상되었다. 세포재순환 에탄올 발효에서 최적 세포농도를 발효액의 유변학적 성질, 막의 투과 성능, 그리고 에탄올 생산성을 고려하여 결정하였다. 또한 최적의 세포 농도에서 최대 에탄올 생산성과 최대 포도당 전환율을 위한 희석 속도를 최적화하였다. 최적의 세포 농도는 130g/L이었고 이 농도에서 최적의 희석 속도는 $1.3h^{-1}$이었다.

NOMENCLATURE

D : dilution rate [h^{-1}

k : consistency index [cPsn]

n : flow behavior index

So : glucose concentration in feed [g/L]

Greek Letters

τ : shear stress [mPa]

$\dot{\gamma}$: shear rate [sec^{-1}]

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