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Continuous Alcohol Fermentation by a Tower Fermentor with Cell Recycle Using Flocculating Yeast Strain

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Flocculating 효모균주의 재순환에 의한 Tower 발효조를 이용한 연속알콜발효

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ABSTRACT

A study on the continuous fermentation with cell recycle by a tower fermentor to produce ethanol has been carried out. ethanol fermentation was conducted with flocculating yeast strain, Saccharomyces cerevisiae TS4, to compare the ethanol productivity with conventional continuous process. Employing a 15% glucose feed, a cell density of 50 g/l was obtaind. The ethanol productivity of the cell recycle system was found to be 26.5g EtOH/l-hr, which was nearly 7.5 times higher than the conventional continuous process without cell recycle. A cell recycle ratio of 7 to 8 resulted in the highest ethanol productivity and cell concentration. Thus the cell recycle ratio was found to be a key factor in controlling the production of clarified overflow liquid. An aeration rate above 3.8 x 10^3 VVM seemed to decrease the ethanol productivity. The continuous fermentation with cell recycle was successfully used in the separation of cells from fermentation broth with enhancement of mixing in the tower fermentor.

INTRODUCION

Oil and its derivatives are the source of the vast proportion of fuels and chemicals used today. However, increase in the real price of oils since 1973 and the approaching exhaustion of this desirable fuel has promoted an extensive evaluation of alternative sources of fuels and chemicals. Amongst these alternatives is the production of ethanol by microbiological processes using the biomass(1). More recently, the use of biomass derived ethanol as a gasoline substitute or supplement is receiving a renewed attention and has been used successfully as an automobile fuel on a massive scale in Brazil (2).

In the fuel alcohol production, it is favorable to produce the ethanol with a high productivity. However, the conventional batch fermentation showed the low overall ethanol productivity of 1.8 to 2.5 g/l-hr(3). In continuous fermentation, the high ethanol productivity may be achieved by employing a high cell concentration within a bioreactor using the cell recycle of cell immobilization(4, 5).

This paper presents to develop the continuous tower

fermentor with cell recycle using flocculating yeast strain in order to increase alcohol productivity.

MATERIALS AND METHODS

Organism.

The yeast used in the experiments was Saccharomyces cerevisiae TS4, which can flocculate, produce a high alcohol concentration and withstand a high temperature up to 40℃.

Fermentation Media.

The composition of fermentation medium was 15% glucoe (anhydrous), 1% yeast extract, 0.1% KH_2PO_4 0.1% $(NH_4)_2SO_4$ and 0.05% $MgSO_4$ $7H_2O$

Fermentation Procedure

The continuous fermentation took place in a 1.3 1 (working volume) tower fermentor, which was made from a glass cylinder (Fig. 1). The fermentation was carried out at 35℃ and the pH of fermentation broth during operation was maintained at about 4.5 using a pH controller (New Brunswick Scientific). The aeration was employed at 0.5 VVM during the initial cell build up. The antifoam agent (Silicolapse 5000) was added to the fermentor through a pump and a foam controller. The concentrated yeast was recirculated back to the fermentor from the bottom of the settling section, which was made from a 40 cm long glass cylinder with a working volume of 0.5 1. A steady state condition was attained after about 3 cycle of volume medium replacement.

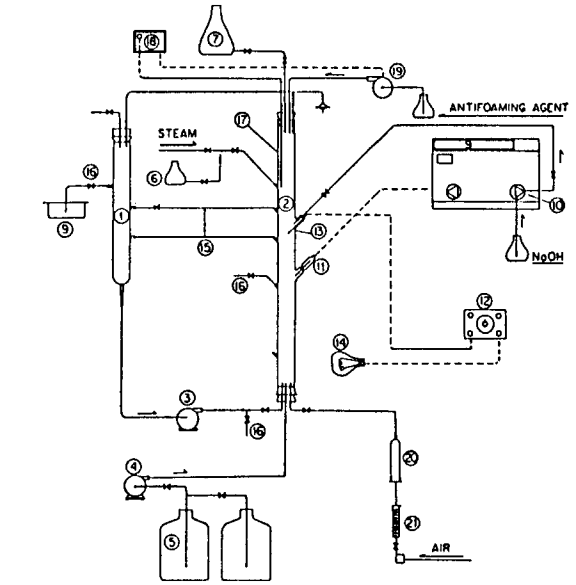

Fig. 1 Diagram of continuous fermentation with cell recycle arrangement

Analytical Methods

The cell concentration was determined by measuring the optical density at 610mm with washing the cells by the centrifugation, and the corresponding dry weight was obtained from an estabished standard curve of optical density versus dry weight. The ethanol concentration was measured by a gas chromatography (Pye Unicam) with a flame ionization detector. The column was packed with Chromosorb 103. The temperature of injector and detector was maintained at 250℃ and the column oven was operated isothermally at 150℃. The internal standard was used with 2% v/v n-propanol. The glucose concentration was measured by the Somogyimethod(6) and a refractometer.

RESULTS AND DISCUSSION

Continuous fermentation with cell recycle

The fermentor filled with the 1.5 1 of fermentation medium and inoculum. The initial cell build up was achieved by the aeration of 0.5 VVM and continuous feeding of medium at dilution rate of 0.12 hr^{-5} for 48 hrs with cell recycle in order to increase the initial cell concentration up to 40-50 g/l. The fermentation was carried out at various dilution rate and at a cell recycle rate of 6.64 l/hr without aeration.

The cell mass, ethanol and glucose concentration and productivity at various dilution rates were shown in Fig. 2. The typical continous fermentation pattern was observed; the maximum cell concentration of about 50 g/l and ethanol production f 4.02% (w/v) up to a diluton rate of 0.56 hr^{-1}. Above this dilution rate, both cell and ethanol concentration decreased rapidly due to cell washout along the overflow liquid. The maximum ethanol

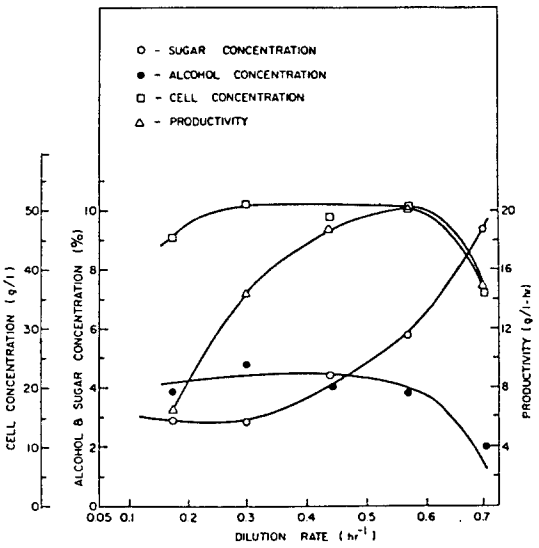

Fig. 2 Results of continuous fermentation with initial cell build up of the yeast strain TS 4.

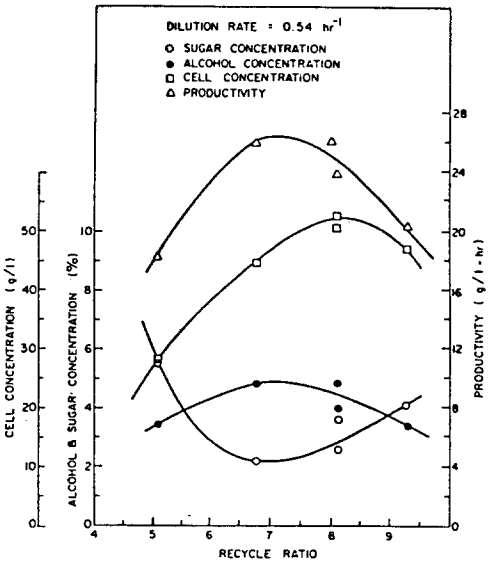

Fig. 3 Effect of recycle ratio on the ethanol production

productivity was found to be 20g EtOH/l-hr at the point before the ethanol production levelled off. Cysewski and Wilke (4, 6) reported the effect of initial sugar concentraction on the ethanol productivity in the conventional continuous fermentation. At 9% sugar in feed, the maximum fermentor productivity obtained was 7g EtOH/l-hr and as sugar concentration was increased to 15% its productivity reduced to 3g EtOH/l-hr. They also reported the maximum ethanol productivity of 29g EtOH/l-hr with 10% sugar feed in the continuous fermentation with cell recycle (6).

Effect of cell recycle ratio on ethanol production

Fig. 3 illustrates the effect of cell recycle ratio on cell mass, ethanol and glucose concentration, and productivity at the dilution rate of 0.54 hr^{-1}. At the cell recycle ratio of about 7, both ethanol concentration and ethanol productivity were at the maximum values of 4.9 g/l and 26.4g EtOH/l-hr, respectivily. It was observed that at a cell recycle ratio above 8, the cell concentration and ethanol productivity declined, because the flow rate in the settling section become higher than the settling rate of the yeast.

As a result, more cells were lost in the overflow stream than produced during the fermentation. According to this investigation, a cell recycle ratio of about 7 to 8 was the most suitable for this strain.

Effect of aeration rate on ethanol production

Fig. 4 shows the effect of aeration rate on continuous fermentation with cell recycle of 6.64 l/hr at the dilution rate of 0.57 hr^{-1}. The aeration rate above 3.8 x 10^{-3} VVM did not improve ethanol fermentation. The ethanol concentration and productivity decreased with increasing the rate of aeration, while Cysewski and Wilk (4) showed that the trace amounts of oxygen stimulated the fermentation rates and maximum ethanol production occurred at 0.07 mmHg oxygen tension. However, Cysewski et al. (6) also reported that when air was sparged into a fermentor, the ethanol productivity decreased substantially because of washout in the fermentor driven by sparging air. Therefore, this suggested that instead of air, pure oxygen be used to maintain a high enough oxygen transfer thus supporting yeast growth.

요 약

Tower fermentor를 이용한 연속 알콜발효에서 cell recycle과 aeration에 대한 영향을 검토하였다. 균주는 flocculationg 효모인 *Saccharomyces cerevisiae* TS 4를 사용하

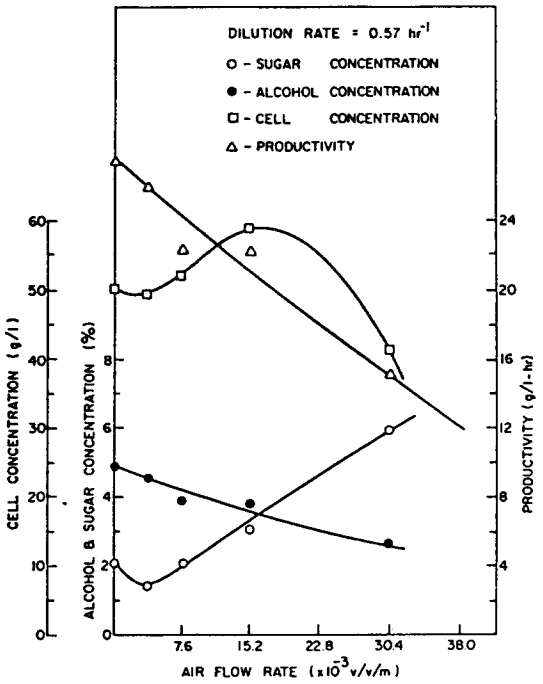

Fig. 4 Effect of air on the ethanol production

였다. 15% glucose를 사용한 cell recycle system의 연속 알콜발효에서 cell 농도는 50g/l였고, ethanol productivity는 26.4g EtOH/l-hr로서 cell ratio는 7−8에서 ethanol productivity와 cell농도가 가장 높은 값을 나타내었으며, aeration rate는 3.8×10^{-3} VVM이상부터는 ethanol productivity가 감소하였다.

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