원통형 깊이 필터를 이용한 전세포 효소 고정화 및 효소반응

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Whole Cell Enzyme Immobilization and Reaction Using a Cylindrical Depth Filter

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INTRODUCTION

Enzyme immobilization was used for the purposes of increasing the stability of the biocatalyst, optimizing the economy of a process by the repeated use of the biocatalyst, and simplifying product isolation. Among them, whole cell immobilization was often selected by its simplicity of immobilization methods and good stability of the enzymes although the enzyme activity was very low. Many whole cell immobilization methods were reported for last 40 years. Among them, some popular methods are entrapment of the cells in the polymer matrix, microencapsulations, and aggregations. However, these methods have same limitations for the strength of beads and complex bead making processes.

In this work, we introduce a simple, and efficient whole cell immobilization method using a cylindrical depth filter that was originally developed for culturing of animal cells[1-2]. This method is quite different with hollow fibers in the point of homogeneous distribution of the cells in whole filter pores. And there is very little concentration polarization. Furthermore, the flow pattern of reaction mixture is nearly plug flow and the pore size of filters are easily controlled by increasing the fiber size. Therefore, any size of microorganisms can be immobilized. At first, the immobilization efficiency of the filters and simple reactions using *Brevibacterium sp.* and yeast were carried out.

MATERIALS AND METHODS

Microorganisms: The model systems employed in this study consists of strains *Brevibacterium* sp. CH2 and recombinant yeast. The *Brevibacterium* sp. CH2[3] has a high nitrile hydratase activity and a high acrylonitrile concentration tolerance. The recombinant yeast (host strain: *Saccharamyces cerevisiae* SEY2102, *MAT* α *ura* 3-52 *leu* 2-3, -112 *his* 4-519) containing the plasmids pSEY210 and pRB58 and producing invertase was kindly donated by professor J.H. Seo (department of foodtechnology, Seoul National Univ., Seoul, Korea). The details for this strain were described in the report of Marten and Seo[4].

Culture Conditions: In the case of *Brevibacterium* sp. CH2, the culture medium contained, per liter: glucose, 15g; yeast extract, 3g; malt extract, 3g; bacto peptone, 5g; KH_2PO_4, 1g; K_2HPO_4, 1g; NaCl, 1g; $MgSO_4 7H_2O$, 0.2g; $FeSO_4 7H_2O$, 0.02g. The initial pH of the medium was adjusted to 7.1 with a 2N NaOH solution. Fermentation was carried out in a 5-liter fermenter (KFC, Korea Fermenter Co.) for 23 h at 30°C, and 500 rpm agitation. The culture broth was inoculated with 100 mL of a preculture grown in an Erlenmeyer flask containing the same medium. Aeration was performed by filtered air into the vessel at 1vvm.

The recombinant yeast cells were grown in selective SD medium[4]: 0.67% (w/v) yeast nitrogen base without amino acids (Difco), 0.5% (w/v) casamino acids (Difco), and 2.0%

(w/v) dextrose. The initial pH of the medium was adjusted to 5.5 with 1.0M HCl solution. Fermentation was carried out with the same conditions mentioned above.

Analytical Methods: Cell mass was estimated by measuring the optical density of the cell broth with a spectrophotometer (Beckman, Du-65, 600 nm). One optical unit for *Brevibacterium* and yeast were equivalent to 0.38 and 0.40 g dry wt/L, respectively.

The concentration of acrylonitrile and acrylamide was determined by gas chromatography as mentioned previously[3]. The concentration of sucrose, glucose and fructose was determined by HPLC (Hitachi) equipped with RI detector.

One unit of nitrile hydratase and invertase was defined as the amount of the whole cells that catalyzed the formation of 1 μmole of acrylamide at 4°C, pH 7.1 and glucose at 30 °C, pH 5.5 per min, respectively.

Preparation of Resting Cells: Cells were harvested from culture broth by centrifugation at 18,000g for 5 min. The harvested cells were washed twice with distilled water. These cells were used for the cell immobilization and resting cell reaction.

Reactor Setup: Whole cell immobilization and fed-batch operation were carried out in a filter unit as shown in figure 1. Filter pore sizes used were 0.5 μm to 7 μm. Continuous operation with entrapped whole cells was carried out in a reactor as shown in figure 2.

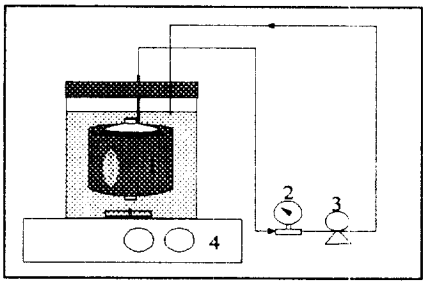

Figure 1. Whole cell immobilization unit. (1: depth filter; 2: pressure gauge; 3: peristaltic pump)

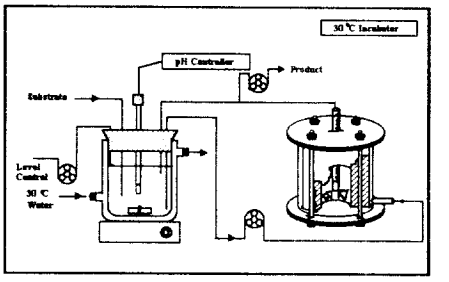

Figure 2. Operational scheme of continuous reaction with depth filter unit.

Results and Discussion

Immobilization efficiency at different pore size of filters: The immobilization efficiency, cell loading capacity and pressure drop are very important parameters in whole cell immobilization by entrapment methods. The simplicity of immobilization process can increase efficiency of whole enzyme reaction process and high cell loading capacity can reduce the total reactor volume. Pressure drop influences the pump capacity and possibility of long-term operation.

Figure 3 shows an immobilization efficiency of yeast cells into a specified pore size depth filter as circulation of cell suspension with constant flow rate of 100 ml/min. The cells were perfectly immobilized in the depth filters having the pore size of below 5 μm in 1 hr. In the 7 μm pore size filter, 28% of the initial cells were not immobilized. The filter pore sizes above 5 μm were too large to sustain all the cells.

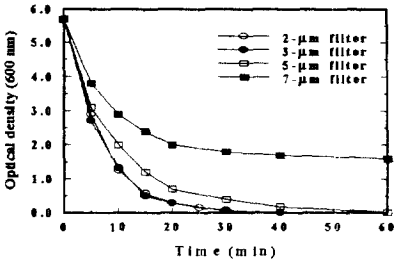

Figure 3. Effect of filter pore sizes on Yeast immobilization.

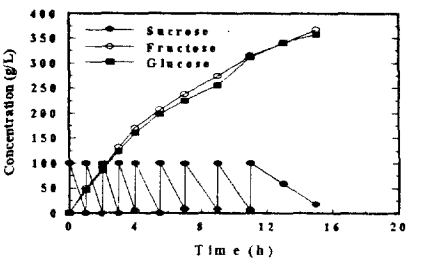

Figure 4. Fed-batch operation using immobilized cells in the depth filter unit.

Fed-batch reactions with resting cells and immobilized cells : Figure 4 shows the results of fed-batch operation using immobilized yeast cells in the depth filter unit. The conversion rate of sucrose was faster than that of in the case of resting cells. Furthermore, product inhibition effect of invertase by glucose and fructose was slightly reduced by immobilization. In this work, we did not reuse the immobilized whole cell enzymes because the invertase is very stable for long period of operation (half life is more than 20 days)[3].

Figure 5 and 6 show the results of fed-batch operation using resting cells and immobilized cells of *Brevibacterium* sp. CH2, respectively. In the case of acrylamide production by nitrile hydratase, most serious problem is product inhibition. Therefore, reducing the product inhibition effect is very important in the process of industrial production of acrylamide. The acrylamide production in the depth filter was 1.2 times higher than that in the resting cells as shown in figures. Considering the cost of immobilization and the effect of product inhibition, depth filters were very useful media for immobilizing the whole cells and using the depth filter was very effective method for producing acrylamide.

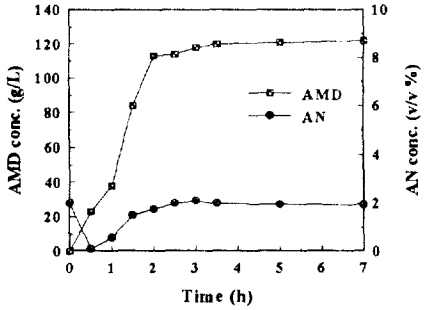

Figure 5. Fed-batch operation using resting cells of *Brevibacterium* sp. CH2.

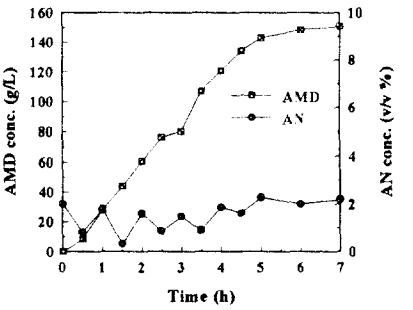

Figure 6. Fed-batch operation using immobilized cells in the depth filter unit (0.5 μm)

Continuous reaction with immobilized cells in the depth filter system: Figure 7 shows the glucose, fructose and sucrose concentrations during the continuous reaction with whole cell invertase in the depth filter unit. Dilution rate was increased stepwise to find the optimum value that shows the maximum productivity. When the dilution rate was below the 0.2 h^{-1}, conversions were nearly 100% and steady-state operation was possible. Otherwise, when the dilution rate was above the 0.2 h^{-1}, the conversion decreased slightly.

However, the maximum productivity of 110 g/L/hr was obtained at the dilution rate of 0.44 h^{-1} as shown in Figure 8. This is 4.7 times higher than that of in the fed-batch operations.

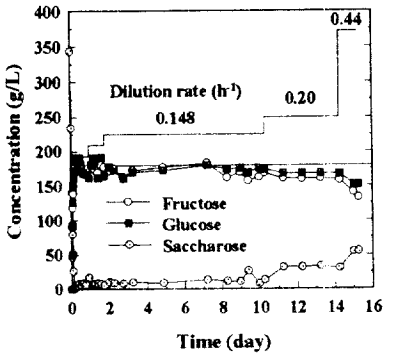

Figure 7. Continuous reaction with whole cell invertase in the depth filter unit. (Working volume=600mL, initial cell conc.=7.9 g/L)

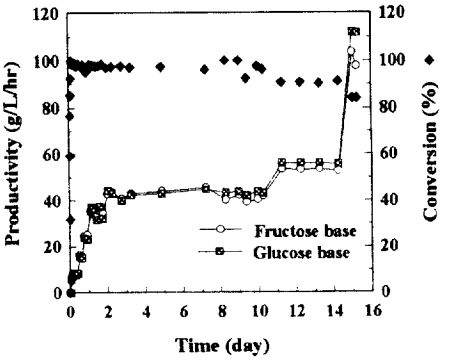

Figure 8. Time course of productivity and conversion in a continuous operation with depth filter unit.

Conclusion

Whole cell enzyme immobilization and reactions using a depth filter were successfully performed. According to the results, optimum filter pore size to immobilize the *Brevibacterium* sp. CH2 and yeast was 0.5 and 5 μm, respectively. Product inhibition effects were improved by using the depth filter immobilizing unit in both cases of yeast invertase and nitrile hydratase. In the continuous operation, high glucose or furctose productivity of 110 g/L/hr was obtained using the depth filter unit.

Accordingly, the depth filter immobilization unit displayed its potential for being one of the promising bioreactor systems for whole cell enzyme reactions.

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