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Continuous Production of Fructo-oligosaccharides by Immobilized Cells of *Aureobasidium pullulans*

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Continuous production of fructo-oligosaccharides employing a packed bed reactor charged with immobilized cells of *Aureobasidium pullulans* was investigated. The optimum conditions for reactor operation were a feed concentration of 860 g/l; a feed rate, expressed as superficial space velocity of 0.2 h^{-1}, and a temperature of 50°C. Under these optimum conditions, the productivity of the reactor was 180 g/l·h. Initial activity was maintained for more than 100 days. The reactor was successfully scaled up to a production scale of 1000l.

The application of immobilization techniques to the enzymatic conversion of sugars is very attractive. The uncharged structure and small size of sugar molecules minimizes unwanted diffusional restrictions and a high substrate concentration prevents contamination problems in an industrial operation (1, 2). An example of such a system, which has a growing industrial importance, can be seen in the production of fructo-oligosaccharides (FOS).

Currently, the industrial production of FOS is carried out using an enzyme system employing either *Aspergillus niger* (4) or *Aureobasidium pullulans* (3, 5).

In previous research, we have reported on the enzymatic behavior of *A. pullulans* cells in the production of FOS by investigating the kinetics of the enzymatic reaction. We proposed the most probable mathematical model for the reaction sequence (6) and an efficient immobilized cell system for the repeated production of FOS in a stirred tank reactor (STR) (9). We reported that a semi-batch process was superior to a continuous process in a STR. The main reason for continuous stirred tank reactor's (CSTR) ineffectiveness for this system was product inhibition caused by a high glucose concentration during the reaction. From a process viewpoint, however, it is obvious that a continuous process has many advantages over a batch or semi-batch process. The packed bed reactor (PBR), therefore, is a process that assures continuous production of FOS while minimizing the product inhibition caused by the accumulation of glucose (8). This study describes the basic kinetic characteristics of immobilized *A. pullulans* cells and application of the PBR to the continuous production of FOS.

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Key words: *Aureobasidium pullulans*, fructo-oligosaccharides, immobilized cell, packed bed reactor, scale-up

MATERIALS AND METHODS

Cell Preparation

Aureobasidium pullulans KFCC (Korean Foundation of Culture Collection) 10245 was cultivated at 28℃ for 36 h in a 2000l fermentor containing 1200l of growth medium composed of 20% sucrose, 2% yeast extract, 0.5% K_2HPO_4, 0.2% $MgSO_4 \cdot 7H_2O$, and 1.5% $NaNO_3$. Cells were harvested by centrifugation and washed twice with deionized water prior to immobilization.

Cell Immobilization

The cell suspension, containing 20%(w/v) wet cells, was mixed thoroughly with a 3%(w/v) sodium alginate (Food grade, Hayashi Pure Chemical Industries, Ltd., Japan) solution in the volume ratio of 1:2. The mixture was extruded through syringe needles (gauge No. 25) to form small beads of about 2 mm diameter before being dropped into a 0.1 M calcium chloride solution. The beads were cured for 2 h and then hardened overnight at 4℃.

Enzyme Assay

The fructosyl transferring enzyme activity in free cells was determined by measuring the released glucose, as previously described (5). One enzyme unit was defined

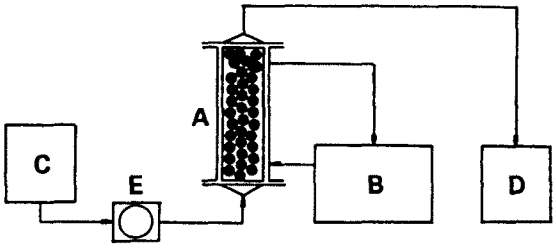

Fig. 1. Schematic diagram of the PBR for the continuous production of FOS. A; packed bed reactor, B; circulating water bath, C; substrate reservoir, D; product reservoir, E; peristaltic pump.

as the amount of enzyme activity required to produce 1 μmole of glucose per minute under the specified conditions described.

Analytical Methods

All reaction products were analyzed by HPLC as previously described (6). To enhance the speed of HPLC analysis the concentration of GF5 and higher oligosaccharides was ignored. These normally account for less than 1% of the total solids in the product mixture.

Reactor Operation

Fig. 1 shows a schematic diagram of the PBR for the continuous production of FOS. The sucrose solution was fed upward continuously to the reactor. The reactor was charged with 600 ml of alginate beads immobilized with 40 g (1,200 units/g) of wet cells. The temperature was kept constant by circulating hot water through the jacket. Unless otherwise specified the reaction was carried out with a sucrose concentration of 770 g/l; a feed rate, expressed as space velocity, of 0.15 h^{-1} and a temperature of 50℃. The pH of the feed solution, which was about 6 to 7, remained essentially constant throughout the operation. An introduction period of 20 to 24 h was required to reach a steady state. Therefore, all samples were taken after 24 hours of operation.

RESULTS AND DISCUSSION

Kinetic Characteristics of Immobilized Cells

Often, the immobilization of biocatalysts causes a degree of change in their kinetic behavior (7). Fig. 2 shows the shift in optimum temperature caused by immobilization. For immobilized cells, the temperature promoting the highest enzyme activity was about 5℃ higher than the temperature for free cells (60℃ for free cells and 65℃ for immobilized cells). This change is due to the increased stability of the immobilized cells, as well as to an increase in the effectiveness factor due to reduced mass transfer resistance as temperature rises. On the other hand, as can be seen in Fig. 3, no significant change in optimum pH was observed as cells were immobilized. However, the immobilized cells became less sensitive to the pH change.

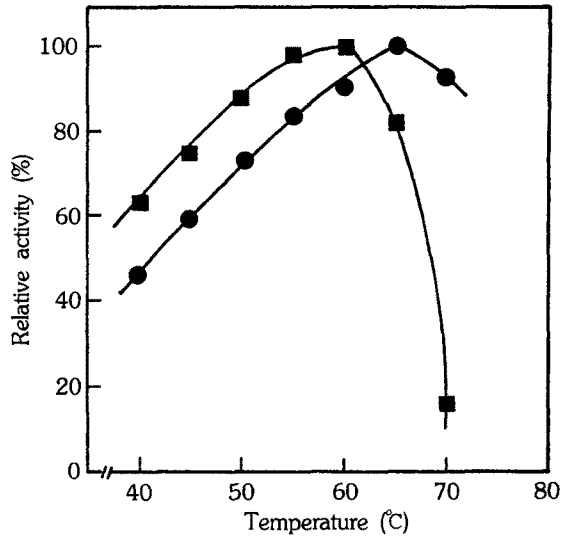

Fig. 2. Effect of temperature on the activity of free and immobilized cells.
The reaction was carried out in 250 ml Erlenmeyer flasks containing 50 ml of 770 g/l sucrose solution and 20 units of free cells or 100 units of immobilized cells. Symbols: (■); free cells, (●); immobilized cells

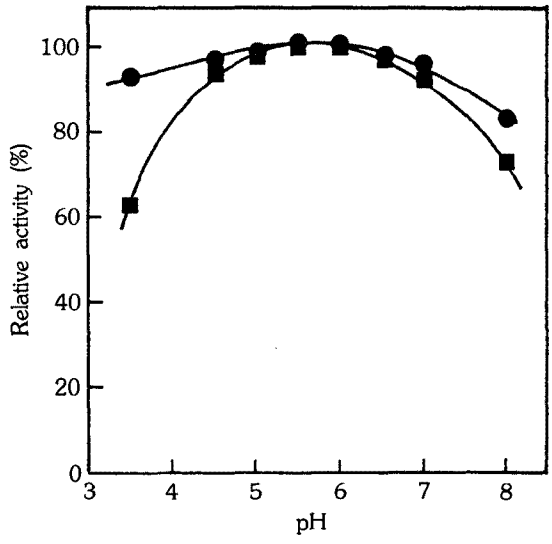

Fig. 3. Effect of pH on the activity of free and immobilized cells.
The reaction condition was the same as in Fig. 2 except that the temperature was 55℃. Symbols: (■); free cells, (●); immobilized cells

Fig. 4 shows a comparison of the thermal stability of the enzyme for free cells, immobilized cells without substrate, and immobilized cells with substrate. The relative

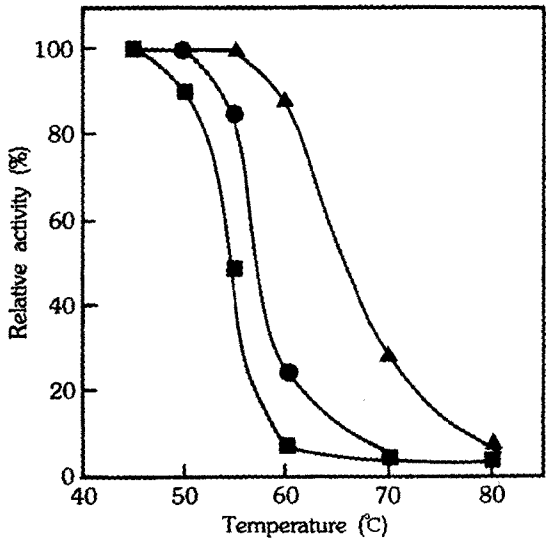

Fig. 4. Thermal stability of free and immobilized cells.
The cell suspensions were kept at various temperatures for 60 min, were then cooled to room temperature. The residual activity was measured. Symbols: (■); free cells without substrate, (●); immobilized cells without substrate, (▲); immobilized cells with substrate (770 g/l sucrose)

activity in Fig. 4 stands for the relative residual activity of cells after 60 min of heat treatment at various temperatures. It is well known that immobilization of cells resulted in significantly improved thermal stability of the cells. Another factor influencing the thermal stability of the cells is the sucrose substrate. Enzyme activity was maintained up to a much higher temperature under the influence of sucrose, which might protect the active sites of the enzyme from thermal deactivation when coupled with the enzyme.

Optimization of PBR Operation

The major operating variables affecting the performance of a PBR charged with well-prepared immobilized cells are flow rate, pH, concentration of feed, and operating temperature.

The performance of the PBR was not influenced by the pH of the feed solution in the range of pH 5 to 7. In determining the optimum temperature there are two conflicting aspects to be considered.

These are the instantaneous enzyme activity and the long-term stability. In general, increased temperature enhances enzyme activity as shown in Fig. 2. However, increased temperature results in reduced long-term stability. Therefore, an optimum temperature, achieving moderately high activity without sacrificing long-term stability, should be chosen. Fig. 5 shows the long-term operational stability of the PBR at 50℃ and 55℃. The reactor maintained its full activity for more than 100 days at 50℃ while a sharp decrease in enzyme activity

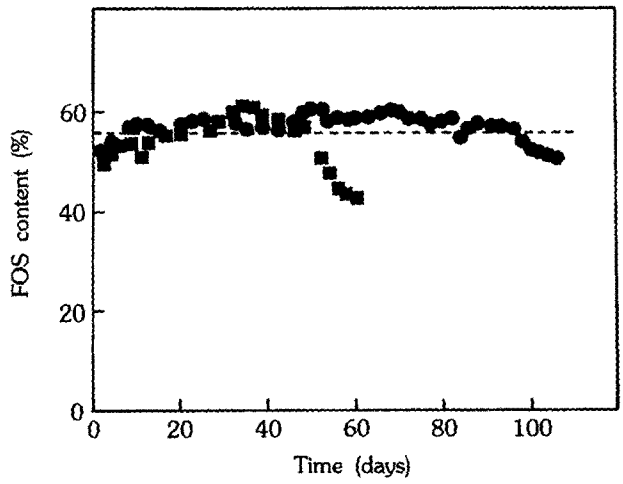

Fig. 5. Operational stability of the PBR for the continuous production of FOS.
The reaction was carried out in a 600 ml PBR fed with 770 g/l sucrose solution at the space velocity of 0.15 h^{-1}. Symbols: (●); 50℃, (■); 55℃

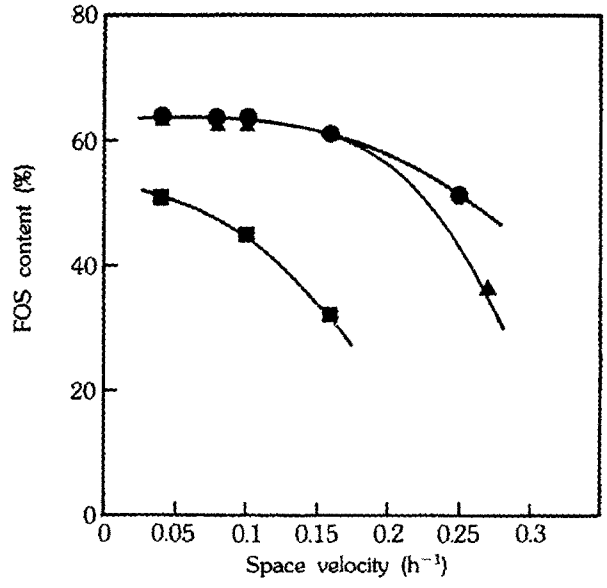

Fig. 6. Effect of flow rate and feed concentration on the continuous production of FOS.
The reaction was carried out in a 600 ml PBR fed with sucrose solution of various concentrations at the space velocity of 0.15 h^{-1} and temperature of 50℃. Symbols: (●); 700 g/l, (▲); 860 g/l, (■); 950 g/l

was observed after 50 days of operation at 55℃. Although enzyme activity was 15% higher at 55℃, 50℃ was selected as the operating temperature because of better long-term operational stability.

Fig. 6 shows the relationship between the feed rate of sucrose and the amount of sucrose converted to FOS for feed concentrations of 700, 860, and 950 g/l. It

Table 1. Comparison of product composition between semi-batch and continuous process (PBR).

Sugars	Semi-batch[a] (%)	PBR[b] (%)
Fructose	0.6	0.6
Glucose	24.9	24.3
Sucrose	14.0	14.0
GF_2	33.0	32.5
GF_3	20.9	21.3
GF_4	6.5	7.3
ΣGF_n	60.4	61.1
Total	100	100

[a] Reaction time 20 h; Bead volume 20%(w/v)
[b] Flow rate (space velocity) 0.2 h^{-1}

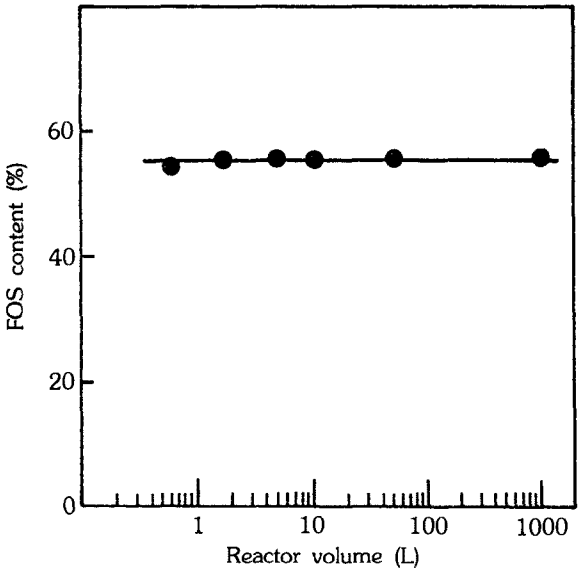

Fig. 7. Scale-up results of the PBR for the continuous production of FOS.
The reaction was carried out in PBR's of various sizes fed with 770 g/l sucrose solution at the space velocity of 0.15 h^{-1} and temperature of 50℃.

appears that a feed concentration of 950 g/l (about 70% on the basis of weight) is too high to produce a commercially acceptable product syrup (more than 55% FOS in the total solid content), even at a low flow rate. A feed concentration of 860 g/l (65% on the basis of weight) was chosen as the optimum concentration. The maximum feed rate, achieving more than 55% FOS content, was about 0.2 h^{-1} expressed as superficial space velocity. At this feed rate the volumetric productivity of the reactor was180 g/l·h, about four-folds higher than that of STR. Table 1 shows a composition of the product mixture produced by a PBR and a STR operated under the above mentioned conditions. There was essentially no difference in product composition between the PBR and the STR.

Scale-up Results of the PBR

The size of the PBR was gradually increased and reactor performance was investigated, as shown in Fig. 7. The height-to-diameter ratio of the reactor was kept constant at 3:1 for all sizes of reactor. As shown in Fig. 7, similar FOS contents were obtained, irrespective of the reactor size. However, as the reactor size was increased, an extra effort to maintain the uniformity of the flow rate throughout the cross section of the reactor was required. Proper design and operation of a PBR is required to achieve uniform radial temperature within the PBR. The difference in solution viscosity, caused by the temperature gradient resulted in unbalanced channelling of the solution. Uniform packing of the calcium alginate beads and proper design of the distributor is important in preventing channelling phenomenon.

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