Biotechnol. Bioprocess Eng. **1996**, 1, 18-21

Continuous Production of Fructooligosaccharides Using Fructosyltransferase Immobilized on Ion Exchange Resin

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A continuous production of fructooligosaccharides from sucrose was investigated by fructosyltransferase immobilized on a high porous resin, Diaion HPA 25. The optimum pH (5.5) and temperature (55°C) of the enzyme for activity was unaltered by immobilization, and the immobilized enzyme became less sensitive to the pH change. The optimal operation conditions of the immobilized enzyme column for maximizing the productivity were as follows: 600 g/L of sucrose feed concentration, flow rate of superficial space velocity 2.7 h^{-1}. When the enzyme column was run at 50°C, about 8% loss of the initial activity of immobilized enzyme was observed after 30 days of continuous operation, during which high productivity of 1174 g/L · h was achieved. The kinds of products obtained using the immobilized enzyme were almost the same as those using soluble enzymes or free cells.

Key words: Fructooligosaccharides, Fructosyltransferase, Immobilized enzyme, Immobilized cells

INTRODUCTION

Recently, sugar industry has faced intense competition from new sweeteners so-called oligosaccharides. Various oligosaccharides such as isomalto-oligosaccharides [1], soybean-oligosaccharides [2], and fructooligosaccharides [3, 4] are known to be promising sweeteners which are beneficial to human and animal health. In particular, fructooligosaccharides (FOS) from sucrose have received a special attention because mass production is easy, like sucrose their taste is similar, and their functional properties are well characterized compared with other oligosaccharides [5, 6].

FOS have been discovered from many plants, such as asparagus [7] and Jerusalem artichoke [8]. The industrial production of FOS, however, uses mainly the microbial fructosyltransferase derived from *Aspergillus niger* [3, 9] or *Aureobasidium* sp. [5, 6, 10]. Current industrial processes for the production of FOS utilize two reaction systems, a batch system with a soluble enzyme [4] or a continuous system with the immobilized cells entrapped in the calcium alginate gels [10]. These two systems, however, could not satisfy an increasing demand for FOS due to the low productivity. Although the continuous system is more effective in operation than the batch one, low volumetric enzyme activity resulted from the diffusional restriction of substrate and product in the bead and cell has reduced the productivity of the reactor. Reflecting this situation, productivity is obviously a critical factor for mass production of FOS. To solve this problem, some researchers have tried to immobilize soluble fructosyltransferase onto several support materials, such as porous glass [11, 12] and DEAE-cellulose [13]. However, a practical system satisfying an industrial application has not been reported up to the present; that is, the immobilization procedures were rather complicated and the operation was not easy to be optimized within the range of actual processing.

In this paper, to achieve a high FOS productivity, the immobilization of the enzyme on a high porous resin was examined. All experiments were performed taking into consideration of scale-up to an industrial scale.

MATERIALS AND METHODS

Chemicals

The sucrose used in this experiment was food-grade, while other chemicals were reagent grade.

Enzyme preparation

Fructosyltransferase was obtained from a flask culture of *Aureobasidium pullulans* KFCC (Korean Foundation of Culture Collection) 10524 as described by Jung *et al.* [21]. To prepare the enzyme solution, log-phase cells were harvested by centrifugation and resuspended in deionized water. The cell suspension was treated with 2% (w/v) lysozyme, Kitalase (Kumiai-kagaku, Japan) for 2 h at 45°C and then filtered using diatomaceous earth. The filtrate was used as crude enzyme solution without further purification.

Enzyme immobilization

A high porous ion exchange resin (Diaion HPA 25, Mitsubishi Chemical Co., Japan) was equilibrated with 0.1 M citrate buffer (pH 5.5) for 24 h, and packed

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into a glass column (2.5× 12 cm) with a bed volume of ca. 50 cm^3. The crude enzyme solution was prepared in 0.05 M citrate buffer (pH 5.5) and then the resulting enzyme solution was employed into the column at a flow rate of 50 cm^3/h. After immobilization for 10 h at an ambient temperature, the column was washed thoroughly using 0.5 L of deionized water. No activation of support was carried out before or after immobilization in order to simplify the immobilization procedure.

Reactor operation

To avoid a channeling problem in actual column operation (due to a density difference between water and sucrose solution), 300 mL of sucrose solutions ranging from 10 to 50% (w/v) were consecutively run. To evaluate a reactor performance, the column was continuously operated at various flow rates using 600 and 770 g/L of sucrose as substrate at 50°C, respectively. Operation of the column was facilitated by the upward flow of substrate such that self-compression of the immobilized enzyme causing a clogging problem was minimized. The effluent concentration of fructo-oligosaccharides was analyzed after 24 h operation, when a steady state was essentially established. Long-term stability of the immobilized enzyme was evaluated using 600 g/L sucrose at a flow rate of 130 cm^3/h (SV 2.7 h^{-1}) at 50°C. Unbuffered sucrose was used throughout the experiments.

Enzyme assay

The activity of soluble fructosyltransferase was determined by measuring the amount of glucose released under the following conditions: reaction mixture consisted of 7.5 ml of 800 g/L sucrose, 2.3 mL of 0.1 M sodium citrate buffer (pH 5.5) and 0.2 ml of enzyme solution; pH, 5.5; temperature, 55°C; reaction time, 1 h. In case of the immobilized enzyme, the enzyme solution was replaced by one gram of the immobilized enzyme. One fructosyltransferase unit is defined as the amount of enzyme activity required to produce one μmol of glucose per minute under the conditions described.

Analytical methods

All reaction products were directly analyzed by HPLC (Varian, USA) using an Aminex HPX-42C column (0.78× 30 cm, Bio-rad, USA) and refractive index detector (Varian, USA). The column temperature was maintained constant at 85°C. Water was used as a mobile phase at a flow rate of 1.0 mL/min. Total amount of FOS was determined by the sum of 1-kestose (GF_2: G, glucose; F fructose), nystose (GF_3), 1^F-fructofuranosyl nystose (GF_4), and 1^F-$(fructofuranosyl)_4$-sucrose (GF_5).

RESULTS AND DISCUSSION

Optimum pH and temperature of the immobilized enzyme

To determine the optimum pH and temperature of the immobilized fructosyltransferase, one gram of the immobilized enzyme was reacted with 7.5 ml of 800 g/L sucrose prepared in a buffer solution within the pH range of 4-8 (for pH 4-6, 0.1 M citrate buffer; for pH 7-8, 0.1 M phosphate buffer) at 55°C for 1 h. The result was represented as a relative production rate of 1-kestose (percent maximum). As shown in Fig. 1 and 2, the optimum pH and temperature of the immobilized enzyme was essentially unaltered compared with those of the soluble enzyme. However, the immobilized enzyme became less sensitive to the pH change. This result was comparable with the case of immobilized cells, where pH optimum (pH 5.5) was unchanged while temperature optimum of the immobilized cells (65°C) was shifted to 5°C higher than that of free cells [10]. This is partly because there exists a microenvironmental difference between the immobilized enzyme and the immobilized cells.

Optimization of the immobilized enzyme column

Common operating variables affecting the performance of the immobilized enzyme column are the flow rate of feed, feed concentration, and operation tem-

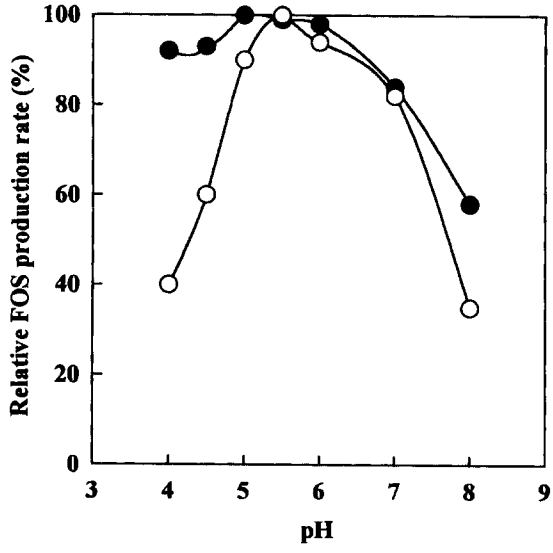

Fig. 1. Effect of pH on enzymatic activities of soluble enzyme (○) and immobilized enzyme (●).

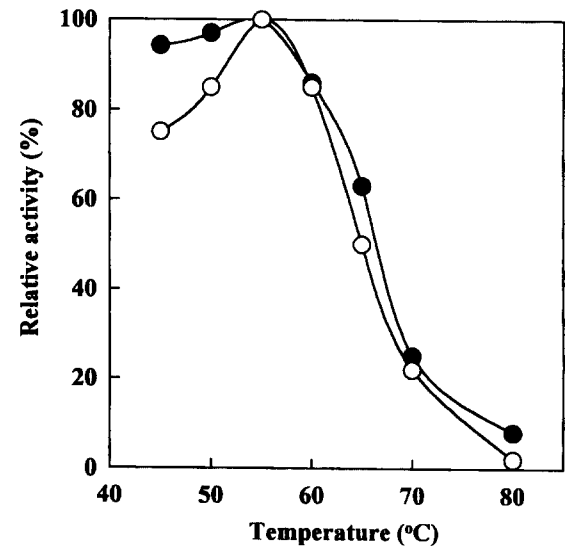

Fig. 2. Effect of temperature on enzymatic activities of soluble enzyme (○) and immobilized enzyme (●).

perature. Considering the optimal pH (5.5) for the activity of immobilized enzyme, the pH of substrate, sucrose solution, was recommended to be naturally maintained at 5.5-6.0 during the processing of unbuffered sucrose to facilitate reactor operation. Because the optimum temperature for the activity of the immobilized enzyme was found to be 55°C, it would be reasonable to operate the column at a lower temperature (50°C) considering a stability during long periods of operation [10, 14, 15]. Fig. 3 presents the relationship between the degree of FOS conversion and feed flow rate (expressed as a superficial space velocity, SV) at two sucrose concentrations. To achieve more than 55% of FOS conversion based upon total solid content (i.e. commercially acceptable level) as well as to maximize the productivity of FOS, the column should be operated at flow rates of SV 1.5 h^{-1} and SV 2.7 h^{-1} with feed concentrations of 770 g/L and 600 g/L, respectively. The result suggests that between the two concentrations 600 g/L sucrose was the appropriate feed concentration to maximize the productivity.

It is well known that as flow rate and substrate concentration increases, the productivity of product proportionally increases. In addition, using highly concentrated sucrose feed solution can minimize the occurrence of microbial contamination. Fig. 3 shows that maximum productivity of 1174 g/L · h is obtained using 600 g/L sucrose solution at a flow rate of 135 mL/h (SV 2.7 h^{-1}). This result is quite remarkable compared with that of immobilized cell system. Much higher productivity can be achieved with more concentrated substrate. In case of immobilized cell column reported elsewhere [10], the maximum productivity was achieved at a flow rate of SV 0.2 h^{-1} using 770 g/L sucrose solution. Compared with the result, the immobilized enzyme column described here is virtually better to achieve a high productivity (see Table 2). The low volumetric enzyme activity in the immobilized cell column was chiefly ascribed to the diffusional limitations of sugars and low concentration of enzyme associated with immobilized cells. On the other hand, the high porosity of the support used in this work allowed more enzymes to be immobilized, yielding a high volumetric enzyme activity.

Operational stability of the immobilized enzyme

The immobilized enzyme activity may be rapidly reduced by the presence of impurities in the substrate. This possibility was partially supported by the fact that the color of the supports were changed into red brown through the column after long periods of continuous operation. The column stability of the immobilized enzyme is well correlated with the spent volume of the substrate. Therefore, an operational column stability of the immobilized enzyme should be evaluated under actual operating conditions.

Hayashi *et al.* [11, 12] reported an excellent system of FOS production by an immobilized fructosyltransferase onto a silanized porous glass, with which they successfully operated the immobilized enzyme column up to 30 days without any loss of initial enzyme activity. Furthermore, they selectively produced 1-kestose at an extremely high flow rate. However, their system was not focused on actual application of the system to an industrial purpose. They used too low concentration of buffered sucrose (below 40%), the preparation of enzyme and support material was somewhat complicated, and operation temperature (i.e. 30°C) was low, where contamination problem might occur in a long-term operation. Figu.4 displays the long-term stability of the immobilized enzyme column at two reaction temperatures. Although the immobilized enzyme was greatly unstable at 55°C, only about 8% loss of the initial enzyme activity of the immobilized enzyme was observed at 50°C after 30 days of continuous operation.

Comparisons between enzyme and cell reactors

Table 1 shows composition of the reaction products catalyzed by different forms of enzyme and different modes of reaction. Although there was no significant difference in product compositions among forms of enzyme, there exists a considerable difference between immobilized enzymes and immobilized cells. The diffusional restriction associated with entrapped cells has resulted in the appearance of an higher oligosaccharide (GF_5). In Table 2, the immobilized enzyme system for FOS production developed in this work was compared with other systems reported in the literatures. The productivity was evaluated by the amount of FOS produced on the basis of unit bed volume and unit time. As illustrated in Table 2, it is

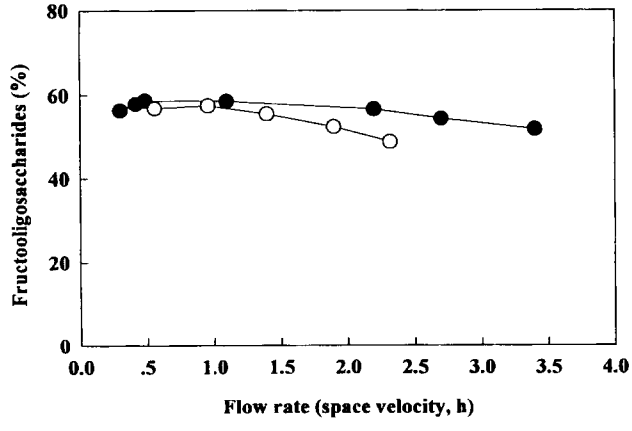

Fig. 3. Effect of feed flow rate and feed concentration on the rate of fructo-oligosaccharide production: (○) 770 g/L sucrose, (●) 600 g/L sucrose.

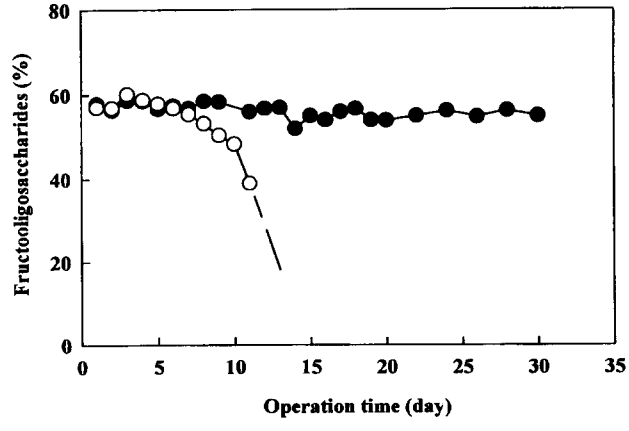

Fig. 4. Operational stability of the immobilized enzyme at different reaction temperatures: (○) 55°C, (●) 50°C. Reactors were operated at flow rate of 2.7 h^{-1} as a superficial space velocity using 600 g/L sucrose.

Table 1. Comparison of fructooligosaccharide compositions produced by different enzyme forms and reaction modes[a]

Carbohydrates[b]	Compositions (%, w/w)[c]					
	enzyme	immobilized enzyme		cells	immobilized cells	
		batch	continuous		batch	continuous
Glucose (G)	26.1	25.7	27.6	27.7	30.0	26.9
Sucrose (GF)	19.0	19.5	15.7	15.5	15.0	15.1
DP3 (GF_2)	40.5	39.0	36.4	39.9	26.0	30.9
DP4 (GF_3)	13.8	14.2	17.5	15.3	19.5	20.2
DP5 (GF_4)	0.6	1.3	2.7	1.3	7.8	5.6
DP6 (GF_5)	0	0	0	0	1.7	1.3
Total FOS (GF_n)	54.9	54.5	56.6	56.7	55.0	58.0

[a]All reactions were conducted at the same residence time of 20 h at 50°C.
[b]DP means degree of polymerization; GF_2, 1-kestose; GF_3, nystose; GF_4, 1^F(1-β-fructofuranosyl) nystose; GF_5, 1^F(1-β-fructofuranosyl)$_4$ sucrose.
[c]Enzyme and cells refer to soluble enzyme and intact cells, respectively.

Table 2. Comparison of the productivity in fructooligosaccharide productions in various immobilized systems

Systems[a]	Enzyme source	Productivity (g/L · h)[b]	Authors
IC(semibatch)	*Aureobasidium pullulans*	45	Yun *et al.* (1990)
IC(continuous)	*Aureobasidium pullulans*	180	Yun *et al.* (1992)
IC(continuous)	*Aspergillus japonicus*	234	Cheng *et al.* (1996)
IE(continuous)	*Aureobasidium* sp.	1190	Hayashi *et al.* (1991)
IE(continuous)	*Aureobasidium pullulans*	1174	This work

[a]IC and IE indicate immobilized cell and immobilized enzyme, respectively.
[b]Productivity was estimated based on the first 30 days of operation of the reactors (total amounts of fructo-oligosaccharides per unit reactor volume per unit time).

clear that the productivity using immobilized enzyme is superior to that using immobilized cells. The productivity described in this work is almost the same as the case of another immobilized enzyme system reported by Hayashi *et al.* [11, 12]. To maximize the productivity and to produce only 1-kestose selectively, they operated the immobilized enzyme column at an extremely high flow rate. Consequently, the yield of FOS (46%) was low compared with the result described in this study (58%).

As shown above, the ion exchange resin used in this study is a useful support for immobilization of fructosyltransferase. Furthermore, because of an excellent productivity and simple processing procedure, the immobilized enzyme system can be recommended as an alternative to a conventional system for FOS production.

Acknowledgment This work was supported by the Korea Science and Engineering Foundation (KOSEF).

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