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Production of Oligosaccharides from Sucrose for Animal Industry

Jae Heung Lee, Hyung Tai Shin, and Soo Won Lee*
Dept. of Food Bio-Technology, Sungkyunkwan University

Introduction

The immobilization of whole microbial cells and their application has been of interest for nearly 30 years [1~4]. Production of L-aspartic acid on an industrial scale using immobilized *Escherichia coli* cells was developed in 1973[5~6]. Cheetham et al.,[2] using immobilized *Erwinia rhapontici* cells, produced isomaltulose, while Yun et al.[3] studied the semibatch production of fructo-oligosaccharides from sucrose by immobilized cells of *Aureobasidium pullulans*. A stirred-tank bioreactor with the immobilized cells was used in this latter process and gave steady production for over 1200 h.

The aim of this study was to develop a novel method for cell immobilization and investigate its potential for the improvement of bioreactor performance. Freeze-dehydration was used to remove water from alginate immobilized *A. pullulans* cells. The kinetic characteristics of the production of fructo-oligosaccharides using the dehydrated beads with sucrose as substrate were investigate

Materials and Methods

A. pullulans ATCC 9348 was grown at 28℃ for 72 h at 100 rpm in a shaking incubator in the medium. Twenty ml of the culture broth was centrifuged, washed with deionized water and then resuspended to 20 ml in deionized water. The cell suspension was then used for the cell immobilization or as a free cell source.

Twenty ml of about 20% (w/v) wet cells was mixed thoroughly with 40 ml of 3% (w/v) sodium alginate (Junsei Chemical, Japan) at the room temperature. The mixture was extruded as small beads into a 1% (w/v) calcium chloride solution. The immobilized cells (i.e. hydrated beads) were cured at the room temperature for 2 h and then hardened overnight at 4℃. Tubes with 30 hydrated beads in 10 ml of water were placed in a freezer compartment (- 15℃) for 6~24 h to induce the freeze-dehydration process. After thawing at the room temperature, the shrunken beads were withdrawn, blotted gently with a tissue paper to remove external moisture, and then used in enzyme kinetic studies. The size and geometry of the hydrated and dehydrated beads were examined by using a video microscope system(Sometech Inc., Korea).

Fructosyl transferase (EC 2.4.1.9) activity was determined by measuring the release of glucose. One unit

was defined as the amount of enzyme activity required to produce one μ mole of glucose per min under the following condition: temperature 55℃, pH 6.2, in a reaction mixture consisting of 10 ml of 60% (w/v) sucrose and 30 hydrated or dehydrated beads. The enzyme reaction was stopped by heating the suspension at 100℃ for 15 min and then released glucose was measured using a glucose oxidase-peroxidase test kit as per manufacturers recommendations. Unless otherwise stated, the enzyme reactions were carried out for 1 h under the conditions described.

Kinetic studies of the enzyme was done by Jung et al.[7] The enzyme reaction products such as GF_2, GF_3, and GF_4 were analyzed using an HPLC (Hitachi L-6200, Japan) equipped with the Kromasil 100-10 NH_2 (250 × 4.6 mm) column. A mixture of acetonitrile/distilled water (7:3, v/v) was used as the mobile phase at a flow rate of 1 ml/min. The column temperature was controlled at 40°C. Fructo-oligosaccharides standards were purchased from Wako Pure Chemical Industries (Osaka, Japan).

Results

Comparison of characteristics of hydrated and dehydrated beads

Relative sizes and geometry of beads before and after the freeze-dehydration process were investigated. The dehydrated beads showed both a reduction in size and a change in shape to an irregular form. The effect of freezing time on the weight loss of the hydrated beads was investigated. Tubes with 30 beads were subjected to slow freezing to -15℃. Water removal resulted in an 85% reduction in final bead weight. During the freezing-dehydration process no loss of cells from the bead mass was observed and enzyme activity was not found in the aqueous phase. A comparison of the characteristics of the hydrated and the dehydrated beads is summarized in Table 1.

Kinetic characteristics of dehydrated beads

When free cells were placed at - 15℃ for 0~24 h, there was no change in the enzyme activity or cell morphology. It can be seen in Table 1 that the relative enzyme activity for the dehydrated beads compared to the hydrated beads decreased significantly. A likely explanation for this finding is that increased diffusional resistance to substrate permeation due to shrinkage of the beads occurred.

Table 1. Comparison of characteristics of hydrated and dehydrated beads

	Hydrated	Dehydrated
Bead size	2.9±0.2 mm	1.8±0.3 mm
Bead geometry	Spherical	Irregular
Relative bead weight	100%	152 %
Relative packed volume of beads	100%	182%
Relative enzyme activity	100%	355%

The freeze-dehydration was carried out for 12 h at - 15℃.

Application of dehydrated beads for production of oligosaccharides

Table 2 shows the enzyme reaction data obtained with the hydrated and the dehydrated beads after 24 h incubation. The lower relative enzyme activity of the dehydrated beads compared to the hydrated beads, resulted in the lower enzyme loading per g sucrose and thus lower total fructo-oligosaccharide (GF_2+GF_3+GF_4) production (Table 2). The lower enzyme activity with the dehydrated beads means that increased enzyme dosage levels (i.e. more dehydrated beads) would be required for similar bioreactor productivity.

The enzyme reactions were carried out in 10 ml cap tubes for 24 h with 60% (w/v) sucrose as a substrate.

Table 2. Batch enzyme reaction data with hydrated or dehydrated beads at 55℃ and pH 6.2

		Hydrated	Dehydrated	
Number of beads used		30	30	75
Enzyme loading (U per g sucrose)		4.4	1.8	4.5
Composition of reacrton products(%)				
Mono- and	G	22	13	26
disaccharides	GF	34	64	30
Fructo-	GF_2	31	17	28
oligosaccharides	GF_3	12	6	13
	GF_4	1	-	3
	GF_2+GF_3+GF_4	43	23	44
	Total(%)	100	100	100

Discussion

The immobilization of cells often causes some changes in kinetic patterns of the resulting product. A shift in optimum temperature for immobilized cells compared to free cells was observed in this study. This change was probably due to the increased stability and integrity of the immobilized cells. Similar results were also reported previously [5, 8].

During slow freezing of alginate beads, ice forms outside the beads which lowers the vapor pressure and hence pulls water from inside of the beads. This phenomenon known as freeze-dehydration, can be used to effectively reduce both the weight and volume of the resulting biocatalyst. Although the relative enzyme activity with the dehydrated beads was only 35% (Table 1) compared to the hydrated beads, it is possible to pack considerably more into a fixed volume bioreactor since the relative volume of the former is only 18%. This means that over 5 times as much biocatalyst can be packed into the equivalent volume reactor leading to a potential gain in bioreactor productivity of nearly 2 fold. This potential for improvement in reactor performance may offer significant commercial advantages in some bioconversion processes. In addition, the relatively smaller size of the dehydrated beads may make it possible to adopt alternative

fluidized-bed bioreactor designs to further increase productivity parameters. Thus, this simple process for making dehydrated beads may enable improvement in immobilized system performance and wider design applications.

Summary

The purpose of the present investigation was to develop a novel method for cell immobilization. *Aureobasidium pullulans* cells were mixed with an alginate solution, and the mixture was extruded to form small gel beads as hydrated- immobilized cells. The beads were then placed at -15°C for 6-24 h to induce freeze-dehydration. The freeze-dehydration resulted in shrinkage of beads due to water removal reducing bead volume by 82% and bead weight by 85%. The dehydrated beads were successfully used for the production of fructo-oligosaccharides in a model reactor system. This study showed that bioreactor performance can be improved up to 2 times by the use of the dehydrated beads.

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