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PRODUCTION OF SHORT CHAIN FRUCTOOLIGOSACCHARIDES

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Pectinex Ultra SP-L, a commercial enzyme preparation with fructosyl-transferase activity and ability to produce short chain fructooligosaccharides, was immobilized onto anionic ionexchange resin by a combined method. During the work this solid-phase biocatalyst was used for the production of fructooligosaccharides from saccharose, where glucose was formed as inhibiting by-product. In the experiments the optimal biocatalyst/matrix ratio and the optimal immobilization conditions: concentration of cross-linking agent, immobilization time and optimal operational conditions: temperature and pH were determined. Moreover an integrated reactor system was constructed for simultaneous fructooligosaccharides production synthesis and glucose elimination to enhance the product yield.

Keywords: fructosyl-transferase, glucose-oxidase

Introduction

Recently maintaining physical health and well-being has become more and more important worldwide, which requires careful nutrition including wholesome food products and food additives. The so-called functional foods contain useful components that have beneficial effects on health conditions [1].

Typical representatives of functional foods are the fructooligosaccharides (FOS). Their significance have risen recently in human and animal nutrition, primarily because of their advantageous effects on the intestinal bacterial population and general health conditions in the body [2]. FOS are not decomposed in the small intestine by the digestive enzymes so reach the colon where they are fermented by the microbial flora (e.g. *Bifidobacteria sp., Lactobacillus sp.)* to lactate and short chain fatty acids, like acetate, propionate, butyrate. Consequently, FOS stimulate the growth and vitality of these microbes and prevent spreading of the harmful pathogens. In addition, they have low sweetness intensity, their caloric value is low, approximately 8–9 kJ g^{-1} and cause no caries. So they can be applied as alternative sweeteners and a part of diet [3, 4].

Short chain FOS are mainly composed of 1-kestose (GF_2), nystose (GF_3) and fructosyl-nystose (GF_4), in which two, three and four fructose units are bound to one unit of glucose, respectively.

They can be found in plants and vegetables, including onion, asparagus, rice, sugar beet, wheat, etc. but generally in low concentration. The industrial scale recovery from these plants is not economical since their low concentration, for this reason, FOS are produced commercially *via* biosynthetic as well as hydrolytic methods using fructosyl-transferase (FTF) enzyme. The raw material of this reaction is sucrose and the product mixture contains unconverted sucrose besides GF_2, GF_3 and GF_4 and glucose as a by-product [5]. The latter component is a strong competitive inhibitor of the synthesis [6]. Elimination of the formed by-product component can result an increase in the product yield. For this purpose several methods can be applied: e.g. membrane separation [7-9], chromatographic separation, or enzymatic method like elimination by glucose oxidase.

The partial hydrolysis of inulin is also used practically for fructooligosaccharide production. Inulin recovered from Jerusalem artichoke (*Helianthus tuberosus*) and chicory (*Cichorium intybus*) species is used currently as substrate of endoinulinases (EC 3.2.1.7) by the industry to produce GF_2-GF_4 FOS [10].

The immobilization of the biocatalysts offers a lot of practical advantages, e.g. easy separation of enzyme and product, the opportunity to realize a continuous process, the enhancement of volumetric productivity of the reactor, etc [11]. Therefore the objects of our work were as follows: to develop an immobilization procedure of a commercial enzyme solution having significant FTF activity, to examine and establish the optimal immobilization conditions, to test the operational possibilities during shake flask experiments, to study the production of FOS with the immobilized biocatalyst in lab scale and to investigate the operation of an integrated system developed for simultaneous by-product elimination. In this paper part of the results of our work are summarized.

Materials and methods

A commercial grade complex enzyme preparation originated the production of short chain fructo-oligosaccharides, originated from *Aspergillus aculeatus* – Pectinex Ultra SP-L (Novozymes, Denmark) – containing fructosyl-transferase activity beside other enzyme activities (pectinase, cellulose, β-galactosidase) [12]. For the glucose elimination glucose oxidase (GOD) enzyme (FLUKA) with 215 U cm^{-3} and catalase (SIGMA) with 830 U cm^{-3} activities were used.

Short chain fructooligosaccharides as 1-kestose, nystose and fructosyl-nystose were obtained from Wako Pure Chemical Industries (Japan) Ltd. All other reagents were reagent grade.

For the immobilization of biocatalysts an anionic ion exchange resin, Amberlite IRA 900 Cl (Rohm and Haas, Germany) was applied. It is a styrene-divinylbenzene copolymer matrix, with 650–720 μm mean particle diameter, 40–75 nm pore diameter and 25 m^2 g^{-1} specific surface area.

In the reaction catalyzed by FTF, the same molar quantity of glucose is formed as the total moles of the different FOS molecules produced. That is why the activity of the enzyme was determined measuring *glucose formed* in the reaction. One unit of FTF activity was defined as the quantity of enzyme that liberated one μmol of glucose per minute [5].

The catalytic activity of Pectinex Ultra SP-L was determined on the basis of HANG & WOODAMS [12]. The applied reaction conditions were 2 M initial sucrose concentration, at 55 °C, pH 5.6 and 2 h incubation time. It has been found that Pectinex Ultra SP-L has 10.9 U cm^{-3} fructosyl-transferase activity.

The catalytic activity of GOD-catalase enzyme product was determined regarding to the GOD activity of the biocatalyst. One unit of GOD activity was defined as the amount of enzyme that transformed 1 μmol of glucose per minute [1].

The glucose concentration was determined with o-dianisidine method [13] in the case of FTF-activity determination. When glucose oxidase activity was measured we used the o-toluidine method [14].

Fructooligosaccharides were analysed by HPLC under the following conditions: Merck-Hitachi L-6000A HPLC apparatus, RI-71 refractive index detector, Aminex HPX-42A column of 300×7.8 mm, 25 °C, distilled water eluent, 0.2 cm^3 min^{-1} flow rate.

Results

During our work a solid-phase biocatalyst (15.6 U g^{-1}) for the production of fructooligosaccharides was developed. The exact method and process were presented earlier [15].

For the elimination of glucose a co-immobilized fine chemical grade glucose oxidase-catalase solid-phase biocatalyst was manufactured. Hydrogen peroxide formed in the reaction is an inhibitor for the enzyme, so it should be removed from the reaction mixture. In our system catalase was applied for the decomposition of H_2O_2. The GOD activity of the prepared biocatalyst was 40.4 U g^{-1} and the catalase activity was 39.5 U g^{-1}.

An immobilization procedure was elaborated for these biocatalysts onto an anionic ion exchange resin, Amberlite IRA 900 Cl, using a combination of adsorption and cross-linking by glutaraldehyde treatment. The immobilization parameters and operational conditions have been optimized for the biocatalysts *(Table 1).*

Table 1: Optimal immobilization conditions and operational parameters

	Pectinex Ultra SP-L	GOD-catalase
Immobilization parameters		
Cross-linking time (h)	15	60
Concentration of glutaraldehyde (%)	0.25	0.5
Operational parameters		
Temperature (°C)	53	30
pH	5.6	5.1

In our studies shaken flask experiments were carried out for the production of fructooligosaccharides with soluble and immobilized Pectinex Ultra SP-L also.

In the first case 3 cm^3 *soluble* Pectinex Ultra SP-L was added to 30 cm^3 sucrose solution (2 M, pH=5.6, 0.05 M acetate buffer). The reaction was conducted at 45°C and 150 rpm. Results are summarized in *Fig. 1.*

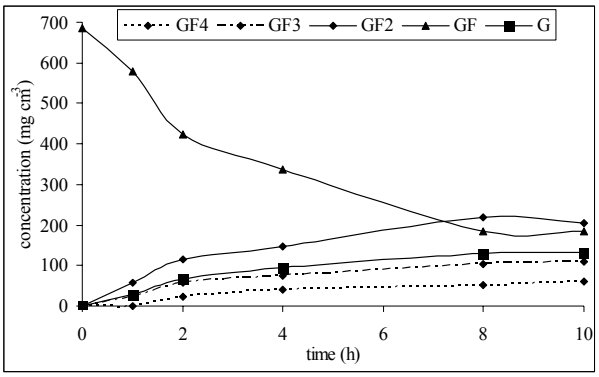

Figure 1: Production of fructooligosaccharides with soluble Pectinex Ultra SP-L

During the applied 8 h reaction time equilibrium of the reaction was reached and ~54 % product yield was achieved. Product yield was calculated as the amount of the formed fructooligosaccharides related to the amount of the initial substrate.

In the second case 6 g *immobilized* Pectinex Ultra SP-L was added to 80 cm^3 sucrose solution (2 M, pH = 5.6, 0.05 M acetate buffer). The reaction was conducted at 53 °C and 150 rpm. Results are summarized in *Fig. 2.* In this experiment the reached product yield was ~60 % under the applied conditions.

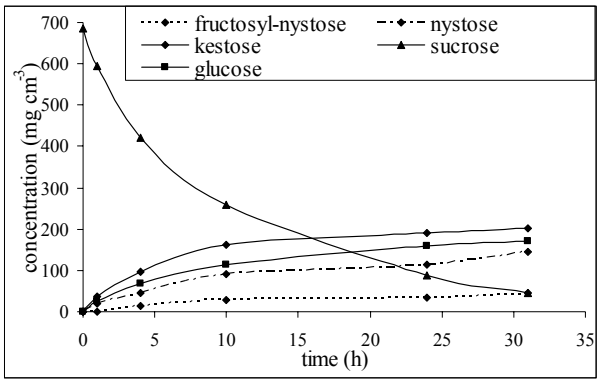

Figure 2: Production of fructooligosaccharides with immobilized Pectinex Ultra SP-L

Based on the results of the shaken flask experiments an integrated reactor system was constructed for the integrated fructooligosaccharides production and glucose elimination. The scheme of it is shown in *Fig. 3.*

The reactor unit for fructooligosacharides production was tempered to 53 °C, the other was operated at 25 °C.

In this system two reactor units were connected. One of them (I.) was filled with 10 g immobilized Pectinex Ultra SP-L, the other (II.) was filled with 5 g co-immobilized glucose oxidase-catalase, and 130-130 cm^3 sucrose solution (2 M, pH = 5.6, 0.05 M acetate buffer) was added.

The substrate solutions were changed between the two reactor units in every second hour, so the reaction was followed in 10 cycles. We measured the remaining/formed glucose concentrations after each cycles *(Table 2).*

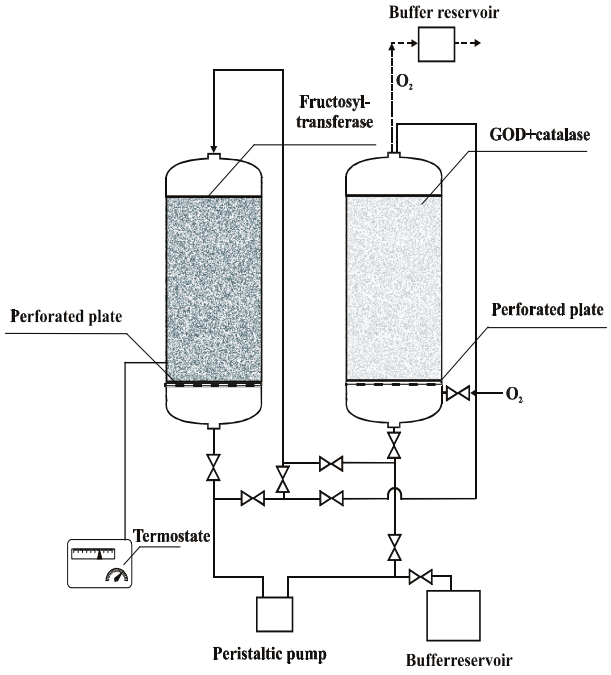

Figure 3: The scheme of the integrated reactor system for the production of fructooligosaccharides

Table 2: Glucose concentrations after several cycles in the integrated reactor system

	I.		II.	
	Reactor 1	*Reactor 2*	*Reactor 1*	*Reactor 2*
cycles	glucose concentration (mg cm^{-3})		glucose concentration (mg cm^{-3})	
1	20.33	-	19.23	-
2	22.41	0.14	23.62	0.54
5	24.32	1.89	22.61	2.12
10	35.33	2.33	41.74	3.66

As it can be seen from the data of the table in the first cycle the capacity of reactor 2 was high enough to eliminate glucose completely, but later (in the further cycles) some glucose appeared in the reactor, though its concentration was quite low.

After the examined cycles the amount of the formed short chain fructooligosaccharides *(Table 3)* was determined and – as it can be seen from the table – more than 74 % product yield was achieved.

Table 3: Product composition and yield in integrated system

	I.	II.
GF (mg cm^{-3})	67.00	75.23
GF_2 (mg cm^{-3})	245.36	237.98
GF_3 (mg cm^{-3})	181.23	169.47
GF_4 (mg cm^{-3})	82.58	75.36
Yield (%)	74.44	70.59

Summary

The production possibilities of FOS were examined in shaken flask experiments with immobilized FTF and ~60 % product yield was reached.

Glucose was found as a strong inhibitor therefore an integrated reactor system was constructed for the simultaneous enzymatic production of short chain FOS and elimination of glucose by-product by a co-immobilized glucose oxidase-catalase enzyme pair in order to reduce its inhibition. In this system ~74 % product yield was achieved.

It can be seen that higher product yield could be reached by application of by-product elimination.

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