UNIVERSITY OF PANNONIA
DOCTORAL SCHOOL OF CHEMICAL ENGINEERING AND MATERIAL SCIENCES

PH.D. THESES

ENZYMATIC SYNTHESIS OF PREBIOTIC FRUCTOOLIGOSACCHARIDES IN INTEGRATED SYSTEM

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Introduction

Healthy living and nutrition including the wholesome food products and food additives have become more and more important in the last years. The so-called functional foods contain useful components that have beneficial effects on health conditions.

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. 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 fermentation of these microbes and his way, prevent spreading of the harmful pathogens. In addition, they have low sweetness intensity, their caloric value is low, approximately 8 kJ g^{-1} (2 Kcal g^{-1}) and cause no caries. So they can be applied as alternative sweeteners and a part of diet.

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. The latter component is a strong competitive inhibitor of the synthesis. 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, chromatographic separation, or enzymatic method like elimination with glucose oxidase.

The immobilization of the biocatalysts offers a lot of practical advantages, e.g. the easy separation of enzyme and product, the opportunity to realize a continuous process, the enhancement of volumetric productivity of the reactor, etc.

Therefore the objects of my work were as follows:

1. Development of an immobilization procedure of a commercial enzyme solution having significant FTF activity;
2. Examination and establishment the optimal immobilization conditions, test of the operational possibilities during shaken flask experiments;
3. Examination of the production of FOS with the immobilized biocatalyst in lab scale;
4. Development a solid phase biocatalyst for the elimination of the formed by-product, glucose;
5. Elaboration of two kind of solid phase biocatalysts for the production of fructooligosaccharides and elimination of the formed by-product and study their operation in an integrated system.

Theses

I. I immobilized a commercial grade enzyme product, Pectinex Ultra SP-L having fructosyl-transferase activity to enhance the stability of the biocatalyst. I found applicable the *Amberlite IRA 900 Cl* type anionic ion exchange resin as immobilization matrix. I established a new two step immobilization method for this biocatalyst: In first step the enzyme solution was adsorbed onto the activated resin particles and ionic bounds were formed. The second step was a covalent bound formation with glutaraldehyde between the protein molecules and enhanced the stability of the solid phase biocatalyst. (*2. publication*)

II. I determined the optimal immobilization parameters. They were as follows: 16.7 g g^{-1} biocatalyst/matrix ratio, 0.25 % covalent binding agent concentration and 15 min cross-linking time. I determined the optimal operational parameters of the developed biocatalyst, they were pH=5.6 and 53°C temperature. I studied the stability of the established solid phase biocatalyst and I appointed 40 day half-time period, which value is appropriate from the practical point of view. I studied the production of fructooligosaccharides in shaken flask experiments with the application of the developed solid phase biocatalyst. In these experiments I reached 64.4 % product yield beside the former determined optimal operational parameters. (*2. publication*)

III. I established a co-immobilized glucose oxidase – catalase solid phase biocatalyst with the former applied two step immobilization method for the elimination of glucose formed as

an inhibiting by-product in the synthesis reaction. I determined the optimal immobilization and operational parameters for this biocatalyst. They were as follows: 0.5 % covalent binding agent concentration and 60 min cross-linking time, pH=5.1 and 30°C temperature. (*1. and 3. publication*)

IV. I constructed an integrated reactor system for the parallel product preparation and by-product elimination. I studied the production possibilities of fructooligosaccharides in this system and I reached 74.4 % product yield, which value was substantially higher than one without glucose elimination. (*1. and 5. publication*)

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