Immobilization and characterization of enzyme for production of lactulose-based oligosaccharides

The theses of Ph.D. dissertation

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2017

Ph.D. School

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Field: Food Science

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1 INTRODUCTION AND OUTLINE

1.1 Introduction

Oligosaccharides play an important role in biological systems such as influence the microbial composition in the gastrointestinal tract, the adhesion of microorganisms to the epithelium, gut maturation, and cell surface glycosylation, systemic effects after intestinal absorption and association studies in humans. The biological functions of oligosaccharides depend on their specific structures. The food-grade oligosaccharides can be classified into 12 main groups including galactooligosaccharides, lactulose, lactosucrose, fructooligosaccharides, palatinose or isomaltulose oligosaccharides, glycosyl sucrose, maltooligosaccharides, isomaltooligosaccharides, cyclodextrins, gentiooligosaccharides, soybean oligosaccharides and xylooligosaccharides. Among the oligosaccharides, the group of non-digestible ones that are not digested by enzymes of the small intestine and considered as soluble dietary fibers, after reaching the large colon stimulate the growth and/or active of one or limited number of beneficial bacteria, thus they have a beneficial effect on human health and are classified as prebiotics. Furthermore, the non-digestible oligosaccharides also have many other beneficial effects in health of the consumers including support the synthesis of K and B type vitamins, such as B1, B2, B3, B6, B9, B12 vitamin; stimulate and enhance absorption of minerals such as calcium and magnesium; improve blood glucose and triglycerides level; has anti-cariogenic activity; relief the symptoms of diabetes mellitus and lactose intolerance; prevent colon cancer, inhibit diarrhea, protect against infection on the gastrointestinal, respiratory and urogenital tracts, reduce risk of gut cancer. With many beneficial functions, non-digestible oligosaccharides are considered to be valuable materials for application in food, feed, pharmaceutical, cosmetic, etc. industries.

Among the oligosaccharides, lactulose is one of the industrial interesting compounds with the significant impact on human nutrition. Prebiotic lactulose has been used in a wide variety of food as a bifidus factor or as a functional ingredient for intestinal regulation. In addition, prebiotic lactulose is used mainly for treatment of constipation, hepatic encephalopathy, a complication of liver disease and maintenance of blood glucose and insulin level. Unfortunately, at high doses, the lactulose has laxative effects and the fact that fermentation occurs mainly in the proximal colon, which results in uncomfortable gas

production, thus the use of prebiotic lactulose could be limited. Otherwise, the lactulose-based oligosaccharides are more competitive than lactulose in prebiotic activity due to the higher degree of polymerization (DP). Fermentation of these oligosaccharides is going slowly and their metabolism takes place more distally in the colon. In other words, lactulose-based oligosaccharides show a better anti-inflammatory profile than lactulose in a model of experimental colitis. Thus, the studies on lactulose-based oligosaccharides, such as production, structure, functions, as well as the application of them are currently attracting the attention of the scientific community.

The oligosaccharides are already occurred naturally such as in human milk, bovine milk, plants, roots, fruits and vegetables, biomass, etc., and can also be synthesized by chemical methods, as well as biotechnological methods. Generally, the chemical methods have many disadvantages such as the formation of by-products, the high cost of product purification, hard to get oligosaccharides with a high degree of polymerization, many steps required in order to achieve region-selectivity etc., thus, these methods are rarely applied in the synthesis of oligosaccharides for food and pharmaceutical applications. Otherwise, due to regio- and stereospecificities of biocatalysts, the enzymatic synthesis of oligosaccharides has been preferred much because it can be done in one step with very high efficiency and without any by-products. This technology could be done by using native (also called soluble or free enzyme) as well as immobilized enzymes or insoluble enzymes.

Recently, the researchers focused on immobilized enzyme because of its advantages over free enzyme such as enhanced stability of enzyme, reuse or continuous use of a bioreactor, convenience in separation from the reaction mixture, possible modulation of the catalytic properties, contamination prevention in the product and easier prevention of microbial contaminations. It is thus apparent that enzyme immobilization can have immediate effects on the cost of process or the quality of the products. Furthermore, it has potential tuning of properties of the enzymes through immobilization. For example, the immobilization of enzyme can change the thermal behavior of the enzyme such as it could potentially extend the operating capabilities of the process and allow the reactor to operate at even higher temperature for long time period, thus increasing the reaction rates and product yields. However, the immobilization of enzyme faces some disadvantages. One of the most common drawbacks often encountered is the partial loss of enzymatic activity. It was withered during the process of immobilization or due to denaturation at the final immobilized enzyme conformation. In the last few years, such methods for enzyme immobilization were advised where the immobilized enzyme

suffered minimal loss of activity, but commercialization of immobilized enzymes is yet to be realized on a large scale. In a literature, there are four fundamental categories of enzyme immobilization, which are non-covalent adsorption or deposition, covalent attachment or carrier binding, entrapment in a gel, matrix or membrane and crosslinking; each of them has unique advantages and disadvantages. Recently, the covalent binding of the enzyme is an important method because of minimum enzyme leakage from the matrix, compared with the other methods of enzyme immobilization. In addition, the use of very stable and ready-to-use activated support would also be very convenient.

1.2 Outline of the dissertation

The increase of the enzyme stability is much important in biotechnology and could be got by covalent immobilization of enzyme onto the suitable insoluble carriers. With this expectation, the main goal of this study was to develop new immobilized biocatalysts for the synthesis of lactulose-based oligosaccharides. The crude enzyme (commercial Pectinex Ultra SP-L preparation) containing the *β*-galactosidases was used. The experimental setup of this thesis was focused on two directions of enzyme immobilization: covalent immobilization on magnetic micro- and nanoparticles, and chitosan microparticles. Another important aim was to develop a continuous system using immobilized enzyme preparations for the synthesis of lactulose-based oligosaccharides. Detailed tasks are following:

+ preparation of carriers: activated chitosan and magnetic particles;
+ immobilization of Pectinex Ultra SP-L (*β*-galactosidase activities) by the covalent binding method;
+ optimization of some factors which affect the yield of enzyme immobilization;
+ characterization of the resulting immobilized enzyme preparations including the effect of temperature and pH on enzyme activity, thermal stability, reusability, catalytic properties for lactulose-based oligosaccharides synthesis, as well as the kinetic model of the immobilized enzyme preparations;
+ development of the bio-system for continuous synthesis of lactulose-based oligosaccharides: setting up a continuous system of bio-reactor; investigation of main factors affected (dilution rate, the initial concentration of substrate) on the yield of the oligosaccharides synthesis.

2 MATERIALS AND METHODS

2.1 Materials

Pectinex Ultra SP-L commercial enzyme preparation from *Aspergillus aculeatus* containing β-galactosidase was purchased from Novozymes A/S (Denmark). Chitosan (low molecular weight), chitin particles (No. C7170), and sodium tripolyphosphate were from Sigma – Aldrich (Hungary). Lactulose was from Panreact Applichem (Germany). All the other chemicals and reagents were of analytical grade and purchased from either Sigma-Aldrich, Reanal (Hungary) or VWR (Hungary).

2.2 Preparation of activated solid carriers

Firstly, the chitosan microparticles were formed by heterogeneous acidic deacetylation of chitin particles. Otherwise, the magnetic Fe_3O_4 nanoparticles, after being formed by co-precipitating of Fe^{2+} and Fe^{3+} ions in the conditions of pH 10 and temperature 70 °C, were coated with chitosan solution by supporting of tripolyphosphate solution. Secondly, both two solid carriers were activated with glutaraldehyde solution. The chitosan microparticles were collected and washed with the same buffer solution by using vacuum filter. On the other hand, a magnetic field was applied in the case of magnetic nanoparticles by using a permanent magnet.

2.3 Immobilization of enzyme with optimization of three main factors

The Pectinex Ultra SP-L was immobilized on the activated chitosan microparticle, and magnetic nanoparticles carriers as well, by covalent binding methods. Three main factors (protein content, time of immobilization, and pH of buffers solution) were optimized by response surface methodology to get maximum yield of enzyme immobilization. The immobilization yield was determined in term of expressed activity, and enzyme activity recovery also was calculated. A set of 20 experiments, obtained from Central Composite Design of the experiment, was conducted at different values of these three factors.

Both of first- and second-order models were built up and compared. Statistical analysis was conducted by using R statistical software version 3.1.3 (www.r-project.org). The fit of regression models was analyzed by the determination coefficient (R-square), adjusted R-square coefficient, Lack of fit (LoF), and AIC score (from Stepwise Algorithm model selection by

Akaike Information Criterion) as well. Three-dimensional surface and counter plots were drawn to the interaction effects of the independent variables on the dependent variable, being described by a polynomial equation, which was fitted to the experimental data. The stationary point was determined also from the selected model.

2.4 Effect of pH and temperature on enzyme activity

Firstly, the enzyme activity was determined under conditions of a constant temperature of 60 °C, the amount of immobilized enzyme, and initial substrate concentration, but various pH values (from 3 to 6.5 with a step of 0.5 unit). Secondly, the enzyme activity was determined under conditions of constant pH (which was got from the previous step), the amount of immobilized enzyme, and initial substrate concentration, but various temperature, which was changing from 40 to 75 °C by a step of 5 °C.

2.5 Stability of free and immobilized enzyme

Thermal stability determination. Briefly, the same amount of the enzyme preparations (about 100 U), including the native, chitosan-immobilized, and magnetic-immobilized enzyme, were transferred into the test tubes containing 5 ml 0.1M McIlvaine buffer solution pH 5.0. The test tubes were incubated at an operational temperature (60 ^{o}C). Samples were periodically taken day by day, and the enzyme activity was assayed.

Storage stability of the enzyme preparations was determined by the same way of operational stability determination but at 5 °C.

2.6 Reusability of immobilized enzyme preparations

Each batch with identified conditions was considered as one time of use of the immobilized enzyme preparations. Relative oligosaccharides formation yields were calculated and compared to that of beginning. The magnetic-immobilized enzyme was applied on the batch bioconversion with lactulose substrate in 0.1M McIlvaine buffer solution pH of 5.0 and temperature of 60 ^{o}C, for 4 h of reaction time. Otherwise, the chitosan-immobilized enzyme was conducted for 12 h of reaction time.

2.7 Lactulose-based oligosaccharides synthesis, and model of enzyme kinetic

Lactulose-based oligosaccharides were synthesized from lactulose substrate under catalysis of two resulted immobilized enzyme preparations in batch reactions with a volume of 2 mL at a temperature of 60 ^{o}C. The initial concentrations of substrate solution (in 0.1M McIlvaine buffer at pH of 5.0) was varied from 20 to 80 % w/v. The samples were taken at regular interval time of reaction, then stored in a freezer for sequence analysis by HPLC.

The kinetic model of lactulose-based oligosaccharides synthesis was considered based on the resulted oligosaccharides formation and referred to the model which was given by previous publication with some modification. Several chemical models included either the lactulose hydrolysis or the trans-galactosylation reactions (prebiotic lactulose substrate plays roles as a donor as well as acceptor), with an assumption of negligible enzyme inhibition by fructose, were supposed. In addition, the products including mono-sugars (fructose and galactose), or lactulose-based oligosaccharides (DP3 and DP4) were presented in the model into both ways of separately or incorporated in single species. Consequently, several sets of several nonlinear ordinary differential equations that define the rate of reactants change on time course including the constant rates of reactions, which were considered to the kinetic parameters of this model or this kinetic mechanism. For kinetic parameter estimation, the multi response nonlinear regression for the whole set of experiments was applied by using the LSODA solver of COPASI software version 4.16. After that, the best model was chosen from the several candidates based on the minimal root mean square error.

2.8 Development of continuous system

The bio-system including the main components of immobilized enzyme packed column, with a jacket of water flow for incubating, a pump which is able to change flow rate, water incubator, big and small tubes. The column with the dimensions of 40 cm height, 6 cm outer diameter and 1.9 cm inner diameter has been filled up with the chitosan-immobilized enzyme

Effects of the initial substrate concentration as well as the dilution rate on the yield of oligosaccharides formation have been investigated. Firstly, the initial concentration of substrate (20 %) and temperature (50 ℃) were kept constantly, while the flow rate was set and changed at three levels 16, 27 and 60 $mL.h^{-1}$, consequently, the dilution rates were 0.14, 0.24 and 0.53 h^{-1}, respectively. The samples were collected at time periods. Secondly, the temperature and

the dilution rate of the substrate solution have been set constantly to 50 ºC and 0.24 h^{-1} (getting from the previous step), respectively. The substrate solutions (substrate dissolved in 0.1M McIlvaine buffer pH 4.5) has been set and changed to 4 %, 20 % and 30 % (w/v) of initial lactose concentration after the end of every reaction. The samples were taken at different time intervals.

2.9 Analytical methods

- The size of particle carriers was measured by SEM.
- The protein content was determined by the Bradford dye-binding procedure (Bradford, 1976) using Bio-Rad Protein Assay Kit (Bio-Rad, USA).
- The carbohydrates were analyzed by TLC and HPLC-RID.
- The hydrolytic activity of β-galactosidase was assayed using artificial *p*-NPGal as substrate at constant conditions. One unit of hydrolytic activity of β-galactosidase was defined as the amount of enzyme required releasing 1 µmol of *p*-nitrophenol under one minute at 60 ºC and pH 4.5.
- The galactosyltransferase activity of β-galactosidase was assayed towards lactulose substrate at certain conditions. One unit of galactosyltransferase activity of β-galactosidase was defined as the amount of enzyme required to syntheses 1 µmol of adjusted lactulose-based oligosaccharides formation (calculated by adjusted-DP3, and 1 µmol of DP4 was equivalent 2 µmol of adjusted-DP3) under one minute at the relevant reaction conditions.

3 RESULTS AND DISCUSSION

The hydrolysis and trans-galactosyl activities of commercial enzyme preparation (Pectinex Ultra SP-L) were investigated on the prebiotic lactulose substrate. Either smaller molecules than lactulose substrate which would be monosaccharides formed due to hydrolytic activity of enzyme preparation, or larger molecules that could be OS synthesized by the trans-galactosyl activity of this enzyme were found in TLC and confirmed by HPLC results.

The chitosan-coated magnetic particles, as well as chitosan particles, were prepared and activated by glutaraldehyde. The SEM results illustrated that the sizes of magnetic particles were range from nanometers to less than around 5 µm (so-called magnetic nanoparticles)

compared to more than several dozen µm for the size of chitosan (so-called chitosan microparticles). Two these activated carriers were successfully applied for covalent immobilization of Pectinex Ultra SP-L contained β-galactosidase with high immobilization yield about 98.8 % and 71 % in term of enzyme activity, respectively. This could be explained by the difference in the size of magnetic particles and chitosan particles; hence, it makes a difference in surface area of solid carriers. The optimization of enzyme immobilization was conducted on three main factors including protein content, pH and time of immobilization, and they were evaluated to be 0.45 mg.mL-1, 4.9 and 4.9 h, respectively.

Both two immobilized enzyme preparations were characterized for the synthesis of oligosaccharides from lactulose and lactose as well, focused on specific activities of the enzyme. Several obtained results were interesting and contributed to the advantages of covalent enzyme immobilization method.

The results of TLC and HPLC as well showed that both trans-galactosylation and hydrolytic activities of β-galactosidase (Pectinex Ultra SP-L) on lactulose substrate remained after immobilization.

The optimal temperature was constant at 60 °C after enzyme immobilization onto both two carriers. However, it is so interesting that the magnetic-immobilized enzyme showed much better operational stability referred to a broader thermal range than that of the other immobilized enzyme, as well as compared to free enzyme. In the range of temperature from 45 to 65 °C (approximately 20 units), the activity of the immobilized enzyme was remained well at over 98 % of original, compared to a temperature range from 55 to a bit over 60 °C (approximately 5 units) of free enzyme case. It was considered to decide that we can totally get 100 % relative activity of the magnetic-immobilized enzyme when applying at around 50 °C instead of 65 °C. This is an amazing thing because helping us to save money such as for energy, as well as extend the lifetime of enzyme preparation. In addition, even at high temperature (70 °C), the activity of the immobilized enzyme remained at 80%, it was much higher than 20%, approximately, for the case of free enzyme and chitosan-immobilized enzyme as well. Otherwise, this advantage of the magnetic-immobilized enzyme could be useful to control the structure of oligosaccharides synthesis because of temperature effect on the direction of the enzymatic reaction.

The optimal pH of the magnetic-immobilized enzyme was kept at around pH 4.5, while shifted from pH 4.5 to 5.5 for the case of the chitosan-immobilized enzyme. Similarly to effect

of temperature on enzyme activity, the magnetic-immobilized enzyme showed higher stability than that of the native enzyme referred to the broader range of operational pH. When pH was changing from 3.5 to 6.0, the relative activity of this immobilized enzyme was retained above 95 % of original, compared to below 70 % for free enzyme case. In other words, the magnetic-immobilized enzyme showed high activity in a wide range of pH (from pH 3.5 to pH 6.5) instead of narrow pH range (about pH 4.5) for the case of the native enzyme. This changing after immobilization was considered to be good when using this immobilized enzyme at neutral pH 6.5 instead of acidic pH 3.5 – 4.0, because chitosan coating layer of this immobilized enzyme could be proposed more stable (less dissolved) in neutral pH environment. The future experiment should be conducted.

Besides the higher operational stability, immobilized enzyme showed greater thermal stability after immobilization when they were both stored at optimum operational conditions of pH and temperature (60 ºC) for a long term. The half-life time of enzyme increased from 2.5 days (in the case of free enzyme) to over 6 days, appropriate 3 folds higher (in the case of magnetic-immobilized enzyme), and 14 days, appropriate 6 folds greater (in the case of chitosan-immobilized enzyme). The increased stability observed in the immobilized enzyme should be attributed to a reduction in the enzyme structure mobility, due to anchorage to the support promoted by the covalent bonds and subsequent translation of the rigidity at each anchorage point to the whole enzyme structure, thus shielding it from damaging effects of the environment. These finding revealed, one more time, that covalent immobilization of enzymes makes them greater stability. Furthermore, the results also illustrated that in term of storage, the stability of the immobilized enzyme was so high. The specific activity of immobilized enzyme preparations was not significantly different to initial one after 14 days of storage at 5 ºC

Higher stability making better reusability. In the case of the magnetic-immobilized enzyme, a batch conduction at optimal pH and temperature (60 ºC) for 4 h was considered to one time of enzyme use. The relative of lactulose-based oligosaccharides formation by the total of carbohydrates was remained at around 85 % after eight times of reuses. In the case of the chitosan-immobilized enzyme, a cycle was finished after 12 h of applying immobilized enzyme at optimal conditions pH and temperature (60 ºC). After thirty-three recycles, the relative of lactulose-based oligosaccharide by the total of carbohydrates was remained at around 60%. These results of immobilized enzyme improved the advantages of enzyme immobilization.

The batches bioconversion of lactulose to lactulose-based oligosaccharides using immobilized enzyme preparations were successfully carried out at optimal condition pH and temperature for a long term. The resulted lactulose-based oligosaccharides were evaluated to be DP3 and DP4. The effect of initial lactulose concentration, as well as converted lactulose on the yield of oligosaccharide formation was investigated. The higher yield of oligosaccharides formation took place at the highest initial lactulose concentration. In the case of the magnetic-immobilized enzyme, at initial lactulose concentration of 80% w/v (the saturated concentration at 60 ºC), the oligosaccharide formation reached over 26% when lactulose substrate was converted at 60%.

Eight chemical models including several (four or five) pathways of bio-reactions were proposed for a simplistic understanding of the bioconversion mechanism. Consequently, eight candidates of mathematic models were given containing different equations of the nonlinear ordinary that define the rate of reactants changes on the time-course including the constant rates of bio-reactions, which were considered to the kinetic parameters of the model. The kinetic model was estimated using the method of multi response nonlinear regression for the whole set of experimental data. Based on the minimal root mean square error, the best-fitted model for both cases of immobilized enzyme preparations were selected from the eight ones, and well validated. The kinetic models for two cases of immobilized enzyme preparations, including magnetic-immobilized and chitosan-immobilized enzyme, were not same although the number of pathways was equal of four.

A packed-bed (40 cm height, 6 cm outer diameter and 1.9 cm inner diameter) continuous system using chitosan-immobilized enzyme preparation for the synthesis of lactose-based oligosaccharide was successfully developed. The effect of two main factors including dilution rates and initial lactose concentrations on the yield of oligosaccharide formation were investigated. In term of effect of dilution rates, the yield of continuous bioconversion of 20 % w/w lactulose in McIlvaine buffer got maximum (10.5 %) at the dilution rate of 0.24 h^{-1}. Lower (0.14 h^{-1}) or higher (0.53 h^{-1}) dilution rates made a decrease of oligosaccharides formation yield. These results could be explained by the need of time for specific properties of multi-reaction of GOS synthesis with catalysis of β-galactosidase including hydrolysis, intra-molecular trans-galactosylation (making disaccharide of galactose-galactose), inter-molecular trans-galactosylation (making OS with DP $\geq$ 3). The retention time for both hydrolytic and trans-galactosylation reactions (in the case of higher dilution rate) may be not enough. It means the shorter retention time will result in a lower yield of galactooligosaccharides formation.

For investigation of effect of initial lactose concentration on bioconversion yield, three different initial concentrations (4 % w/v, 20 % w/v and 30 % w/v) of lactose in the 0.1M McIlvaine buffer solution pH 5.0 were conducted at constant conditions of optimal pH and temperature, the dilution rate at 0.24 h^{-1}. The results showed that the yield of galactooligosaccharides formation increased with the initial lactose concentration from 9.8 % to 11.3 %. This could be explained by a higher probability of the β-galactosyl groups attaching to lactose (forming galactooligosaccharides) than water as an acceptor at increasing lactose concentration. In contrast, in a diluted lactose solution, water can be more competitive to be an acceptor for the binding with the enzyme-galactose complex forming galactooligosaccharides. However, there was a limitation of this increasing linear may be because of the catalysis capacity and high viscosity of the bio-reaction solution.

4 NOVEL SCIENTIFIC RESULTS

1) The Pectinex Ultra SP-L (β-Galactosidases) was successfully immobilized onto chitosan-coated magnetic nanoparticles, as well as chitosan microparticles by the covalent binding method. High yield of immobilization and recovery of activity were obtained. In the case of magnetic nanoparticles, protein content, pH and time were optimized using response surface methodology for enhancement of enzyme immobilization yield. The optimal factors were determined to be 0.45 $mg.mL^{-1}$, pH 4.9 and 4.9 h, respectively. The yields of enzyme immobilization in the cases of magnetic nanoparticles and chitosan microparticles were calculated to be 98.8% and 71%, respectively.

2) Effects of pH and temperature on catalytic and stability of the immobilized enzyme preparations were determined. In the case of chitosan microparticle carrier, the optimal temperature of the immobilized enzyme was 60 °C and was similar to that of a free enzyme; while in the case of magnetic nanoparticles, the optimal temperature was in the range from 45 °C to 65 °C and much broader than that of the free one. Both immobilization methods did not affect significantly optimal pH values. The immobilized enzymes exhibited extremely stable in storage at 5 °C and half-life times of magnetic-immobilized enzyme and chitosan-immobilized enzyme were 6 days and 14 days, respectively at pH 4.5 and 60 °C. The reuses of immobilized enzymes were investigated and more than 50 % of their activities were retained after 30 cycles of use.

3) Kinetic constants of bioconversion with magnetic-immobilized enzyme were estimated by the four-step pathway of trans-galactosylation using different initial lactulose concentrations (20, 30, 40 and 50 w/v %). In the case of the chitosan-immobilized enzyme, kinetic constants of both hydrolytic and galactosyltransferase activities were determined. The *K*m and *V*max of hydrolytic activity of the chitosan-immobilized enzyme in lactulose substrate were 187 mM and 227 µM Gal/min/g support, respectively. The kinetic constants of bioconversion with chitosan-immobilized enzyme were estimated by the four-step pathway of trans-galactosylation using 0.101, 0.134, 0.172 and 0.249 $mol.L^{-1}$ of initial lactulose concentrations.

4) System using chitosan-immobilized enzyme preparation was set up for continuous bioconversion to produce the galactooligosaccharides. The working dimensions of the packed-bed column were 40 cm height, 6 cm outer diameter, and 1.9 cm inner diameter. Maximum yield of oligosaccharides on lactose substrate was obtained at 27 $mL.h^{-1}$ of flow rate and 30 % of initial substrate concentration.

5 PUBLICATIONS

Articles in reviewed journals

Bujna E, Rezessy-Szabó JM, Nguyen DV, Nguyen DQ (2016) **Production and some properties of extracellular phytase from *Thermomyces lanuginosus* IMI 096218 on rice flour as a fermentation substrate**. *Mycosphere* 7:1576-1587. (IF 0.654)

Vuong D. Nguyen, Gabriella Styevkó, Linh P. Ta, Anh M. Tran, Erika Bujna, Petra Orbán, Mai S. Dam, Quang D. Nguyen (under revision). **Immobilization and some properties of commercial enzyme preparation for production of lactulose-based oligosaccharides**. *Food and Bioproducts Processing*. (IF 2.687).

Vuong D. Nguyen, Gabriella Styevkó, Erzsébet Madaras, Gökçe Haktanırlar, Erika Bujna, Mai S. Dam, Quang D. Nguyen (manuscript under review). **Optimization in immobilization of β-galactosidase on magnetic nanoparticles and its application on the synthesis of oligosaccharides from lactulose.** *Process Biochemistry* (IF 2.529)

Oral presentations

Vuong D. Nguyen, Gabriella Styevkó, Linh T. Phuong, Erzsébet Madaras, Gökçe Haktanırlar, Quang D. Nguyen (2016). **Immobilization and characterization of immobilized β–galactosidase preparations**. *Chemical Engineering Days '16. Veszprém, Hungary*.

Vuong D. Nguyen, Gabriella Styevkó, Erzsébet Madaras, Quang D. Nguyen (2016). **Bioconversion of lactulose to f-GOS with immobilized enzyme preparation**. *Fiatal Biotechnológusok II. Országos onferenciáján (FIBOK 2016) in Szent Istvan University, Hungary.*

Poster presentations

Vuong D. Nguyen, Gabriella Styevkó, Linh T. Phuong, Quang D. Nguyen (2014). **Lactulose Base Galactooligosaccharides Produced by Immobilized Commercial Enzyme Preparation**. *The 3rd Asia Pacific Symposium on Postharvest Research, Education, and Extension (APS2014), Ho Chi Minh, Vietnam.*

Vuong D. Nguyen, Erzsébet Madaras, Gabriella Styevkó, Quang D. Nguyen (2015). **Effect of some environmental parameters on the preparation of immobilized β-galactosidase for the synthesis of galactooligosaccharides**. *Food Science Conference '15, Budapest, Hungary.*

Styevkó Gabriella, Nguyen Duc Vuong, Hoschke Ágoston, Nguyen Duc Quang (2014). **Oligosaccharide synthesis on different substrates by commercial enzyme preparation of Pectinex Ultra SP-L from *Aspergillus aculeatus***. *Conference of Chemical Engineering Days '14, Veszprém, Conference proceeding, 167.*

Styevkó Gabriella, Nguyen Duc Vuong, Hoschke Ágoston, Nguyen Duc Quang (2014). **Mannobiose and mannotriose synthesis by a commercial enzyme preparation of Pectinex Ultra SP-L from *Aspergillus aculeatus***. *A Magyar Mikrobiológiai Társaság 2014 évi Nagygyűlése, Keszthely, Absztraktfüzet, 67-68.*