DEVELOPMENT OF FRUCTOSYLTRANSFERASE NANOBIOCATALYST FOR APPLICATION IN SYNTHESIS OF BIOACTIVE FRUCTO-OLIGOSACCHARIDES

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Abstract

Fructo-oligosaccharides (FOS) are recognized as prebiotic compounds which have the ability to stimulate the growth of gut microbiota, microorganisms that positively affect human health. Additionally, they have excellent nutrition- and health-relevant properties such as low caloric, non-cariogenicity, the ability to reduce total serum cholesterol levels, and many others. FOS can be found in various vegetables and fruits, but in low concentrations, which represents the main reason for their production on an industrial level. Production of FOS is predominantly based on the enzymatic transformation of sucrose using enzymes with transfructosylation activity such as fructosyltransferases or β-fructofuranosidases at the higher sucrose concentrations. In recent years, FOS growing market demand mainly as ingredients for food applications (bakery products, sweets, different types of beverages) has been increased, so there is a necessity for the development of new enzymatic systems for production of FOS with high yields and productivities. Enzyme immobilization techniques have been proposed as one of the potential solutions. Thus, this work aimed to evaluate the potential of amino-modified and cyanuric chloride functionalized amino-modified nonporous fumed silica nanoparticles (AFNS and CCAFNS, respectively) for the development of efficient nanobiocatalysts for application in the biosynthesis of FOS. Selected modified nanocarriers were applied for the immobilization of fructosyltransferase (FTase) from commercial enzyme preparation Pectinex® Ultra SP-L whereby the effects of immobilization parameters like initial enzyme concentration, immobilization time and pH were analysed. Among both used nanocarriers, the one with chloride groups (CCAFNS) exhibited the highest FTase binding capacity of 89 mg/g of support with the efficiency of 35 % at an initial enzyme concentration of 250 mg/g of support, pH 6.0, and immobilization time of 2.5 h. By examining the influence of immobilization time, it was found that the highest activity of 1576 IU/g of support was demonstrated by FTase immobilized on AFNS after 5 h, while FTase covalently immobilized on CCAFNS, exhibited activity of 1122 IU/g of support. According to the achieved activity, both obtained nanobiocatalysts were further applied in FOS production which was performed at 50 °C and sucrose concentration of 500 g/l during 48 h. Apparently, with CCAFNS preparation FOS production of 14 g/l/h was achieved as compared to 5.9 g/l/h by AFNS preparation. Since in both cases, high sucrose conversion of 89 % was accomplished, it can be concluded that covalent immobilization of FTase on CCAFNS has the better catalytic capability for FOS production than FTase immobilized on AFNS via electrostatic interactions.

Key words: *nanoparticles, immobilization, fructosyltransferase, transfructosylation, FOS, prebiotics*

Introduction

In recent years, there is increasing attention toward production of functional food which have in addition to their nutritive value disease preventing and health promoting effect (Aquino et al., 2017). The prebiotics are considered as functional food components, among which fructo-oligosaccharides (FOS), in addition to galacto-oligosaccharides (GOS), have been studied to the most extent. FOS are carbohydrates composed of fructose units linked by a β-(2,1)-glycosidic bonds and a terminal glucose unit (Flores-Maltos et al., 2016). FOS cannot be digested by the enzymes present in the human small intestine tract, therefore, they reach the colon where represent food for intestinal microbiota (Flores-Maltos et al., 2016). As a result of their activity, selective stimulation of intestinal bacteria occurs, contributing to health beneficial effect not only in the gastrointestinal tract but also in the whole organism (Davani-Davari et al., 2019). They can be found in natural sources like as bananas, onion, tomato, chicory and many others (Murari, 2016). However due to their low content in natural sources, for large-scale industrial production, they can be manufactured by using chemical reagents or enzymes. Enzymatic production is often approached because this process does not require the use of toxic substances and enable obtaining FOS of defined composition (Sánchez-Martínez et al., 2020). Although, the inulin can be used as a substrate, sucrose represents a more economical choice for the synthesis of these oligosaccharides, requiring the use of enzymes with transfructosylate activity such as fructosyltransferase (FTase, EC 2.4.1.9) and β-fructofuranosidase (FFase, EC 3.2.1.26) only under specific conditions (Bali et al., 2015). By the transfructosylation reaction, mentioned enzymes hydrolyse a sucrose molecule and transfer the fructose unit to another sucrose molecule producing FOS 3 with the release of glucose units (Dominguez et al., 2014). By the further duration of the reaction, the newly synthesized FOS 3 represents the substrate for obtaining FOS 4 which is responsible for the production of FOS 5.

Taking into account the growing application of FOS in many products, the great attention of the scientific community is focused on the development of new enzyme systems which are based on enzyme immobilization by various methods such as adsorption, covalent immobilization, entrapment or encapsulation in sol-gel matrices and cross-linking (Mohamad et al., 2015). Even though enzymes can be immobilized on a variety macro-sized supports, nano-sized materials, such as nanoparticles, nanowires, nanofibers, are considered very attractive for use since they possess ideal features (large specific surface area, low mass transfer resistance and effective enzyme loading) for promoting biocatalysts efficiency (Fathi et al., 2019). So far, nanobiocatalysts have been successfully used in medicine, pharmacy and other fields (Rudramurthy and Swamy, 2018).

Lately, the use of silica based nanoparticles is becoming more common due to their excellent physical characteristics, chemical and thermal stability, large surface area biocompatibility and other advantages (Banjanac et al., 2016). In order to achieve better performance and extend the field of their application, surface modification of these nanoparticles is often approached using various reagents (Banjanac et al., 2016). There are data in the scientific literature that silica nanoparticles have been used successfully as a support for immobilization of different enzymes such as laccase (Bebić et al., 2020), β-galactosidase (Banjanac et al., 2016), lipases (Banjanac et al., 2016) (Banjanac et al., 2016), inulinase (Karimi et al., 2014) and many others. In the case of FTases, it was reported that this enzyme is immobilized on chitosan-magnetic nanoparticles (de Oliveira et al., 2020), while there are no data on the use of silica based nanoparticles for this purpose. Hereby, in this study, it will be consider the use of non-modified silica nanoparticles, amino-modified (AFNS) and cyanuric chloride activated amino-modified silica nanoparticles (CCAFNS), for immobilization of FTase from the commercial enzymatic mixture Pectinex® Ultra SP-L derived from *Aspergillus aculeatus*, with the aim of creating immobilized preparation with improved catalytic properties for FOS synthesis.

Materials and Methods

Materials

Commercial enzyme preparation from *Aspergillus aculeatus* (Pectinex® Ultra SP-L) as a source of fructosyltransferase (FTase) was purchased from Novozymes (Bagsvaerd, Denmark). Hydrophilic fumed silica nanoparticles AEROSIL® 380 (FNS) were purchased from Evonik Industries (Essen, Germany). Sucrose and reagents used for FNS modification such as aminopropyltrimethoxylsilane (APTMS) and cyanuric choride (CC), were purchased from Fluka Analytical (USA). Coomassie Brilliant Blue G-250, bovine serum albumin and substances used for preparation buffer solution were obtained from Sigma Chemical Co (St. Louis, USA). Chemicals used for HPLC analysis such as acetonitrile and water HPLC grade, were purchased from Thermo Fisher Scientific (Waltham, USA).

FTase immobilization procedure

The immobilization of FTase on fumed silica nanoparticles (FNS), amino-modified silica nanoparticles (AFNS) and cyanuric chloride activated amino-modified silica nanoparticles (CCAFNS) was examined. The nanoparticles used as supports for FTase immobilization such as AFNS and CCFNS were prepared by modifying the FNS according to a procedure thoroughly described by Banjanac et al. (2016). For all examined supports, the some immobilization procedure was applied: the reaction mixtures consisted of support, buffer solution and enzyme. In order to optimize FTase immobilization process, the influence of different pH, concentration of enzyme and immobilization time was examined. For determination optimal pH, the buffer solutions with different pH (4.0-7.0) were incubated with 10 mg of support (AFNS) and 50 µl of enzyme. After choosing the adequate pH, the impact of different initial enzyme concentrations (10, 20, 50, 100 and 250 mg/g support) and immobilization time (1, 2.5 and 5 h) on immobilization process was simultaneous examined. For AFNS and CCAFNS, the some procedure was applied: the amount of support was varied (2, 5, 10, 25 and 50 mg) while amount of enzyme (50 µl) and buffer (950 µl), was constant. Thus prepared reaction mixtures were put on roller shaker (IKA® Roller 6 basic, Werke GmbH and Co. Germany) at 25 ^{o}C. After defined immobilization time (1, 2.5 and 5 h), the immobilization mixtures were centrifuged for 10 min at 13000 rpm, then the separated supernatants were used for protein analysis, while the immobilized preparations were washed with appropriate buffer and used for determination of activity.

FTase activity assay

The enzyme activity assay was carried out by adding of free or immobilized enzyme in 5 ml of substrate solution (aqueous solution of sucrose concentration of 500 g/l), at 50 ^{o}C under shaking (150 rpm). At predefined time, the samples volume of 100 µl were taken, diluted with distilled water and in order to deactivate the enzyme treated for 5 min in ThermoMixer® C (Eppendorf, Germany) at 99 ^{o}C. Afterwards, the samples are analysed on HPLC. The activity of free or immobilized FTase was pronounced in international units (IU) where one unit is defined as amount of enzyme that converts 1 µmol of substrate (sucrose) into FOS per minute under defined conditions. The activity immobilization yield (IYa) was calculated according to the following equation (Eq. 1):

$$\text{IYa (\%)} = \frac{\text{The expressed activity of immobilized FTase}}{\text{The initial activity of FTase}} \times 100 \qquad (1)$$

Protein assay

The protein loading (mg of bound proteins per g of support) and the initial protein concentration (mg of offered proteins per g of support) were determined according to the Bradford's method (Emami Bistgani et al., 2017). The immobilization parameters such as protein immobilization yield and specific activity were calculated. The protein immobilization yield (IYp, %) was defined as the ratio of the protein loading (which represents the difference between the introduced proteins in the immobilization process and the proteins retained in the supernatant after immobilization process was finished) and the initial protein concentration (Eq. 2). The specific activity (IU/mg of proteins) was determined as the quotient of the expressed activity of immobilized FTase (IU/g support) and the protein loading (mg/g support) (Eq. 3).

$$\text{IYp (\%)} = \frac{\text{Protein loading}}{\text{Initial enzyme concentration}} \times 100 \qquad (2)$$

$$\text{Specific activity} = \frac{\text{The expressed activity of immobilized FTase}}{\text{Protein loading}} \qquad (3)$$

The production of FOS

The production of FOS was carried out by incubating immobilized enzyme in Erlenmeyer flasks with the 20 ml of aqueous sucrose solution concentration of 500 g/l at 150 rpm and 50 ^{o}C for 48 h. In the reaction mixtures were added the amounts immobilized enzymes (FTase immobilized on AFNS or CCAFNS) that are calculated in order to ensure that identical number of units of immobilized enzyme was introduced in reactions. The samples were taken at predefined time, diluted ten times with distilled water, filtered and analysed by HPLC.

HPLC analysis

The samples were analysed by HPLC system (Dionex Ultimate 3000 HPLC system (Thermo Fisher Scientific, Waltham, USA)) equipped with amino column (Hypersil™ APS-2, 250 mm × 4 mm, 5μm) at 35 °C. Aqueous solution of acetonitrile (80 % v/v) was used as the mobile phase with the flow rate of 1 ml/min. Detection of target compounds was done by RI detector (RefractoMax 520, ERC, Riemerling, Germany) while Chromeleon software was used for data management.

Results and Discussion

Selection of support for FTase immobilization

In this study, the suitability of commercial fumed silica nanoparticles Aerosil®380 (FNS) as support for immobilization of FTase from commercial enzyme preparation Pectinex® Ultra SP-L was chosen to be investigated due to the fact that they possess favorable features such as uniform particle size (diameter of 7 nm), large specific surface area of 380 m^2/g, thermal and mechanical stability, and their surface is rich with silanol groups that could be easily modified with different reagents under middle conditions. Moreover, it is well known that the enzymes could be attached onto the surface of FNS nanoparticles *via* the formation of the weak hydrogen and Van der Waals interaction, as well as *via* non-specific electrostatic interactions between enzyme molecules and silanol groups present on FNS surface (Fig. 1), meaning that the modification of FNS surface is necessary in order to introduce groups that could form more stable interactions with enzymes. So, the FNS surface has been modified with aminopropyltrimethylsilane (APTMS) which resulted in the introduction of amino groups on FNS surface. By introducing

the amino groups on FNS surface, more stable electrostatic interactions with nucleophilic groups (thiol, carboxyl, or even hydroxyl) of side chains of enzymes could be formed (Fig. 1). Furthermore, amino-modified fumed silica nanoparticles (AFNS) were activated with cyanuric chloride, and as result free chloride groups of triazine ring on the surface of AFNS were introduced. The enzymes could be attached on cyanuric chloride activated amino-modified FNS (CCAFNS) by forming strong covalent bonds (Fig. 1).

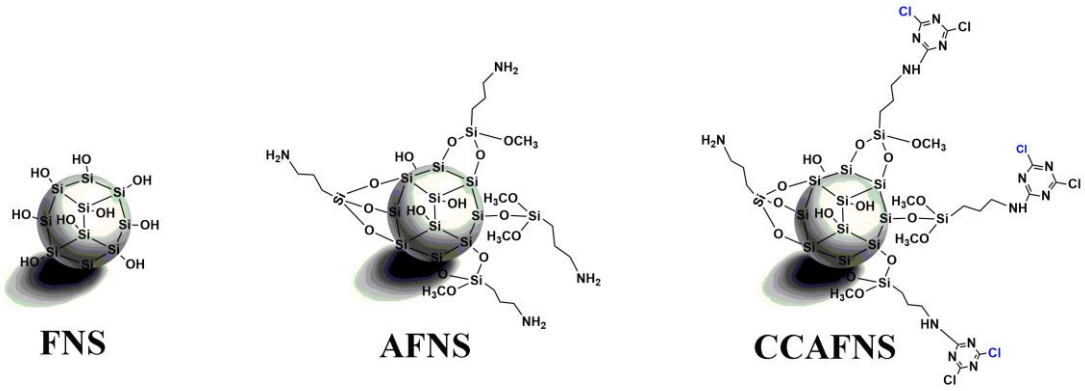


Figure 1. Selection of nano-sized support for FTase immobilization.

In our previously reported studies, fumed silica nanoparticles, amino-modified fumed silica nanoparticles as well as cyanuric chloride activated silica nanoparticles proved to be efficient supports for immobilization of lipase from *Candida rugosa*, *β*-galactosidase from *Aspergillus orzyae* and laccase from *Myceliophthora thermophila* expressed in *Aspergillus oryzae* (Novozym® 51003). Taking into account all these facts, the selected nanocarriers were applied for the immobilization of FTase whereby the effects of immobilization parameters like initial enzyme concentration (mg per g of support), immobilization time and pH were analyzed.

FTase immobilization on amino-modified silica nanoparticles (AFNS)

Firstly, non-modified FNS nanoparticles for immobilization of FTase were applied. The immobilization process was performed at different initial enzyme concentrations in range of 10-250 mg/g of support for 2.5 h in 0.1 M buffer (pH 4.0-7.0). The results showed that FNS did not have adequate surface chemistry for FTase immobilization since obtained immobilized preparations did not expressed activity and achieved protein loadings were very low (data not shown). It could be presumed that under this experimental conditions, the formation of repulsion forces between negatively charged FTase molecules and negatively charged silanol groups present on FNS surface (pI of FNS is determined to be 4.2) occurs, enabling attachment of FTase on FNS surface (Banjanac et al., 2016).

In order to overcome this obstacle and promote bonding between the nanoparticles and the FTase, amino-modified silica nanoparticles (AFNS) were used as carrier. As result of FNS modification with aminopropyltrimethylsilane (APTMS), the high amount of amino groups (4.58 μmol/mg support) was introduced on the FNS surface and the success of modification of FNS to AFNS was confirmed by the recorded FT-IR spectra (Bebić et al., 2020).

Since AFNS has favorable positively charged amino groups on the surface, only at appropriate immobilization pH values, the electrostatic interactions between negatively charged amino acid residues from the surface of FTase molecules and support functional groups will be established. So, in order to determine optimal pH for FTase immobilization on AFNS, the activity immobilization yield was monitored for the pH values ranging from 4.0 to 7.0 (Fig. 2A). The immobilization for each pH value is carried out at initial enzyme concentration of 100 mg of

offered proteins per g of support for 2.5 hour. The results showed that the immobilized FTase on AFNS expressed the highest activity yield of 87 % at pH 6.0. At pH values lower and higher than 6.0, the activity yields were around 40 %. The optimized immobilization at pH 6.0 was adopted for further studies on the kinetics of immobilized FTase.

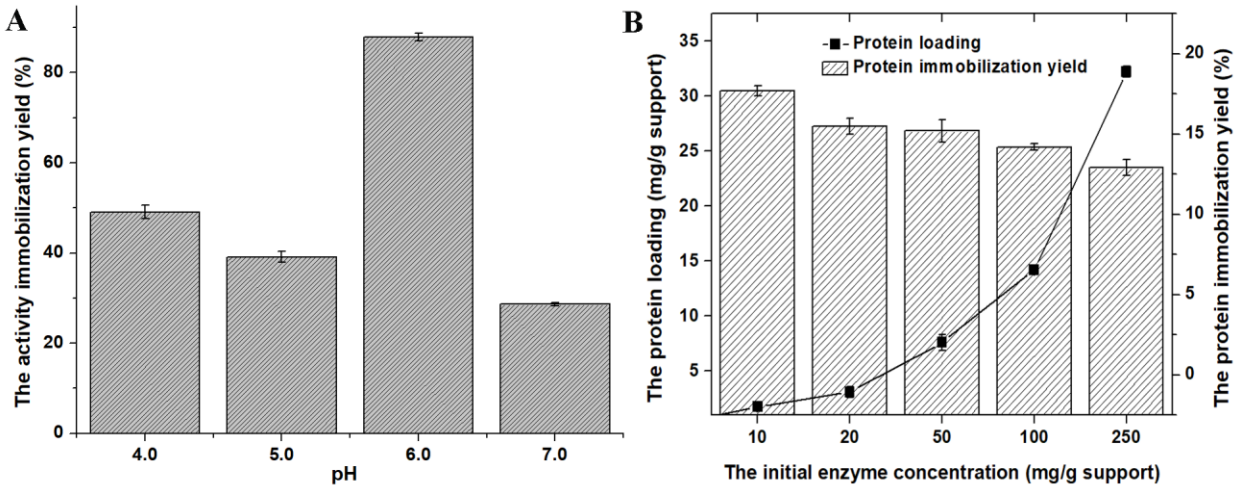


Figure 2. The immobilization pH optimal (A). The effect of initial enzyme concentration (mg/g support) on protein loading (mg/g support) and protein immobilization yield (%) (B).

The kinetics of the FTase immobilization on AFNS was analyzed by performing immobilization at different initial enzyme concentrations of 10 mg, 20 mg, 50 mg, 100 mg and 250 mg of offered protein per g support and protein loading (mg/g support) was monitored for 2.5 hours. With the increase of the initial FTase concentration, the concentrations of immobilized enzymes increases up to 32 mg/g support obtained at highest initial enzyme concentration (Fig. 2B). Also, the maximum protein immobilization yield of 17 % was achieved at lowest initial enzyme concentration (10 mg/g of support), while at highest initial enzyme concentration the activity yield was 13 %. From all present results regarding the achieved protein loadings and protein immobilization yields, it could be concluded that immobilization of FTase should be performed at initial enzyme concentration of 250 mg/g support for 2.5 hours in 0.1 M Na-phosphate buffer pH 6.0. Additionally, these results indicate that chemically modified FNS with APTMS (AFNS) have great prospects in application as support for FTase immobilization.

Furthermore, to complete optimization of immobilization process, the effect of initial enzyme concentration on the immobilized FTase activity was investigated. For different initial protein concentrations of 10 mg up to 250 mg/g support, the immobilized FTase activity per g of support was determined (Fig. 3A). The activity of the immobilized enzyme increased with the increase of initial protein concentration up to 250 mg/g support meaning that the same trend as with enzyme loading (Fig. 2B) was observed. The highest activity of FTase immobilized on AFNS carrier (1502 IU/g support) was determined for the highest initial protein concentration of 250 mg/g support. It should be noted that at 2.5 times lower initial enzyme concentration (100 mg/g support), FTase expressed the activity of 1448 IU/g support which is drop of only 5 % in comparison to 250 mg/g support. The lowest activity of 108 IU/g support FTase expressed at 10 mg/g support. Moreover, on Fig. 3B, it can be seen that similar results for specific activity (IU/mg proteins) and activity immobilization yield (%) are achieved when immobilization was performed at 50 and 100 mg/g support, while the step drop in specific activity and activity yield at 250 mg/g support was observed. At the highest initial protein concentration (250 mg/g support), no significant increase of enzyme activity was detected (Fig. 3A), but there is relevant change in the concentration of active immobilized proteins above 100 mg/g support (Fig. 3B).

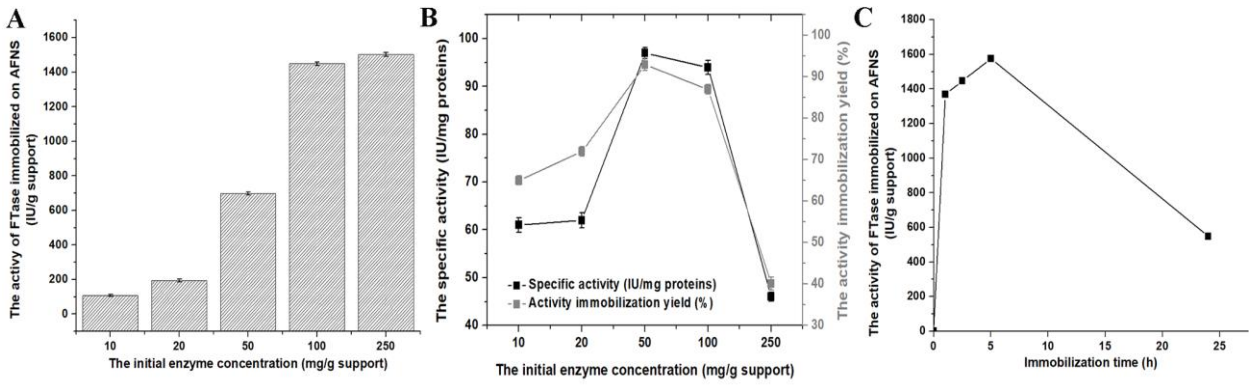


Figure. 3. The effect of initial enzyme concentration on activity (IU/g support) (A) and specific activity (IU/mg protein) and activity immobilization yield (%) of immobilized FTase on AFNS (B). The effect of immobilization time on the expressed activity of FTase immobilized on AFNS at 100 mg/g support in 0.1 M Na-phosphate buffer pH 6.0.

At 50 mg/g support the activity of FTase immobilized on AFNS was 2 times lower than at 100 mg/g support. It should be noted that at 100 mg/g support, activity yield was 87 % while specific activity was determined to be around 90 IU/mg proteins (Fig. 3B). By summarizing all presented results on Fig. 1 and Fig. 3, it could be concluded that optimal initial enzyme concentration for immobilization FTase on AFNS is 100 mg/g support. The influence of immobilization time on activity of immobilized FTase on AFNS at 100 mg/g support is also investigated (Fig. 3C). The kinetics of FTase activity immobilization showed that maximum activity of 1576 IU/g support was achieved after 5 h, which is slightly higher than after 2.5 h. In the context of immobilization process efficiency, the immobilization time of 2.5 h is adopted as optimal for future experiments.

As already stated, it can be presumed that the electrostatic attraction forces govern the interaction between the negatively charged protein residues and positively charged amino groups of AFNS under the experimental conditions. The expressed high activity of the immobilized FTase on AFNS can be explained by the fact that negatively charged protein groups, engaged in the adsorption to the support are opposite to the FTase active site, hence enabling readily access of substrate. This statement is confirmed by high values of parameters that describe catalytic efficiency of immobilized preparation (specific activity and activity immobilization yield). Therefore, the immobilized FTase on AFNS obtained under determined optimal conditions (100 mg offered proteins per g of support in 0.1 M Na-phosphate buffer pH 6.0 and immobilization time of 2.5 h) was further used for investigation of possible usage in synthesis of FOS.

FTase immobilization on cyanuric chloride activated amino-modified silica nanoparticles (CCAFNS)

After successful immobilization of FTase on amino-modified silica nanoparticles (AFNS) by adsorption, the cyanuric chloride activated amino-modified silica nanoparticles (CCAFNS) were further investigated, for possible covalent enzyme-support immobilization which is a much-preferred immobilization technique over adsorption. The CCAFNS support possess chloride groups of triazine ring on surface, these groups could form strong covalent bonds with amino groups present on surface of enzyme molecule. For complete investigation of this support suitability, the optimal initial enzyme concentration for the immobilization process was evaluated by monitoring the protein loading (mg/g support), activity (IU/g support), specific activity (IU/mg proteins) and activity immobilization yield (%), like in the case of amino-modified carrier (AFNS), and results are presented on Fig. 4. The immobilization process for all tested initial enzyme concentration is performed in 0.1 M Na-phosphate buffer pH 6.0 for 2.5 h.

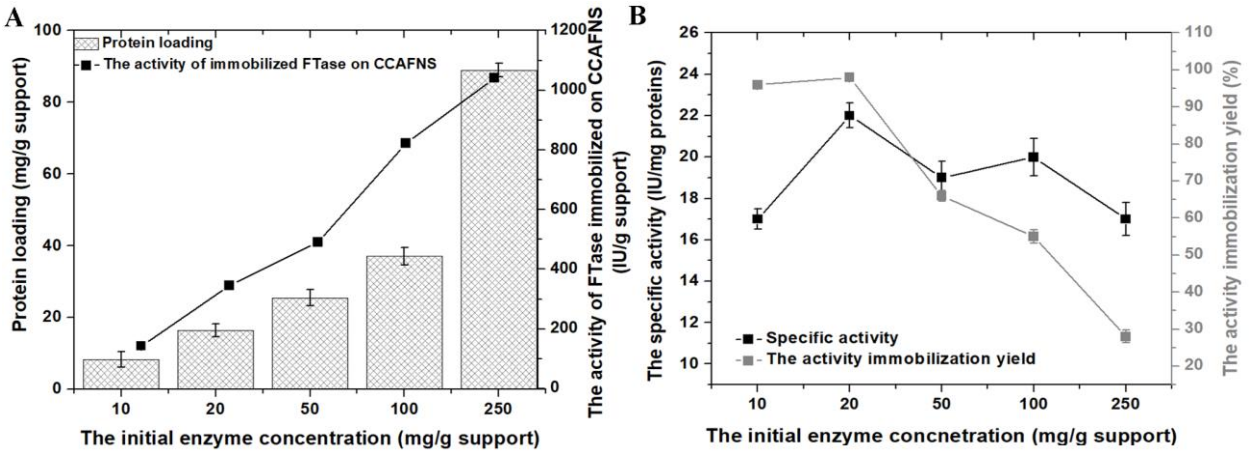


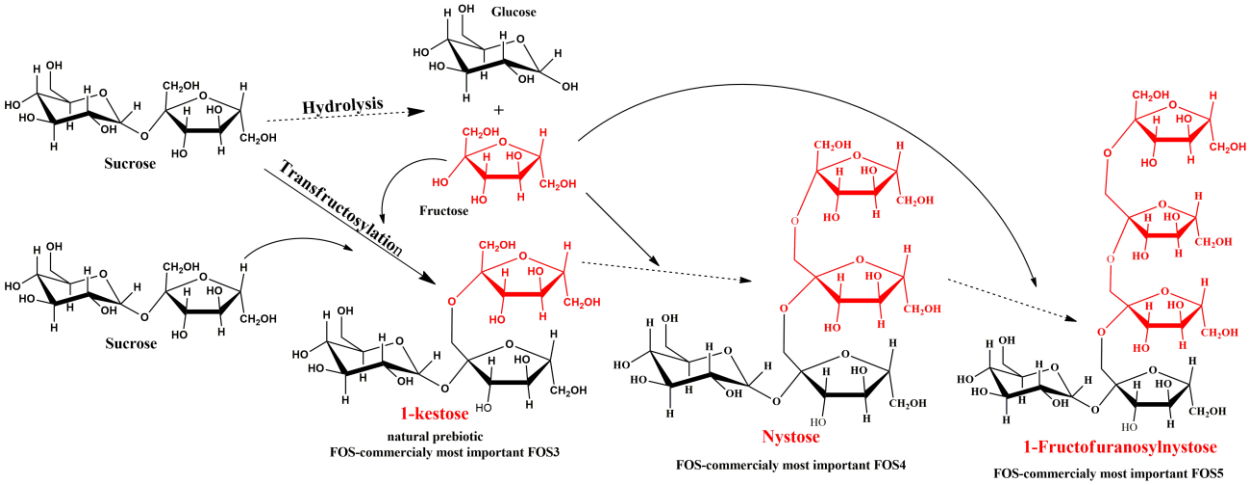
Figure. 4. The effect of initial enzyme concentration on protein loading (mg/g support) and activity (IU/g support) (A) and specific activity (IU/mg protein) and activity immobilization yield (%) (B) of immobilized FTase on CCAFNS.

As in case of AFNS, the initial enzyme concentration was varied in a wide range of 10 mg/g support to 250 mg/g support. From the results obtained, it can be observed that with increase of initial enzyme concentration, the steep increase in protein loading and activity of immobilized preparations occurs. For the highest initial enzyme concentration of 250 mg/g support, the highest protein loading of 89 mg/g support and activity of 1041 IU/g support were achieved (Fig. 4A). On the other hand, by analyzing specific activity and activity yield of immobilized preparations (Fig. 4B), it can be observed the step decrease of these two parameters at 250 mg/g support. The highest specific activity (22 IU/mg proteins) and activity yield (98 %) was obtained during immobilization of FTase at 20 mg/g support, while the activity of this preparation was only 400 IU/g support. Based on all results presented, it can be seen that at initial enzyme concentration of 100 mg/g support gives optimum immobilized preparation with activity of 822 IU/g support and specific activity of 19 IU/mg proteins, meaning that high yield of enzymes are bound in their active conformation, accessible to the substrate. The influence of immobilization time on activity of immobilized FTase on CCAFNS at 100 mg/g support is also determined (data not shown). The results showed that maximum activity of 1122 IU/g support was achieved after 5 h, which is 1.5 times higher than at 2.5 h. So, in case of FTase immobilization on CCAFNS, the immobilization time of 5 h is adopted as optimal.

By comparing the results obtained for immobilization of FTase on CCAFNS with ones obtained for AFNS, it can be noted that CCAFNS support is more favorable in terms of protein loading, while immobilized enzyme on AFNS expressed 1.4 times higher activity. Taking into account that on AFNS, FTase is bound *via* adsorption while on CCAFNS strong covalent bonds are formed, both immobilized preparations were chosen to be applied for synthesis of FOS.

Production of FOS using FTase immobilized on modified silica nanoparticles

In this study, developed nanobiocatalysts (FTase immobilized on AFNS and FTase immobilized on CCAFNS) were further evaluated in bioconversion of sucrose into fructo-oligosaccharides (FOS) (Fig. 5). In this way, short-chain FOS are formed, which represent a mixture of oligosaccharides whose number of fructose units varies from 2 to 4 (Scheme 1). They are formed by a transfructosylation reaction which involves the simultaneous cleavage of the β-(2,1)-glycosidic bond in the sucrose molecule and transfer of the fructose unit to another donor which may be sucrose (forming 1-kestose) or the obtained FOS (forming nystose or 1-fructofuranosylnystose) releasing glucose units as a secondary product. Scheme 1 gives a simplified view of the mechanism of the transfructosylation reaction.



Scheme 1. Production of FOS from sucrose.

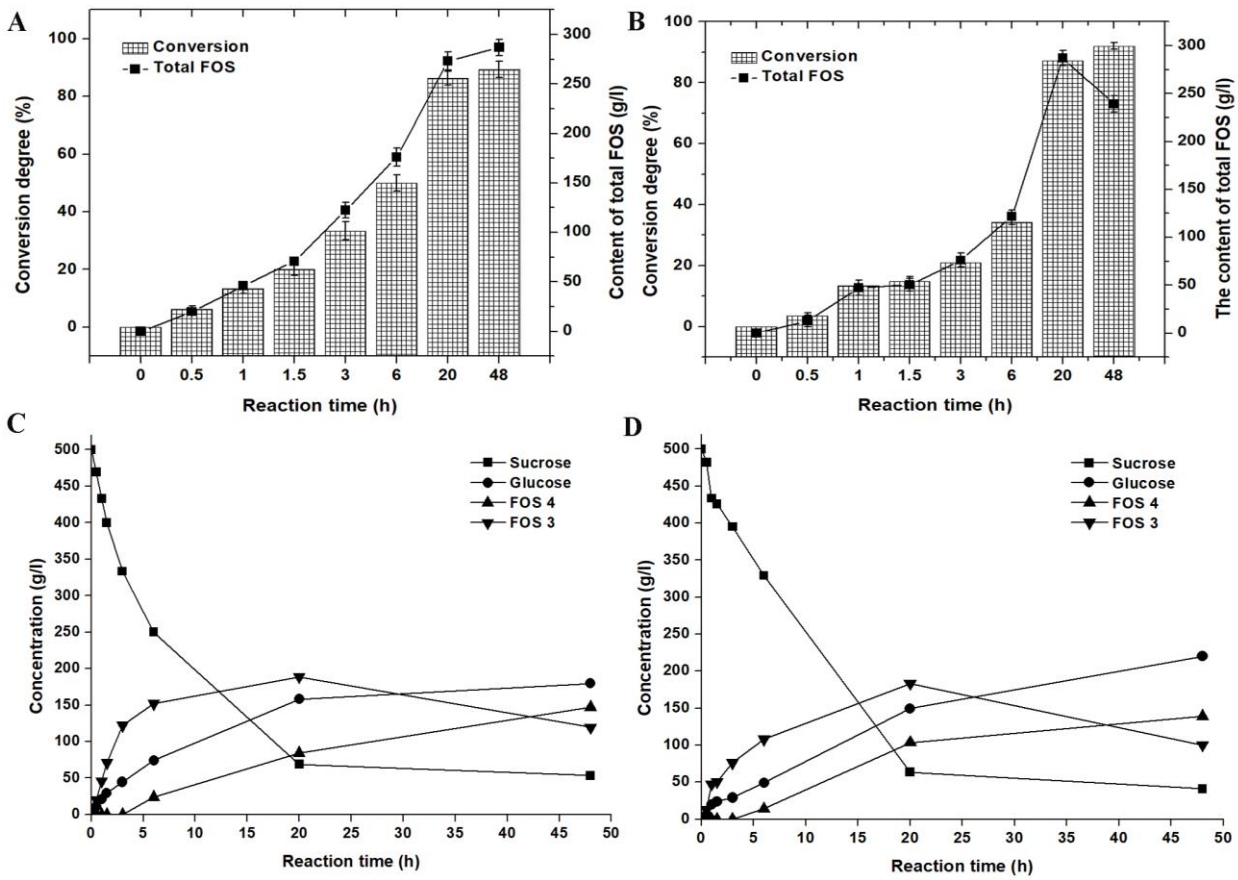


Figure 5. Time course of total FOS formation (-■-) and sucrose conversion (bars) using FTase immobilized on AFNS (A) and CCAFNS (B). Reaction mixture concentration changes in the product stream at different reaction time for FTase immobilized on AFNS (C) and CCAFNS (D).

The FOS synthesis was performed at initial sucrose concentration of 500 g/l and 50 ºC during 48 h since these reaction conditions are determined as optimal (Martins et al., 2019) (Fig. 5). For adequate estimation of FTase affinity towards transfructosylation, the FOS synthesis was carried out with the amounts of nanobiocatalysts that provide equal activity (7.88 IU) of FTase immobilized on AFNS and CCAFNS. The concentration of total FOS produced by both FTase

immobilized preparations and the sucrose conversion degree was monitored during 48 h (Fig. 5A and 5B). As observed, total FOS production increased after a certain degree of sucrose conversion. In case of CCAFNS preparation, after 6 h, conversion degree of sucrose was 34 % and content of FOS was 122 g/l, while maximum FOS (286 g/l) was produced at sucrose conversion of 87 % after 20 h. For AFNS preparation, the corresponding value of 287 g/l was obtained after 48 h at sucrose conversion of 89 %.

Considering that different types of interactions are formed between enzyme and these two nanoparticles, presented results confirm that enzyme immobilization on AFNS or CCAFNS did not lead to change in specificity and selectivity of FTase toward FOS synthesis. Also, usage of immobilized preparations is interesting since very high sucrose conversion degrees are achieved, which is beneficial for FOS downstream processing. The concentration of reaction species (sucrose, glucose, FOS 4 and FOS 3) of sucrose bioconversion as a function of reaction time is depicted at Fig. 5C and 5D. In both cases, it can be observed that after 20 h, the concentration of FOS 3 decreased and the production of FOS 4 become dominant. Also, during first 3 hours, only FOS 3 was synthetized. Apparently, with CCAFNS preparation FOS production of 14 g/l/h was achieved as compared to 5.9 g/l/h by AFNS preparation.

Conclusions

In this study, amono-modified and cyanuric chloride activated amino-modified silica nanoparticles were successfully applied for the immobilization of fructosyltransferase (FTase) from commercial enzyme preparation Pectinex® Ultra SP-L. CCAFNS preparation exhibited 1.4 time higher hydrolytic activity than AFNS preparation. In both cases, it has been determined that optimal conditions for immobilization were at initial enzyme concentration of 250 mg/g support, pH 6.0, and immobilization time of 5 h. In terms of FOS production, which was performed at 50 °C and sucrose concentration of 500 g/l, AFNS and CCAFNS nanobicatalysts produced 289 g/l of FOS was with sucrose conversion of 89 %. The FTase covalently immobilized on CCAFNS has the better catalytic capability for FOS production than FTase immobilized on AFNS *via* electrostatic interactions, since CCAFNS preparation accomplished maximum FOS yield twice faster than AFNS preparation.

Acknowledgments

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