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Sorbitol Galactoside Synthesis Using β-Galactosidase Immobilized on Functionalized Silica Nanoparticles

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Abstract : Nowadays, considering the growing awareness of functional food beneficial effects on human health, due attention is dedicated to the research in the field of obtaining new prominent products exhibiting improved physiological and physicochemical characteristics. Therefore, different approaches to valuable bioactive compounds synthesis have been proposed. β-Galactosidase, for example, although mainly utilized as hydrolytic enzyme, proved to be a promising tool for these purposes. Namely, under the particular conditions, such as high lactose concentration, elevated temperatures and low water activities, reaction of galactose moiety transfer to free hydroxyl group of the alternative acceptor (e.g. different sugars, alcohols or aromatic compounds) can generate a wide range of potentially interesting products. Up to now, galacto-oligosaccharides and lactulose have attracted the most attention due to their inherent prebiotic properties. The goal of this study was to obtain a novel product sorbitol galactoside, using the similar reaction mechanism, namely transgalactosylation reaction catalyzed by β-galactosidase from Aspergillus oryzae. By using sugar alcohol (sorbitol) as alternative acceptor, a diverse mixture of potential prebiotics is produced, enabling its more favorable functional features. Nevertheless, an introduction of alternative acceptor into the reaction mixture contributed to the complexity of reaction scheme, since several potential reaction pathways were introduced. Therefore, the thorough optimization using response surface method (RSM), in order to get an insight into different parameter (lactose concentration, sorbitol to lactose molar ratio, enzyme concentration, NaCl concentration and reaction time) influences, as well as their mutual interactions on product yield and productivity, was performed. In view of product yield maximization, the obtained model predicted optimal lactose concentration 500 mM, the molar ratio of sobitol to lactose 9, enzyme concentration 0.76 mg/ml, concentration of NaCl 0.8M, and the reaction time 7h. From the aspect of productivity, the optimum substrate molar ratio was found to be 1, while the values for other factors coincide. In order to additionally, improve enzyme efficiency and enable its reuse and potential continual application, immobilization of β-galactosidase onto tailored silica nanoparticles was performed. These non-porous fumed silica nanoparticles (FNS)were chosen on the basis of their biocompatibility and non-toxicity, as well as their advantageous mechanical and hydrodinamical properties. However, in order to achieve better compatibility between enzymes and the carrier, modifications of the silica surface using amino functional organosilane (3-aminopropyltrimethoxysilane, APTMS) were made. Obtained support with amino functional groups (AFNS) enabled high enzyme loadings and, more importantly, extremely high expressed activities, approximately 230 mg proteins/g and 2100 IU/g, respectively. Moreover, this immobilized preparation showed high affinity towards sorbitol galactoside synthesis. Therefore, the findings of this study could provided a valuable contribution to the efficient production of physiologically active galactosides in immobilized enzyme reactors.

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