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Improvement of Activity of β-galactosidase from Kluyveromyces lactis via Immobilization on Polyethylenimine-Chitosan

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Abstract : β-galactosidases (E.C. 3.2.1.23) are enzymes that have attracted by catalyzing the hydrolysis of lactose and in producing galacto-oligosaccharides by favoring transgalactosylation reactions. These enzymes, when immobilized, can have some enzymatic characteristics substantially improved, and the coating of supports with multifunctional polymers is a promising alternative to enhance the stability of the biocatalysts, among which polyethylenimine (PEI) stands out. PEI has certain properties, such as being a flexible polymer that suits the structure of the enzyme, giving greater stability, especially for multimeric enzymes such as β-galactosidases. Besides that, protects them from environmental variations. The use of chitosan support coated with PEI could improve the catalytic efficiency of β-galactosidase from Kluyveromyces lactis in the transgalactosylation reaction for the production of prebiotics, such as lactulose since this strain is more effective in the hydrolysis reaction. In this context, the aim of the present work was first to develop biocatalysts of β-galactosidase from K. lactis immobilized on chitosan-coated with PEI, determining the immobilization parameters, its operational and thermal stability, and then to apply it in hydrolysis and transgalactolisation reactions to produce lactulose using whey as a substrate. The immobilization of β-galactosidase in chitosan previously functionalized with 0.8% (v/v) glutaraldehyde and then coated with 10% (w/v) PEI solution was evaluated using an enzymatic load of 10 mg protein per gram support. Subsequently, the hydrolysis and transgalactosylation reactions were conducted at 50 °C, 120 RPM for 20 minutes, using whey supplemented with fructose at a ratio of 1:2 lactose/fructose, totaling 200 g/L. Operational stability studies were performed in the same conditions for 10 cycles. Thermal stabilities of biocatalysts were conducted at 50 ºC in 50 mM phosphate buffer, pH 6.6 with 0.1 mM MnCl2. The biocatalyst whose support was coated was named CHI_GLU_PEI_GAL, and the one that was not coated was named CHI_GLU_GAL. The coating of the support with PEI considerably improved the parameters of immobilization. The immobilization yield increased from 56.53% to 97.45%, biocatalyst activity from 38.93 U/g to 95.26 U/g and the efficiency from 3.51% to 6.0% for uncoated and coated support, respectively. The biocatalyst CHI_GLU_PEI_GAL was better than CHI_GLU_GAL in the hydrolysis of lactose and production of lactulose, converting 97.05% of lactose at 5 min of reaction and producing 7.60 g/L lactulose in the same time interval. QUI_GLU_PEI_GAL biocatalyst was stable in the hydrolysis reactions of lactose during the 10 cycles evaluated, converting 73.45% lactose even after the tenth cycle, and in the lactulose production was stable until the fifth cycle evaluated, producing 10.95 g/L lactulose. However, the thermal stability of CHI_GLU_GAL biocatalyst was superior, with a half-life time 6 times higher, probably because the enzyme was immobilized by covalent bonding, which is stronger than adsorption (CHI_GLU_PEI_GAL). Therefore, the strategy of coating the supports with PEI has proven to be effective for the immobilization of β-galactosidase from K. lactis, considerably improving the immobilization parameters, as well as, the catalytic action of the enzyme. Besides that, this process can be economically viable due to the use of an industrial residue as a substrate.

Keywords : β-galactosidase, immobilization, kluyveromyces lactis, lactulose, polyethylenimine, transgalactosylation reaction, whey
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