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Immobilization of β-Galactosidase from Kluyveromyces Lactis on Polyethylenimine-Agarose for Production of Lactulose

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Abstract : Galactosidases are enzymes responsible for catalyzing lactose hydrolysis reactions and also favoring transgalactosylation reactions for the production of prebiotics, among which lactulose stands out. These enzymes, when immobilized, can have some enzymatic characteristics substantially improved, and the coating of supports with multifunctional polymers in immobilization processes is a promising alternative in order to extend the useful life of the biocatalysts, for example, the coating with polyethyleneimine (PEI). PEI is a flexible polymer that suits the structure of the enzyme, giving greater stability, especially for multimeric enzymes such as β-galactosidases and also protects it from environmental variations, for example, pH and temperature. In addition, it can substantially improve the immobilization parameters and also the efficiency of enzymatic reactions. In this context, the aim of the present work was first to develop biocatalysts of β-galactosidase from Kluyveromyces lactis immobilized on PEI coated agarose, determining the immobilization parameters, its operational and thermal stability, and then to apply it in the hydrolysis of lactose and synthesis of lactulose, using whey as a substrate. This immobilization strategy was chosen in order to improve the catalytic efficiency of the enzyme in the transgalactosylation reaction for the production of prebiotics, and there are few studies with β-galactosidase from this strain. The immobilization of β-galactosidase in agarose previously functionalized with 48% (w/v) glycidol and then coated with 10% (w/v) PEI solution was evaluated using an enzymatic load of 10 mg/g of protein. Subsequently, the hydrolysis and transgalactosylation reactions were conducted at 50 °C, 120 RPM for 20 minutes, using whey (66.7 g/L of lactose) supplemented with 133.3 g/L fructose at a ratio of 1:2 (lactose/fructose). 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 biocatalysts whose supports were coated were named AGA_GLY_PEI_GAL, and those that were not coated were named AGA_GLY_GAL. The coating of the support with PEI considerably improved immobilization yield (2.6-fold), the biocatalyst activity (1.4-fold), and efficiency (2.2-fold). The biocatalyst AGA_GLY_PEI_GAL was better than AGA_GLY_GAL in hydrolysis and transgalactosylation reactions, converting 88.92% of lactose at 5 min of reaction and obtaining a residual concentration of 5.24 g/L. Besides that, it was produced 13.90 g/L lactulose in the same time interval. AGA_GLY_PEI_GAL biocatalyst was stable during the 10 cycles evaluated, converting approximately 80% of lactose and producing 10.95 g/L of lactulose even after the tenth cycle. However, the thermal stability of AGA_GLY_GAL biocatalyst was superior, with a half-life time 5 times higher, probably because the enzyme was immobilized by covalent bonding, which is stronger than adsorption (AGA_GLY_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 enzyme, catalyzed reactions. In addition, the use of whey as a raw material for lactulose production has proved to be an industrially advantageous alternative.

Keywords : β-galactosidase, immobilization, lactulose, polyethylenimine, whey
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