Effect of Additives as Glycerin, Polyetilenoglycol and Trehalose on the Stabilization/Reactivation of Immobilized Glucoamylase from Aspegillus niger

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INTRODUÇÃO: Glucoamylases hydrolyze α-1,4 and α-1,6 linkages of starch and related polymers to produce glucose as the sole end product. Glucoamylases also hydrolyze other starch-related oligo- and polysaccharides, and show a preference for maltooligosaccharides of at least six residues. MATERIAL and METHODS: glucoamylase from Aspergillus niveus was produced by submerged fermentation using soluble starch as carbon source, at 40 ºC, for 72 hours. After that, the dialyzed crude extract containing the active glucoamylase was purified on two successive steps using DEAE-fractogel and sephacryl S 200 chromatographic columns. The purified enzyme was immobilized by covalent attachment using activated CNBr-agarose and ionic interaction on sepharose Q. The parameters of the immobilization procedures were defined as: yield of immobilization (YI) which is the ratio between the amount of immobilized enzyme and the amount of enzyme offered to immobilization. The activity recovery (RA) which is the ratio between the measured derivative activity and the theoretical immobilized activity (difference between the initial activity and the activity measured in the final supernatant).

RESULTS and DISCUSSION: Soluble glucoamylase presented a half-life of 90 min, at 55ºC, while the Sepharose Q and CNBr derivatives presented a half-life of 140 and 270 minutes respectively. The additives glycerol, polyethylene glycol (PEG) and trehalose were used in different concentrations to improve the stability of the enzymatic derivatives. The additive glycerol decreased the stability of the derivatives CNBr and Sepharose Q, whereas the additive PEG increased the stability of derivative CNBr and decreased derivative sepharose Q. The additive trehalose increased the stability of the derivative CNBr in all concentrations tested, however, at 20% the immobilized enzyme was more stable. In order to verify the effect of trehalose on the reactivation of the enzymatic activity after denaturation, the derivative CNBr was subjected to a treatment with guanidine 8M. Trehalose 20% was added together with denatured derivative CNBr and the reactivation of enzyme activity was more effective than using only acetate buffer.

Keywords: glucoamylase, immobilization, stabilization

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