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IMMOBILIZATION OF *Rhizomucor miehei* LIPASE ONTO CHITOSAN HYBRIDS

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The specificity of enzymes and their ability to catalyze reactions make them attractive for applications in biochemical and industrial fields. One major advantage of this biocatalyst specificity is the achievement of targets free of side products. Immobilization offers some operational advantages over free enzymes, such as choice of batch or continuous processes, rapid termination of reactions, controlled product formation, ease of removal from the reaction mixture and adaptability to various engineering designs. By careful selection of the matrix, it is also possible to vary the nature of the immobilized derivative in order to improve enzyme activity and stability and also to allow easier handling and storage. Lipase has been immobilized by several methods, namely adsorption, cross-linking, adsorption followed by cross-linking, covalent attachment and physical entrapment. However, the selection of an immobilization strategy should be based on process specifications for the catalyst, including such parameters as overall enzymatic activity, effectiveness of lipase utilization, deactivation and regeneration characteristics, cost of the immobilized procedure, toxicity of immobilization reactants and the desired final properties of the immobilized derivative on an industrial scale requires their immobilization and re-usability. This work aims to obtain high activity and thermal stable immobilized derivatives of *Rhizomucor miehei* lipase using hybrid matrixes of chitosan and different copolymers as k-carrageenan and sodium alginate activated by glycidol, epichlorohydrin or glutaraldehyde analyzing some parameters such as immobilization yield, recovered activity and thermal stability at 60ºC. Glutaraldehyde activation was made by contacting hybrid-chitosan beads with sodium phosphate buffer 0.1M, pH 7.0 containing glutaraldehyde 5% (m/v) using a ratio V_{beads}/V_{total} of 1/10 during 1.0 h at 25℃, and washing with distilled water to remove the excess of the activating agent. Glyceryl-supports were prepared by mixing beads under stirring with an aqueous solution containing NaOH 1.7M and $NaBH_4$ 0.75M (glycidol)[1] or NaOH 2M and $NaBH_4$ 0.12M (epichlorohydrin) in ice bath. Then, 0.48 mL of glycidol or 2.00 mL of epichlorohydrin per gram of bead were added, kept under mechanical stirring for 18 h and washed until neutrality. Glyoxyl/oxirane-supports were obtained by contacting beads with 2.00 mL of 0.1M $NaIO_4$ solution per gram of gel for 2.0 h at room temperature. Afterwards, they were washed with excess of distilled water until neutrality [2]. Lipase ($200U_{pNPB}$ of enzyme per gram of bead) in sodium bicarbonate buffer 0.1M, pH 10.0 was added to the activated support (ratio m/v of 1/10). The preparation was kept under mild stirring at 25℃ and 5h. Enzyme activity was assessed via spectrophotometer using pNPB. The product released by the hydrolysis, in 0.1M sodium phosphate buffer, pH 8.0 at 25ºC was measured at 410 nm. One unit (pNPB) corresponds to the amount of enzyme that hydrolyzes 1µmol of pNPB per minute at pH 8.0 and 25℃. Table 1 presents preliminary results with different activants and varying chitosan concentration.

Table 1: Influence of the polymer composition on the immobilization of lipase at pH 10.0, 25℃, for 5 h. Enzyme load: $200U_{pNPB}$ of enzyme.g^{-1} of gel. Immobilization parameters: immobilization yield (I_Y), recovered activity ($\mathbf{R_A}$), half-life ($t_{1/2}$) and stabilization factor (S_F) at 60℃. half-life is not in the table

Support	*Activation*	$I_Y(\%)$	$R_A(\%)$	S_F
Chitosan 2.5%	Glu	93	7	94
Chitosan 5.0%	Glu	95	3	n.d
Chitosan 2.5%	Gly	79	31	37
Chitosan 5.0%	Gly	59	n.d	n.d
Chitosan 2.5%	Epi	68	25	200
Chitosan 5.0%	Epi	59	11	68

It's possible to notice the effect of the activation method on the stability of the lipase. Thus, it's possible to improve its characteristics aiming optimal lipase stability.

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