STABILIZATION OF NEUTRASE USING DIFFERENT METHODS OF IMMOBILIZATION ONTO CHITOSAN HYBRIDS

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The specificity of enzymes and their ability to catalyze reactions at low temperatures and neutral pH make them attractive for applications in biochemical and industrial fields. However, they are expensive, have low operational stability and the recovery of the free enzyme from the medium is not economically viable, what has hindered their application in large scale processes. The enzyme immobilization on insoluble supports allows not only the reuse of the protein but also to modulate the catalyst properties. Besides, the use of an insoluble enzyme derivative enables the operation of continuous processes, fast ending of reactions, controlled product formation, easiness of reaction mixture removal and adaptability for several engineering purposes and making possible its use in a larger number of processes. Neutrase, a bacterial endoprotease produced by *Bacillus subtilis* presents considerable interest due to a wide variety of possible applications, for example, transformations of cheese whey proteins in high value food, as protein hydrolysates with low contents of phenylalanine that are an important issue for chemical and pharmaceutical industries. Thus, the aim of this work will be developing a neutrase immobilization protocol using chitosan and their hybrids using 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 75ºC and comparing to soluble enzyme. Chitosan beads will be prepared by dissolving in a solution of acetic acid 5% v/v. The obtained solution will be dropped into a gently stirred NaOH 0.1M solution. Supports will be activated with glutaraldehyde, glycidol and epichlorohydrin before enzyme immobilization. Glutaraldehyde activation will be 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 will be 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 18h 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 [2]. Neutrase (5mg enzyme.g of support $^{-1}$) in 0.1M tris-borate buffer at pH 8.0 or sodium bicarbonate buffer 0.1M pH 10.0 will be added to the activated support and kept under mild stirring at 25℃ for different process times. Solub le and immobilized neutrase activities will be assessed via spectrophotometer analysis at 700 nm according to the TCA- Lowry assay [3]. One unit of activity is defined as the amount of enzyme that hydrolyzes casein to produce equivalent color to 1 µg of tyrosine per minute at pH 8.1 and 50 ℃.

Table 1. Influence of the polymer composition on the immobilization of lipase at pH 10.0, 25℃, for 5 h .

Support	*Activation*	I_Y*(%)*	R_A*(%)*	S_F
Chitosan 2[P1]**.5%**	Glu	74	56	2
Chitosan 5.0%	Glu	74	68	2
Chitosan 2.5%	Gly	22	100	n.d
Chitosan 5.0%	Gly	45	75	2
Chitosan 2.5%	Epi	17	100	n.d
Chitosan 5.0%	Epi	51	11	n.d

According to preliminary results (Table 1), it was obtained a good immobilization yield and recovered activity, however low thermal stability was reached due to enzyme complexity that is a metalloprotein.

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REFERENCES

1. Tardioli, P.W.; Pedroche, J.; Giordano, R.L.C.; Fernández-Lafuente, R.; Guisán, J.M. Biotechnology Progress, (2003), 19, 352-360.
2. Adriano, W. S., Mendonça, D. B., Rodrigues, D. S., Mammarella, E. J.,Giordano, R. C. L., Biomacromolecules
3. Ortega, N.,Perez-Mateos, M.,Pilar, M. C., Busto, M. D. Journal of Agricultural and Food Chemistry.(2009),.57, 109-115.

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