IMMOBILIZATION OF *Aspergillus niger* LIPASE ON CHITOSAN-COATED MAGNETIC NANOPARTICLES USING TWO COVALENT-BINDING METHODS

Yolanda Osuna[1], Karla M. Gregorio-Jauregui[1], Guillermo López[2], José Sandoval[1], José L. Martínez[1], Hened Saade[2], and Anna Iliná[*1]

[1] Facultad de Ciencias Químicas, Universidad Autónoma de Coahuila, Boulevard V. Carranza y José Cárdenas Valdés, 25280 Saltillo, COAH, México.

[2] Centro de Investigación en Química Aplicada (CIQA), Boulevard Enrique Reyna No. 140, 25294 Saltillo, COAH, México

*Corresponding author. E-mail: anna_ilina@hotmail.com

Key words: covalent-binding immobilization, chitosan-coated magnetic nanoparticles, Aspergillus niger lipase.

Introduction. Lipases have potential applications in many areas and industries. To completely exploit the economic and technical advantages of these enzymes, their use in immobilized state is recommended. Recently, nanostructured magnetic materials have been employed as supports for enzymes immobilization due to many advantages such as easy separation of catalysts under a magnetic field [1].

The goal of the present study is to immobilize *Aspergillus niger* lipase on chitosan-coated magnetic nanoparticles (NP) activated with glutaraldehyde and glycidol, as well as to characterize immobilized derivatives.

Methods. NP were prepared according to a previously reported procedure [2] and activated with glutaraldehyde and glycidol according to the described methodology [3], followed by lipase immobilization. Immobilization yields were calculated from protein balance. Free and immobilized lipase activity was determined using *p*-nitrophenyl propionate as substrate. The morphology of immobilized derivatives was determined by a high-resolution transmission electron microscopy (HRTEM). Kinetic and operational parameters (K_m, V_{max}, pH, temperature, and thermal stability) were compared.

Results. Higher lipase activity and immobilization yield were detected for the enzyme immobilized using glutaraldehyde (Table 1). This behavior can be explained considering more reactivity of glutaraldehyde groups than the glyoxyl groups [4].

The red lines in the image of HRTEM micrographs of the immobilized derivatives (Fig. 1) marks non-crystalline area probably corresponding to the bound enzyme.

Comparison of operational and kinetic parameters of free and immobilized lipase demonstrates the advantages of glutaraldehyde immobilized derivative in terms of higher affinity and stability (Table 2).

Table 1. Immobilization parameters: lipase activity and immobilization yield (IY).

Covalent binding	IU/g of NP	IY (%)
Glutaraldehyde	309.54±2.01	90.10±1.15
Glycidol	129.78±2.04	62.2±0.89

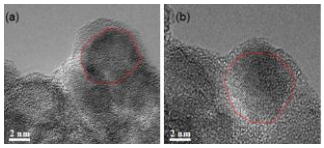

Fig 1. HRTEM micrographs of the lipase immobilized on NP activated with glutaraldehyde (a) and glycidol (b).

Table 2. Kinetic and operational parameters of free lipase and immobilized derivatives: NP-GLY-E – obtained with glycidol; NP-GLU-E obtained with glutaraldehyde.

Parameter	NP-GLY- E	NP-GLU-E	Free E
Optimal pH	8	8	8
Optimal temperature, °C	40	40	40
K_m, mM	21±0.2	17±0.7	12±1.2
V_{max}, µM/min	2630± 0.8	2440±0.5	2260± 0.7
k_{in} at 40°C, h^{-1}	0.0171± 1.2	0.0074±1.3	0.0393± 1.4
k_{in} at pH 8, h^{-1}	0.0287± 1.6	0.0196±0.8	0.0416± 0.8

Conclusions. Lipase immobilization using glutaraldehyde and glycidol as coupling agents was successful**.** The best results, as well as kinetic and operational properties were demonstrated for lipase immobilized on chitosan coated magnetic nanoparticles activated with glutaraldehyde.

Acknowledgements. The authors would like to thank CIQA and SRE-CONACYT 187488 grant.

References.

1. Xie, W. and Ma, N. (2009). Energy & Fuels. (23):1347-1353.

2. Osuna, Y., Gregorio-Jauregui, K.M., Gaona-Lozano, J. G., de la Garza-Rodríguez, I. M. et al. (2012). J. Nanomater. (2012): 1-7. doi:10.1155/2012/327562

3. Rodrigues, D. S., Mendes, A. A., Adriano, W. S., Gonçalves, L. R. B. and Giordano, R. L. C., (2008), J. Mol. Catal. B: Enzym. (51): 100-109.

4. Silva, J. A., Macedo, G. P., Rodrigues, D. S., Giordano, R. L. C. and Gonçalves, L. R. B., (2012). Biochem. Eng.(60): 16-24.