IMMOBILIZATION OF CARBOXYLESTERASES PRODUCED BY A*spergillus nidulans* IN DIFFERENT SUPPORTS TO BE USED IN BIODIESEL PRODUCTION

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Key words: immobilization, carboxylesterases, supports

Introduction. Carboxylesterases are enzymes that show high regio- and stereospecificity, which makes them attractive biocatalysts for the production of optically pure compounds in fine-chemicals synthesis and they have great potential for application in many other biocatalytic processes, including biodiesel production. *A. nidulans* encodes almost 70 genes for these enzymes and our group has characterized four of them (1-4). Enzyme immobilization ensures recycling of the biocatalyst, allows easy product separation, and may improve performance of the enzyme. The immobilization often results in greatly increased resistance to various denaturation factors, like extreme pH and temperature or the use of solvents and results in a longer storage time.

In this work, different supports were evaluated to determine which can be the best for the immobilization of each of the four carboxylesterases from *A. nidulans*, which will be assayed for biodiesel production.

Methods. Carboxylesterases in crude extracts were obtained according to described protocols (1-4). Concentration and buffer exchange of supernatant was done by ultrafiltration through a membrane having 10-kDa NMWL. The concentrated solution was used to verify enzymes presence and its esterase activity (5). Esterase activity in the crude extract was detected by spectrophotometry at 410 nm, using *p*-NPA as substrate as described by Ejima et al. (5). Protein analysis was done with a SDS-PAGE at 12% and the confirmation of its in situ esterase activity 149 was carried out by zymography as described by Peña-Montes *et al.* [1]. The enzymes were adsorbed on the supports (Accurel MP1000, Celite 545, Chitosan and Nylon) previously treated, and the mixture was incubated at 4°C with a shaking speed of 200 rpm on an orbital shaker at different times. The immobilization process was evaluated by taking aliquots from the initial and final solutions to quantify the protein content and enzyme activity as described before.

Results. Every enzyme has different interactions with the supports. The protein concentration in the sample has an important effect in the process of adsorption because it may result in steric hindrance. Most of them showed high yields in Accurel MP1000, a hydrophobic support, but Chitosan could be an adequate support as a second choice (Table 1). Lipases are selectively adsorbed onto Accurel MP1000 due to their hydrophobic domains, a feature that results in the purification of the lipase as well as in its immobilization. The behavior shown here after adsorption of NstcI and ANCUT2 might be due to low protein yields, while immobilization efficiency was high (Table 2). Chitosan has important advantages as it is cheaper than Accurel MP1000 and it is a residue of shrimp processing. To achieve immobilization with Nylon 6 a covalent bond is formed, so the active site may be altered. In fact, it had the lowest activity among the assayed supports.

Table1. Yields of the immobilization of Carboxylesterases

Enzyme	Immobilization conditions (Supports)	Total Protein (mg)	Total Activy (U)	Specific Activity (U/mg of protein)
NStcI	Accurel MP1000	4.00	3552.80	412.50
	Celite 545	1.39	957.21	180.63
	Chitosan	1.33	1009.78	495.92
	Nylon 6	3.87	2377.34	332.5
ANCUT2	Accurel MP1000	1.37	2669.93	1702.49
	Celite 545	0.39	222.00	356.82
	Chitosan	0.42	138.88	1505.99
	Nylon 6	1.39	1613.69	388.42
PrtA	Accurel MP1000	1.16	2441.08	2022.44
	Celite 545	0.40	570.05	209.49
	Chitosan	0.35	79.83	691.16
	Nylon 6	1.21	2850.37	180.37
Cocktail Carboxylesterases	Accurel MP1000	1.13	799.67	2079.49
	Celite 545	0.42	321.15	399.88
	Chitosan	0.32	309.54	1727.15
	Nylon 6	1.25	570.82	2014.42

Table 2. Immobilization efficiency (E%) and protein yield (P%) of immobilized carboxylesterases from *A. nidulans* on different supports.

Enzyme	Immobilization conditions (Carrier)	P (%)	E (%)
NStcI	Accurel MP1000	42.48	81.94
	Celite 545	48.92	73.59
	Chitosan	47.05	77.63
	Nylon 6	40.95	54.83
ANCUT2	Accurel MP1000	68.26	88.35
	Celite 545	64.76	24.49
	Chitosan	69.93	15.32
	Nylon 6	69.22	53.40
PrtA	Accurel MP1000	58.51	76.33
	Celite 545	70.60	59.42
	Chitosan	61.88	8.32
	Nylon 6	61.23	89.13
Cocktail Carboxylesterases	Accurel MP1000	59.66	40.95
	Celite 545	74.62	54.82
	Chitosan	55.58	52.84
	Nylon 6	65.8	29.23

Conclusions. We found that the Accurel MP1000 was the best support for the carboxylesterases evaluated. The immobilization process is simple. However, chitosan leads to acceptable immobilization yields and is a cheap support. The use of Nylon 6 affects the catalytic activity.

Acknowledgements. CONACYT 153500 and PAPIIT IN

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