Title: CHARACTERIZATION OF PURIFIED LIPASES FOR IMMOBILIZATION IN MAGNETIC NANOPARTICLES

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Abstract:

Lipases are serine hydrolases defined as triacylglycerol acylhydrolases (E.C. 3.1.1.3). They catalyze in nature the hydrolysis of the ester bond of tri-, di- and mono-glycerides of long-chain fatty acids into fatty acids and glycerol. In favorable thermodynamic conditions (for instance low thermodynamic water activity), they are able to catalyze reactions of synthesis such as esterification or amidation. This is made possible thanks to their resistance to organic solvents. They are well appreciated in industry because they are able to catalyze chemoselective, regioselective and stereoselective reactions [1]. The high cost for its production makes it necessary to use an immobilized form to enable reuse. Therefore, the present study aims to develop a new enzymatic biocatalyst from the immobilization of *Yarrowia lipolytica* (IMUFRJ 50682) lipase on magnetic nanoparticles. The production of the lipase conducted in 3 L benchtop bioreactor produced, after 24 h of culture, a crude enzyme extract (CEE) with hydrolytic p-nitrophenyl laurate activity of 58 U/ml. Partial purification of CEE by tangential ultrafiltration with hollow fiber membrane with a molecular cut of 50 kDa doubled the hydrolytic activity (128.15 U / ml), but did not allowed the reduction of proteolytic activity. The biphasic aqueous system (BAS) and protein precipitation with cold acetone and kaolin kept the same hydrolytic activity of lipase. However these two purification methods virtually eliminated the protease activity. The use of polyethylene glycol 1500 and potassium phosphate buffer, 20% w / w (BAS) favored the specific activity, allowing to obtain a very high purification factor (41,39). The characterization of crude and purified extracts showed that they support a wide temperature range (20 to 60 C °) and pH (3 to 9) with activities higher than 50% for 90 min incubation. Assays of isotherm of adsorption with bovine albumin in the magnetic nanoparticles (produced by precipitation process of Fe2+ and Fe3+) showed a definition of process of protein immobilization in the support in relation to the temperature and the saturation adsorptive capacity. The best magnetic nanoparticle biocatalyst was obtained with the extract purified by ultrafiltration, which allowed a best hydrolytic activity.

Keywords: enzyme adsorption; enzyme biocatalyst; palm oil; partial purification.

[1] Fickers et al. (2011) The lipases from *Yarrowia lipolytica*: genetics, production, regulation, biochemical characterization and biotechnological applications, Biotechnol Adv. 29(6):632-44