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Lipolytic biocatalyst based on recyclable magnetite-polysiloxane nanoparticles

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ABSTRACT

This work presents a novel hydrophobic magnetizable nanosupport able to load and valorize the lipase derived from *Candida cylindracea* (CCL). Nude magnetite nanoparticles (MP) were coated by covalent binding with an ester-polysiloxane (PS). The chemical composition, dimensions, morphology and magnetic properties of the resulted core–shell nanoparticles (MP-PS-CCL) are analyzed. The amount of immobilized lipase increase when loaded from aqueous solutions of up to 12.8 mg/mL CCL, when a lipolytic activity of 74.76 U/g is achieved. For higher concentrations of the loading solution, the activity of immobilized lipase decreases, probably due to the enzyme steric hindrance.

MP-PS-CCL exhibits a good lipolytic activity against 4-nitrophenyl laurate (4-NPL), which allows the kinetic study of lipolysis reaction by measuring the amount of released 4-nitrophenol (4-NP), when working at room temperature, in TRIS buffer (pH 8.2). Even after three months of storage, the product is able to sustain up to 4 reusing cycles.

1. Introduction

Lipases (triacylglycerol acylhydrolases, EC 3.1.1.3) are serine hydrolases which catalyze lipid hydrolysis, interesterification, esterification, alcoholysis, acidolysis and aminolysis [1,2]. Their immobilization on supports is a commonly used technique to increase the operational stability of the enzymes [3,4] and to facilitate their recovery for reuse [5,6], in order to make the biotechnological processes economically advantageous.

Several methods for lipase immobilization have been reported [7,8], but adsorption is the cheapest in preparing biocatalysts [8,9]. It is based on the physical adsorption and/or ionic bonding of the lipase to the surface of the support. The weak interactions between enzyme and the support can only be of Van der Waals, hydrogen bonding and hydrophobic types, which minimally affect its catalytic activity [10]. Such interactions make often possible the regeneration of immobilized biocatalyst by the enzyme desorption from the support.

The affinity of a lipase for an adsorbent increases with the hydrophobicity of the support surface, whereas hydrophilic substrata facilitate the desorption [11]. Efficient adsorption depends on the enzyme concentration in the loading system. The aim of immobilization procedures is to ensure high immobilization yields, measured through the equivalent enzyme activity of the biocatalyst. The activity of adsorbed lipases varies from zero to fairly high values, and sometimes exceeds the activity of soluble enzymes [12]. Actually, most of the lipases have a better activity when they are adsorbed on hydrophobic supports. Such a peculiarity has been related with conformational changes of the enzymes during adsorption processes, which induce the exposing of substrate-accessible active sites, beside an overall interfacial activation effect [13].

Inorganic substrata are often used to immobilize enzymes, due to their chemical and superficial properties [14–16]. Magnetic (nano)particles have been reported as good supports for enzymes immobilization [17,18] because of several advantages, such as: the improvement of mass-transfer properties of the immobilized enzymes [19], reusability due to the easier separation from the reaction milieu using magnetic fields (which minimizes the loss of enzyme) [20,21], low toxicity [22], simple way of biocatalyst purification [23], minimized operating expenses [24,25] and waste disposal costs [26]. Among the many types of iron oxides,

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