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Immobilized Lipases: An Old-Fashioned Twist for a New Generation of Industrial Biocatalysts

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Introduction

Lipase catalysed reactions are bio-inspired processes, serving the requirements to integrate environmental welfare with economical demands of modern industry [1]. Microbial lipases are especially prominent industrial biocatalysts with wide array of applications, owing to cost-effective production, pronounced chemo-, regio- and stereoselectivity, high catalytic efficiency in reaction systems with different water content (aqueous solutions to nearly anhydrous systems) and the possibility of tailoring them according to one's need [2-5]. However, their application is often hampered by the lack of long-term stability and difficulties with biocatalyst recovery and recycling.

Elaboration of the right stabilization protocol for lipases is a true work of art, because myriad of opportunities available. Nonetheless, carefully executed immobilization still represents an indispensable tool to improve almost all lipase properties, required for industrial practice [6]. Due to the phenomenon of interfacial activation, lipases are traditionally immobilized on hydrophobic supports, leading to enzyme hyperactivation [7]. Recently, by simple physical adsorption of Candida rugosa lipase (CRL) on hydroxyapatite, a biocompatible biocatalyst with improved thermal stability and excellent stability in methanol was designed, for innovative biosynthesis of short-chain methyl-aroma esters [8]. Two-step covalent immobilization of lipase B from Candida antarctica (CALB) on epoxy-activated Purolite A109 has yielded an immobilized preparation with higher thermal stability compared even with commercial immobilized CALB – Novozyme435 [9]. Similar strategy was implemented for covalent attachment of CRL on Eupergit C, via lipase carbohydrate moiety. As a result, half-life of immobilized CRL at 75°C was 18 times higher, compared to free enzyme [10]. By immobilizing CRL, CALB and *Rhizopus oryzae* lipase in polymer networks, different membrane reactors for hydrolysis of olive oil, synthesis of butyl-butyrate and esterification of lauric acid (respectively) were obtained [11-13]. Extremophylic lipase from *Pseudomonas aeruginosa* san-ai strain, used in detergents, leather manufacturing and for production of fine chemicals, was immobilized on alginate-type exo-polysaccharide, co-produced and co-secreted with the enzyme [14]. This cost-effective, time-saving approach for simultaneous production, purification and immobilization of lipase, showed a great potential of improving reusability of this enzyme.

Nanoparticles have also emerged as efficient immobilization supports, because of high specific area, effective enzyme loading and resistance to mass transfer effects [15]. Upon immobilization on magnetic cellulose nanocrystals, significant improvements of *Pseudomonas cepacia* lipase pH, temperature, organic solvent and storage stability were observed. Moreover, the same immobilized enzyme preparation was used for high-yield asymmetric ketoprofen-ethyl ester hydrolysis [16]. Also, it was revealed that immobilization of CRL on chemically modified silica nanoparticles results with novel biocatalyst with increased thermal stability, operational stability and esterification activity [17,18].

Carrier-free immobilized preparations are relatively new self-assembled systems, based on covalent crosslinking of enzyme molecules in different forms (crystals, aggregates or spray-dried). This proved to be very convenient method for CRL immobilization, giving rise to improved thermal stability and enantioselectivity of immobilized biocatalyst [19].

Constant development in the field of industrial catalysis has given rise to novel applications of lipases as important group of industrial enzymes. Even though it is considered as an old-school technique, immobilization still remains an inexhaustible source of opportunities for making lipases suitable for industrial transformations of a new generation.

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