Enzyme Immobilization and Biocatalysis

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Enzymes are catalysts with outstanding properties. Their use in industry offers several environmental and economic advantages over the chemical route, including mild operational conditions (pH and temperature), high selectivity and specificity towards substrates, avoidance of side reactions, easier separation, and product recovery with the elimination of treatment costs associated with product and side stream purification, catalyst biodegradability, and environmental acceptability. However, several technical challenges need to be overcome to make an enzymatic process economically feasible: the high cost of the enzymes, their low stability causing a loss of activity during the process, the inhibition by reactants and products, and difficult recovery [1–3].

Enzyme immobilization often allows for improving these unfavorable factors. Enzyme immobilization is the process of confining enzymes physically or chemically to a solid support while preserving their activity, in order to exploit the advantages of heterogeneous catalysis. It allows multiple use of the catalyst and continuous processes, easy separation of the products from the reaction mixture, rapid termination of the enzyme-substrate reaction by removing the enzyme from the reaction solution, and low proteinaceous contamination of the products [4]. Besides, enzyme immobilization often results in the stabilization of enzymes, sometimes increasing or modifying their activity [5]. Immobilization can increase pH and temperature tolerance, and resistance to proteolytic digestion and denaturants. For all these reasons, the use of immobilized enzymes is industrially preferred over free enzyme. The key issues for enzyme immobilization are the selection of the appropriate support and of the immobilization technique.

A field of application of choice for enzymatic catalysis, thanks to its high racemic resolution power, the purity of the products, and the use of substances that are not toxic to human health, is undoubtedly the pharmaceutical industry. For example, UDP-glycosyltransferase Bs-YjiC mutant M315F and sucrose synthase AtSuSy were co-immobilized on heterofunctional supports (resin LX1000HG modified with glyoxyl-metal-chelate bifunctional groups) to promote the one-pot reaction of ginsenoside biosynthesis from fructose and protopanaxadiol (PPD) [6]. Rare ginsenoside Rh2 exhibits diverse pharmacological effects, such as anti-oxidation, hepatoprotection, and anti-diabetes and it is a promising candidate for cancer prevention and therapy. Using the two-step process of specific adsorption and multipoint covalent attachment, the co-immobilized enzymes showed improved binding stability, improved pH and thermal stabilities, and good operation stability. Interestingly, a higher yield was achieved using co-immobilized enzymes by allowing the bioreaction at a high initial concentration of PPD by alleviating inhibition. This study has established a green and sustainable approach for the production of ginsenoside Rh2 in a high level of titer, which provides promising candidates for natural drug research and development. Racemization is an important process in the pharmaceutical industry to produce chiral drugs since enantiomers interact differently in living organisms due to their distinctive spatial arrangement. One approach is the production of a racemic mixture,

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followed by a resolution step, for instance, chiral chromatography. The amino acid racemase (AAR) (EC 5.1.1.10) from *Pseudomonas putida* KT2440 was covalently immobilized on the carrier Purolite ECR 8309F, a methacrylate polymer, functionalized with amino groups on an ethylene spacer, and pre-activated with glutaraldehyde [7]. AAR has a wide substrate specificity that makes it particularly valuable. AAR immobilized in an enzymatic fixed bed reactor (EFBR) coupled with enantioselective chromatography. The racemization of L- or D-methionine was studied for different temperatures, pH values and fractions of organic co-solvents. The long-term stability of the immobilized enzyme at operating and storage conditions was found to be excellent and recyclability was demonstrated. To indicate the large potential of the AAR, racemization rates were studied for lysine, arginine, serine, glutamine and asparagine. Significant AAR activities were confirmed for these amino acids.

Enzymatic catalysis is also widely used in the food industry. For example, specific enzymatic processes for generating a natural smoke flavor at mild reaction conditions without the risk of formation of unwanted and toxic by-products can be applied. A feruloyl esterase from *Rhizoctonia solani* and ferulic acid decarboxylase ScoFAD from the edible fungus *Shizophyllum commune* were covalently immobilized on agarose to enable reusability in a fixed-bed reactor [8]. The first enzyme liberated ferulic acid from cellulosic material, the second promoted the decarboxylation of ferulic acid to 4-vinylguaiacol, that can be used as smoky flavor. The two-enzyme cascade showed high conversion rates and retained activity for nearly 80 h of continuous operation.

Some enzymes, i.e., laccases and lipases, find application in many fields.

Lipases are used in a variety of biotechnological fields such as food and dairy, pharmaceutical, agrochemical, oleochemical, cosmetic industries, and detergents. Among the materials that can be used for lipase immobilization, mesoporous silica nanoparticles represent a good choice due to the combination of thermal and mechanical stability with controlled textural characteristics. Moreover, the presence of abundant surface hydroxyl groups allows for easy chemical surface functionalization. This latter aspect has main importance since lipases have a high affinity with hydrophobic supports. A review summarizing the main results obtained in the immobilization of lipase on several mesoporous silica supports was published [9].

The chemical functionalization of supports by using monoclonal antibodies (MoAbs) as specific receptors is a novel approach for specific enzyme binding. Anti-lipase MoAbs, chemically conjugated on the surface of polymeric nanoparticles (poly-(D,L-lactic-co-glycolic) acid) as specific lipase binding receptors, were used to selectively immobilize *Candida rugosa* lipase through by physical adsorption [10]. Hydrolytic enzymatic assays evidenced that such immobilization technique led to a significant enhancement of enzymatic activity in comparison to the free enzyme.

Laccases catalyze the oxidation of a variety of phenolic and non-phenolic compounds. They find applications in biological pulping and bleaching in the paper industry; decontamination of industrial waste and contaminated soil or water, dye decolorization, degradation of polyaromatic hydrocarbons; in the food and textile industries; and for biosensors and diagnostics. CotA laccase variant T480A-CotA was expressed and purified on *Bacillus subtilis* spores [11]. The enzyme is attached to the inert surface of the spore coat. Enzymes displayed on the *B. subtilis* spore coat showed several features useful for catalysis. The protein stability was increased, and they were resistant to environmental changes. Furthermore, they can be easily removed from the reaction solution and reused.

In conclusion, although enzymatic immobilization is an established technology, further studies are needed to overcome the present technological challenges and to lower the enzyme costs, to make it competitive in industrial production. In particular, the design and development of innovative and inexpensive immobilization techniques is needed.

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References

1. Mohammad, J.T.; Keikhosro, K. Enzyme-based hydrolysis processes for ethanol from lignocellulosic materials: A review. *BioResources* **2007**, *2*, 707–738.
2. Christopher, L.P.; Kumar, H.; Zambare, V.P. Enzymatic biodiesel: Challenges and opportunities. *Appl. Energy* **2014**, *119*, 497–520. [\[CrossRef\]](#)
3. Chapman, J.; Ismail, A.E.; Dinu, C.Z. Industrial applications of enzymes: Recent advances, techniques, and outlooks. *Catalysts* **2018**, *8*, 238. [\[CrossRef\]](#)
4. Sheldon, R.A. Enzyme immobilization: The quest for optimum performance. *Adv. Synth. Catal.* **2007**, *349*, 1289–1307. [\[CrossRef\]](#)
5. Mateo, C.; Palomo, J.M.; Fernandez-Lorente, G.; Guisan, J.M.; Fernandez-Lafuente, R. Improvement of enzyme activity, stability and selectivity via immobilization techniques. *Enzym. Microb. Technol.* **2007**, *40*, 1451–1463. [\[CrossRef\]](#)
6. Chu, J.; Yue, J.; Qin, S.; Li, Y.; Wu, B.; He, B. Biocatalysis for Rare Ginsenoside Rh2 Production in High Level with Co-Immobilized UDP-Glycosyltransferase Bs-YjiC Mutant and Sucrose Synthase AtSuSy. *Catalysts* **2021**, *11*, 132. [\[CrossRef\]](#)
7. Harriehausen, I.; Bollmann, J.; Carneiro, T.; Bettenbrock, K.; Seidel-Morgenstern, A. Characterization of an immobilized amino acid racemase for potential application in enantioselective chromatographic resolution processes. *Catalysts* **2021**, *11*, 726. [\[CrossRef\]](#)
8. Günther, T.; Schoppe, L.; Ersoy, F.; Berger, R.G. Alternative Routes for the Production of Natural 4-Vinylguaiacol from Sugar Beet Fiber Using Basidiomycetous Enzymes. *Catalysts* **2021**, *11*, 631. [\[CrossRef\]](#)
9. Costantini, A.; Califano, V. Lipase immobilization in mesoporous silica nanoparticles for biofuel production. *Catalysts* **2021**, *11*, 629. [\[CrossRef\]](#)
10. Chronopoulou, L.; Sayalero, V.C.; Rahimi, H.; Rugghetti, A.; Palocci, C. Polymeric nanoparticles decorated with monoclonal antibodies: A new immobilization strategy for increasing lipase activity. *Catalysts* **2021**, *11*, 744. [\[CrossRef\]](#)
11. Sheng, S.; Farinas, E.T. Laccase and its mutant displayed on the bacillus subtilis spore coat for oxidation of phenolic compounds in organic solvents. *Catalysts* **2021**, *11*, 606. [\[CrossRef\]](#)