NANOMATERIALS FOR BIOCATALYSIS

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CHAPTER 1

Nanobiocatalysis: an introduction

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1.1 Introduction

The most common enzymes are made of protein structure mostly used in the biocatalysis industry owing to their high specificity, activity, and selectivity under mild conditions (Garcia-Galan, Berenguer-Murcia, Fernandez-Lafuente, & Rodrigues, 2011; Kumar et al., 2019; Sharma, Sharma, Meena, Kumar, & Kanwar, 2018). Enzymes are highly efficient in their action and can increase the rate of reaction up to 10^{19} folds and employed at low concentrations compared with chemical catalysts (Osbon & Kumar, 2019). In the last decades, there is a rise in the potential use of enzymes in fishery waste valorization, pharmaceutical research, food modification, agroindustry, biofuel production and laundry (Choi, Han, & Kim, 2015; Narancic, Davis, Nikodinovic-Runic, & OC, 2015; Sharma & Kumar, 2021; Sharma et al., 2019). In brief, enzymatic processes are cost-effective, ecofriendly, and more sustainable. However, applications of enzymes are limited by their low stability and the requirement of reusability properties when they are applied in industrial processes (Kumar, Wu, & Liu, 2018; Patel, Singh, & Kumar, 2017; Sharma, Meena, & Kanwar, 2018). Fortunately, in the last few years, immobilization becomes a powerful tool that allows increased industrial applications of enzymes in many fields. After immobilization, the biocatalyst is expected to tolerate harsh conditions including extreme pHs, high temperature, high ionic strengths, solvents with enhanced activity, and reusability (Kumar, Patel, & Mardan, 2018; Kumar, Gudiukaite, & Gricajeva, 2020; Sharma, Sharma, Kamyab, & Kumar, 2020). However, various methodologies for recovering enzymes from the reaction system are poorly developed. Hence, it is important to choose the enzyme proper matrix with robust, separable, and biocompatible features. Among the various carriers used for enzyme immobilization, magnetic nanoparticles could be simply segregated from the reaction mixture by the use of external magnets (Sharma, Verma, Kumar, & Kamyab, 2018).

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With the continuous growth in nanotechnology, nanomaterials have been evolved and widely used for enzyme immobilization (Rahman, Culsum, Kumar, Gao, & Hu, 2016). The range of nanomaterials was established by considering quantum mechanics approach that ranges between 1 and 100 nm with exceptional electric, magnetic, optical, and structural properties. The living cells are "nanometric entities," and the size of cell metabolic components exceeds several times 100 nm. Some nanomaterial occurs naturally, and some are engineered to perform specific functions and utilized at industrial level. The size of nanomaterial and their ability to engineer nanomaterial according to specific requirements offers various advantages compared to other traditional bulk materials used for enzyme immobilization (Indu, Mandal, & Dubey, 2020). The development of nanomaterial with exceptional features and functions comprises (1) addition of functional group on the nanomaterial surface for immobilizing enzyme, (2) creation of a special structure for facilitating substrates and products diffusion, increasing the surface area, reusing nanomaterial (3) improving thermal and mechanical stability of nanomaterial. Moreover, nanomaterials can be in the form of tubes, fibers, rods, or particles. Nanoscale materials such as nanotubes, nanofiber, nanowires, nanoparticles, and nanosheets have been reported for immobilization of several enzymes (Kumar, Park, & Patel, 2019; La, Truong, & Pham, 2020; Sharma et al., 2017; Yogeswaran & Chen, 2008). As emerging materials, efforts have been made to design a nanomaterials with various components such as metal oxides (Fe_3O_4, SiO_2, Cu_2O), noble metals (Au), polymers (i.e., polycaprolactone, polylactic glycolic acid, aldehyde-derived pluronic polymers) carbon-based (carbon nanotubes, carbon dots) and complex compounds such as ($Mn_3(PO_4)_2$, $Cu_3(PO_4)2{\cdot}3H_2O$, $Cu_4(OH)_6)SO_4$, $Ca_8H_2(PO_4)_6$, and $Co_3(PO_4)_2{\cdot}8H_2O$) (An, Li, & Zhang, 2020). In recent years, various nanomaterials reported for enzyme immobilization are summarized in Table 1.1.

Nowadays, nanomaterials have been broadly utilized in chemistry, medicine, environmental monitoring and remediation, pharmacy, and microelectronics. Advances in nanoscience develop various nanomaterials, and enzymes immobilization on these nanomaterials has been considered as a propitious method to increase enzyme performance. Generally, the enormous benefits of nanomaterials as immobilization matrices can be expected as their huge specific surface area/volume ratio, unique mechanical, physicochemical, and cost-effective features (Kumar, Kim, Patel, & Lee, 2018; Liu & Dong, 2020; Nadda & Kanwar, 2012; Rahman et al., 2016). It must be noted that it is crucial to control the matrix -biocatalyst interactions to know about the modifications that could be made and its possible applications. Nanomaterials can be expected to meet the necessities of the immobilization matrix, while some may not be appropriate for the immobilization of some enzymes, because of enzymes tend to aggregate displaying detrimental properties and/or just by denaturing the protein structure. Furthermore, the linking between the nanomaterial and enzyme has been reported by physical methods such as encapsulation, entrapment, absorption, and/or by chemical methods like covalent attachment, ionic bonds, hydrophobic interactions and

Table 1.1 Various nanomaterials reported for enzyme immobilization.

Nanomaterial	Enzyme	Immobilization method	Immobilization yield (%)	Application	Reusability	Reference
Multiwalled carbon nanotubes (MWCNTs)	Asparaginase	Adsorption	90.0	Food industry, biosensor	–	Cristóvão, Almeida, and Barros (2020)
Polyacrylic acid coated magnetic silica nanocomposite	Lipase	Covalent	–	*p*-NPP hydrolysis	5	Esmaeilnejad-Ahranjani, Kazemeini, Singh, and Arpanaei (2016)
Chitosan nanoparticles	Lysozyme	Ionic	–	Antimicrobial activity	8	Wang, Li, and Jin (2020)
Magnetic nanoparticles	Asparaginase	Covalent	–	L-asparagine hydrolysis	8	Orhan and Aktaş Uygun (2020)
Superparamagnetic few-layer graphene oxide	Lipase	Electrostatic	–	Biodiesel production	5	Nematian, Shakeri, Salehi, and Saboury (2020)
Hollow core-mesoporous shell silica nanospheres	Protease	Physical and covalent	75.6	Laundry detergent formulations	12	Ibrahim, Al-Salamah, and El-Toni (2016)
Gold nanoparticles	Horseradishperoxidase	Chemical and absorption	–	Biosensor	–	Luo, Xu, Zhang, Yang, and Chen (2005)
MWCNTs	Laccase	Covalent	76.7	Removal of diclofenac	7	Xu, Tang, Zhou, Li, and Zhang (2015)
Iron oxide nanoparticles	Glucosidase	Covalent	93.0	Biofuel production	16	Verma, Chaudhary, Tsuzuki, Barrow, and Puri (2013)
ZnO nanowires	Lipase	Adsorption	–	Esterification of phytosterols with oleic acid	12	Shang, Li, and Zhang (2015)
Chitosan-MWCNTs	Galactosidase	Covalent	95.0	Aroma enhancement of tea extract	10	Çelik, Dinçer, and Aydemir (2016)
MWCNTs	Laccase	Covalent	–	Dye decolorization	10	Othman Abdelmageed, González-Domínguez, Sanromán, Correa-Duarte, and Moldes (2016)
Graphene oxide	Horseradish peroxidase	Electrostatic	–	Phenolic compounds removal	7	Zhang, Zheng, and Zhang (2010)

(*Continued*)

Table 1.1 (Continued)

Nanomaterial	Enzyme	Immobilization method	Immobilization yield (%)	Application	Reusability	Reference
Amino-functionalized magnetic nanoparticles	Lipase	Covalent	62.0	*p*-NPB hydrolysis	11	Hu, Pan, Yu, Liu, and Xu (2009)
PEGylated graphene oxide nanosheets	Cellulase	Covalent	70.5	Saccharification of lignocellulose	3	Xu, Sheng, and Wang (2016)
Gold nanoparticles	Cholesterol oxidase	Covalent	–	Biosensor	-	Saxena, Chakraborty, and Goswami (2011)
Organic-inorganic nanocomposites	Laccase	–	75.3	Dichlorophenol removal	8	Qiu, Qin, Xu, Kang, and Hu (2019)
Molybdenum sulfide nanosheets	β-amylase	Covalent	92.0	Industrial processes	10	Li, Nandgaonkar, and Wang (2017)
Polyethyleneimine-modified superparamagnetic Fe_3O_4 nanoparticles	Lipase	Covalent and adsorption	91.7	Ethyl valerate synthesis	12	Khoobi et al. (2015)
Polymeric materials	Glucosidase	Entrapment	93.0	Cellobiose hydrolysis	6	Javed, Buthe, Rashid, and Wang (2016)
MWCNTs	Glucosidase	Adsorption	–	Lactose hydrolysis	6	Ansari, Satar, Kashif Zaidi, and Ahmad (2014)

combined or other more sophisticated methods. Moreover, the nanoporous materials provide an excellent surface area for the covalent binding or entrapment of biocatalyst, substrate and products diffusion through the matrix, drugs, and biomolecules but the major task is to make the desired structure with appropriate biocompatibility and surface properties. Nowadays, several attempts have been made for the synthesis of mesoporous materials and blend it with other molecules or structures so as to make a matrix having large surface area to volume ratio and suitable pore size (Szczęśniak, Choma, & Jaroniec, 2020). Furthermore, some basic parameters that should be considered during enzyme immobilization on nanomaterials are minimal enzyme deactivation, high immobilization yield, effective catalyst reutilization, high specific activity that keeps the selectivity (i.e., chemo-, stereo- and regio-), and recovered activity (Cipolatti, Valério, & Henriques, 2016; Rana, Sharma, Kumar, Kanwar, & Singh, 2020). In the present chapter, we provide an overview of various nanomaterials utilized in biocatalysis and their applications.

1.2 Metallic nanomaterials

Nanomaterials containing metals are commonly known as metallic nanomaterials. Hence, metallic nanomaterials have been generally utilized for fabricating nanobiocatalysts. Some metallic nanoparticles are also considered to be nontoxic and can be used in drug delivery, radiotherapy enhancement, biosensors, magnetic resonance imaging, and gene delivery (Ren et al., 2011; Yamada, Foote, & Prow, 2015). Since the 1970s, the utilization of magnetic particles has increased in the field of medicine and bioscience. Therefore the applications of metal and metal oxide nanomaterials in immobilizing the enzyme are discussed in this section.

1.2.1 Metal nanomaterials

The first category of nanomaterials made up of platinum, zinc, gold, silver, thallium, iron, and cerium are known as metal nanomaterials. Functional groups (e.g., amino, phosphate, carboxylate, thiolate) are added onto the metal nanomaterial to form a robust interfacial reaction with the biocatalyst, and subsequently to improve enzyme immobilization (Ghodake, Shinde, & Saratale, 2019; Yaqoob, Ahmad, & Parveen, 2020). Metal nanomaterials have been synthesized by numerous methods like coprecipitation, hydrothermal synthesis, thermal reduction, biosynthesis, and micelle synthesis (Misson, Zhang, & Jin, 2015). Among these nanomaterials, gold nanomaterials are mostly utilized for enzyme immobilization because of their good biocompatibility, porous network structure, large surface area, mechanical and thermal stability (Cipolatti et al., 2016). In catalysis, there is growing interest in immobilizing enzymes on gold nanomaterials, even though these are not considered as viable matrix due to economic issues for most of applications at industrial level. Previously, α-amylase

immobilized on gold nanorods by hydrophobic and electrostatic binding was employed for starch processing and the immobilized α-amylase exhibited improved thermal and storage stability (Homaei & Saberi, 2015). Saxena et al., (2011) functionalized gold nanoparticles using N-hydroxysuccinimide and N-ethyl-N'-(3-dimethy laminopropyl carbodiimide) for immobilization of cholesterol oxidase and successfully used the immobilized enzyme for detection of cholesterol in human serum sample. Moreover, the immobilization of various enzymes on nanoporous gold resulted in an enhanced enzyme stability and biocatalytic performance (Wang et al., 2011).

Furthermore, silver nanoparticles have been proved to be beneficial for medical applications because of exceptional antimicrobial effect against bacteria, viruses, and complex microbial structures such as biofilms (Yaqoob et al., 2020). Previously, lactose peroxidase was immobilized on silver nanoparticles showing enhanced antimicrobial activity as compared to free lactoperoxidase (Sheikh, Yasir, & Khan, 2018). The most known antimicrobial mechanism of metal nanoparticles is metal ion release, redox properties, and interference with molecular transport and metabolism, denaturing molecules and/or inhibiting central metabolism, etc., (Sangaonkar & Pawar, 2018). The antimicrobial activity of metallic nanoparticles could be because of their nanosize and high surface area to volume ratio, residual charges in surface which allows them to penetrate inside the bacterial membrane.

1.2.2 Metal-oxide based nanomaterials

Metal oxide nanomaterials including iron oxide, zinc oxide, and titanium oxide are mostly used to immobilize biocatalysts. With large surface area, high biocompatibility, TiO_2 nanocarriers have been mostly utilized for immobilizing biocatalyst and in the various applications such as wastewater treatment, biosensor, and so on. The most common methods for synthesis of TiO_2 nanomaterials are hydrothermal, direct oxidation, solvothermal, sol-gel, and chemical vapor decomposition methods (Liu & Dong, 2020). Lipase was immobilized on phenylaminopropyl trimethoxysilane functionalized mesoporous TiO_2 by hydrophobic interactions and used for the synthesis of cinnamyl acetate. In addition, immobilized enzyme retained good operational stability after ten successive runs and showed improved thermal stability (Gao et al., 2018). Furthermore, horseradish peroxidase was immobilized on TiO_2 nanotube coated with polydopamine for constructing a novel photoelectrochemical bio-sensing platform (PEC). The PEC exhibited good selectivity as well as sensitivity for the biosensing of H_2O_2 (Li, Li, & Zhao, 2018).

In recent years, ZnO with different nanostructures are a promising matrix for enzyme immobilization, as they are nontoxic, biocompatible, chemically stable having high catalytic efficacy, strong absorption ability, and displays antimicrobial activity. The wet chemical route is mostly used to fabricate ZnO nanostructures, including nanorods, nanosheets and nanoparticles. The ZnO nanostructure shows high

isoelectric point with negatively charged surface in the reaction medium, and appropriate for electrostatic absorption of the biocatalyst. Ahmad et al., showed a new method for making glucose biosensor using glucose oxidase absorbed on a single ZnO nanofiber (Ahmad, Pan, Luo, & Zhu, 2010). The result showed that a single ZnO nanofiber is a good matrix for immobilizing the enzyme and could be utilized in numerous applications. On the other hand, Fe_3O_4 nanoparticles are frequently utilized for the development of iron oxide magnetic nanoparticles, because they have a large surface area, less mass transfer resistance, and easy separation from the reaction medium (Orfanakis, Patila, & Catzikonstantinou, 2018). Previously, the lipase immobilized on polyethyleneimine functionalized super magnetic Fe_3O_4 by absorption and covalent attachment was used for the synthesis of ethyl valerate. The study showed that immobilized enzyme retained 80% of original activity after 12 reaction cycles (Khoobi et al., 2015). Despite various reported methods for immobilization of the enzyme on a metallic nanomaterial, there is still demand for efficient and cost-effective methods.

1.3 Carbonaceous nanomaterial

Carbonaceous materials such as graphene, reduced graphene oxide, and carbon nanotubes (CNT) have been widely used for enzyme immobilization because of their superior benefits. These nanomaterials have engrossed wide attention in various applications (Table 1.1), including drug delivery, catalysis, bioremediation, and contaminant biotransformation (Anwar, Kim, & Kumar, 2017; Czepirski et al., 2016). With the recent development in nanotechnology, the role of carbonaceous materials will be more and more significant in the future.

1.3.1 Graphene and graphene oxide

Graphene is one atom thick two-dimensional honeycomb crystal structure of carbon atoms, which seems to be an ideal matrix for immobilization owing to its large surface area ($\sim 2630\ m^2\ g^{-1}$) that helps to enhance enzyme loading, strong mechanical strength increases the reusability of biocatalysts, and extraordinary thermal, optical and electrical features. Graphene-based nanomaterials mostly interact with enzymes by VanderWaals forces, hydrophobic and electrostatic interactions. In addition, graphene immobilized biocatalysts are mostly used in biosensors because of their exceptional electric conductivity (Ahmad et al., 2010). In a previous study, glucose oxidase was immobilized on polyvinylpyrrolidone-protected graphene for the creation of an electrochemical glucose biosensor. The biosensor exhibited a broader linearity range (2−14 mM), that ensured blood sugar detection (Shan et al., 2009). On the other hand, graphene oxide is the precursor of graphene, has been widely used for enzyme immobilization as they exhibit ultrahigh surface area, thermal and mechanical properties. Graphene oxide has oxygen-containing functional groups like carboxyl, hydroxyl,

and epoxy which are useful for enzyme immobilization (Compton & Nguyen, 2010). The three most popular methods reported for the synthesis of graphene oxide are Staudenmaier, Hummers and Brodie (Compton & Nguyen, 2010). Xu et al., (2016) covalently immobilized cellulase on graphene oxide nanosheets and used for saccharification of lignocellulose. After immobilization cellulase retained 70% of its original activity. Various authors reported that enzyme immobilized in graphene oxide has shown different operational stability, catalytic efficiency, and application potential.

1.3.2 Carbon nanotube

CNTs are one-dimensional hollow tubes made up of carbon atoms. CNTs are of two types of single-wall carbon nanotubes (SWCNTs) with three isoforms and multiwalled carbon nanotubes (MWCNTs) that have been mostly used for enzyme immobilization. CNTs exhibit extraordinary electrical, thermal, and mechanical properties. Arc discharge, chemical vapor deposition and laser ablation methods are used for the synthesis of CNTs. CNTs show increasing application possibilities in enzymes immobilization, especially for the synthesis of biofuel cells and biosensors (Feng & Ji, 2011). Previously, laccase was immobilized on to MWCNTs for the biodegradation of diclofenac. The results of this study showed that immobilized laccase exhibits better storage stability, reusability and high biotransformation efficiency of diclofenac (Xu et al., 2015). Also, using the carbodiimide coupling technique, laccase was covalently immobilized on MWCNTs and used for dye decolorization. Immobilized laccase showed enhanced thermal stability and retained 95% of activity after 12 cycles of repetitive use (Othman Abdelmageed et al., 2016). Furthermore, β-glucosidase immobilized on MWCNTs and used for aroma enhancement of tea extracts. The immobilized enzyme showed better reusability, thermal and storage stability (Çelik et al., 2016). Moreover, to completely examine the potential of CNTs enzyme complex, it is important to find an optimum technique for enzyme immobilization.

1.4 Other nanomaterials

Besides the nanomaterials discussed here, other nanocarriers such as silica, cellulose, metal-organic frameworks (MOFs), chitosan, and other bio-based nanomaterials have also been utilized for enzyme immobilization, some of them will be analyzed in detail in the following chapters. Moreover, silica can be modified using a silane blocking agent to add reactive groups including carboxyl, epoxy, thiol, and amino groups for immobilizing the biocatalyst. According to morphological and physicochemical features silica can be categorized into natural, micro, meso, macro, and synthetic material. Nowadays, mesoporous silica also known as nanosilica is mostly studied and has various benefits such as huge surface area ($300-1500\ m^2\ g^{-1}$), uniform pore diameter (2−40 nm), inert and stable at higher temperature (Zhou & Hartmann, 2013). Besides silica, chitosan and

cellulose-based nanomaterials are mostly combined with other materials for enzyme immobilization. Particularly, chitosan, a biopolymer obtained by partial deacetylation of chitin, and can be regarded as an attractive matrix for enzyme immobilization because of its low cost, biodegradability, and presence of functional groups (Tang, Qian, & Shi, 2007). Recently MOFs are getting attention because of their large pore volume, three-dimensional structure, and tremendous absorption ability. Some enzymes including lipase, catalase, and carbonic anhydrase have been successfully immobilized onto MOFs (Ren, Feng, & Wen, 2018). Integration of enzyme as guest species into MOFs would provide an artificial biocatalytic system promising for biosensing, biomedical devices, and biocatalysis (Sharma A. et al. 2020; Sharma T. et al., 2020).

1.5 Conclusion

Nanomaterial immobilized biocatalysts generally exhibit improved activity, stability, and reusability by creating a unique microenvironment. Although large-scale production of immobilized enzyme is a vital matter for their applications because of technical and economic issues. However, at the same time, it cannot deny that immobilization nanotechnology could makes enzymes commercially available in reactions where selectivity and purity of enzymes are important like in delicate processes of chemical precursor's synthesis, biomedicine, and pharma. With efforts from many academic fields like biochemistry, bioprocess engineering, molecular biologyt, and others, the production of immobilized enzyme reactors including microchannel, packed bed, and fluidized bed reactors could be successfully commercialized in the future.

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