



Magnetic nanocomposites as multifunctional carriers for enzymes immobilization: a review

Li Zaixing^{1,2} · Li Chao¹ · Zhang Qinqin¹ · Hong Chen³ · Zhang Wenjing¹ · Xu Bingbing¹ · Qin Xue¹ · Li Guixia¹ · Ning Zhifang¹

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Abstract

The practical application of enzymes as a biocatalyst still faces serious challenges, namely inactivation and loss. Immobilized enzyme technology can significantly improve the recoverability, stability and environmental adaptability of enzymes. Magnetic nanocomposites are widely used in enzyme immobilization because of their magnetic properties and the composite properties of various nanomaterials. In this work, the research progress of magnetic nanocomposites as enzyme carriers is reviewed and the future development direction is prospected.

Keywords Magnetic nanocomposites · Enzyme immobilization · Biocatalysts · Supporting materials · Green chemistry

Introduction

Enzymes are natural machineries for molecular processing and powerful synthetic tools for building complex molecules *in vivo* (Van Beilen et al. 2002). Studying nature and ourselves is an important step forward in science and technology, so many researchers are committed to using enzymes for other purposes. Owing to their exceptional regio-, stereo- or chemospecificity and selectivity, enzymes can carry out very precise reactions (Van Beilen et al. 2002). Enzymes has been extensively used in numerous biochemical processing industries, including fine and bulk chemicals, organic syntheses, healthcare and pharmaceuticals, as well as cosmetics, foods, textiles, paper and pulp (Huang et al. 2022; Liang et al. 2021) (Fig. 1).

However, nothing can be perfect. Enzymes are expensive and easy to be inactivated, the three-dimensional structure of them is predominantly maintained by fragile non-covalent interactions, free enzymes are difficult to recover and susceptible to some extreme conditions (such as strong acid, strong alkali, high temperature), especially when the industrial processes are too complex and the processes often use the non-natural substrates of the biocatalysts (Sheldon et al. 2021; Zahirinejad et al. 2021). The recycling of free enzymes is also a big problem, they are often discharged from the reaction equipment with the product and difficult to separate (Gan et al. 2021). The above series of problems hinder a more widespread implementation of enzyme catalysts across different industries.

To solve the aforementioned drawbacks, several strategies, such as artificial modification, directed evolution of enzymes, solvent engineering, protein and enzyme engineering and immobilization, have been adopted (Aggarwal et al. 2021). Enzyme immobilization is an enabling technology, which refers to enzymes directionally being embedded or fixed in the specific region of selected carriers via bonding, cross-linking, adsorption or encapsulation, the technology not only improve the operational stability and recoverability of enzymes, hence is instrumental in continuously reuse and cost savings, but also combine the catalytic reactions among them (Sharma et al. 2021a, b; Arana-Peña et al. 2021; Nunes et al. 2021).

✉ Li Zaixing
li_zaixing@163.com

✉ Qin Xue
2058867352@qq.com

¹ Department of Environmental Engineering, Hebei University of Science and Technology, Shijiazhuang 050018, China

² Department of Environmental Engineering, Beijing Institute of Petrochemical Technology, Beijing 102617, China

³ Department of Energy and Environmental Engineering, University of Science and Technology Beijing, Beijing 100083, China

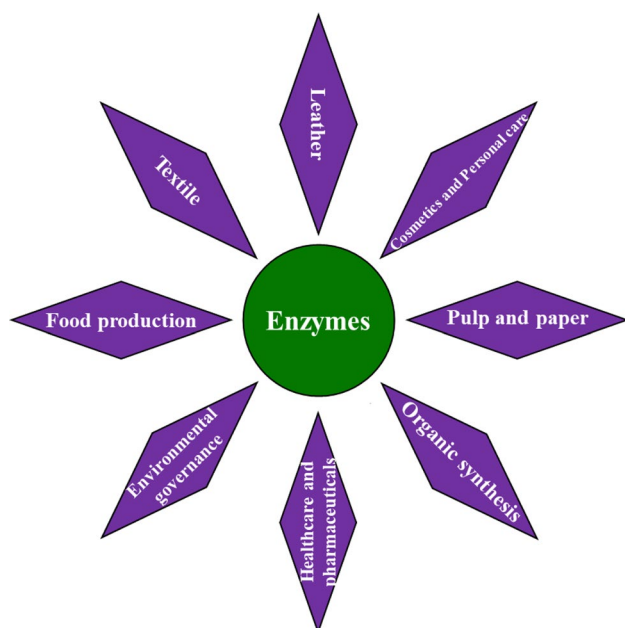


Fig. 1 Application status of enzymes

Despite an array of various carrier materials (graphene oxide, chitosan and polyurethane foam, etc.) have been used for the enzyme immobilization, the exploration and development process of novel high-performance immobilization matrices are still endless (Zdarta et al. 2018). There are many entry points to the problem, one of which is how to improve the current 'false' recoverability. Most carriers still exist in nanometer or micron particles, separating them must be considered a hindrance, no company has the ability to provide nanoscale or micron-scale filters for effective enzyme separation (Homaei et al. 2013).

The most direct way to solve this problem is to make the carrier into millimeter or centimeter level, but this will inevitably have a negative impact on enzyme loading and catalytic reaction. Another strategy is to associate enzymes with magnetic nanocomposites, which can provide guiding force for drugs and biomolecules through external magnetic field, so as to achieve the purpose of fixing and separating magnetic labeled biological entities (Vaghari et al. 2016). Magnetic nanomaterials can achieve a facile separation of the biocatalytic system by the use of an external magnetic field, and therefore quickly terminating the enzymatic reactions and recovering the enzymes for continual uses (Bilal et al. 2018). In addition, magnetic nanocomposites are attracting great attention as promising carriers in enzyme immobilization due to the surface hydroxyl groups which enables their easy strong binding of the enzyme molecule and functionalization (Gennari et al. 2020). This review systematically analyzes the application of magnetic nanocomposites in enzyme immobilization, in

order to provide reference for the application of enzyme immobilization.

Magnetic nanocomposites and their properties

Now there are still many shortcomings that limit the application of bare magnetic nanomaterials, such as poor chemical stability, high aggregation tendency, strong dipole–dipole attraction and low amount of adsorbed enzymes. (Cao et al. 2012, 2016). In order to solve the above problems, researchers have tried to use inorganic or organic materials to form magnetic nanocomposites with bare magnetic nanomaterials, which cannot only improve the chemical stability, but also delay the oxidation of magnetic nanomaterials (Table 1) (Feng et al. 2021).

Magnetic nanoparticles @ silica

Magnetic nanoparticles represented by Fe_3O_4 are easily oxidized and dissolved in acidic environments (Bellova et al. 2010). Silica (SiO_2) has high stability, strong chemical inertness, rich surface groups and easy modification under air or acidic conditions (Jeelani et al. 2020). SiO_2 are biocompatible and hydrophilic in many biosystems, so its standard chemistry protocols can be followed to conjugate various biomolecules to its surface (Luckarift et al. 2004). All the above advantages make the magnetic nanoparticles @ silica favored by many researchers in enzyme immobilization.

Shanmugam et al. (2020) immobilized *trichoderma asperellum laccase* on $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ -chitosan nanosupport for delignification of lignocellulosic biomass and then utilized for biohydrogen production. With sweet sorghum stover as substrate, the immobilized laccase achieved higher delignification ability than the free one, and can maintain high activity in 8 cycles.

Li et al. (2021) used a magnetic host–guest cage structured magnetic nanoparticles@alginate@ SiO_2 with a specific enzyme embedding as its guest in the host matrix. The magnetic nanoparticles compensate for the collapsibility of the alginic acid internal structure, SiO_2 enhances the swelling resistance of the carrier and inhibits the polysaccharides degradation in the natural environment (Fig. 2).

We all know porous materials can provide a better support for covalently binding or entrapping enzymes, while a current challenge facing magnetic nanoparticles@ SiO_2 composites is synthesizing and designing an ideal structure with suitable biocompatibility and properties (Zhang et al. 2020a, b; Zhang et al. 2019a, b, c). Unfortunately, up to now the synthesis of hierarchical mesoporous silica materials is complex in many ways (Feng et al. 2019). In addition, the low specific interaction between silica and enzymes leads to

Table 1 Typical magnetic carriers and their immobilized enzymes, immobilization method and applications

Composite materials	Magnetic carriers	Immobilized enzymes	Immobilization method	Applications	References
Silica	Fe_3O_4 @ SiO_2 -chitosan nanosupport	<i>Trichoderma asperellum</i> laccase	Adsorption method	Sweet sorghum stover biomass delignification and biohydrogen production	Shammugam et al. (2020)
	Magnetic nanoparticles@alginate@ SiO_2	<i>Laccase</i>	Adsorption method	Degradation of tetrachloro-roguaiacol	Li et al. (2021)
Metal-organic frameworks	Fe_3O_4 @MOF core-shell microsphere	<i>Olive oil hydrolase</i>	Covalent binding fixation method	Hydrolysis of olive oil	Wang et al. (2016)
	Fe_3O_4 @MIL-100(Fe)	<i>Peroxidase</i>	Adsorption method	Catalytic oxidation of 3,3',5,5'-tetramethylbenzidine by H_2O_2	Wu et al. (2017)
Polysaccharides	Fe_3O_4 @Chitosan	<i>xylanase</i> and <i>cellulase</i>	Cross-linking method	Preparation of new materials for biotechnology applications	Diaz-Hernández et al. (2018)
Magnetic nanoparticles	Ionic liquids-modified magnetic carboxymethyl cellulose nanoparticles	<i>Lipase</i>	Covalent binding fixation method	Hydrolysis of triacetate	Suo et al. (2020)
	Magnetic alginate-polydopamine microspheres	<i>tyrosinase</i> and β - <i>glucosidase</i>	Cross-linking method	Enhance the inhibitory activity of tea polyphenol extract	Zhang et al. (2020a)
Carbon-based materials	Magnetic carbon nanotubes	<i>glucoamylase</i>	Adsorption, covalent bond and metal ion affinity interaction	Convert starch into glucose	Zhao et al. (2011a, b)
	Magnetic biochar	<i>laccase</i>	Adsorption method	Catechol degradation	Zhang et al. (2020b)
	Modified magnetic graphene oxide	<i>laccase</i>	Embedding and derivative microcapsule method	Degradation of dyes in water	Ariaeenejad et al. (2021)
	Graphene oxide modified by CuFe_2O_4 magnetic nanoparticles	<i>Laccase</i>	Chemical cross-linking	Synthesis of arylsulfonylenzenediol	Rouhani et al. (2018)
Au	Magnetic AuNP@ Fe_3O_4	<i>Trypsin</i>	Embedding and derivative microcapsule method	Digest the protein	Peng et al. (2013)
	Core-shell NPs having magnetite cores and gold shells	<i>laccase</i>	Adsorption method	Catechol degradation	Majouga et al. (2015)

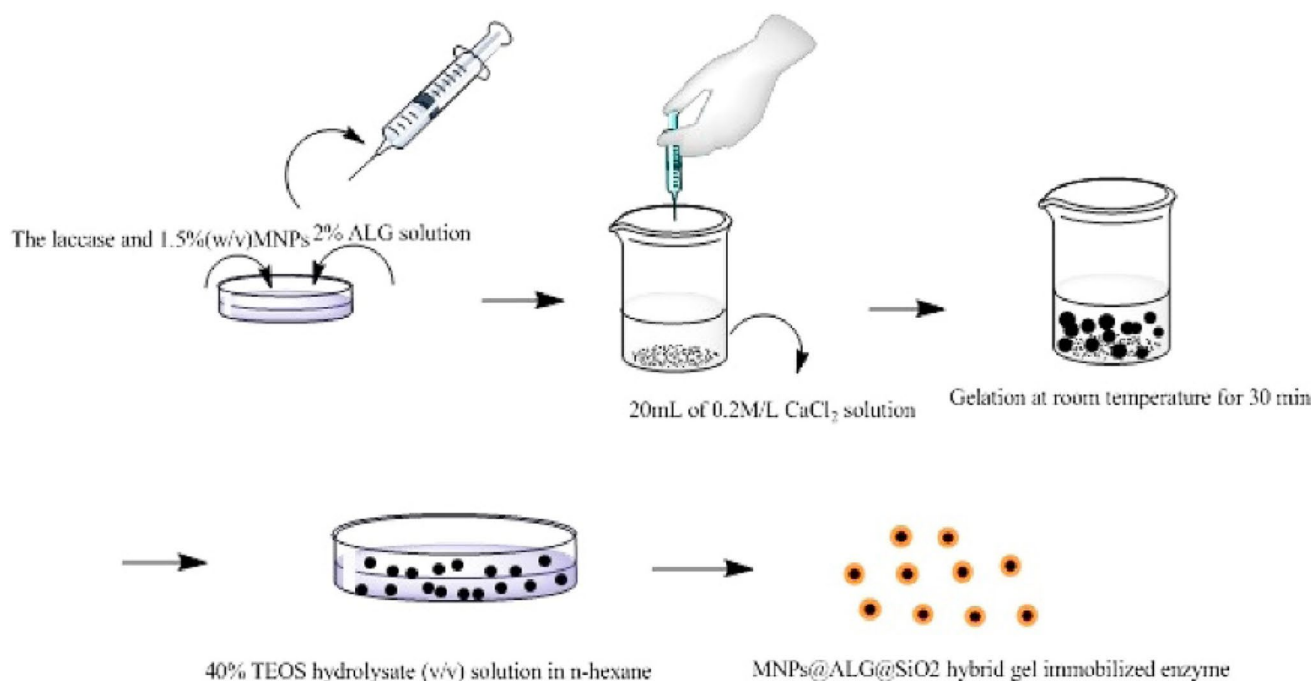


Fig. 2 Schematic diagram of nanoparticles@alginate@SiO₂ preparation process (Li et al. 2021)

leaching, which affects the reusability and recovery (Poorakbar et al. 2018). Due to the diffusion restrictions and deactivation of enzymes during immobilization process, the catalytic performance of enzymes may be negatively affected.

Magnetic nanoparticles @ metal–organic frameworks

Metal–organic frameworks (MOF) is an organic–inorganic hybrid platform for enzyme immobilization, which has promising applications in the vast field drug/enzyme carriers (Nadar and Rathod 2018). It has some excellent properties such as adjustable pore sizes with well-defined cavities, large pore volumes, high surface areas, designable organic ligands and crystallographic structure (Huo et al. 2015). Especially, the large hierarchical surface area and ultra-high porosity lead to high loading capacity and strong affinity for enzyme molecules MOF to avert leaching, which is a perfect feature that does not share with SiO₂ (Zhou et al. 2020).

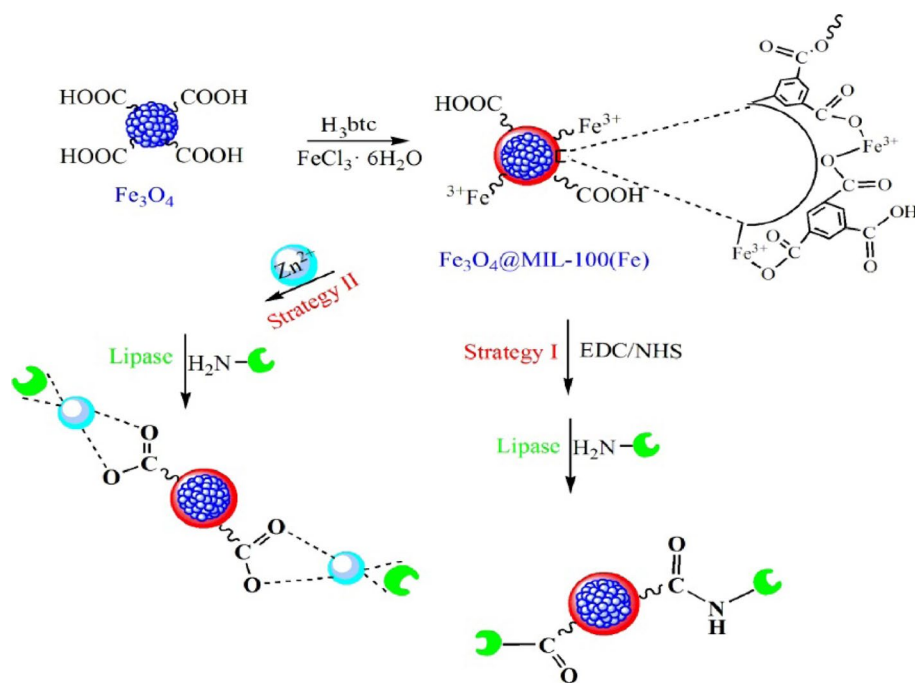
In recent years, a novel strategie incorporating magnetic nanoparticles with enzyme and MOF composite (magnetic-enzyme-MOF) has received attention. These composites have both the diverse properties of MOFs and magnetic properties of metal or metal oxides (Lian et al. 2017). Wang et al. (2016) prepared a Fe₃O₄@MOF core–shell microsphere by growing MIL-100(Fe) and three-dimensional MOFs on Fe₃O₄ nanoparticles. The carrier has both large specific surface area and magnetic characteristics, the *olive*

oil hydrolase immobilized on it showing high stability and recoverability (Fig. 3).

In addition, Magnetic nanoparticles@MOF may show structures and functions similar to natural enzymes, which are commonly referred as nanozymes (Huang et al. 2021). Nanozymes have some unique advantages such as impressive robustness and low-cost production with easy scale-up, which are ideal substitute for natural enzymes (Niu et al. 2020). Due to the presence of abundant redox sites, magnetic-MOF based nanozymes are regarded as a candidate in achieving high sensitivity for the detection of a target analytes (Zhang et al. 2019a, b, c). Wu et al. (2017) prepared a core–shell artificial peroxidase (Fe₃O₄@MIL-100(Fe)), which can significantly enhance peroxidase-like activity, the 3,3',5,5'-tetramethylbenzidine was oxidized by H₂O₂ to blue product.

Although magnetic nanoparticles@MOF have many advantages, there are still many problems to be solved before the practical application. Firstly, the MOF synthetic methods depends on the organic and inorganic counterparts for biocatalytic applications (Gao et al. 2021). Some novel synthetic methods are necessary for designing multifunctional magnetic hybrid materials as a batter platform for enzyme immobilization. Secondly, most of the existing MOFs remain relatively unstable under some certain conditions (e.g., pH, solvents, water, etc.), which seriously hinders magnetic nanoparticles@MOF's practical use (Chen et al. 2017). Therefore, we need to improve the structural stability

Fig. 3 The synthetic route of $\text{Fe}_3\text{O}_4@$ MIL-100(Fe) microspheres and the immobilization of lipase (Wang et al. 2016)



of MOF to give full play to the potential of this immobilization strategy.

Magnetic nanoparticles @ polysaccharides

Due to the flexible chemical modification based on the properties of immobilization, polysaccharides (chitosan, cellulose, alginate, etc.) have become a highly concerned enzyme carrier and carrier intermediate (Sharma et al. 2021a, b). If the enantioselectivity, regional properties and chemical of the immobilized enzymes can be effectively regulated, it will obviously help to reverse any undesired reaction to increase the target end product (Sotelo et al. 2022). In addition, polysaccharides can enable the enzymes to be used in non-aqueous environments, especially considering that natural enzymes are only used in aqueous reaction media, which is very important (Bodakowska-Boczniewicz and Garncarek 2020).

Chitosan, a polysaccharide containing a large number of amino, hydroxyl and other functional groups, is an *N*-deacetylated product of chitin (the main component of crustacean and arthropod shells such as crab, lobster, squid and shrimp) (Jiang et al. 2005). Due to the presence of free amino groups on its molecular, the reactivity and solubility of chitosan are greater than chitin (Pospiskova and Safarik 2013).

Chitosan exhibits many significant biological and chemical properties, namely biodegradable, biocompatibility, hydrophilicity, availability of reactive functional groups for chemical modifications, mechanical stability, polycationic properties, regenerability, ease of preparation in different geometrical configurations suitable for a chosen

biotransformation (Long et al. 2014; Yang et al. 2010; Zang et al. 2014). Especially, chitosan appears economically attractive to prepare low-cost carriers for large scale applications since chitin is the abundant natural polymer next to cellulose (Díaz-Hernández et al. 2018). Coating or surface modification magnetic nanoparticles with chitosan cannot only solve the problem of aggregation in liquid media, but also significantly improve the biocompatibility, so that they can be widely used in biomedical fields, such as protein immobilization, drug delivery systems, wound healing, tissue engineering and magnetic resonance imaging (Dal Magro et al. 2019).

Díaz-Hernández et al. (2018) synthesized chitosan-coated Fe_3O_4 nanoparticles by a one-step alkaline precipitation method, and used the natural biological cross-linking agent genipin to cross-link the synthesized magnetic complex with xylanase and cellulase to prepare an enzyme reactor. The results showed that the synthesized magnetic composite nanoparticles had simple preparation process and superparamagnetism, and could be applied to the field of isomerase.

Hojnik Podrepšek et al. (2020) used different synthesis methods to functionalize maghemite with chitosan, and compared the properties of the product. The results show that the chitosan suspension cross-linking process (MC_2) is most suitable for obtaining the highest activity of immobilized enzyme, and nanoparticles functionalized with chitosan using the covalent binding method (MC_3) also have excellent properties (Fig. 4).

However, pure chitosan in powder shape has low specific surface area and absence of porosity, which can result in inefficient enzyme immobilization, so researchers focused

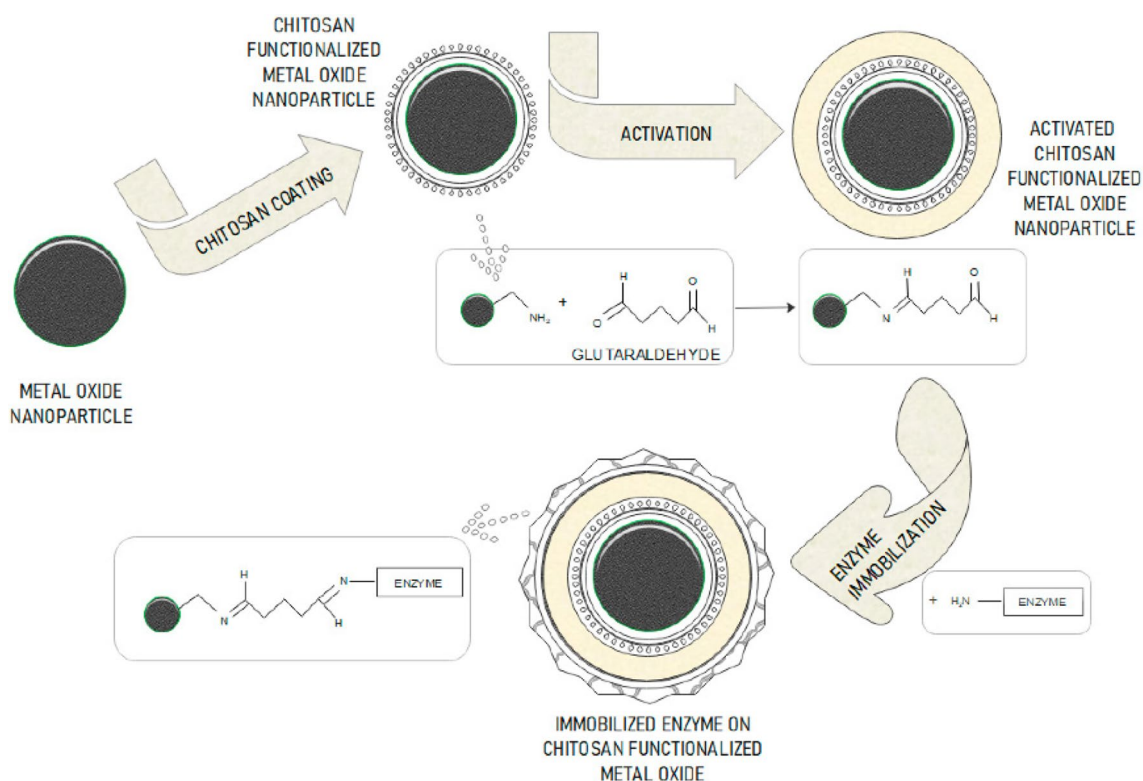


Fig. 4 Schematic representation of the entire process of chitosan functionalization, activation and enzyme immobilization on a metal oxide nanoparticle, with chemistry mechanism (Hojnik Podrepšek et al. 2020)

on chitosan based nanofibers (Zhao et al. 2011a, b; Hwang and Gu 2013). Due to the fiber diameter in the nanometric scale, the nanofibers have large surface area to volume ratio, excellent mechanical behavior and high porosity with interconnected voids (Huang et al. 2018). The poor stability of chitosan under acidic conditions is another concern, as the pH continues to decrease (lower than the pK_a of chitosan), the electrostatic repulsion between the amino groups will increase, eventually leading to the dissolution of the carrier material (Demirkan et al. 2018).

The cross-linking method can overcome the above shortcomings to a certain extent (Gracida et al. 2019). The functional groups of chitosan can be used to form cross-linked structures via intermolecular linkages with cross-linkers (Gracida et al. 2019). In fact, these intermolecular linkages can improve the insolubility of material in acid medium; in addition to enhance its mechanical and thermal properties (Wang and Jiang 2019). The functional groups of chitosan can form cross-linked structures by intermolecular linkages with cross-linking agents, the intermolecular linkages cannot only improve the insolubility in acidic media, but also enhance the mechanical and thermal properties (Qiao et al. 2022).

As the most abundant renewable natural polysaccharide, cellulose has a series of advantages such as sustainability,

low cost and biocompatibility (Gennari et al. 2020). Cellulose also exhibits other important features suitable for enzyme immobilization, such as chemically inert behavior and amphiphilic under physiological conditions (Cao et al. 2014).

The study of magnetic cellulose as a carrier for enzyme immobilization has been increasing in recent years. Researchers have tried to incorporate new modifications into these materials to improve their interaction with enzymes. Suo et al. (2020) prepared ionic liquids-modified magnetic carboxymethyl cellulose nanoparticles for *lipase* immobilization and achieved 2.81 times the specific activity of free enzyme. Water contact angle analysis showed that the introduction of ionic liquids increased the hydrophobicity of the carrier, which in turn induced the opening of the lid of the lipase, making its active sites easier to approach. In addition, the affinity between the lipase immobilized on the prepared carrier and the substrate was enhanced.

Alginate is one of the most profoundly exploited natural polysaccharides, which consists of alternating blocks of (1,4)-linked α -l-guluronate (G) and β -D-mannuronate (M) residues (Hou et al. 2015). Due to its low cost, non-toxicity, biocompatibility and the ability to gelate under mild conditions by adding divalent metal cations such as Ca^{2+} , alginate

plays a key role in the development of enzyme-immobilized carrier materials (Marjani et al. 2021).

Alginate can be encapsulated in different forms, covalently immobilized and adsorbed enzymes, such as microspheres, capsules and hydrogels (Ivanova et al. 2011). Zhang et al. (2020a, b) used in situ TYR-mediated dopamine polymerization and internal stereotype strategy-mediated magnetic alginate-polydopamine gelation reaction to co-immobilize double enzymes (tyrosinase (TYR) and β -glucosidase (β -Glu)) in magnetic alginate-polydopamine (PDA) microspheres. The double network cross-linking of alginate and PDA was induced by D-(+)-glucono- δ -lactone (GDL) and TYR, respectively, which significantly reduced the leakage of enzyme from alginate beads (Fig. 5).

Although most scholars recognize the application value of magnetic nanoparticles @ alginate in enzyme immobilization, some problems also exist objectively. Insufficient mechanical strength and enzyme leakage are two representative problems (Marjani et al. 2021). In order to solve these problems, sodium alginate is usually combined with gelatin, chitosan, starch, pectin and other biological polysaccharides and inorganic materials for enzyme immobilization (Cao et al. 2014).

Magnetic nanoparticles @ carbon-based materials

Immobilizing enzymes on organic materials can maintain their high activity, however, these materials do not have the appropriate chemical and thermal stability required for industrial use, and may even be toxic to enzymes. In order to overcome these shortcomings, inorganic materials, such as

carbon-based materials, can be used, which have excellent heat resistance and better mechanical and microbial resistance than organic materials.

Carbon nanotubes are composed of graphite sheets, which are rolled into a cylindrical shape with a length of microns and a diameter of up to 100 nm. Carbon nanotubes exhibit extraordinary mechanical, electrical and thermal properties as well as biocompatibility, which makes enzyme immobilization a promising biotechnology application for carbon nanotubes.

Zhao et al. (2011a, b) prepared magnetic carbon nanotubes (MCNTs) with necklace-like nanostructures by hydrothermal method. Based on the Michael addition of methyl acrylate and the amidation reaction of the generated ester with a large amount of excessive ethylenediamine (EDA), hyperbranched polyamide-amine (PAMAM) was grafted on the surface of MCNTs, which can achieve generation growth under this uniform step-by-step reaction. The-NH₂ group at the end of the dendritic PAMAM reacts with different functional groups to form functionalized MCNTs. Subsequently, *glucoamylase* was immobilized on the functionalized MCNTs by adsorption, covalent bond and metal ion affinity interaction. The results showed that in addition to being easily recovered by magnetic separation, the immobilized glucoamylase on functionalized MCNTs provided excellent stability and reusability without compromising the substrate specificity of free glucoamylase.

Biochar is a stable, porous and light solid product obtained by thermal decomposition of biomass under anaerobic conditions. It is mainly composed of 70% C and P, and may also contain elements such as Ca, Mg and Si. Biology

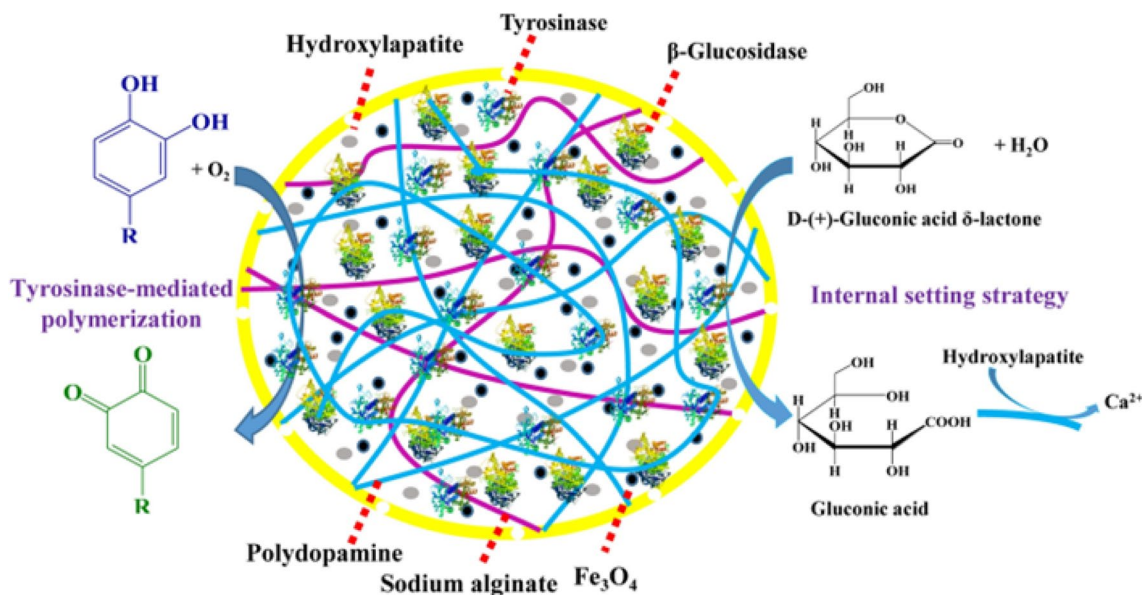


Fig. 5 The schematic of in situ TYR and internal setting strategy-mediated magnetic alginate-PDA hydrogelation (Zhang et al. 2020a, b)

has high surface area, good physical and chemical tolerance, rich surface oxygen-containing functional groups, good dispersion and biocompatibility, which can be used for stable and high-load enzyme immobilization.

Magnetic biochar derived from waste has become an attractive enzyme immobilization carrier due to its low cost, easy availability, lack of enzyme inhibition and porosity. Zhang et al. (2020b) prepared magnetic biochar from biosolids under hydrothermal conditions for laccase immobilization. The specific activity of immobilized *laccase* was 47.1% higher than that of free *laccase*. After 10 times of washing, the immobilized enzyme retained 79.3% activity. In addition, MBC has low acute toxicity and is relatively benign from an environmental perspective.

Graphene exhibits a two-dimensional structure of honeycomb carbon lattice, with a large specific surface area, abundant oxygen-containing functional groups, biocompatibility and excellent structural, electrical, mechanical and thermal properties (Li et al. 2018). In addition, due to its excellent antioxidant properties, graphene can promote the removal of free radicals and protect the enzymes from biocatalytic inactivation. The above series of excellent characteristics make graphene an excellent carrier for loading magnetic nanoparticles and immobilized enzymes (Heidarizadeh et al. 2017). Due to the severe functionalization of the conjugated network, graphene sheets have the characteristics of insulation, the Magnetic nanoparticles@graphene composites not

only has a unique magnetic two-dimensional structure, but also has the characteristics of simple production, low toxicity, strong surface modification and large enzyme loading, which has attracted great attention in enzyme engineering research (Badoei-Dalfard et al. 2019; Wang et al. 2020).

Ariaeenejad et al. (2021) reported bi-functional applications of a novel immobilized enzyme on the modified magnetic graphene oxide for degradation of dyes in water. The results showed that the immobilized process provides more enzyme protection and dye adsorption/degradation ability even in the concentrated dye solutions (Fig. 6).

Rouhani et al. (2018) prepared a graphene oxide modified by CuFe_2O_4 magnetic nanoparticles and immobilized the *laccase* by covalent interaction. The results showed that the immobilized *laccase* has a wider temperature and pH applicability than the free one, there was no significant activity loss after storage for 30 days or repeated 10 times (Fig. 7).

Now some key points still need to be solved. Firstly, the effects of graphene on the structure and function of enzymes (or other proteins) need to be further studied, which is important for optimizing nanobiocatalytic systems (Baghayeri et al. 2019). Secondly, the development of simple and low-cost methods for designing and creating novel graphene-based materials with tailored physicochemical properties and surface functionality is an urgent task (Li et al. 2022). Finally, it is crucial to develop graphene-based nanomaterials with high biocompatibility and negligible environmental

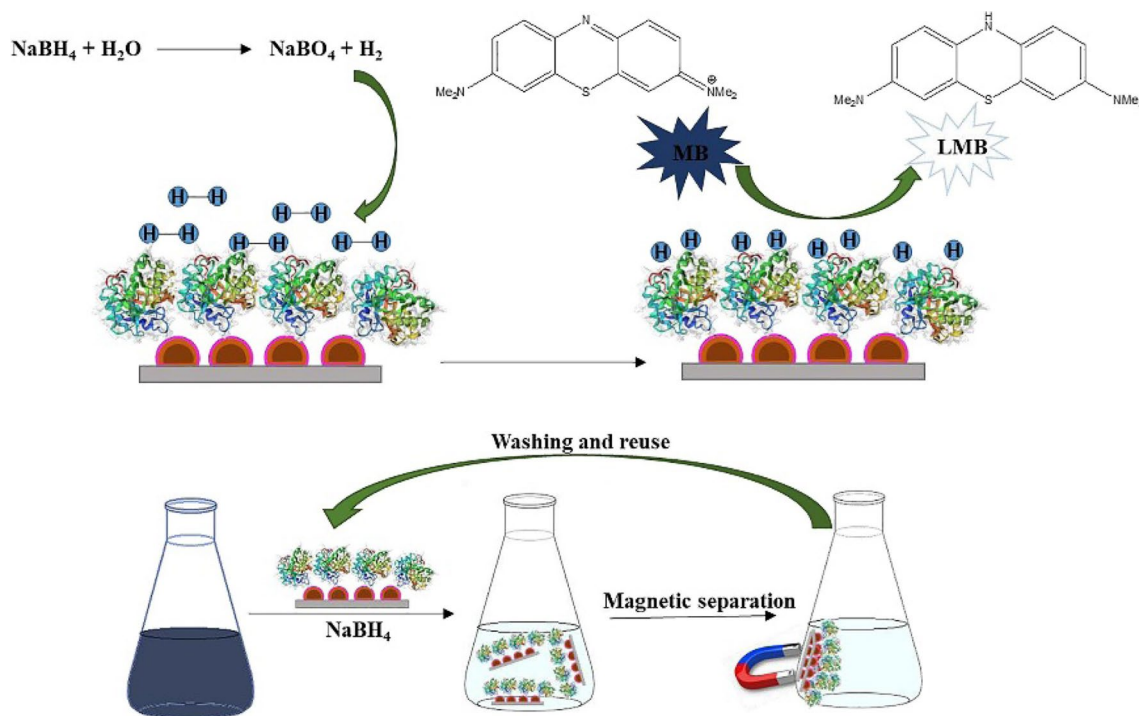


Fig. 6 Schematic representation of plausible mechanism for the reduction of methylene blue by immobilized enzyme nano-biocatalyst (Ariaeenejad et al. 2021)

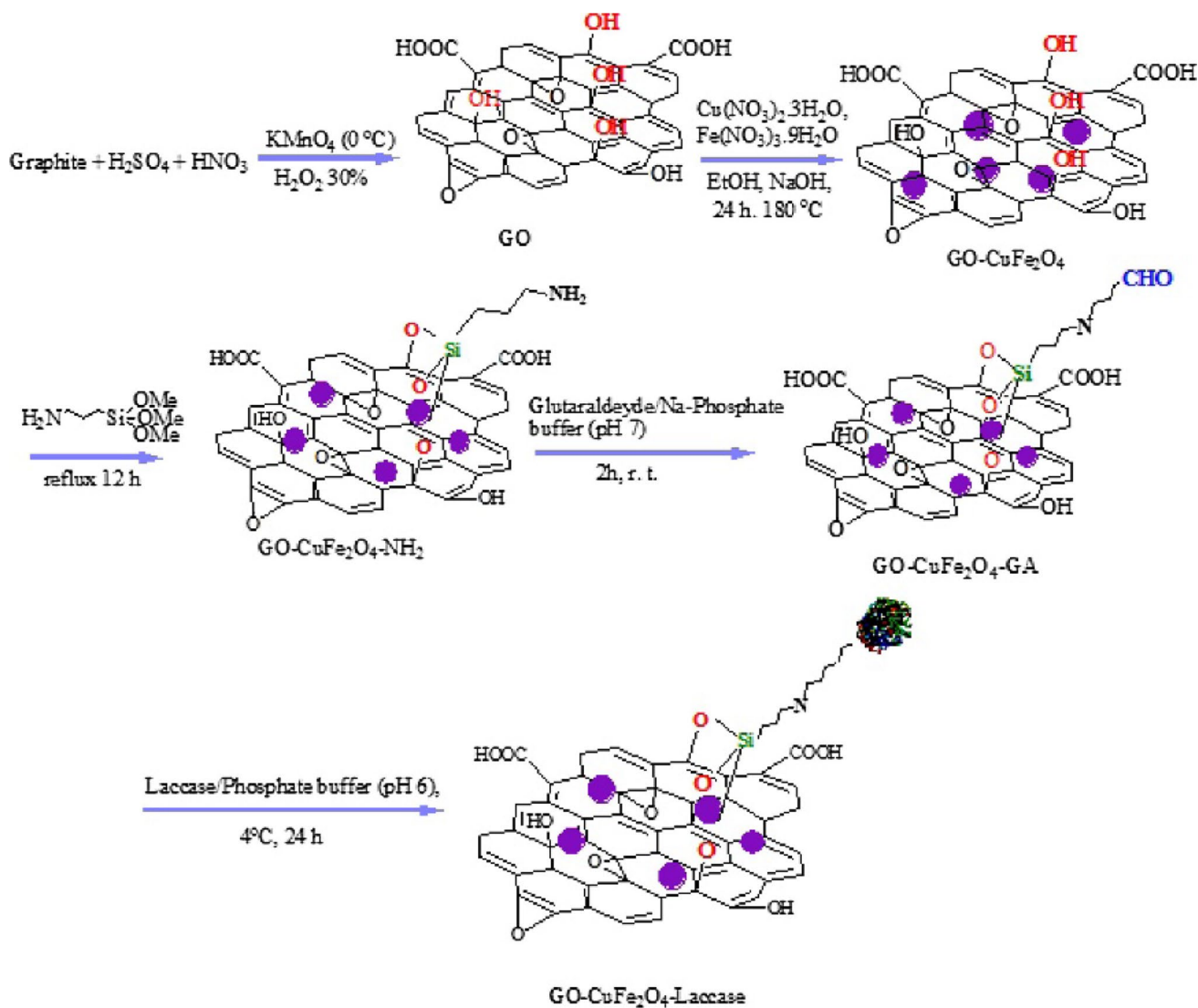


Fig. 7 Synthesis steps of GO-CuFe₂O₄-Laccase (Rouhani et al. 2018)

impact, which will expand the application of graphene-based nano biocatalytic systems in the fields of biocatalytic conversion, enzyme engineering, biofuel and energy production (Paz-Cedeno et al. 2021).

Magnetic nanoparticles @ Au

The surface of Au nanoparticles can be modified to obtain abundant functional groups (such as thiol group and disulfide group), which can provide a good carrier for the enzymes (Zhang et al. 2019a, b, c). The Magnetic nanoparticles@Au nanocomposites have excellent electrical conductivity and biocompatibility, providing an appropriate microenvironment for maintaining enzyme activity (Mohammadi et al. 2019).

Peng et al. (2013) reversibly immobilized *trypsin* on Fe₃O₄ nanoparticles by using Au nanoparticles as intermediate ligands. The results showed that trypsin was immobilized on magnetic AuNP@Fe₃O₄ to form an excellent biocatalyst, which could digest the protein very quickly. After repeated times, it could still maintain high activity and some enzymes could be regenerated (Fig. 8).

Majouga et al. (2015) synthesized core-shell NPs having magnetite cores and gold shells modified with various sulfur containing ligands, the carboxylic groups displayed at the surface of the NPs were utilized for NP conjugation with a model enzyme, the enzyme activity can be manipulated by a remote magnetic field using the proposed enzymes-superparamagnetic NP hybrid structures (Fig. 9).

Fig. 8 Facile preparation of novel core-shell enzyme-Au-polydopamine- Fe_3O_4 magnetic bionanoparticles for glucosensor (Peng et al. 2013)

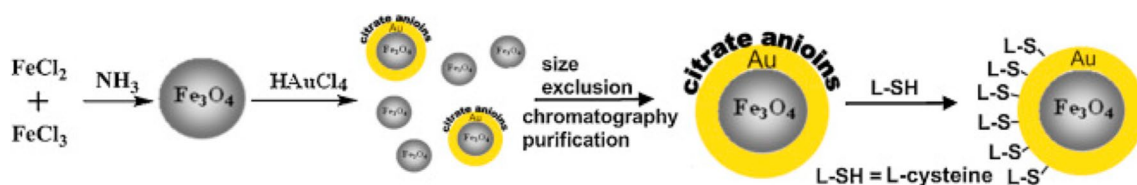
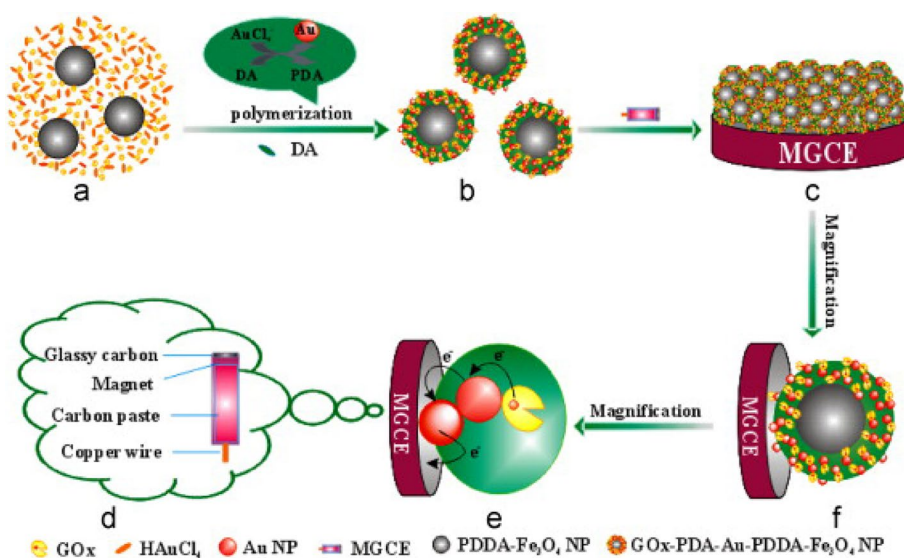


Fig. 9 Synthesis, purification and modification of core-shell magnetite-gold nanoparticles (Majouga et al. 2015)

Immobilized enzymes bioreactors utilizing a magnetic field

Although the research of magnetic nanocomposites as enzyme carriers is not complete, it is necessary to fully understand the development status of their supporting bioreactors. Now the research on immobilized enzymes bioreactors utilizing a magnetic field has made some progress, mainly aiming at realizing the simple recovery of magnetic nanocomposites and inhibiting the agglomeration between nanoparticles (Al-Qodah et al. 2017). In the magnetic field, the induced magnetic force stabilizes the magnetic particles and makes them uniformly arranged along the magnetic field line (Al-Qodah et al. 2017). Another advantage of this stability is the suppression of the formation of large bubbles, which helps to avoid poor contact between different phases (De Cuyper 2018). Other studies have shown that the introduction of magnetic field is beneficial to prolong the life of enzymes on magnetic nanocomposites, because mutual collision and aggregation will not only lead to enzyme shedding but also destroy the microenvironment of enzyme survival (Wang et al. 2022).

Some specific issues have to be considered when building the above bioreactors (even in the laboratory).

Magnetic particle size is the first parameter, too large or too small is wrong. Too large particle size will affect the mass transfer efficiency, and too small will make the agglomeration difficult to control (Takei et al. 2018). The next question is the direction and strength of the magnetic field. At present, the axial magnetic field is still the first choice, because it is beneficial for the magnetic field to exert a uniform influence on the whole reactor (Darwesh et al. 2019). The magnetic field strength should also be controlled within a reasonable range, because studies have shown that high magnetic field strength can easily lead to agglomeration (Sotelo et al. 2022).

Some forward-looking issues also have to be considered. Firstly, it should be noted that the magnetic field can affect the activity of the enzymes by affecting the three-dimensional structure of the active sites, this effect can be positive or negative (Armenia et al. 2019). Therefore, it is better to test the effect of magnetic field on the target enzyme before building the bioreactors. In addition, based on the results of the laboratory to enlarge and scale is the inevitable problem in future. It is therefore an important preparation to try to model these systems to correlate the reactor and particle size with the required conversion rate.

Conclusion and future perspectives

Magnetic nanocomposites have shown obvious advantages in enzyme immobilization due to their easy separation and recovery by magnetic fields, larger surface area and high mass transfer capacity. In this work, the research progress of magnetic nanocomposites as enzyme carriers is reviewed and the future development direction is prospected.

- (1) A variety of inorganic or organic materials have been used to form magnetic nanocomposites with bare magnetic nanomaterials to improve some inherent defects (poor chemical stability, high aggregation tendency, strong dipole–dipole attraction and low adsorption enzyme amount, etc.). Easy preparation, high specific surface area, low cost, strong stability and excellent biocompatibility are the basic requirements for the organic or inorganic materials. Unfortunately, it is difficult to find materials that meet the above conditions at the same time. In fact, our research group does not believe that there is a universal material suitable for all application scenarios. What the front-line engineers need to do is to find the relative best choice according to the actual needs.
- (2) Some novel synthetic methods are necessary for designing multifunctional magnetic hybrid materials as a better platform for enzyme immobilization. The novel synthesis methods must be able to fully combine magnetic nanoparticles with one or more other inorganic or organic materials under the premise of cost control. The interaction between the composite materials and the enzymes is also a matter of concern. In addition to providing an attachment platform for the enzymes, the specific interaction should promote the enzyme activity.
- (3) With the continuous development of magnetic nanocomposites as enzyme carriers, bioreactors based on them are also an inevitable demand. Now the research on immobilized enzymes bioreactors utilizing a magnetic field has made some progress, mainly aiming at realizing the simple recovery of magnetic nanocomposites and inhibiting the agglomeration between nanoparticles. Obviously, a series of emerging issues are worthy of attention. For example, it should be noted that the magnetic field can affect the activity of the enzymes by affecting the three-dimensional structure of the active sites, this effect can be positive or negative.
- (4) Moreover, the current research still faces some problems. For example, the focus of research is limited to the immobilization of single enzyme and ignores the study of immobilized multi-enzyme system; there is a

lack of effective control methods for the interface interaction between carrier and enzyme molecules; there are few studies on the synergistic catalysis of magnetic carrier and enzyme. In addition, concerted efforts are required to analyze the nanoparticles–enzyme binding sites, surface–function relationship and the involvement of conformational changes in the immobilization process.

In a word, the research on enzyme immobilization involves the cross integration of many disciplines such as material science, biological science, catalytic science and process science, and its related research achievements play an important role in promoting the development of many industries such as biology, pharmacy, energy, environment and food.

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Declarations

Conflict of interest No competing financial interests exist.

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