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Original Article

Recent progress in magnetic nanoparticles and mesoporous materials for enzyme immobilization: an update

Progresso recente em nanopartículas magnéticas e materiais mesoporosos para imobilização de enzimas: uma atualização

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Abstract

Enzymes immobilized onto substrates with excellent selectivity and activity show a high stability and can withstand extreme experimental conditions, and their performance has been shown to be retained after repeated uses. Applications of immobilized enzymes in various fields benefit from their unique characteristics. Common methods, including adsorption, encapsulation, covalent attachment and crosslinking, and other emerging approaches (e.g., MOFs) of enzyme immobilization have been developed mostly in recent years. In accordance with these immobilization methods, the present review elaborates the application of magnetic separable nanoparticles and functionalized SBA-15 and MCM-41 mesoporous materials used in the immobilization of enzymes.

Keywords: functioned MCM-41 and SBA-15 mesoporous material, magnetic nanoparticle, enzyme immobilization.

Resumo

Enzimas imobilizadas em substratos com excelente seletividade e atividade apresentam alta estabilidade e podem suportar condições experimentais extremas, e seu desempenho foi mantido após repetidos usos. As aplicações de enzimas imobilizadas em vários campos se beneficiam de suas características únicas. Métodos comuns, incluindo adsorção, encapsulamento, ligação covalente e reticulação, e outras abordagens emergentes (por exemplo, MOFs) de imobilização de enzima, foram desenvolvidos principalmente nos últimos anos. De acordo com esses métodos de imobilização, a presente revisão elabora a aplicação de nanopartículas magnéticas separáveis e materiais mesoporosos funcionalizados SBA-15 e MCM-41 usados na imobilização de enzimas.

Palavras-chave: material mesoporoso funcionado MCM-41 e SBA-15, nanopartícula magnétiaca, imobilização enzimática.

1. Introduction

Due to their excellent ability to boost the rate of chemical and biochemical reactions, enzymes have been widely used as catalysts in various fields, including but not limited to the textile industry, pharmaceutical industry, steal industry, animal feed, daily cosmetics, food industry, environmental governance and bioenergy utilization (Gurung et al., 2013; Jegannathan and Nielsen, 2013; Choi et al., 2015; Dwevedi, 2016; Escamilla-Alvarado et al., 2017). Their extensive application in industrial production is demonstrated by a respectable averaged annual growth rate of 4.6% over the past decades (Datta et al., 2013). The demand for proteases and lipases claims more than 70% share of the global enzyme market, amounting to as much as 5 billion US dollars, and is predicted to increase to 17.5 billion US dollars by 2024 (Han et al., 2015; Mohamad et al., 2015; Mojsov, 2016; Patel et al., 2016; Guerrand, 2017). The inherent characteristics of instability, low reusability and high cost are severe limitations and prevent enzymes from evolving into a cost-effective option (Minteer, 2017). The application of immobilization techniques could alleviate these disadvantages and therefore has emerged and developed gradually since the 1970s (Vaghari et al., 2016; Bernal et al., 2018). Enzyme immobilization offers the advantage of maintaining and developing robust biocatalyst activity under harsh operational conditions for a long time (Landarani-Isfahani et al., 2015; Rueda et al., 2016). Aside from carrier-free immobilization and carrier-bound immobilization as the main methods (Wang et al., 2015), adsorption (Klein et al., 2016; Nguyen and Kim, 2017), covalent attachment (Wu et al., 2015), crosslinking and encapsulation are also frequently used in enzyme immobilization (Bezerra et al., 2015; Majewski et al., 2017; Ma et al., 2018; Singh et al., 2019).

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Aside from optimizing immobilization methods, the selection of suitable support materials is also vital in industrial production (Santos et al., 2015). These materials can input their specific properties upon immobilization, given that the properties of the supported enzyme are determined by both the enzyme and the support material (Bilal et al., 2018). For instance, magnetic nanoparticles (MNPs) are valued for their high surface area, large surface-area-to-volume ratio and easy separation under external magnetic fields (Liu et al., 2018; Shiri et al., 2018). Moreover, porous materials feature large pore diameters and outstanding carrying capacities (Sun et al., 2016; Abe et al., 2017, Cacicedo et al., 2019). Low cost, thermal resistance, high reusability and stability are the desired features of ideal support materials (Zdarta et al., 2018). Since the pore size of mesoporous materials can be mediated continuously from 2 nm to 50 nm, they are suitable and ideal materials for enzyme fixation (Baino et al., 2016).

In recent years, magnetic materials, mesoporous materials, and metal-organic materials have led to many beneficial changes in enzyme immobilization studies, which have the potential to advance their industrial production. This review provides an update on the research on enzyme immobilization by MNPs and the functionalized mesoporous materials SBA-15 and MCM-41 in the last five years and discusses the changes in enzyme properties and the application of immobilization support materials.

2. Magnetic nanoparticles

Bypassing the procedures of catalyst filtration or centrifugation after a finished reaction, magnetic separation is a practical approach to recycle magnetized catalysts (Shokouhimehr, 2015; Zhang et al., 2016). Magnetic materials are extensively used in the separation process after enzyme catalytic reactions because of their excellent adsorption to magnetic substances, which enhances their reusability and reduces the cost of immobilized enzymes (Vaghari et al., 2016; Zhao et al., 2019). We reviewed the application of enzyme immobilization by using MNPs over the past five years and found that organic polymer-functionalized and mesoporous material-functionalized MNPs are frequently used for enzyme immobilization (Table 1) (Bilal et al., 2018). Herein, we elaborate on applications that can improve the stability of enzymes and enhance their catalytic efficiency. Silica-coated nanocomposites provide a core-shell structure to preserve the properties of the enzyme, whereas organic materials prevent the accumulation of immobilized enzyme, and in mesoporous materials, organosilane is introduced into the mesoporous matrix to increase the loading capacity and to prevent leaching.

2.1. Mesoporous material-functionalized MNPs

The formation of functionalized silica coatings improves the chemical stability and biocompatibility of magnetic nanoparticle surfaces, providing outstanding supports by preventing nanoparticle aggregation in solution (Mahto et al., 2016; Gao et al., 2017a). In the application of immobilized enzymes, surface-modified nanomaterials promote particle dispersion and enhance antioxidant capacity, thus modulating the reaction features. In metal nanoparticles and metal magnetic nanoparticles, Fe_3O_4 is the most common material. In the methods of modification, organic molecule modification uses surfactant, a silylation coupling agent (3-aminopropyltriethoxysilane APTES), and an organic polymer, whereas inorganic modification applies silica core-shell particles (Mahto et al., 2016; Gkaniatsou et al., 2018) and mesoporous silica (Gao et al., 2017a). The immobilization of mesoporous material-coated MNPs is shown in Figure 1.

Here, silica coating is a unique method because it helps form a silica shell on the surface of the magnetic core for the functionalization/modification of Fe_3O_4 nanoparticles (Cui et al., 2017, 2018a, b). The formation of the silica shell can protect the magnetic core from aggregation and oxidation, thereby improving its chemical stability. Additionally, it also enhances the characteristics of

Table 1. Enzyme immobilization on MNPs.

Immobilization Method	Support material	Enzyme	Improved Enzyme Properties	Ref
Covalent Bond	Fe_3O_4 nanoparticles	*Candida antarctica* lipase B	Stability	(Gkaniatsou et al., 2018)
Covalent Bond	Fe_3O_4 nanocomposites	Lipase	Hydrophilicity and biocompatibility	(Mahto et al., 2016)
Crossing-linking	Tannic-acid-templated magnetic mesoporous silica nanoparticles (TA-MMSNs)	NHase	Yield	(Gao et al., 2017a)
Crossing-linking	Polydopamine (PDA)-functionalized magnetic nanoparticle	DNA catalytic	Stability and reusability	(Yang et al., 2018)
Crossing-linking	PAL-coated magnetic nanoparticles	Phenylalanine ammonia-lyase (PAL)	Reproducibility	(Ender et al., 2016)
Crossing-linking	Dendritic polymer-modified nanoparticles	Lipase	Yield	(Li et al., 2018)

hydrophilicity and biocompatibility of the material. During silica coating, *Candida antarctica* lipase B was adsorbed to MNPs modified by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide and 1-hydroxy-2,5-pyrrolidinedione. After 10 min of reaction, HPD/ECD aqueous solutions had a strong activating effect on the carboxylic acid group of *Candida antarctica* lipase B at RT. Additionally, the amino groups were attached to the surface of Fe_3O_4 nanoparticles by covalent bonds. Thus, superparamagnetic materials and functionalized nanoparticles that remained dispersive were employed to immobilize the lipase under mild conditions. The enzyme maintained its stability throughout the process (Gkaniatsou et al., 2018).

Additionally, silicon dioxide shells protect nuclei from aggregation and oxidation, improving their chemical stability by increasing biocompatibility and hydrophilicity (Poorakbar et al., 2018). The changed optimum pH values after immobilization were mostly caused by the immobilization of magnetically mesoporous silica nanocomposites with polyaniline functionalized (Pani-MS@Fe_3O_4) by multipoint immobilization to ensure the stability of lipase molecules. Recent studies demonstrated that mesoporous silica-immobilized lipase modified with polyaniline showed high activity across a wide pH range and high temperature stability, which was attributed to the change in the optimal pH in Pani-MS@Fe_3O_4 by multipoint fixation facilitating the stabilization of lipase molecules by Fe_3O_4 nanocomposites (Mahto et al., 2016). Notably, the advantages of immobilized NHase included improvements in the loading capacity, ease of separation and recycling of materials (Gao et al., 2017b). Moreover, tannic acid-modified magnetic mesoporous silica was crosslinked with the enzyme glutaraldehyde to form crosslinked nitrile hydratase aggregates, which were characterized by a high loading rate, easy separation and recovery of materials compared to those of the free enzyme (Gao et al., 2017a). Moreover, the activities of free NHase and tannic acid-templated magnetic mesoporous silica nanoparticle-immobilized NHase (CLNHAs@TA-MSNs) were highest at 30°C and 40°C, respectively. CLNHAs@TA-MSNs had a higher optimum temperature than free NHase. The reason is that the active conformation of NHase molecules could be maintained at high temperatures through multipoint covalent bonds between NHase molecules and TA-MSNs, whereas the destruction of the conformation of CLNHAs@TA-MSNs required much more energy.

The large specific surface area, adjustable pore size and easy surface functionalization make mesoporous silica materials ideal carriers in catalytic reactions. Moreover, MNPs have potential application value in the field of magnetic separation due to their low toxic side effects and special magnetic properties. However, simple magnetic nanoparticles are prone to agglomeration. In future research, additional attention should be paid to the immobilization of MNPs in combination with mesoporous silica materials.

2.2. Organic polymer-functionalized MNPs

Compared with mesoporous silica-immobilized enzymes, organic polymer magnetic material-immobilized enzymes are widely used as lightweight materials due to their low density, structural diversity and easy processing (Figure 2). Generally, in situ modification and ectopic modification are the two methods used for modifying MNPs with organic polymers. During in situ modification, the organic polymer is added as a stabilizer to the precursor solution to form Fe_3O_4 nanoparticles, and the polymer coating is synthesized on the polymerized monomer. However, the repulsive force generated by the polymer coating tends to weaken the magnetic properties. Thus, the enzymes interact with the material via van der Waals interactions to prevent their aggregation and improve the stability and dispersion.

One study described the preparation of a mild and versatile immobilized enzyme reactor by applying DNA-directed immobilization (DDI) to anchor trypsin on polydopamine (PDA)-functionalized magnetic nanoparticles (MNPs). The reactor exhibited outstanding reusability and improved catalytic efficiency. Notably, the immobilized trypsin reactor maintained 55% of its initial activity even after 70 cycles of reaction at pH 9.0 and 37°C. Its outstanding properties are mostly ascribed to immobilization by DDI, which is attached to the vector by a DNA splice that acts as a linkage between the enzyme and the vector. DNA, as a spacer molecule, allows the free use of the enzyme functional units to enhance the conformation of the enzyme. The functional units of the enzyme (e.g., active sites) are freely available to enhance its conformation, thereby boosting its activity and potency (Yang et al., 2018). Ferenc Ender et al. (2016), introduced a novel microfluidic device (Magne-Chip) containing microliter-volume reaction cells filled with PAL-coated magnetic nanoparticles (MNPs), which could be used as a novel efficient and flexible tool for the enzyme-catalyzed

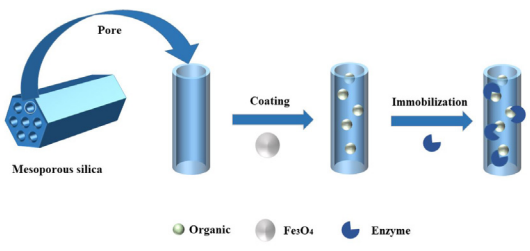

Figure 1. Mesoporous material-functioned MNPs.

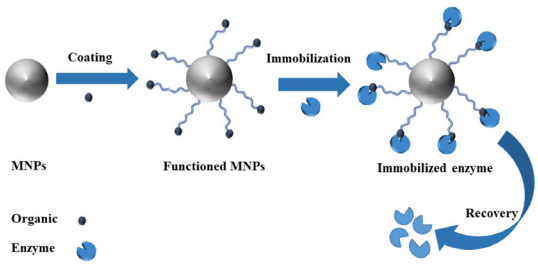

Figure 2. Organic polymer-functioned MNPs.

biotransformation of L-phenylalanine (L-1**a**) and five unnatural substrates (rac-1**b–f**) by phenylalanine ammonia-lyase (PAL). The experimental results showed the excellent reproducibility of enzyme-catalyzed biotransformation in the chip and the high reusability of the enzyme layer during 14 hours of continuous measurement (>98% over 7 repetitive measurements with L-1**a**) (Ender et al., 2016). In this work, a novel melamine-glutaraldehyde dendrimer was first grafted onto amino-magnetic nanoparticles to facilitate the immobilization of lipase on the protein-binding site. Dendritic polymer-modified nanoparticles were used to control the lipase conformation to enhance the potential of contact with the lipase catalytic center. The activity of immobilized lipase was approximately 58.0 times higher than that of BCL powder and 3.0 times higher than that of immobilized lipase from unmodified polymer. Moreover, the time required for the reaction was reduced from 180 mins to 20 mins. However, due to the weak physical adsorption interaction between the enzyme and the carrier, enzyme leakage may occur in each batch of the reaction, resulting in increased costs for separation and purification of downstream products (Li et al., 2018).

Overall, the application of magnetic nanomaterial MNPs for enzyme immobilization has shown satisfactory performance. The protective effect of the MNP material on the enzyme increases the stability of the enzyme under acidic and thermal conditions, and the MNPs, as magnetic materials, provide better recycling performance. In addition, enzymes linked to these polymers by noncovalent or covalent bonds to produce biocatalysts can be used as homogeneous catalysts, and applying appropriate stimuli can result in high reusability of the immobilized enzyme (Singh et al., 2013; Shahrestani et al., 2016; Fletcher et al., 2019; Suo et al., 2020).

3. Mesoporous materials

3.1. Functional group-modified SBA-15

SBA-15 mesoporous materials with tunable pore diameters (from 20 to 50 nm) can be synthesized for various applications (Meléndez-Ortiz et al., 2016). A chemical covalent bond is formed between the functional groups on the enzyme protein molecule and the reactive group on the mesoporous material, forming an immobilized product with a strong binding force to ensure the stability of the enzyme during the catalytic reaction (Gholamzadeh et al., 2017; Yang et al., 2019). After the introduction of different organosilanes (amino, cyano, epoxy or mercapto) onto the surface or the pores of the mesoporous matrix, the presence of these functional groups generates many reaction sites for subsequent attachment of enzymes, thereby improving the loading capacity and preventing the leaching of the enzyme (Zhong et al., 2019). The procedure of enzyme immobilization on mesoporous materials via covalent bonds is illustrated in Figure 3.

SBA-15 materials have a high specific surface area, ordered structure and large pore volume, which provide high immobilization efficiency (Bhanja et al., 2017; Wongvitvitchot et al., 2019). Moreover, SBA-15 can provide pH protection, which is influenced by the pore size of the material (Lynch et al., 2016). In addition, SBA-15 can be optimized by postmodication, and when functionalized or modified SBA-15 is used for immobilizing enzymes, their thermal stability and pH sensitivity are further improved (Rios et al., 2016; Zhong et al., 2019). Immobilization of enzymes by SBA-15 after the introduction of a suitable bridging agent can also improve the enzyme stability and reusability as well as the pH and temperature tolerance (Xiang et al., 2018). In studies of enzyme immobilization with mesoporous materials as carriers, lipase accounts for a large fraction (Gholamzadeh et al., 2017). Based on production requirements, lipase immobilized with the mesoporous material SBA-15 is used to catalyze chemical reactions, including esterification and transesterification (Zhao et al., 2015; Gholamzadeh et al., 2017). Here, we summarized the recent progress on lipases immobilized with various support materials (Table 2).

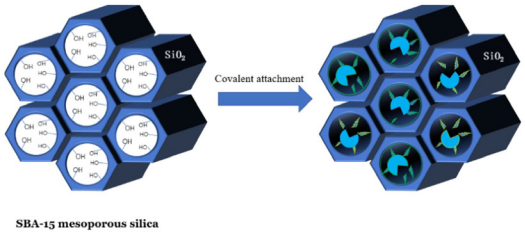

Figure 3. Enzyme immobilization on mesoporous materials.

3.1.1. Functionalized SBA-15 for lipase immobilization

Over the last 20 years, SBA-15 has been widely used for immobilizing lipase. In recent years, organically modified and ionic liquid-modified SBA-15 have shown satisfactory performance in enzyme immobilization (Abolghasemi et al., 2016; Kong et al., 2016; Yuan et al., 2016). The immobilization of *Candida antarctica* lipase B (CALB) by using mesoporous SBA-15 resulted in improved enzymatic activity and stability. The thermal stability of CALB after immobilization greatly depends on its moisture content; when it reached 3.22%, and its thermal stability was significantly improved (Cai et al., 2016). Moreover, higher protein rigidity contributed to 4-12 times higher thermal stability of immobilized lipase at 60°C than that of free lipase (Živković et al., 2015).

As imidazole-based ionic liquids (ILs) boost enzyme bioactivity, the immobilization of CALB on a carrier (IL-SBA-15) could be achieved by modifying the mesoporous silica SBA-15. The hydrophilic carrier promoted the enzyme activity because it retained the essential aqueous layer of lipase and hence prevented the impairment of the catalytic activity (Zhong et al., 2018). Moreover, recombinant *Candida antarctica* lipase B (LIPB) expressed in Pichia pastoris was immobilized in SBA-15 to produce the biocatalyst SBA-15-LIPB-GA via glutaraldehyde (GA) crosslinking. The biocatalyst showed excellent thermal and solvent stability. Hydrophobic interactions were probably the main driving force of the adsorption (Rios et al., 2018). Notably,

Table 2. Enzyme immobilization on SBA-15 mesoporous materials.

Immobilization Method	Support material	Enzyme	Improved Enzyme Properties	Ref
Cross-Linking	Epoxy functionalized SBA-15	Carbonic anhydrase	Storage stability	(Fei et al., 2016)
Cross-Linking	Aminoy Functionalized SBA-15	Carbonic anhydrase	Storage stability	(Fei et al., 2016)
Encapsulation	Mesoporous Silica SBA-15	Alkaline protease	Enzyme Activity	(Kumari et al., 2015)
Cross-linking	Mesoporous Silica SBA-15	Lysozyme	PH Resistance	(Lynch et al., 2016)
Covalent bond	Aminofunctionalized SBA-15	Laccase	Biodegradation	(Bautista et al., 2015)
Adsorption	Mesoporous Silica SBA-15	*Candida rugosa* lipase	Affinity and thermostability	(Živković et al., 2015)
Adsorption	Mesoporous Silica SBA-15	*Candida antarctica* Lipase B	Enzyme activity and stability	(Cai et al., 2016)
Covalent bond	Amino acid modified S BA-15	Porcine pancreases lipase	Heat resistance	(Zou et al., 2013)
Adsorption	Magnetic Fe_3O_4/SBA-15	*Candida rugosa* lipase	Easily separated	(Rios et al., 2016)
Adsorption	Mesoporous Silica SBA-15	*Candida antarctica* lipase B	Thermal stability	(Rios et al., 2016)
Cross-linking	SBA-15-LIPB-GA	*Candida antarctica* lipase B	High activity and stability	(Rios et al., 2018)
Cross-linking	NH_2-SBA-15	Acetylcholinesterase	Immobilization efficiency	(Rui et al., 2018)
Covalent bond	Mesoporous SBA-15 silica	Polyphenol oxidase	Enzyme efficiency	(Escuin et al., 2017)
Covalent bond	Ionic Liquids Modified SBA-15	*Candida antarctica* Lipase B	Enzymatic activity	(Zhong et al., 2018)

Rios NS et al., have shown that the surface of SBA-15 can be treated with 3-amino-propyltriethoxysilane (APTES) to form SBA-15-APTES. After activation, incubation (at pH 10.2) and divinyl sulfone (DVS) treatment, the biocatalyst SBA-15-APTES-DVS-LIPB can be obtained, which combines multipoint covalent bonds between the enzyme and its carrier at 50°C and pH 7.0; SBA-15-LIPB-GA was reused for five cycles, and retained 76.80% of its initial activity after the third cycle (Rios et al., 2016).

All these above studies indicated that lipase immobilized using the mesoporous material SBA-15 showed improved thermal stability and enzymatic bioactivity. In addition, the immobilization of lipase into the modified mesoporous material SBA-15 exhibited superior properties compared to unmodified mesoporous material in terms of improved thermal stability, activity and solvent stability of the immobilized lipase.

3.1.2. Functionalized SBA-15 for immobilization of other enzymes

In addition to their ability to be applied to the immobilization of lipases, SBA-15 mesoporous materials used in immobilizing other enzymes have also been reported. The conversion rates of aminobutyl-based laccase to naphthalene, phenanthrene and anthracene were 82%, 73% and 55%, respectively, which is close to the conversion rate of free enzymes. However, laccase technology based on aminopropyl-modified SBA-15 led to satisfactory covalent immobilization. In addition, the aminopropyl-grafted laccase on SBA-15 exhibited the best reusability, and after 4 cycles, it had a higher activity than the aminobutyl-based laccase after 4 cycles (Bautista et al., 2015). Moreover, the method of acetylcholinesterase (AChE) immobilized on the amino-functionalized SBA-15 mesoporous sieve NH_2-SBA-15 was reported and applied to the determination of organophosphorus and carbamate pesticides. With regard to different immobilization techniques, we found that the adsorption-crosslinking technique was the best method. Specifically, the results indicated that AChE-NH_2-SBA-15 had a high immobilization efficiency of 95% (Rui et al., 2018). Escuin PC et al., also compared and analyzed the immobilization of polyphenol oxidase (PPO) by various mesoporous SBA-15 silica materials at different pH values. The results showed that the pore size and volume of the carrier were the main structural characteristics affecting PPO immobilization. When the pH value reached 4.0, the carrier achieved a maximum load of PPO

(Escuin et al., 2017). Two functionalized SBA-15 materials with different types of functional groups, amine-functionalized SBA-15 (AFS) and epoxy-functionalized SBA-15 (GFS), were prepared by treatment with APTES and 3-glycidyloxypropyltrimethoxysilane (GTMS), respectively, for post functionalization. Specifically, carbonic anhydrase (CA) reached its maximum activity at pH 10.0 (Fei et al., 2016). Kumari et al., proposed a new procedure to prepare immobilized alkaline proteases on the surface of silanol-functionalized SBA-15, providing a promising platform for application as milk coagulants in cheese-making and further research. In this process, the method was applied to immobilize alkaline protease on SBA-15. SBA-15 has a surface silanol group, to which protease can be attached, resulting in a high stability. The enzyme stability was enhanced, and protease immobilization on SBA-15 exhibited a residual activity as high as 70% after 12 reapplications (Kumari et al., 2015).

In conclusion, in some cases, the enzymes immobilized with SBA-15 had high recoveries, such as aminopropyl grafted-laccase on the SBA-15 material mentioned before. Covalent immobilization of SBA-15 with enzymes using amino-modified SBA-15 via glutaraldehyde crosslinking can improve the immobilization efficiency of immobilized enzymes and reusability, and the use of divinyl sulfone (DVS) crosslinked with amine-modified SBA-15 for immobilizing enzymes can further enhance the thermal stability of immobilized enzymes. Epoxy-functionalized SBA-15 (GFS) has a more suitable pore size than amine-functionalized SBA-15 (AFS). Modifications in the pore volume, specific surface area and immobilized enzyme could all improve the CA stability at high pH values during immobilization (Fei et al., 2016).

3.2. Modified MCM-41 materials

Kresge et al., reported an ordered mesoporous material named MCM-41, a novel nanostructured material featuring ordered hexagonal arrangement and continuous adjustment of pore diameter ranged from 2 to 10nm, a size smaller than that of SBA-15 mesoporous materials (Xu et al., 2019). Currently, efforts have been made to analyze the utility of MCM-41 as a catalyst carrier (Jiang et al., 2016; Molaei et al., 2018; Polikarpova et al., 2018; Brezoiu et al., 2019). The pure silicon MCM-41 mesoporous material has low surface activity and poor stability, and is only used as a general carrier or adsorption material. In order to improve its performance, so that it can be used in a wider range of areas, it is necessary to modify it. At present, there are mainly the introduction of metal heteroatoms, organic modification or functionalization and load-type modification methods (Sang et al., 2013; Wu et al., 2016; Fellenz et al., 2017; Costa et al., 2020). The loading modification is a common method in immobilized enzymes by loading the active components (such as Fe_3O_4) in the pore channel to improve the performance of the immobilized enzyme. The methods and materials for the above enzyme immobilization are listed in Table 3.

Recent reports have shown that the application of aqueous-organic two-phase systems to MCM-41 mesoporous materials enhances enzyme activity, among these cases, Chen et al., reported that Candida rugosa lipase immobilized on MCM-41 mesoporous molecules showed excellent performance during the resolution of racemic naproxen methyl ester. Immobilized lipase was found to have increased activity relative to free lipase. In the range of pH 6.5-8.5, higher pH increases activity but decreases selectivity of lipase enantioselectivity in the reaction (Chen et al., 2015).

Appaturi and Selvaraj immobilized catalyst on the surface of DL-Alanine functionalized MCM-41. The durability of catalyst and rate of production (3-(2-furylmethylene)-2, 4-pentanedione) were significantly enhanced than that of the free one. Studies have shown that the alanine molecule has been successfully immobilized on MCM-41. 16Alanine-MCM-41 can be effective for the condensation reaction of furfural with acetylacetone under solvent-free conditions. The catalyst exhibited excellent performance, i.e., 92.41% of the main product 3-(2-furylmethylene)-2, 4-pentanedione, due to its high selectivity for substrates (Appaturi et al., 2018). Of most recent, Xie et al., reported a structured Fe_3O_4-MCM-41 nanoparticles, which provided a protective layer on the surface of immobilized lipase. The compound involved covalent linking using glutaraldehyde as the cross-linking reagent onto the surface of materials, demonstrating excellent enzyme kinetic properties. The catalytic properties for the bound lipase carried out in the esterification of lard and soybean oil, showing that higher catalytic activity of immobilized lipases for the interesterification reaction, lower melting point of the final product than the original mixture (Xie and Zang, 2016). Besides, MCM-41 nanoparticles were

Table 3. Enzyme immobilization on MCM-41 mesoporous materials.

Immobilization Method	Support material	Enzyme	Improved Enzyme Properties	Ref
Covalent Bond	Alanine Functionalized MCM-41	DL-Alanine functionalized MCM-41 catalyst	Substrate Selectivity	(Gkaniatsou et al., 2018)
Encapsulation	CPS/GO-Fe_3O_4@MCM-41	*Porcine pancreas* lipase	Reusability	(Mahto et al., 2016)
Covalent Bond	MCM-41 coated with polyethylenimine	*Rmomyces lanuginosa* lipase	Stability and reusability	(Gao et al., 2017a)
Encapsulation	Fe_3O_4 -MCM-41	*Candida rugosa* lipase	Enzyme Activity	(Yang et al., 2018)

coated with polyethyleneimine (MCM-41@PEI) and chelated by further modification of divalent metal ions (M = Co^{2+}, Cu^{2+} or Pd^{2+}) to produce metal-chelated silica nanoparticles particles (MCM-41@PEI-M). Thermomyces lanuginosa lipase (TLL) was immobilized by physical adsorption on MCM-41@PEI. The results showed that the highest biocatalytic activity at very acidic and basic pH (pH = 3.0 and 10.0) values were achieved by MCM-41@PEI-Co and MCM-41@PEI-Cu. While for different catalytic temperatures, MCM-41@PEI-Co maintained the highest activity at 75°C (Sadighi et al., 2017)

4. Conclusions

We summarized recent studies on the role of MNPs and the functionalized mesoporous materials SBA-15 and MCM-41 in immobilized enzyme applications. The traditional process of adsorption--immobilized enzymes is relatively simple, and the conformation of the enzyme is rarely affected, but the immobilization effect is not obvious. The reusability of immobilized enzymes can be improved due to the high adsorption of magnetic nanomaterial MNPs to magnetic substances. When mesoporous material-modified MNPs are applied to immobilized enzymes, due to the increased protection of the enzyme by the silica gel shell, the immobilized enzyme shows stability under harsh conditions. In addition, MNPs functionalized with organic polymers in immobilized enzymes can change multipoint covalent bonds, thus further promoting the catalytic efficiency. Furthermore, the specific structure of SBA-15 and MCM-41 enhances the binding capacity between the enzyme and substrate due to the increased spatial resistance and thus contributes to the excellent loading capacity of the immobilized enzyme.

Although several efforts have been made in the past few years to obtain versatile immobilization insights, joint studies are still necessary to evaluate the relationship of modified functional groups and carrier materials, binding sites between nanomaterials and enzymes and conformational shifts in the reactions. From previous successful studies, it can be concluded that the application of modified carrier material provides a vital approach for enzyme immobilization and several other fields.

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