Transactions on Materials, Biotechnology and Life Sciences
ISSN: 2960-1932, eISSN: 2960-2319 | Volume 4
NSCBM 2024

Enzyme Immobilization Based on Metal-Organic Frameworks and Its Biosensing Applications

Feilu Sun *

One Direction Academy, Ontario, Canada

* Corresponding Author Email: kongyan@ldy.edu.rs

Abstract. Many studies have shown that metal-organic frameworks (MOFs) that immobilized enzymes have broad application prospects. As highly efficient biological catalase, enzymes are easily inactivated under some special conditions, which becomes a major obstacle to industrial catalysis and hinders their wide application. However, when the enzyme is immobilized, its disadvantages such as instability and low recyclability are improved, resulting in higher catalytic efficiency and cost savings. The use of flexible, stable, and efficient MOFs as platforms for immobilized enzymes is considered to be a better scheme. In this research, four methods of enzyme solidification in MOFs, namely, in situ encapsulation, surface attachment, covalent conjugation and entrapment, are introduced, including their principles, applications, main advantages and disadvantages. At the same time, the applications of enzyme-multiple organ function (enzyme-MOFs) biosensors in the fields of environment, food and medicine are reviewed, including environmental contaminant detection, contaminant degradation, food safety detection, glucose detection and drug transportation. Finally, some current technical problems are briefly listed, and the technological development in the field of enzyme-MOFs biosensor is prospected.

Keywords: metal-organic frameworks; enzyme immobilization; enzyme-MOFs biosensors.

1. Introduction

Due to high efficiency, selectivity and specificity, enzymes are widely used as important biocatalases in food modification, agriculture, chemicals and pharmaceuticals, environmental monitoring and biosensing. However, the chemical composition of most enzymes is protein, which is prone to unfolding, denaturation and inactivation under high temperature, high pressure, extreme pH, high-frequency vibration and heavy metals. At the same time, the enzyme will not be consumed as the chemical reaction proceeds, so the residual free enzyme will also exist in the product as a contaminant, and the separation and purification of the mixed product may require complex processes and high costs [1]. These problems hinder the wide application of enzymes to a certain extent.

In order to overcome these challenges and maintain the activity of fragile enzymes, three methods can be used to improve the stability of the enzyme, namely immobilization, chemical modification, or non-covalent modification. Among them, the immobilization method has become the most practical method because of its applicability, convenience and great prospect. Immobilize fragile enzyme molecules on good carriers to provide biocompatible microenvironment for enzymes and improve enzyme stability and recyclability in a low-cost manner.

Although there are many kinds of carriers to immobilize enzymes, such as resins, hydrogels, organic polymers, polymers, graphene oxide, and mesoporous silica, the above substrates have some problems, such as poor designability, poor modifiability, inflexible pore size, small surface area, low loading efficiency, or enzyme limitation, which cannot make the immobilized enzymes reach the ideal state [2]. In addition, a variety of enzyme properties can also be altered during immobilization due to improper material selection or manipulation, which can affect the catalytic efficiency. Therefore, finding appropriate fixation materials and reasonable fixation methods is the key to curing enzyme technology, which is also the goal of researchers in this field.

Metal-organic frameworks (MOFs) are excellent solid substrates. MOFs are porous skeleton nanomaterials formed by the coordination of metal nodes and organic ligands, which have good ductile properties and can be adjusted according to different ligands and metal nodes to form different topological structures, so as to make different designs according to a variety of targets and realize reasonable application [3]. Due to their topological structure, there may be a variety of interaction forces between MOFs and enzymes, such as hydrogen bonds, covalent bonds and coordination bonds, which make their binding more stable. In addition, MOFs have high surface area, pore volume, and high porosity, which enables them to immobilize more enzymes with high loading efficiency. The ordered crystal structure and adjustable height of MOFs can create a more favorable microenvironment for enzymes. It can prevent substances larger than the pore size from contacting the enzyme, thus limiting the size of the substance [4]. It can prevent the enzyme from being exposed to adverse harsh environments, thereby protecting the fragile enzyme [4]. Meanwhile, the tiered porous structure induces more efficient delivery of the enzyme, thus improving the efficiency of the system [4]. These effectively improve the selectivity, stability and activity of the enzyme [4]. Overall, MOFs have outstanding advantages over conventional vectors. The diverse structure and function of MOFs make the enzyme immobilization step simple, and their easy modification indicates that they can be a good enzyme immobilization scheme. From the perspective of detection ability, they are high-quality materials for making enzyme biosensors.

This research will provide a concise summary and review of the current research results of MOFs as enzyme immobilization substrates. Firstly, four enzyme immobilization methods using MOFs as carriers (in situ encapsulation, surface attachment, covalent conjugation and entrapment) are highlighted. Secondly, the applications of enzyme-MOFs biosensors in the environment (including environmental pollution monitoring and pollutant degradation applications), food (including food safety detection applications), and medicine (including glucose detection and drug delivery applications) are highlighted. Finally, the technical development in this field of enzyme-MOFs biosensors and the discovery of new curing materials and the exploration of new curing modalities are prospected.

2. Methods for enzyme immobilization by MOFs

For immobilization materials, MOFs stand out from traditional enzyme substrates due to their various advantages. In terms of immobilization methods, there are four mainstream methods for solidifying enzymes in MOFs, which can be divided into two categories: one is adding and solidifying enzymes at the source of MOFs synthesis, including in situ synthesis. Another category is the introduction and solidification of enzymes in the finished product of MOFs, including surface immobilization, covalent binding, and entrapment [1]. In the following section, these methods will be discussed in turn, and the principle, application, advantages and disadvantages of each method will be introduced. Fig. 1 shows an overview of the four enzyme immobilization methods [5].

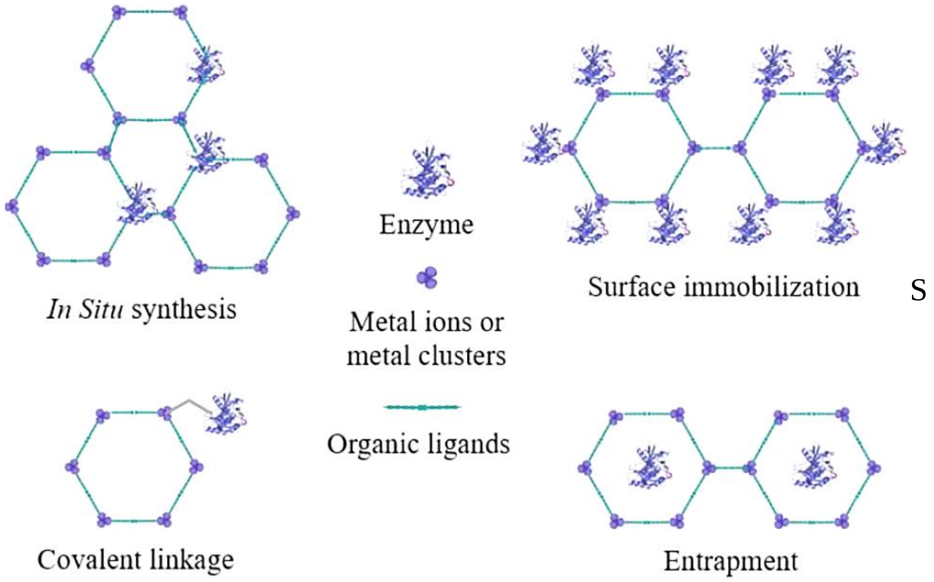

Fig. 1 Four enzyme immobilization methods [5].

2.1. In situ encapsulation

This approach is the latest approach to packaging enzymes in MOFs. In this method, metal particles and organic ligands that have not yet synthesized MOFs need to be put into a suitable solution under mild and suitable conditions, the desired target enzyme is added and mixed. In this way, the nucleation and enlargement of MOFs and enzyme immobilization will occur simultaneously, and enzyme molecules with radii larger than the outer channels of the MOFs will be embedded in the MOFs.

Wang et al. used zeolite imidazole salt framework (ZIF)-8 as substrate to package Candida Antarctic lipase B (CalB) in organic solvent at suitable temperature [6]. The stability of the system was then tested, and it was concluded that both the stability and activity of the immobilized enzyme in the system were higher than in its free state. This indicates that the immobilization of the enzyme has a good protective effect on the enzyme, which can enable the fragile enzyme to maintain good performance in poor solvent environment. It also revealed the feasibility of using the co-precipitation method to solidify the enzyme.

Although in situ synthesis plays a great role in protecting the enzyme properties and the enzymes in MOFs can be easily recycled, this method requires simple biocompatible synthesis conditions, so only a few MOFs can meet the requirements [1]. At the same time, the different random spatial position of the enzyme will also affect the later reaction [7]. Therefore, the addition of the concentration of metal particles and organic ligands and the localization of the enzyme need to be studied in the future.

2.2. Surface attachment

This is the most commonly used method, and the enzyme is physically adsorbed on the surface of MOFs mainly through weak interaction forces such as hydrogen bonding, electrostatic force, and van der Waals forces [8].

Pang et al. used a surface immobilization method to immobilize laccase on a novel bimodal microporous-mesoporous zirconium MOFs (Zr-MOFs) [9]. The laccase also does not inactivate or cause channel blockage at high concentrations. Moreover, the state of immobilized laccases was much more stable than that of free laccases in harsh environment. At the same time, the enzyme in this system can maintain a certain activity after repeated use or long-term storage. Therefore, it can be concluded that Zr-MOFs is a good laccase curing material, and also reveals the feasibility of using physical adsorption method to cure enzymes. Although this method is less costly, does not require reagents, does little damage to the enzyme, and is easy to perform, it can also achieve good results.

However, the external environment has a great influence on this system. Once the factors affecting the enzyme such as temperature, pressure or pH value fluctuate, the enzyme may desorb from the carrier. And because of its weak force, there will be a problem of low adsorption efficiency. All these problems need to be improved.

2.3. Covalent conjugation

This is one of the strongest enzymes solidifying chemical effects. The amino group on the enzyme can often synthesize peptide bonds with the functionalized carboxyl bond of the chemically modified MOFs. Thus, unlike physical adsorption, covalently linked enzymes generally exhibit better stability [1].

Lou et al. used UiO-66-NH_2 MOFs as a substrate to decure soybean epoxide hydrolase (SEH), resulting in a novel nano-catalyst SEH@U10-66-NH_2, which exhibited extremely high SEH loading and enzyme activity recovery [10]. Immobilized SEH can maintain higher activity and stability than its free form. It can be concluded that UiO-66-NH_2 MOFs is a good SEH curing material, and also reveals the feasibility of using covalent binding method to immobilize the enzyme.

The covalent binding method has many advantages, such as increasing the stereospecificity of the enzyme and improving its stability. The enzyme can be better reused. The multi-point covalent bond is fixed, the chemical force is strong, the possibility of enzyme leakage is very low, and the enzyme can work normally in some adverse environments [11]. However, covalent binding also has limitations, such as requiring longer time and more complex steps. Sometimes it is necessary to modify the enzyme to expose functional groups available for binding [11]. In addition, the mobility of the enzyme will be limited, which may limit the occurrence of some conformational changes during catalysis, making the catalysis less efficient [10].

2.4. Entrapment

This method is used a lot between small objects. This is an irreversible process by which the enzyme is confined inside the MOFs by diffusion [1]. The polymer concentration used in MOFs can be adjusted to achieve the purpose of adjusting the pore size to prevent enzyme leakage.

Li et al. encapsulated organophosphatase hydrolase (OPAA) in mesoporous zirconia-MOFs [12]. The results showed that the synthesized vector had good enzyme carrying ability and good stability in many aspects. Therefore, it can be concluded that porous zirconium MOFs is a good OPAA curing material, and it also reveals the feasibility of using entrapment to cure enzymes.

The entrapment method has many advantages, including the large number of enzymes that can be loaded, the relatively low cost, the enzyme does not leak easily, and the ability to adjust the microenvironment in which the enzyme is located [11]. However, there are many disadvantages, for example, the choice of substrate has certain conditions. Because the extension of polymerization thickens the matrix, the mass transfer is reversed, so the degree of diffusion is not as good, and the enzyme does not work well [1].

3. Application of enzyme-MOFs biosensors

As a good biometric element, enzyme has the characteristics of high sensitivity, high efficiency and strong selectivity. However, some restrictive properties of enzymes prevent their wide application. MOFs-modified immobilized enzymes can effectively improve some application defects of free enzymes, making them show high activity, good stability and recyclability. In reality, enzyme-based biosensors are widely used in many fields. The applications of enzyme-MOFs composites in environmental, food and pharmaceutical fields are highlighted below.

3.1. Applications in environment

Environmental pollution has great harm to human living conditions and is not conducive to human health, which has been a problem that needs to be improved and solved for many years. Therefore, the monitoring, decomposition and removal of pollutants have become the focus of related research.

3.1.1. Environmental pollutant detection

Organophosphate compounds and phenols are major environmental pollutants [13], although there are many methods to detect these pollutants, such as quantification based on chromatographic principles [14]. However, these traditional techniques may have the disadvantages of troublesome sample pretreatment and complex technology.

Several biosensor devices that use oxidases, including peroxidase, laccase, and aldehyde dehydrogenase have developed, which can be well applied to the detection of contaminants after immobilization with MOFs. These enzymes can be used for detection not because of their catalytic effect, but because the substrate inhibits the activity. That is, the substrate can be converted from inactive to having optical or electrochemical properties, so that the enzyme activity can be known [3]. This method is relatively less complicated to operate and has the prospect of replacing the traditional methods.

3.1.2. Pollutant degradation

Organic pollutants can be treated by diluting them and adding enzymes capable of hydrolyzing the waste. By solidifying these enzymes with MOFs, they can play a monitoring role. Because enzymes are specific and MOFs are also highly tunable, this technique can be used for specific compounds. Ahuja et al. pointed out that some contaminants including organochlorines, hexane, toluene, polycyclic aromatic hydrocarbons, naphthalene, metals, and some radioactive substances can be detected using this enzyme-MOFs biodevice [15]. Compared with traditional technology, this technology has a wide detection range and relatively low cost, so it can be widely used to achieve the purpose of efficient pollutant removal.

3.2. Applications in food

Because of the gradual improvement of living and consumption levels, food safety issues closely related to people’s health have attracted more and more attention. Bacterial contamination, excessive or substandard food additives and the addition of heavy metal ions can lead to food contamination [16]. Overuse of pesticides and pesticides can also lead to excessive chemical residues. Accurate detection of food safety is an important and urgent issue. There is a need for more sensitive, faster, more ubiquitous and cheaper methods. Enzyme-MOFs complex has been widely used in monitoring and screening food contamination due to its high sensitivity. For example, an enzyme-MOFs electrochemical sensor was used to detect lead content in leafy vegetables [17]. MOFs have a very good prospect as a curing enzyme material, and its high adaptability and adjustability are of great value in the field of food safety.

3.3. Applications in medicine

3.3.1. Glucose measurement

Glucose measurement is used to evaluate the glucose metabolism status of the body, help determine the causes of glucose metabolism disorders, assist in diagnosis and guide treatment. Liu et al. immobilized GOx on a Zr-based multilayer porous biomimetic MOFs HP-PCN-224 (Fe) and utilized a simulated multienzyme system as a glucose biosensor [18]. The modulator dodecanoic acid (DA) was introduced to reduce the input ligand to cause MOFs to form defects, and then hydrochloric acid was used to remove DA. The porous structure of HP-PCN-224 (Fe) was prepared. The formation of pores in MOFs was closely related to the amount of DA. The intrinsic peroxidase-like activity of HP-PTN-224 (Fe) cascade showed a clear linear relationship in a certain concentration range in glucose detection, which proved the good feasibility of glucose biosensor application.

3.3.2. Drug delivery

The development of macromolecular encapsulation technology can solve the problem of drug delivery *in vivo* [19]. That is, the biomolecules themselves can be combined with the MOFs to form porous capsules containing drugs [20]. As the MOFs degrades or the pore size increases, the drug disconnects from the complex and reaches the corresponding transport site [19]. This is obviously efficient because MOFs have many pores and can also be well controlled and regulated. The problem with this technique is that the metabolism of the compound may lead to toxic metal release. Therefore, minimizing the generation of such toxic effects will be one of the follow-up research goals of this technology.

4. Conclusion

This research analyzed the necessity of enzyme immobilization based on the characteristics of enzymes and the importance of immobilized materials and illustrates some advantages of MOFs as promising materials. In situ encapsulation, surface attachment, covalent conjugation and entrapment are introduced in this research, and the applications that can prove their feasibility are listed. The strengths and weaknesses of these methods are subsequently analyzed. At the same time, the applications of enzyme-MOFs biosensor in three aspects of environment, food and medicine, such as environmental pollutant monitoring, pollutant decomposition, food safety detection, biological index (glucose) detection and drug transportation, are listed. The advantages and prospects of the application technology are pointed out.

Although MOFs have many advantages in the fabrication of enzyme biosensors, the technology is not yet mature, and there are still some obstacles to be overcome, such as diffusion of enzymes during immobilization, low stability of enzymes, and challenges in transduction steps. However, enzyme-MOFs biosensors can be potentially applied to a variety of technical fields in the future, which is worthy of in-depth research. Finally, it will look forward to the improvement of immobilization technology in the future, and more optimal enzyme immobilization materials can be discovered.

References

[1] Xia H, Li N, Zhong X, et al. Metal-organic frameworks: a potential platform for enzyme immobilization and related applications. Frontiers in Bioengineering and Biotechnology, 2020, 8: 695.

[2] Feng Y, Xu Y, Liu S, et al. Recent advances in enzyme immobilization based on novel porous framework materials and its applications in biosensing. Coordination Chemistry Reviews, 2022, 459: 214414.

[3] Souza J E S, Oliveira G P, Alexandre J Y N H, et al. A comprehensive review on the use of metal–organic frameworks (MOFs) coupled with enzymes as biosensors. Electrochem, 2022, 3(1): 89-113.

[4] Wei T H, Wu S H, Huang Y D, et al. Rapid mechanochemical encapsulation of biocatalysts into robust metal–organic frameworks. Nature communications, 2019, 10(1): 5002.

[5] Silva A R M, Alexandre J Y N H, Souza J E S, et al. The chemistry and applications of metal–organic frameworks (MOFs) as industrial enzyme immobilization systems. Molecules, 2022, 27(14): 4529.

[6] Wang Y, Zhang N, Tan D, et al. Facile synthesis of enzyme-embedded metal–organic frameworks for size-selective biocatalysis in organic solvent. Frontiers in Bioengineering and Biotechnology, 2020, 8: 714.

[7] Wu X, Yang C, Ge J. Green synthesis of enzyme/metal-organic framework composites with high stability in protein denaturing solvents. Bioresources and bioprocessing, 2017, 4: 1-8.

[8] Cui J, Ren S, Sun B, et al. Optimization protocols and improved strategies for metal-organic frameworks for immobilizing enzymes: Current development and future challenges. Coordination Chemistry Reviews, 2018, 370: 22-41.

[9] Pang S, Wu Y, Zhang X, et al. Immobilization of laccase via adsorption onto bimodal mesoporous Zr-MOF. Process Biochemistry, 2016, 51(2): 229-239.

[10] Cao S L, Yue D M, Li X H, et al. Novel nano-/micro-biocatalyst: soybean epoxide hydrolase immobilized on UiO-66-NH2 MOF for efficient biosynthesis of enantiopure (R)-1, 2-octanediol in deep eutectic solvents. ACS Sustainable Chemistry & Engineering, 2016, 4(6): 3586-3595.

[11] Maghraby Y R, El-Shabasy R M, Ibrahim A H, et al. Enzyme immobilization technologies and industrial applications. ACS omega, 2023, 8(6): 5184-5196.

[12] Li P, Moon S Y, Guelta M A, et al. Encapsulation of a nerve agent detoxifying enzyme by a mesoporous zirconium metal–organic framework engenders thermal and long-term stability. Journal of the American Chemical Society, 2016, 138(26): 8052-8055.

[13] Salouti M, Khadivi Derakhshan F. Biosensors and nanobiosensors in environmental applications. Biogenic nanoparticles and their use in agro-ecosystems, 2020: 515-591.

[14] Cruz-Navarro J A, Hernandez-Garcia F, Romero G A A. Novel applications of metal-organic frameworks (MOFs) as redox-active materials for elaboration of carbon-based electrodes with electroanalytical uses. Coordination Chemistry Reviews, 2020, 412: 213263.

[15] Ahuja S K, Ferreira G M, Moreira A R. Utilization of enzymes for environmental applications. Critical reviews in biotechnology, 2004, 24(2-3): 125-154.

[16] Liu C S, Sun C X, Tian J Y, et al. Highly stable aluminum-based metal-organic frameworks as biosensing platforms for assessment of food safety. Biosensors and Bioelectronics, 2017, 91: 804-810.

[17] Bhardwaj R, Sharma T, Nguyen D D, et al. Integrated catalytic insights into methanol production: Sustainable framework for CO2 conversion. Journal of Environmental Management, 2021, 289: 112468.

[18] Liu X, Qi W, Wang Y, et al. Rational design of mimic multienzyme systems in hierarchically porous biomimetic metal–organic frameworks. ACS applied materials & interfaces, 2018, 10(39): 33407-33415.

[19] Falcaro P, Ricco R, Doherty C M, et al. MOF positioning technology and device fabrication. Chemical Society Reviews, 2014, 43(16): 5513-5560.

[20] Filik H, Avan A A. Nanostructures for nonlabeled and labeled electrochemical immunosensors: Simultaneous electrochemical detection of cancer markers: A review. Talanta, 2019, 205: 120153.