Enzyme Immobilization Technology to Treat Emerging Pollutants

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Abstract Human health has been adversely affected by widespread industrialization and urbanization contaminating the environment with xenobiotics. Although there are many physical and chemical methods for managing xenobiotic pollution, bioremediation appears to be a sustainable solution from an environmental perspective. An inexpensive, environmentally friendly method of converting xenobiotics into less hazardous or nontoxic forms is to use microorganisms, plants, or their enzymes. Compared to microorganisms or phytoremediation, enzyme-based bioremediation is more effective at breaking down pollutants with less waste generated. However, enzymes have a number of drawbacks, including low stability (storage, pH, and temperature), limited reuse potential, and difficulty separating them from reaction media. It may be possible to solve the issues and increase reusability by facilitating the separation process and immobilizing enzymes without compromising their activity by immobilizing enzymes without impeding their activity under various environmental conditions. This chapter discusses the benefits and drawbacks of enzyme immobilization and its future in bioremediation, as well as carrier selection, immobilization techniques, as well as immobilization techniques and their application to bioremediation. For the immobilized systems to maximize their potential for extensive industrial wastewater treatment, this assessment identifies upcoming trends and problems that must be addressed quickly.

Keywords Enzyme immobilization · Bioremediation · Hazardous pollutants · Wastewater treatment

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1 Introduction

As a result of human activities, ecosystems have become contaminated, which has negatively affected humans and other animals (Yaashikaa et al. 2022). Many types of pollutants have been found in most water sources, such as pesticides, dyes, heavy metals, medicines, and other emissions from personal items. Uncertainty surrounds the presence of these contaminants in water sources, but they are quantifiably present, changing the quality of the water and raising the danger to humans and other creatures who ingest polluted water. The aforementioned pollutants may be categorized as emerging pollutants and fall under WHO Category 1 carcinogens due to their lethal characteristics. Lack of current technologies to remove or destroy pollutants from the sources is the main contributor to this problem (Saravanan et al. 2021). Traditional methods for removing these dangerous contaminants have led to the pollutants' development of resistance to these techniques and their ineffective removal. These procedures were less effective since they generated waste byproducts along with additional waste materials. Traditional methods require using a lot of power, which is expensive to produce, to address these restrictions (Chauhan et al. 2012; Kaur et al. 2021).

Secondary waste products are produced as a result of methods such as chemical flocculation and filtering. As a result, it is critical to develop new innovative processes which are environment friendly, feasible, and waste-free. Microorganisms are used in biological treatments to remove pollutants. This approach entails using bacteria, fungi, algae, yeasts, and enzymes for bioremediation (Mahmoodi et al. 2020). Nevertheless, microbiological technologies, while environmentally beneficial, have downsides such as sluggish processing, time consumption, inefficiency in surviving under dangerous pollutant concentrations, and the need for upkeep. Immobilized enzymes are currently employed to remove hazardous contaminants from the environment, which is a significant advancement. Different enzymes, such as peroxidases, laccases, manganese, tyrosinase, and others, performed remarkable good in waste removal because they are easier to handle, monitor, and manage. The use of enzymes has several advantages, including less waste generation, a low energy input demand, decreased toxicity, and the capacity to work at optimal and moderate circumstances. Due to their instability and poor reusability, the employment of flowing freely enzymes is constrained in large-scale industries (Zhang and Wang 2021). Immobilized enzymes are commonly used to increase enzyme stability, catalytic activity, and efficiency. Immobilizing enzymes results in heterogeneous catalysts with improved stability under unfavorable circumstances, leads to better and effective results and a significant reduction in cost (Jenjaiwit et al. 2021). The effectiveness of immobilized oxidoreductases in removing rising contaminants has been demonstrated. However, the choice of substrate and the immobilization method needs to be carefully selected to minimize the negative effects of the process on the enzyme's catalytic function. Biocatalyst deactivation is the main source of dispute in enzymatic treatment procedures. This is brought on by alterations in the enzyme binding site brought on by enzyme denaturation at high temperatures or pH. The enzyme loses its ability to bind to the substrate when an active site is changed. Both competitive and noncompetitive situations can result in inhibition. When inhibitors bind to allosteric sites, they cause noncompetitive inhibition, while competitive inhibition occurs when the inhibitor competes with the substrate for binding to the enzyme's active site (Ariaeenejad et al. 2021).

This book chapter shows how immobilized enzymes may be used to remove harmful and hazardous contaminants from the environment. Researchers have recently been interested in the removal of environmental contaminants utilizing immobilized enzymes. This study also looks at the many types and classifications of backbone materials to immobilize enzymes. A comparison of the difficulties and developments of immobilized enzymes with unbound enzymes is explored. The elimination of various environmental toxins using immobilized enzymes is also demonstrated. The combination of the aforementioned problems sets this review apart from other pieces of literature.

2 Water Pollution's Origins and Consequences

Both point sources and nonpoint sources of pollution can affect water sources. Point source contaminants can originate from a single location, such as a manufacturing outlet, an oil spill, and the release of industrial waste. Point sources can include industrial and municipal effluents. Pollutants in nonpoint sources originate from a variety of sources and enter water supplies in a variety of ways, where they remain undetected in the ecosystem. Transboundary pollutants (e.g., radioactive waste) are present in the environment and have longer consequences. Organic or inorganic contaminants can be found in ground or surface water (Ferreira et al. 2016). Diseases, chemical substances, bacterial communities, pesticides, herbicides, and other substances fall within the organic pollutants category. Heavy metals, dyes, fertilizers in agricultural runoff, and chemical leakage from industrial wastes are other examples of inorganic pollutants. Urbanization is another source of pollutant discharge, resulting in increased quantities of phosphorous deposition, particularly in metropolitan areas. The major causes of this emission include industrial and municipal discharges, as well as urban runoff.

Solid waste is another problem (Delgado-Povedano and De Castro 2015). The large output of organic and biodegradable wastes resulted in inefficient solid waste management. As a result, most rubbish is not properly disposed of, resulting in the release of significant amounts of harmful contaminants into the environment. Sewage may sometimes be used as fertilizer since it contains a large number of nutrients such as phosphorus and nitrogen, which are essential for plant and animal growth. Chemical fertilizers and pesticides are also a factor in the infiltration of minerals into the ground in agricultural areas, which promotes the growth of algae and reduces the oxygen content of the water, ultimately killing aquatic life forms (Kujawa et al. 2021). Paper, leather, and steel are examples of industrial sectors located on the banks of water sources.

Industries use a large amount of water for numerous operations and discharge effluents including colors, acidic solutions chemical substances, and bases into flowing water supplies. Chemical businesses that primarily manufacture aluminum discharge significant fluoride levels into water and, in certain cases, into the atmosphere. Similarly, steel plants emit cyanide, while fertilizer plants emit ammonia. There is a change in the release of large amounts of chromium salts into water as these salts are used to make sodium dichromate (Zamel and Khan 2021). These toxins are all released into the water stream from various water sources, harming both people and other living things.

The agricultural sector contributes significantly to the pollution of the rivers. To boost crop output, chemical-based fertilizers, herbicides, and insecticides are utilized. Excessive pollutant concentrations are deposited in water bodies as a result of improper handling and removal procedures for these chemical pesticides from agricultural areas (Pokorna and Zabranska 2015). Several operations, such as runoff from the fields, spraying, and washing, pollute and degrade the quality of the water. There are many pollutants that are persistent, biodegradable, and affect the environment for a long time.

The human food chain is contaminated with toxic substances, which leads to biomagnification. Any variation in temperatures of the water has an impact on water quality and puts aquatic life in peril. Anthropogenic activity is mostly to blame for pollution deposition and water contamination. Industrial boilers, steel melting, electric and nuclear power plants, and petroleum refineries are some of the sources of heat that release high temperatures into water sources and alter their physical and chemical properties (Rahmani et al. 2020). Low oxygen level in hot water affects aquatic species' physiological processes such breathing, reproduction, digestion, and digestion.

3 Traditional Treatment Methods

Physical, chemical, and mechanical treatments are all used in traditional wastewater treatment systems. Coagulation, sedimentation, and filtration are examples of physical techniques. Precipitation and adsorption are used in chemical procedures, whereas screening and filters are used in mechanical methods. The physical technique includes removing contaminants from wastewater without affecting the biological characteristics of the pollutant. This approach is straightforward, practicable, and produces less solid waste; nonetheless, it necessitates the use of people and energy. For pollution removal, the chemical approach employs chemical substances. Adding target substances separates the dissolved contaminants. Although chemical treatment systems may efficiently handle industrial and agricultural waste, the deposition of large amounts of chemical substances in agriculture area degrades fertility of soil and mixes with flowing water, generating contamination.

4 Microbial Treatment Methods

Utilizing microorganisms to remove toxins is part of the biological remediation process. The creation of by-products and secondary wastes, the usage of hazardous chemical compounds, the expensive cost of equipment, and other issues make physical and chemical cleanup methods less desirable (Peng et al. 2021). As a result, increased emphasis is being placed on biological approaches to address these limitations. Biological treatment technologies are tested in both laboratories and large-scale companies to ensure their efficiency and effectiveness. This treatment strategy can be applied in the following manners: either by employing enzymes to remove toxins or by using microorganisms to treat pollutants. The most frequently utilized microbial populations for remediation are *Streptomyces* and *Pseudomonas*, whereas *Pleurotus* and *Trametes* are the most frequently used fungal populations. The cleanup procedure can either be aerobic in nature or anaerobic, or an amalgamation of both, according to the type of microorganism engaged. When oxygen is not used during the anaerobic phase, poisonous aromatic amines may be produced. In comparison, the aerobic process does not produce extremely hazardous chemicals but takes longer to complete (Wang et al. 2022).

Microorganisms aid in the removal of harmful contaminants found in wastewater; however, they are unsuccessful in the removal of colors, phenolic, medicinal drugs, and other chemicals. This might be owing to the substance's resistance to microbiological treatment procedures. Second, the use of enzymes such as peroxidases, lactases, and tyrosinase as an alternate tool for increasing the efficacy of the biological therapy process. In aromatic molecules, specially phenolic and nonphenolic compounds, these enzymes catalyze an oxidation-reduction process. The catalytic activity of enzymes is primarily responsible for breaking down developing pollutants (Awad and Mohamed 2019). The decrease of extremely dangerous chemicals caused by the catalytic function of enzymes leads to the creation of products with fewer hazards and a significant degree of contaminant removal. The mixture's less dangerous elements are hard to separate from one another. The instability and restricted reusability of free-moving enzymes is a problem that also exists with their utilization. The solution selected to solve these drawbacks is immobilization. This method increases the reliability and versatility of the enzyme over multiple cycles (Primožič et al. 2020).

In addition to membrane bioreactors, enzyme immobilization methods can be used to develop biodegradation and bioremediation approaches. The foundation that is used for immobilization determines the procedure efficiency. Materials that are organic, inorganic, hybrid, and composite can be used. Materials should have characteristics like biocompatibility, resistance to mechanical stress, strength, and structural resistance.

4.1 Disadvantage of Microbial Treatment Methods

It has been established that using microbial remediation for dangerous pollutant decontamination has drawbacks, such as preventing microbial growth and possibly eliminating the microbe. Additionally, a number of factors, such as pH levels, amount of nutrients, and the outside temperature, have an impact on microbial development and are crucial for achieving the best possible growth of microorganisms and, as a result, the efficient decontamination of dangerous chemicals. In addition, the kind and chemical content of the waste, its solubility, and its interaction with bacteria all have a significant role in the decontamination of dangerous contaminants by microorganisms (Bharagava et al. 2018).

5 Plant-Based Treatment Methods

In order to reduce pollution in polluted areas, phytoremediation is the process by which plants engage in chemical, biochemical, physical, microbiological, and biological activities. Depending on the type of pollutant, combustion, cleaning, deterioration, and stabilization techniques take place. Pollutants might be organic or elemental. As opposed to organic contaminants like chlorinated compounds and hydrocarbons, which are typically removed using combustion, rhizoremediation, stabilization, and deterioration, elemental pollutants like radionuclides and toxic heavy metals are typically removed using transformation, sequestration, and extraction methods (Thakare et al. 2021). When plants like willow and lucerne are employed, mineralization is also a possibility. A variety of factors must be considered when selecting a phytoremediation, including the type of root system, biomass above ground, plant survival and development rate, environmental conditions, the toxicity of the pollutants, and, most importantly, the length of time required for the bioremediation process. The plants employed are also resistant to diseases and pests. The passive absorption of contaminants by the roots, which are subsequently transferred to the shoot via the xylem, is the basic process of phytoremediation. Subsequent accumulation and transport are affected by xylem and adjacent tissue division, transpiration rate, plant type, and contaminated environment features (Thakur et al. 2016). The success of phytoremediation is dependent on increasing native plant remediation capacity by bio-stimulation or bio-augmentation using exogenous or endogenous plant rhizobacteria. Plants that grow in contaminated environments are typically considered to be superior phytoremediators. Plant growth-promoting rhizobacteria (PGPR) improve plant resistance to heavy metals and edaphic conditions while boosting biomass production. Exogenous plant growth-producing rhizobacteria have also been demonstrated to stimulate stem and root development in *Festuca ovina* L. and *Brassica napus* L. plants, which in turn increases plant length and lowers growth inhibition. Similar to this, *Spartina maritima* plants used in the bioaugmentation of endogenous PGPR in heavy metal-contaminated estuaries led to an increase in metal accumulation and removal (Azubuike et al. 2016). Plants have been discovered to be the most cost-effective and long-lasting phytoremediation alternatives; however, field research has revealed that the bioremediation process is extremely sluggish and plant tolerance is minimal. To circumvent the limitations of native plants, transgenic plants with higher bioremediation effectiveness were developed (Rai et al. 2020).

5.1 Disadvantage of Plant-Based Treatment Methods

The main disadvantages of plant-based bioremediation methods, according to Shukla et al. (2019), are that only a limited number of compounds are biodegradable, that the end products of the bioremediation processes are occasionally more toxic than the original pollutant, that the reaction processes are specific and thus require specific biological agents, that scaling up plant-based bioremediation is difficult and time consuming, and that issues can arise when bioremediation is not a component of phytoremediation; it mostly includes bioaccumulation. In addition to the right growing conditions, plants need a variety of growth nutrients.

6 Immobilization of Enzymes: Characteristics and Approaches

6.1 Properties

Enzymes are a more effective way to get around the drawbacks of using microorganisms. Enzymes function as effectors in all transformation processes and have a variety of beneficial qualities (Masjoudi et al. 2021). Enzymes can be used as catalysts for a variety of compounds because of their broad or narrow specificity. These enzymes alter the toxicological and structural characteristics of pollutants, aid in the complete transformation of harmful compounds into less dangerous forms, and result in the production of a variety of by-products. Enzymes offer more advantages than conventional and microbiological methods. Figure 1 depicts the mechanism of pollution removal by an immobilized enzyme. Microbial metabolism inhibitors do not block enzymes. In bad conditions, the inhibitors can be used to limit microbial activity. Enzymes are more effective at lower dangerous dosages and are more active when microbial competitors are present. Enzymes can function in free or immobilized form, intracellular (in the presence of originating cells) or extracellular (in the absence or presence of originating cells). The laboratory testing of immobilized enzymes showed that both the temperature, time and stability of immobilized enzyme have significantly impact on xenobiotic compounds transformation. The enzyme activity is kept constant throughout the process. When enzymes are immobilized,

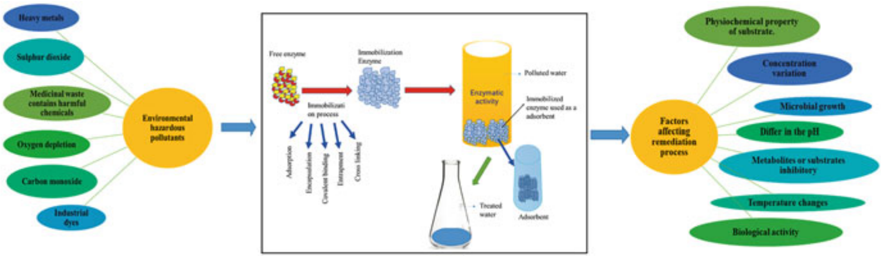

Fig. 1 Mechanism of pollutant removal by immobilized enzymes (Adapted from Yaashikaa et al. 2022)

the support material affects how the enzymes behave. These modifications in the properties of the immobilized enzyme may result from an interaction between the enzyme and the support material that is distinct from the reaction between the substrate and the enzyme in bulk solution. The multidimensional configurations of the protein, which are linked to the support material, may also be to blame for the modifications. Enzyme immobilization improves both the stability and ability to load of the enzymes, leading to controlled diffusion. The stability is improved by the number of bonds that are formed between the support material and the enzyme (Kahoush et al. 2021). Enzyme activity is destroyed when immobilized enzymes bind to substrates because their active sites are unable to bind to the substrate. It is claimed that throughout the process, the free enzyme's activity is maintained at normal conditions. On the other hand, the enzyme may lose its activity if it is immobilized on the support material. An enzyme's interaction with the support substance may be the cause of this.

6.2 *Methods*

Traditional approaches are excellent at removing toxins in large amounts, but unsuccessful at removing pollutants at ppm or ppb concentrations. During the immobilization process, enzymes connect to the support material in two ways: first, by binding to the surface of the support material, and second, by trapping (Zhu et al. 2020). The polymer materials used to bind the enzymes to the matrices in the first scenario can include biopolymers (such as agarose, chitosan, gelatin, and albumin), organic and inorganic polymers (such as alumina, silica, and zeolite), synthetic polymers (such as acrylic resins and beads), smart polymers (thermally stable and biocompatible), hydrogels, and others. The entrapment operation in the second method commonly employs solgel (metal alkoxide). Enzymes can diffuse over porous solid matrices without interacting with other molecules (Martínková et al. 2016). This approach of immobilization improves enzyme structural stability without sacrificing functional, thermal, or pH tolerance.

Ionic bonding, trapping, metal-linked, covalent bonding, adsorption, encapsulation, and other methods can be used to immobilize enzymes. Adsorption is the most adaptable, fundamental, and straightforward strategy for achieving outstanding results. This approach aids in the removal of contaminants even at ppm or ppb levels. The solid matrix is generated during the adsorption process utilizing a range of materials such as ion-exchange resins, calcium alginate, activated charcoal, and others. The matrix and enzymes are held together by weak forces like hydrogen bonds, van der Waals forces, hydrophobic bonds, or ionic interactions. Desorption processes also allow for the regeneration of adsorbent materials. Covalent bonding allows enzymes to be directly attached to matrices (such as porous silica, polyacrylamide, and porous glass) for increased stability and solid connections (Abdel-Shafy and Mansour 2016). The enzymes are confined within polymer membranes (natural or synthetic membranes) in the entrapment approach, enabling the product and substrate to flow through while inhibiting the enzyme inside the solid matrix. When compared to alternative immobilization procedures, this approach, which may be obtained by gel or fiber encapsulation, is rapid and easy. The primary disadvantages are enzyme deactivation and mass limitation.

Through a reversible ionic bonding process, the solid matrix and the enzyme establish a salt link. The two steps involved in affinity binding are the stimulation of the support material with the ligand for enzyme addition and the interaction of a modified enzyme with the matrix. Warming the metal ions during metal-linked immobilization causes metal salts to build on the matrix's surface (Zdarta et al. 2022). This approach has several advantages, including its simplicity, high enzyme activity, and reversibility. Adsorption appears to be a simple, inexpensive, and active immobilization approach for removing contaminants such as heavy metals from water sources when compared to other methods. Table 1 summarizes the advantages, disadvantages, and applications of various immobilization techniques.

7 Parameters Affecting the Activity of Immobilized Enzymes

Numerous factors, including pH, temperature, the method used to immobilize the enzymes, the choice of support material, the quantity of enzyme used in the procedure, and others, might have an impact on how well the immobilized enzymes work. pH and temperature are important because changes in these factors can impact total enzyme activity. Variations may result in enzyme deactivation or release from immobilized carriers. As a result, depending on the type of enzyme used, optimal pH and temperature must be maintained throughout the process. Methods for immobilizing enzymes have both advantages and disadvantages. If a proper approach is not employed, bonds may be broken, enzymes may be unbound from the substrate, recovery may be poor, or product yield may be low. Carrier materials are another factor that influences the activity of an immobilized enzyme. These

Table 1 Comparison of advantages, disadvantages, and applications of enzyme immobilization techniques (Adapted from Yaashikaa et al. 2022)

Immobilization method	Advantages	Disadvantages	Applications	References
Entrapment	• Operate in mild conditions. • Low cost and simple method.	• Leakage of enzyme. • Low level of pore diffusion.	• Food industry.	Sharma and Singh (2020)
Adsorption	• Less conformational changes during reaction. • High catalytic process. • Expansive materials are reusabale.	• Low level of efficiency. • Stability can be low. • Desorption of enzyme.	• Biotransformation occurs in the molecule.	Vo et al. (2020), Zhang et al. (2021)
Encapsulation	• Cell density maintains constant. • Expene-sive materials are usable. • Low MW compounds are easily movable.	• Small molecules can be easily cross from the membrane. • Limitation of pore size. • Mass transfer will be low.	• Biomedical field.	Li et al. (2020), Moreira et al. (2021)
Cross linking	• Strong binding of biocatalyst and leakages to be prevented. • Decrease in desorption.	• Low enzyme activity. • Diffusion may be less. • Formation of unwanted product.	• Production of secondary metabolite.	Lim et al. (2020), Olejnik et al. (2020)
Covalent binding	• Simple method and wide applicability. • Prevent the elution of catalytic activity. • Flexible enzyme and substrate concentration.	• Low effective process. • Low functional conformational. • Carrier molecules are not regenerated.	• Textile industry.	Sutanto et al. (2020), Gatou et al. (2021), Vasseghian et al. (2022)

compounds must be less toxic, more freely accessible, and enzyme-compatible. The immobilized enzymes may be affected by the characteristics and structure of the carrier materials. Enzyme inactivation occurs when there are too many enzymes present, which restricts the stretching of enzyme production. The performance of the immobilized enzyme is directly influenced by the quantity of enzymes immobilized on the support materials. Therefore, caution must be used when creating immobilized enzymes.

8 Advantages and Disadvantages of Immobilized Enzymes Versus Free Enzymes

8.1 Stability of Immobilized Enzymes

The main issue when employing enzymes in labs and large-scale operations is maintaining stability in adverse circumstances such as high and low pH and temperature, as well as the presence of oxidizing chemicals, solvents, and ionic liquids. Therefore, the most effective methods for tackling these stability difficulties are immobilization techniques and enzyme engineering (Boonnorat et al. 2016). Enzymes that are immobilized are less stable than unbound enzymes. Immobilized enzymes are found to have a larger portrait with little improvement and to be unaffected by pH variations. Horseradish peroxidase was immobilized using a magnetic graphene oxide composite that was developed by Ji et al. (2017) and its stability was investigated. The thermal stability of this enzyme was shown to increase when it was immobilized. Another research demonstrated the covalent stability of immobilized amylase onto organic frameworks. Using a simple adsorption immobilization approach, the stability of the immobilized enzyme was found to be higher than that of free enzyme. Covalent binding and adsorption were used to immobilize the laccase enzyme on a magnetic metal-organic framework (Zdarta et al. 2022). The enzyme showed great activity, enhanced resistance to changes in physicochemical parameters, and stability during heating and storage. After being immobilized and stored in ethanol, acetonitrile, and methanol, laccase activity was discovered. Immobilized laccase is simply removed from the reaction solution since it was coupled to a magnetic organic framework. The stability of immobilized laccase was similarly increased at elevated levels of salt. The ability of the enzyme to breakdown was unaffected by increasing the salt content, although it did affect the magnetic framework's adsorption. It was shown that immobilization increased structural stability. Recent studies have demonstrated that ionic liquids have a number of advantages over dangerous, flammable organic solvents, including stability, solubility, and catalytic activity. The stability of ionic liquids is significantly impacted by immobilization techniques.

Ionic liquids have been shown in studies to be effective at removing pollutants from wastewater. The extraction technique has recently gained attention; it is dependent on the type of solvents employed in the remediation operation. The interaction of the solute and solvent determines the effectiveness of the process, which is facilitated by properties such as viscosity, vapor pressure, and so on. In wastewater treatment operations, tricaprylmethylammonium methylthiosalicylate, tricaprylmethylammonium thiocyanate, methyltrioctylammonium chloride, trihexyltetradecylphosphonium methylthiosalicylate, and other ionic liquids are used. Ionic liquids have further advantages, but their high cost and laborious extraction prohibit them from being extensively employed. However, efficient optimization approaches for using ionic liquids as a medium for successfully removing pollutants from wastewater can be developed in the future.

8.2 Enzyme Evolution and Engineering

The creation of specific and advanced enzymes is now possible because of recent discoveries and breakthroughs in enzyme engineering. Enzyme physicochemical properties and catalytic activity are being improved by the introduction of mutations (Miri et al. 2021). These changes improved stability in adverse conditions, including low pH, high temperature, excessive amounts of salt, solvent content, and the amount of substrate. Direct evolution and rational design are the two basic strategies used in enzyme engineering. Changes in the direct evolution approach were caused by either random mutations or gene shuffles. This approach has the benefit of requiring no structural knowledge and allowing alterations to be made anywhere, whether near to or far from an enzyme's active site (Ameri et al. 2021). Apart from the advantages, the practical application of this technology is time utilization, the requirement for high-throughput testing, and dependability. In rational design, site-directed mutagenesis is employed to boost the probability of finding advantageous mutations. In these techniques, the structure of enzymes is used to identify the site of the mutation. De novo enzyme design is the result of this method, which creates novel enzymes with greater activity than native enzymes. To boost the activity, the active site of an enzyme can be altered or modified (Shchemelinina et al. 2021). Oxidoreductases are the enzyme that is most frequently employed to clean up filthy water. This enzyme can catalyze oxidation reactions in both organic and inorganic substrates due to its high redox potential. For instance, peroxidase uses hydrogen peroxide as an oxidizing agent to oxidize a variety of substrates.

8.3 Immobilized Enzyme Reusability and Recycling

The immobilization of enzymes onto the membranes as a foundation has been the subject of recent research. Benefits of membranes include their composition, permeability, large surface space for efficient enzyme enforceable, and pore size. These characteristics facilitate the accessibility of the enzyme's active regions to the components of the reaction solution. To suit the demands of the user, the setup and design may be altered. The reuse of immobilized enzymes through multiple reactions is made possible by membranes. Due to their inertness, affordability, nontoxicity, durability against microbial community and degradation, and environmental friendliness, hard support structures can also be employed for enzyme immobilization (Chen et al. 2016). Throughout the operation, these tough materials support and preserve the enzyme immobilized on them from severe environmental influences. Immobilization can be achieved using minerals, carbon and metal oxides, aluminum oxide, quartz, and other inorganic elements. Because of its features, including its large surface area, the presence of surface hydroxyl groups, and chemical and heat tolerance, silica is the most often utilized inorganic material for immobilization. Enzymes can be immobilized on silica gel, solgel silica, and fumed silica. Pollutant breakdown was considerably increased when the soybean peroxidase enzyme was immobilized on titanium inorganic oxide (Sohrabi et al. 2016). Another work exhibited manganese peroxidase immobilization onto ferric oxide composite for dye degradation, including reactive orange and methylene blue. This backing material was recycled around five times before a magnet was used to pull it out. Another study found that the immobilized peroxidase enzyme's first cycle of reusability for removing pollutants with nanomaterials achieved 93%. No enzyme activity was seen during the sixth or seventh reusability cycles. The second elimination step with a 50% reduction in reuse material (Torres et al. 2018).

As a result, when the accumulation response serves as a support, the enzyme activity denied the enzymatic reaction's product. As a result, in the subsequent cycle phase, the immobilized enzyme peroxidase has less ability. It may result in higher ability in recycling, according to several research on the reusability of the immobilized laccase enzyme (Xu et al. 2017). 95% of items are reusable the first time, whereas 66% are used more than seven times. According to the investigation, the more efficiently polluted effluent was removed utilizing nano-copper (Adamian et al. 2021).

8.4 Cost Comparison

Enzymes are valuable biocatalysts used in a variety of industrial operations. They are a superior option to chemical catalysts because of features such as resource availability, substrate selectivity, and low waste creation. At mild temperature and pH conditions, industrial enzymes outperformed standard catalysts (Vareda et al. 2016). The cost-effectiveness of enzyme-linked biological catalysts in industrial applications is still a subject that has to be explored. Enhancing stability is crucial to boost cost-effectiveness since adverse harsh conditions like high temperature and pressure as well as changing pH can cause enzymes to become inactive. Indirect measures of overall product yield such as enzyme specificity, reaction kinetics, and the number of cycles the enzyme has been utilized determine the cost of an immobilized enzyme. The main problem with using immobilized enzymes at the industrial level is the difficulty of calculating the entire economics of the process, including all expenses like liberated enzyme, ingredients for immobilization, recuperation, bioreactors, and carriage regeneration. As a result, both technical and economic aspects must be improved in order to use immobilized enzymes as biocatalysts for waste removal. The lifecycle assessment (LCA) is used to analyze the sustainability and overall cost.

LCA may also be used to assess the environmental effect by accounting for energy use, harmful product discharge, and chemical compound addition (Richardson et al. 2016). Techno-economic analysis can be used to look into the use of enzymes in large-scale companies. Utilizing enzymes that have been immobilized can improve cost-effectiveness. Despite being costly, the immobilization approach yields enzymes with higher stability, recyclability, and recovery.

8.5 Scaling-up of Bioreactors

Environmental toxins are being removed using bioreactors. The biological system being researched should be the major emphasis of the bioreactor design, along with determining the elements that need to be handled, such as service and expenditures on capital, scaling-up, security, and other factors (Jeon and Cha 2015). A few prerequisites must be satisfied before constructing an immobilized enzyme bioreactor. Maximum catalytic activity of the enzyme is required, and other requirements include resistance to force, recuperation and reuse ability, expense for manufacturing and construction, temperature of the reaction, and simplicity and ease of operation of the process. Mixed tanks bioreactors, static beds or stacked row bioreactors, and fluidized bed bioreactors are the most common bioreactor forms for constructing an immobilized enzyme bioreactor.

8.6 Current Advances in Designed Enzymes

The enzyme immobilization process could be applied with nanospace material to enhance the capability, security, and efficacy of industrial wastewater treatment. Recent studies have shown that the organic system may operate in a variety of ways, including covalent organic systems, metal-organic systems, and hydrogen-bonded organic systems (Wilson et al. 2022). In the meantime, immobilization based on the food business was introduced employing cutting-edge and various technology. To migrate with great effectiveness and performance, they use a carrier such as nanomaterials and agricultural waste products. Due to its effectiveness, large surface area, security, active site, and permeability, which would enable the transporter of an immobilized enzyme to pass through, the metal-organic system has drawn a lot of interest (Chauhan and Saxena 2016; Chauhan and Gupta 2017a; Chauhan et al. 2017b; Chauhan 2020; Xie et al. 2022).

9 Role of Immobilized Enzymes in Metal Removal

9.1 Lipases

The bulk of fats are digested by hydrolase enzyme-like lipases, with the largest source of lipase enzyme being the human pancreas. Lipase is found in a wide range of industries, including cosmetics, medicines, organic synthesis, detergents, and food (Niu et al. 2016). Many studies on immobilized lipases enzyme heavy metal removal have been carried out. Nickel ions were entangled by the nanoparticle chitosan after nickel was removed from the effluent throughout the experiment (Yang et al. 2016). Ion precipitation was achieved using entrapment, and the ceramic matrix was appropriately mixed. Lipase enzymes are immobilized using a ceramic doped with nickel ions (Ni-CP). The study discovered that 99.4% of the enzymes could be extracted with maximal efficiency, whereas 65% of the activity of the immobilized enzyme could be seen under optimal conditions. In the aforementioned doped ceramic nickel lipase, the enzyme responsible for recycling the doped nickel more than 20 times has great temperature stability and may produce an adsorbent with a 98% reusability (Kureel et al. 2016). The entire inquiry was improved, and it was discovered that the lipase enzyme is engaged in nickel ion elimination.

9.2 Ureases

Urease, a separate enzyme, has catalyzed the hydrolysis of urea, resulting in CO_2 and NH_3. It is a metalloenzyme that is nickel-dependent. Plant seeds, soybeans, and jack beans are the primary suppliers of the enzyme, in addition to animal tissues and gut microorganisms (Gupta et al. 2021). The urease enzyme's primary role is assumed to be therapeutic. There has been little study on the immobilized urease enzyme in metal removal, and few studies have evaluated the fluctuations in metal-binding capacity and enzyme activity during the inhibitory period (Yan et al. 2013). Several studies have demonstrated that immobilized urease enzyme may remove heavy metals. *Bacillus badius* enzyme was isolated and immobilized utilizing hydrosol gel technology and a nylon membrane. Lead ions are present in the milk sample, and the absorbent can persist up to 2 months (Jegannathan and Nielsen 2013). In another research, the stability of the immobilized urease enzyme is investigated when elements such as copper ions and mercury are removed. It was proved that it was employed with the magnetic nanomaterial that was treated with the thiol and amino group under the name of siloxane layers employing solid matrix and the surface grafting technique of immobilization. The experiment improved the enzyme's stability following immobilization (Li et al. 2013). After immobilization, 65% of the predicted enzymatic activity remained, whereas trace amounts of copper and mercury ions remained. The activity of the natural enzyme is lowered in the presence of copper ions and mercury. Cadmium, copper, silver, mercury, and zinc ions are hazardous to the extremely stable immobilized urease enzyme (Morshed et al. 2021). Glutaraldehyde-pretreated urease is kept in place by membrane chitosan. The findings demonstrate that urease is stable enough to remove heavy metals as the immobilization process progresses. Some enzymes are limited, whereas unrestricted enzymes are fully inert, such as ions of zinc, copper, cadmium, mercury, and silver. The concentration of immobilized urease in the metal solution that has not been activated is the same in both cases. In the water purification experiment, solgel adsorbents were utilized to extract cadmium and copper (Gosset et al. 2016).

The urease enzyme was immobilized as the porous material in the presence of magnetic nanoparticles, and DTPA, or diethylene triamino pent acetic acid, was utilized to prevent the enzyme from remaining dormant in a heavy-metal contamination mixture. Every physiochemical property characterization was researched and recorded. The absorbent can detect the DTPA group as well as cadmium and copper, according to the study. Furthermore, the Langmuir adsorption model performed admirably (Deng et al. 2016). The urease enzyme's activity remained stable throughout and while it was present in the metal solutions due to the DTPA group, indicating that it will continue to function during the restoration process.

9.3 Laccases

Copper is used to treat another oxidase enzyme, laccase. The major source is enzymes found in fungi, microbes, and plants and this enzyme work only in the presence of oxygen (Sirisha et al. 2016; Chauhan et al. 2017b). Phenolic molecules, like lignin, can oxidize by laccase enzyme. This enzyme's major applications include food businesses, wine production, etc. It have been less investigations on immobilized laccases in the bioremediation of toxic ions and metals. The study used the catechol adsorption technique on immobilized laccases (Girelli et al. 2021). Laccases were immobilized using oxide materials such as GO, MMT, and PAN. In order to extract catechol from various aqueous solutions, an adsorbent substance is used. The solution contains immobilized enzyme laccases, according to the scanning electron microscope examination (Chatterjee et al. 2016). However, a lot of assimilation took place in the oxides, affecting things like the pH and ideal temperature. The catechol-containing solution had a 40% COD level of removal. The immobilized laccases enzyme allows for the extraction of catechol from the alloy liquid.

9.4 Papain

It is also known as papaya proteinase and is classified as a proteolytic enzyme. It aids in the fragmentation of protein particles (Petronijević et al. 2021). It has commercial applications in areas such as food, cosmetics, and textiles. Research suggests that after the immobilization procedure is finished, mercury may be removed from an aqueous solution. Under optimum experimental conditions, it focused on the removal of heavy metals including Hg and Pb from a stirred fluid comprising calcium alginate bead-immobilized papain (Hu et al. 2017). This study's absorbent was immobilized papain based on alginate, which was well-optimized and capable of removing Hg and Pb from the stimulated aqueous solution. It was found that Pb and Hg had an impact on the enzyme activity of immobilized enzymes, and that the outcome was effective because a specific amount of papain was immobilized (Ariaeenejad et al. 2021). As a result, the kinetic investigation was also completed in part and resulted in a model. They were modeled using several sorts of kinetic models. The immobilized papain enzyme had a bond with mercury and lead, according to desorption and X-ray spectrometry analyses, which allowed the absorbent to reuse it. Response surface technique studies were used to confirm adsorbent reuse.

9.5 Bromelain

Bromelain, a protein-digesting enzyme, was discovered in pineapple stems (Pandey et al. 2021). The enzyme is categorized as a cysteine or sulfhydryl enzyme. Its primary application is in medicine, where it is used to treat blood clotting, burns, and cancer formation. It also has anti-inflammatory, anticancer, and antibacterial properties. Arthritic patients can utilize this enzyme as tablets because it has no side effects and can be taken orally. Because it has the potential to reduce natural inflammation, it lowers joint and knee discomfort (Jiang et al. 2022). Because bromelain serves as a digestive enzyme, it is employed in the beer and bread sectors. In its active site, bromelain has one free sulfhydryl group and two disulfide linkages. Due to its affinity for heavy metals, it can be used in bioremediation. It will get rid of heavy metal pollution and work as an enzyme-based test technique and a protease inhibitive assay approach. On charcoal, the bromelain immobilization method was used to remove metal. According to the study, activated charcoal and bromelain are used with tannery effluent and stimulating solution, which contain chromium and lead to process immobilization. The response surface approach was employed to achieve the appropriate conditions for the immobilization procedure (Chen et al. 2022). All parameters, including concentration, pH, temperature, and weight of the charcoal, were first set up in accordance with the experimental chart. The author claims that the current circumstance, sometimes referred to as CIB (charcoal immobilized bromelain), has reached the immobilization rate. Both before and after treatment, all physiochemical tests for both heavy metals were run. Heavy metal binding in the adsorbent was identified by energy-dispersive X-ray spectroscopy (EDS) (Swain et al. 2021). The experiment was conducted using bromelain that had been immobilized in charcoal under the same conditions. The findings show that compared to free enzyme substrate, the immobilized enzyme bromelain has a high specific affinity. As a result, immobilized bromelain and activated charcoal work

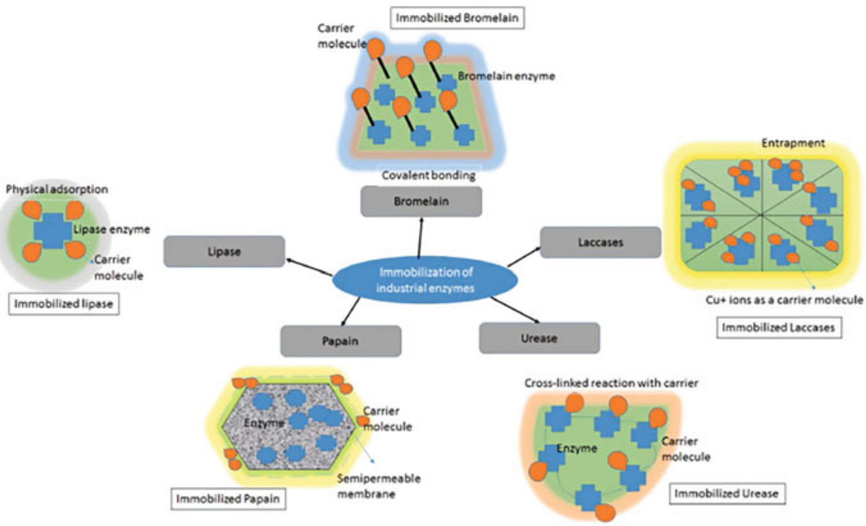

Fig. 2 Removal mechanism of immobilized industrial enzymes (Adapted from Yaashikaa et al. 2022)

well together in this condition's research. In 5 min or fewer, the responses reach the point of nearly full exclusion. In the ensuing 10 min, the separation process attained equilibrium (Aggarwal et al. 2021). Figure 2 shows how the main industrial enzymes are eliminated.

10 Conclusion and Perspectives for the Future

Enzyme immobilization is a technique used in a variety of industrial industries, including textiles, bioremediation, food, and pharmaceuticals. The immobilization of enzymes with distinct characteristics in support materials and their use in large-scale processes have been reported (Zhang et al. 2021). Since immobilization lends stability to enzymes, this approach reduces the cost required. Nevertheless, there are a number of difficulties with enzyme immobilization approaches that must be addressed before these processes may be scaled up from lab to pilot scale. The cost of enzyme production and it immobilization can be lowered through the invention of novel procedure and the optimization of existing technologies. (Zhang et al. 2020). Enzyme nature and its characteristics must be improved in an unfavorable hostile environment. Novel techniques for enhancing enzyme reusability must be established. An enzyme reactor and membrane bioreactor with enzyme beads immobilized at the surface for the treatment of aqueous and industrial wastewater (Pekgenc et al. 2022). To improve the effectiveness of pollution degradation and removal, unique and notable approaches will be evaluated. Implementing immobilized enzymes in real-world industrial wastewater treatment processes by designing and developing novel ways to scale up small-scale processes. It is also known that enzyme activity is decreased when enzymes are immobilized. Material and method costs for immobilization were lower. In addition to preventing enzyme deactivation, this method also makes enzymes more reusable (Zhou et al. 2021). For the industrial treatment of wastewater, immobilized enzymes have evolved into a practical, cost-effective, and efficient solution.

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