Tuijin Jishu/Journal of Propulsion Technology
ISSN: 1001-4055
Vol. 44 No. 5 (2023)

Polymer-Based Strategies for Enzyme Immobilization: A Comprehensive Review

[1]Gupta D, [2]Modi G

Abstract: Enzyme immobilization refers to the process of attaching or confining enzymes onto a solid support or within a matrix, often made of polymers or other materials. This immobilization creates a stable and controlled environment for the enzyme to interact with substrates and perform catalysis. The primary goal of enzyme immobilization is to enhance enzyme stability, reusability, and activity under specific conditions, making them more practical and efficient for various biotechnological, industrial, and medical applications.
Immobilization methods can vary widely, including physical adsorption, covalent bonding, entrapment within matrices, encapsulation, crosslinking, and more. These methods provide a means to control the interactions between the enzyme and the surrounding environment, affecting factors such as substrate accessibility, enzyme orientation, and stability.
Due to their ease of fabrication and superior structural adaptability, polymer compounds in a variety of physical forms, including beads, films, fibers,and coatings,have become popular as supportive materials for enzyme immobilization. For enzyme immobilization, a number of natural polymers, including agar, agarose, alginate, dextran, chitosan,and carrageenan, as well as synthetic polymers, such as polyamides, polystyrene, and polyacrylamide, are often employed as a carrier system. The immobilization offers a cost-effective system for various applications in biotechnology, industry, and research.
Keywords: Enzyme Immobilization, Polymers, Cost-effective Production, Natural Polymers, Synthetic Polymers.

1. Introduction

Enzymes play a crucial role in various industrial processes due to their remarkable catalytic properties and specificity. In the food industry enzymes are extensively used to improve product quality, enhance production processes, and reduce costs [1]. The most common enzymes used in this industry are Amylases, Proteases, Lipases, Pectinases, Cellulases, Lactase, Transglutaminase, Invertase, Catalase, Glucose Isomerase, Xylanase, Transglucosidase, etc.[2]These enzymes help achieve various goals in food and beverage production, such as improving texture, flavor, and shelf life, reducing production time, and making products more suitable for specific dietary needs [3]. They are carefully selected and applied to ensure that the desired enzymatic reactions take place during the manufacturing process, ultimately resulting in high-quality food and beverage products [4].

Like food and beverage, enzyme have been successfully utilized in various other industries like; pharmaceutical, drug manufacturing, textile, paper and pulp, biofuel production, detergent, environmental remediation, textile and leather processing, agriculture industries, etc.Enzymes offer numerous advantages in these industrial applications, including high specificity, mild reaction conditions, reduced environmental impact, and increased process efficiency. As a result, they continue to be essential tools across a wide range of industries [5, 6].

Due to their great efficiency, specificity, and selectivity as well as the ability to carry out activities in accordance with the principles of Green Chemistry, enzymes are utilized in a wide variety of industries and biotechnological applications. Unfortunately, using enzymes has a number of problems since they stop functioning in environments outside of their normal habitat. Enzymes can therefore become denaturized by chemical deterioration, physical unfolding, and aggregation brought on by changes in temperature or pH, organic solvents, or even the activity of other enzymes [7, 8]. For the effective implementation of enzyme-based operations in industry, immobilization is a significant approach, especially for the generation of renewable and environmentally friendly energy or food products from biomass-derived enzymatic transformation [9].For several applications, including biomedicine, organic synthesis, biosensing, and bioremediation, it has been beneficial to immobilize the enzymes to fabricate functional, stable, and strong biocatalytic hybrid materials (nanoparticles, capsules, hydrogels, or films) [10].The simplicity of separation and ease of recovery for reuse, while preserving activity and selectivity, are the benefits of stabilized enzyme systems. Because stabilized

enzyme systems are anticipated to be environmentally friendly, reasonably priced, and simple to use for enzyme-based industrial applications, Recent studies have been conducted on various enzymes immobilized on various support materials, which have enormous potential for biosensor, antibiotic production, food industry, biodiesel production, and bioremediation [11, 12].*Aspergillusoryzae* amino cyclase for the biosynthesis of L-amino acids, pectinase, cellulases, and other enzymes have been immobilized on appropriate carriers and are used in the food sector (baking, dairy products, jams and jellies), beverage processing (wine, beer, fruit and vegetable juices), and other industries. The choice of support material utilized is critical to the immobilization process due to the significant influence of these materials on the characteristics of the resulting catalytic system. For effective immobilization of enzymes, a wide range of inorganic oxides, minerals, and organic, hybrid, and composite materials of synthetic or natural origin may be employed [13].

2. Methods of enzyme immobilization using polymers

There are several methods of enzyme immobilization using polymers. The choice of method depends on factors such as the enzyme's properties, the intended application, and the desired immobilization outcomes. Each method has its advantages and limitations, and the optimal strategy is to be selected based on the specific requirements of the enzyme and the application. Some common methods of enzyme immobilization using polymers are discussed here in detail.

Physical approaches are advantageous for preserving the catalytic activity of immobilized enzymes, whereas chemical methods are advantageous for maintaining stability. Combining physical and chemical methods might be a promising technique for producing immobilized enzymes with high catalytic activity and good stability [14].

Recent advances in the development of a plethora of diverse nanostructured support materials and immobilization approaches have enabled precise immobilization of biocatalysts, which has resulted in a heightened interest in recent years in the flourishing of nanocatalysts-mediated industrial bioprocesses. Functionalized nanoparticles, in particular, have a strong influence on the inherent mechanical characteristics of the enzyme and provide great biocompatibility and distinct nano-environments around it [15, 16, 17].

2.1 Physical Adsorption:

Enzymes are attached to a polymer matrix through weak non-covalent interactions such as electrostatic forces, hydrogen bonding, or hydrophobic interactions.Simple and quick method, but enzyme stability and reusability can be limited.Suitable for applications where temporary or short-term immobilization is needed.Enzyme activity and stability can be increased by immobilizing them on a newly synthesizedpolypyrrole-methyl anthranilate-titanium oxide nanocomposite containing amine groups. We proved this by effectively immobilizing lipase from *Rhizopusoryzae* via physical adsorptionand a glutaraldehyde-activated covalent coupling method on a Ppy-MA/TiO2 NC.In comparison to the adsorbed homolog, the covalently immobilized lipase had a much greater activity yield (effectiveness factor of 0.97) [18].The combination of immobilization techniques increases the binding force between enzymes and support, minimizing enzyme leakage from the support. Lipase adsorption on hydrophobic support results in lipase interfacial activation during immobilization. The adsorption approach also results in little or no change in enzyme structure, particularly in the active region. As a result, this approach is the most commonly utilized in industrial immobilization [19].

The immobilization approach of physical adsorption coupled with covalent crosslinking was developed to overcome the drawbacks of both noncovalent and covalent coupling methods. TYR was immobilized on Fe3O4-NH2 using the described technique, demonstrating improved pH and temperature durability, reusability, storage stability, and better affinity to the substrate. The immobilized TYR was used to screen 11 TCMs for enzyme inhibitors [20].

The enzyme laccase was physically confined on the synthesized meso-MIL-53(Al) and used for catalytic TCS degradation. Lac-MIL-53(Al) retained the MOF framework structure and had roughly similar activity to free laccase. The immobilizedlaccase was more adaptable to a wider pH and temperature range, and it had better storage stability and reusability [21].

2.2 Covalent Binding:

Enzymes are chemically linked to polymer matrices through covalent bonds formed between functional groups on both the enzyme and the polymer.Provides strong and stable attachment, ensuring long-term activity and reusability.Requires chemical modification of the enzyme or the polymer surface.Common covalent coupling methods include carbodiimide activation, glutaraldehyde coupling, and epoxy activation.Zhang and Sun generated SNPs-pCBMA, a new nanoparticle-based carrier modified by grafting a highly hydrophilic zwitterionic polymer, poly(carboxybetaine methacrylate) (pCBMA), on silica nanoparticles (SNPs).To create SNPs-pGMA, an uncharged polymer, poly(glycidyl methacrylate) (pGMA), was additionally grafted onto SNPs. The two types of polymer-grafted SNPs were thoroughly characterized and employed for the covalent bonding of two enzymes, catalase, and lipase (pGMA-CAT, pCBMA-CRL, pGMA-CRL, pCBMA-CRL, and pCBMA-CRL). Due to the protective effects of the zwitterionic polymer, both immobilized enzymes on SNPs-pCBMA displayed greater thermal and storage stabilities than those on SNPs-pGMA [22].

2.3 Encapsulation:

Enzymes are encapsulated within polymer microspheres, microcapsules, or nanoparticles.Offers protection to enzymes, controlled release, and stability.Can be achieved using techniques like emulsion polymerization, solvent evaporation, or nanoprecipitation.Provides a controlled environment for enzyme activity.The efficiency of immobilized enzymes is considerably influencedby the arrangement and kind of support. One-dimensional fibrous materials might be one of the most suitable supports for enzyme immobilization. This is owing to their high surface area to volume ratio, internal porosity, ease of handling, and great mechanical stability, all of which allow for increased enzyme loading, release, and, ultimately, superior catalytic effectiveness. Fortunately, the enzymes may live within individual nanofibers to remain encapsulated and keep their three-dimensional structure. These qualities may safeguard the enzyme's tolerance against extreme circumstances such as pH changes and high temperatures, which may improve the enzyme's stability [23].

2.4 Entrapment:

Enzymes are physically trapped within a porous polymer matrix, such as a hydrogel.Offers protection and stability to enzymes while allowing for substrate diffusion.Can be prepared through polymerization of monomers in the presence of enzymes.Entrapping an enzyme in a support material has the potential to provide versatility, simplicity, and dependability. To accomplish immobilization, entrapment does not require any direct interactions with the enzyme. Changes in the protein within an enzyme library are unlikely to render an entrapment approach outdated.Entrapped enzymes have regularly showed strong enzyme activity, with better temperature and pH stability, as befits a successful immobilization approach, as demonstrated by the finest chemical procedures, and there are instances [24].

2.5 Polymer Beads or Resins:

Enzymes are immobilized onto or within polymer beads or resins.Beads can be packed into columns for continuous flow processes.Beads provide a high surface area for enzyme loading.

By creating Candida rugosa lipase (CRL)/inorganic hybrid nanosheets on sulfonatedmacroporous resins (SMRs), a new immobilized enzyme was created.After ten recycling cycles at pH 7.0 and 30°C, the immobilized lipase used in the batch hydrolysis of olive oil emulsion maintained 81.6% activity as a biocatalyst. This suggests that the carefully thought-out carrier materials will be extremely useful and significant in commercial enzyme catalysis [25].

Poly(aspartic acid), alginate, and silica gel composite beads have been used to immobilize the lipase B enzyme from *Candida antarctica* (CaLB). Adsorbed CaLB was first on functionalized mesoporous silica gel particles, which were subsequently caught in the interpenetrating network of zinc-ion-crosslinked alginate and thiolatedpoly(aspartic acid). Finally, a bisepoxide cross-linker called poly(ethylene glycol) diglycidyl ether chemically stabilized the beads. This allowed for the production of spherical biocatalysts with a diameter of 3-5 mm. The kinetic resolution of racemic 1-phenyl ethanol was used to assess the biocatalytic activity of the catalysts. Similar to when CaLB was physically adsorbed on silica gel particles, the activity of CaLB in the

beads was also comparable. Even after five test reactions, there was no discernible loss of biocatalytic activity in the composite beads, and they were simple to recover from after usage [26].

The diffusion of lipase CalB in two distinct resins during immobilisation was examined using a technique called Fourier transform infrared (FT-IR) microscopy. According to the findings, lipase CalB exhibited a strong preference for immobilisation on a hydrophobic carrier, demonstrating increased enzyme activity in the bead's outer region. In order to minimise the hydrophobic contact at the expense of reduced enzyme activity, a more hydrophilic resin (ECR8204M) was utilized. This resin allowed the CalB to enter deeper into the resin beads [27].

Research was done on the immobilization of free pectinase onto polystyrene resin beads using glutaraldehyde crosslinking. Confocal laser scanning microscopy and Fourier transform infrared spectroscopy were used to characterize the immobilized pectinase. The optimal pH and temperature of the immobilized pectinase changed from 8.0 to 8.5 and 45 to 60 °C, respectively, when the immobilization conditions were optimized. This indicates that the immobilized pectinase is more stable at these temperatures and pH levels than free pectinase [28].

2.6 Layer-by-Layer (LbL) Assembly:

Layer-by-layer assembly (LbL) is a general method of applying functional materials to surfaces with the goal of enhancing their characteristics. Enzymes and polymer layers are alternately deposited onto a solid support, forming a multilayer film.This offers precise control over enzyme loading and release.Suitable for creating thin films with tailored properties.Using positively charged poly(ethyleneimine) (PEI) and alternative electrostatic adsorption, multilayered films of cellulose nanoparticles (NFCs) and modified multi-walled carbon nanotubes (MWCNTs) were formed onto cellulose support. The NFC's and MWCNT's free carboxylic groups were joined using ethylenediamine.The Schiff base reaction between the free amino sites of the proteins and the aldehyde groups of glutaraldehydeimmobilized glucose oxidase and laccase.The specific activity of the immobilized enzymes on the nanoparticle surface is greater than that of the enzymes immobilized directly on the cellulose surface, suggesting that the nanoparticles stabilize the proteins [29, 30, 31].

In order to prepare thin films by fabricating enzymatic coatings by layer-by-layer (LbL) assembly for air or effluent decontamination, encasing an enzyme in halloysite nanotubes is a feasible path toward stable bioactive coatings. This method could be readily modified to entrap other types of biomacromolecules [32].

2.7 Magnetic Polymer Immobilization:

Due to their outstanding features, including their huge surface area, high mass transference, mobility, and large surface-to-volume ratio, magnetic nanoparticles (MNPs) have garnered special attention as adaptable carriers and supporting matrices for immobilization applications. More significantly, they are also readily recoverable and separable when an external magnetic field is used [33].

Fe3O4 nanoparticles were entrapped within the cross-linked ionic liquid/epoxy-type polymer to create a magnetic nanocomposite. Cellulase was covalently immobilized using the resultant support by reacting with epoxy groups. When compared to free cellulase, the activity, thermal stability, and reusability of cellulase were enhanced by the ionic surface and covalent binding of the enzyme onto the support [34, 35].The physical and mechanical characteristics of biopolymers have been enhanced by the application of various materials with nanometric sizes, including metallic nanoparticles, carbon nanotubes, nanofibers, and nanocellulose, thanks to advancements in nanotechnology. Magnetic nanoparticles (MNPs) are one of these materials that have been receiving greater attention because of their enormous surface area and larger size, which give them better magnetic characteristics [36].

By acting as an intramolecular cross-linker, polyamine has the potential to both stabilize the structure of the produced polymer layer and greatly speed up the deposition of TA on supports. Furthermore, under ideal circumstances, CALB, a typical lipase for the synthesis of biodiesel, was effectively immobilized on this nanocomposite with a 132.8 mg g−1 support loading capacity and 56.7% activity recovery. In addition, compared to free CALB, the physicochemical characteristics of immobilized CALB showed several noteworthy benefits, such as enhanced temperature tolerance, methanol tolerance, and pH stability [37, 38, 39].

2.8 Hydrogel Immobilization:

Using N-hydroxysuccinimide (NHS) as a moderate chemical cross-linker, researchers created and characterized a strong agarose-chitosan hydrogel. With over 90% of the horseradish immobilization efficiency achieved, the hydrogel provided a straightforward, efficient, and environmentally friendly support material. Horseradish peroxidase immobilized in agarose–chitosan hydrogel (ACH–HRP) demonstrated pH and temperature stability across a broad range and showed promise for reusability in substrate oxidation. The activity of the ACH-HRP was better retained in acidic surroundings (pH 4.0; 38 vs. 5.9%), and it was well stabilized in alkaline circumstances (pH 10.1), where it was 3.9 times more active than in free environments [40].

Using the example of the kinetic resolution of rac-1-phenylethanol with vinyl acetate, a raw extract of CalB was encapsulated in poly(VEImBr) and evaluated with regard to solvent, temperature, the quantity of enzyme, leaching behavior, and reusability. When compared to the non-immobilized enzyme, the CalB raw extract's enzyme activity was higher thanks to this immobilization technique [41].The chromoionophore ETH 5294 (CI) and pectin hydrogel membrane as the indicator pH have been successfully used to create a novel and straightforward optical biosensor to detect triglycerides (TGs), with lipase acting as the catalyst [42].The gelatin-based hydrogel was shown to be an efficient carrier for invertaseimmobilization in a study comparing the effects of two natural hydrogel matrices, alginate and gelatin. Thus, a very stable and reactive biocatalyst might be created by employing moderate conditions and a pro-ecological, biodegradable substrate. The selection of the invertase preparation immobilized in gelatin was in line with the hypotheses derived from the molecular models of the hydrogel matrix and the enzyme employed [43, 44, 45].

2.9 Crosslinking within Polymer Matrices:

Enzymes are crosslinked within a polymer matrix to create a stable network forenhanced enzyme stability and reusability.A potential approach for resolving these problems is to use cross-linked enzyme aggregates (CLEAs), such as magnetic CLEAs, porous CLEAs, and combi-CLEAs. By utilizing cross-linking chemicals like glutaraldehyde to form numerous links between the molecules of the enzymes, cross-linking techniques can stabilize and immobilize the enzymes. High catalytic activity is ensured by the high catalyst density and microporous assembly of CLEAs, which, when combined with their extended shelf life, operational stability, and reusability, offer an affordable substitute for matrix-assisted immobilization techniques [46].

The co-immobilization of α-amylase and glucoamylase on crosslinked gelatin porous supports was the subject of research. Two co-immobilization techniques based on crosslinking with glutaraldehyde (Ggta) or calcium chloride (CaCl2) in the presence of alginate (Gcal) were suggested for this purpose. Based on the FTIR study and thermal characteristics, the support's characterization showed a porous microstructure with good component interaction. After determining that 60 °C and pH 6.0 were the ideal conditions for the Gcal co-immobilized enzymes, they showed an enzymatic activity of 120 μmol·mL·min−1. Furthermore, up to eight hydrolysis cycles may be completed on both supports. Furthermore, over time, co-immobilized enzymes were more effective than free enzymes in the long-term starch saccharification process [47].

2.10 Modification of Polymer Surfaces:

Polymer surfaces are modified to introduce functional groups for enzyme binding which allows enzymes to be immobilized through covalent or non-covalent interactions.This requires surface modification techniques like plasma treatment or chemical grafting.After integrating favorable surface functional groups through plasma treatments (atmospheric pressure-AP or cold remote plasma-CRP (N2 + O2)) and/or chemical grafting of hyperbrancheddendrimers (poly-(ethylene glycol)-OH or poly-(amidoamine)), the glucose oxidase (GOx) enzyme was successfully immobilized on poly(ethylene terephthalate) nonwoven fabric (PN). Comparative research was done on the absorption, stability, catalytic activity, and reusability of the resulting fibrous bio-catalysts.According to the findings, modified polyester with functional groups attached to the amine terminals had superior surface properties, allowing for up to 31% enzyme loading and 81% active immobilized enzymes. Using a colorimetric test, the enzyme's activity was quantified in terms of how well GOx interacted with it in a certain amount of time to make hydrogen peroxide. After being utilizedsix(06) times, the

immobilizedGOx maintained 50% of its initial activity and showed better stability in response to temperature than the unbound enzyme [48, 49].

3. Types of Polymers Used in Enzyme Immobilization

Various types of polymers are used for enzyme immobilization due to their diverse properties, biocompatibility, and ease of modification. The choice of polymer depends on factors such as the enzyme's properties, the intended application, and the immobilization method. The specific choice of polymer depends on the immobilization method, the enzyme's characteristics, the desired properties of the immobilized enzyme, and the intended application. Different immobilization techniques include entrapment, covalent binding, adsorption, crosslinking, and encapsulation, and each polymer may excel in a particular technique or application.Polymer chemistry provides several chances for successfully combining enzymes with diverse natural or synthesized polymers. The creation of functional, stable, and resilient biocatalytic hybrid materials (nanoparticles, capsules, hydrogels, or films) has proved beneficial in a variety of applications including biomedicine, organic synthesis, biosensing, and bioremediation [50].

Here are some common types of polymers used in enzyme immobilization:

3.1 Natural Polymers

Various naturally occurring polysaccharides like; alginate,carrageenan,cellulose, chitin, pectin,starch, etc. are currently being utilized as base materials by combining multiple immobilization strategies such as adsorption, affinity immobilization, covalent binding, encapsulation, entrapment, and so on. Polysaccharides have been increasingly popular as a resource for green and sustainable products. Such base supports are widely accessible, have a simple construction method, are insoluble in an aqueous environment, are biocompatible, non-toxic, biodegradable, and physiologically inert, and are biocompatible, non-toxic, biodegradable, and physiologically inert [51].Two commercial asparaginases and lipase B from Candida antarctica (CaLB) covalently immobilized, on rice husk exhibited an increased efficacy in the solvent-free polycondensation of dimethylitaconate. Because of its unique combination of low density and exceptional mechanical toughness, CaLB on rice husk seems especially well-suited for use in very viscous processes. Though the rice husk loaded less protein, the biocatalyst immobilized on the rice husk performed in an aqueous solution for the two asparaginases at least as efficiently as the enzyme immobilized on methacrylic resins [52].

Using ethanol as the terminal reductant, covalent immobilisation of yeast alcohol dehydrogenase on an amino-functionalized polymeric resin improved stability for furfural hydrogenation to furfuryl alcohol. Studies using scanning electron microscopy, thermogravimetric analysis, nitrogen physisorption, and Fourier transform infrared spectroscopy verified that the resin had been successfully functionalized and that YADH had been immobilized. After 20 cycles, a residual activity analysis showed 94% activity retention. When compared to soluble YADH, immobilized YADH exhibited superior reusability, three times longer lifespan, and four times greater substrate consumption during fed-batch and repeated-batch tests conducted over a 48-hour period for the catalysis of ethanol-dependent furfural reduction to furfuryl alcohol.

3.1.1 Alginate: Derived from algae, alginate forms hydrogels when crosslinked with divalent cations. It is used for encapsulating enzymes and cells due to its biocompatibility and mild gelation conditions [53].

3.1.2 Chitosan: Obtained from chitin, chitosan is biocompatible, biodegradable, and offers various functional groups for enzyme binding. It can be used as films, beads, or nanoparticles for immobilization.Outstanding characteristics of chitosan-based nanofibers can increase the stability and effectiveness of enzyme immobilization under a variety of operating situations. These characteristics include high surface area to volume ratio, improved porosity and mechanical qualities, simple separation and reusability, biodegradability, antibacterial activity, non-toxicity, presence of functional groups (amino and hydroxyl), and big surface area to volume ratio [54, 55].

3.1.3 Gelatin: Derived from collagen, gelatin is widely used for encapsulation due to its protein nature. It forms a hydrogel matrix that provides a supportive environment for enzymes.Since the SulE activity recovery of cross-linked gelatin/chitosan (GLT/CTS) was greater than that of γ-polyglutamate/gelatin (γ-PGA/GLT) and γ-polyglutamate/chitosan (γ-PGA/CTS), it was chosen as the enzyme immobilization carrier. The effects of pH and temperature on GLT/CTS-SulE and free SulE activities to degrade tribenuron-methyl and metsulfuron-

methyl were studied, and the immobilization conditions were further optimized. The findings demonstrated that GLT/CTS-SulE exhibited superior pH and temperature adaptabilities as well as greater degradation efficiencies for both herbicides in the soil, particularly tribenuron-methyl when compared to free SulE [56].

Aspergillusawamori β-glucosidase, exhibiting a molecular weight of 180 kDa, was generated in solid-state cultures by the utilization of a blend including wheat bran and pineapple crown leaves. The enzyme produced 820 ± 30 U/g of substrate at its maximum after 8 days of incubation at 28 °C and 80% initial moisture. On commercial gelatin that had been cross-linked with glutaraldehyde, the crude enzyme was effectively immobilized.When the free and immobilized forms of the enzyme were compared, it was found that the immobilized form was less glucose-inhibited and more thermostable [57].

3.2 Synthetic Polymers

It has been observed that immobilizing enzymes on organic support nanomaterials greatly increases their thermal and storage durability while also providing protection from the inhibitory effects of metal ions. For the immobilization of enzymes, organic support nanoparticles are an excellent option since they are biocompatible and pose less health hazards. When compared to macroscopic supports, organic nanomaterials—and particularly organic-inorganic hybrids—can greatly enhance the kinetic and thermodynamic characteristics of immobilized enzymes. Additionally, organic nanoparticles are more environmentally friendly for use in medical applications like biosensors and prodrug carriers [58].

3.2.1 Polyacrylamide: Often used in gel form for creating thin layers or gels, polyacrylamide offers excellent control over pore size and provides a suitable environment for immobilizing enzymes.Trametesversicolor laccase enzyme was immobilized on polyacrylamide-alginate cryogel (PAG) functionalized with glycidyl methacrylate (GMA). Fourier transform infrared spectroscopy, environmental scanning electron microscopy, energy dispersive X-ray analysis, surface area analysis using the Brunauer-Emmett-Teller technique, and swelling tests were used to characterize the cryogel. The laccase enzyme was covalently immobilized, and at pH 3.0 and 25 °C, the maximum loading (68.7 ± 1.45 mg/g) happened.It has been shown that immobilizedlaccase enzyme is more stable than free laccase enzyme when exposed to temperature increases, prolonged storage, and frequent usage [59].

3.2.2 Polyvinyl Alcohol (PVA): PVA hydrogels are known for their biocompatibility and resistance to swelling in water. They are used for enzyme immobilization in various applications. Polyvinyl alcohol/metal-organic framework Using freeze-thaw cycles, cryogel-immobilized laccases, or MOF/PVA/Lac, were made and then utilized to extract the anthraquinonecolor alizarin green. The samples with the highest relative laccase activity and acid tolerance were MIL-68(Al)/PVA/Lac. The MIL-68(Al)/PVA/Lac demonstrated improved pH, thermal, and operational stabilities in comparison to free laccase. For the elimination of alizarin green, the MIL-68(Al)/PVA/Lac exhibited remarkable efficiency (95.86%) in less than 12 hours [60].

3.2.3 Polyethylene Glycol (PEG): PEG can be functionalized to introduce specific groups for enzyme binding. It offers a non-fouling surface and is widely used in biocompatible enzyme immobilization.Enzymes modified with poly(ethylene glycol) (PEG) may solubilize in aqueous and nonaqueous conditions since PEG is an amphiphilic polymer that is soluble in both water and organic solvents. The synthesis of ornithine-ß-alanine, a derivative of a salty peptide, was carried out using the aminolysis reaction of PEG-modified papain in organic solvents. In hydrophilic organic solvents, the immobilized enzymes catalyze peptide synthesis, transesterification, and esterification. They are stable [61].

Polymers containing magnetic nanoparticles can be used for magnetically assisted enzyme immobilization and easy separation from reaction mixtures using external magnetic fields.The design, production, and application of nanoparticles coated with biopolymers in their natural state as well as after chemical alterations are evolving at a rapid pace. Enzymes are still the most often immobilized proteins on magnetite particles, most likely because of the possibility of reuse in the catalytic cycle.

4. Significance of enzyme immobilization by using Polymers

Polymers can provide a protective environment for enzymes, shielding them from harsh conditions such as temperature, pH extremes, and organic solvents. This improved stability increases the lifespan and efficiency of the enzymes, allowing for longer and more consistent usage.Immobilizing enzymes onto polymers

allows for their reuse in multiple reaction cycles. The polymers act as a physical barrier, preventing enzymes from diffusing away or being lost during reactions [9]. This reusability not only reduces the cost of enzyme production but also minimizes waste generation.Polymers can regulate enzyme activity by influencing the microenvironment around the immobilized enzyme. This control can lead to improved catalytic efficiency, selectivity, and substrate conversion rates. The polymer matrix can be engineered to optimize enzyme-substrate interactions.Immobilized enzymes can be easily separated from reaction mixtures using filtration or sedimentation methods. This simplifies downstream processing, purification, and product recovery, especially in continuous or batch processes [10].

Polymers can be designed to allow specific substrates to diffuse into the polymer matrix while excluding others. This enables selectivity in enzyme-catalyzed reactions, making them suitable for complex reaction mixtures.Immobilizing enzymes on polymers enables their use in environments where free enzymes would be deactivated or denatured. This is particularly useful for industrial applications involving extreme temperatures, organic solvents, or aggressive chemicals.Polymers can be functionalized with specific groups to allow enzyme engineering and modification. This includes attaching cofactors, inhibitors, or other compounds that can enhance enzyme performance or confer new properties.Immobilized enzymes are more practical for large-scale industrial processes due to the ease of separation, reuse, and stability. Polymers provide a consistent and controlled environment for enzymes, ensuring reliable and efficient production.Polymers can be combined with other materials to create hybrid systems for enzyme immobilization. For example, combining polymers with nanoparticles, magnetic materials, or carbon nanotubes can further enhance the immobilization process and the properties of the resulting biocatalysts.Many polymers used for enzyme immobilization are biocompatible and do not interfere with the catalytic activity of the enzymes. This is crucial when enzymes are used in medical applications, such as biosensors or drug delivery systems [11-13].

5. Conclusion

Overall, polymers provide a versatile platform for immobilizing enzymes, allowing for optimization of their performance in a wide range of applications. The choice of polymer and immobilization method depends on the specific requirements of the enzyme, the desired application, and the conditions under which the enzyme will be used.This review might explore the various applications of enzyme immobilization using polymer-based strategies, including biocatalysis, biosensors, drug delivery, and wastewater treatment.

References

[1] Garcia-Galan C, Berenguer-Murcia Á, Fernandez-Lafuente R, Rodrigues RC. Potential of different enzyme immobilization strategies to improve enzyme performance. Adv Synth Catal. 2011 Nov;353(16):2885-904. Doi: https://doi.org/10.1002/adsc.201100534

[2] Datta S, Christena LR, Rajaram YR. Enzyme immobilization: an overview on techniques and support materials. 3 Biotech. 2013;3:1-9.Doi: https://doi.org/10.1007/s13205-012-0071-7.

[3] Homaei AA, Sariri R, Vianello F, Stevanato R. Enzyme immobilization: an update. J Chem Biol. 2013;6: 185–205. Doi:https://doi.org/10.1007/s12154-013-0102-9.

[4] Hwang ET, Gu MB. Enzyme stabilization by nano/microsized hybrid materials. Eng Life Sci. 2013; 13(1):49-61. Doi: https://doi.org/10.1002/elsc.201100225

[5] Bezerra CS, de Farias Lemos CM, de Sousa M, Gonçalves LR. Enzyme immobilization onto renewable polymeric matrixes: Past, present, and future trends. Inc. J. Appl. Polym. Sci. 2015; 132(26): 1-15. Doi: https://doi.org/10.1002/app.42125

[6] Zdarta J, Meyer AS, Jesionowski T, Pinelo M. A general overview of support materials for enzyme immobilization: characteristics, properties, practical utility. Catalysts. 2018;8(2):92.Doi: https://doi.org/10.3390/catal8020092.

[7] Sneha HP, Beulah KC, Murthy PS. Enzyme immobilization methods and applications in the food industry. InEnzymes in food biotechnology 2019; 645-658. Academic Press.Doi: https://doi.org/10.1016/B978-0-12-813280-7.00037-2.

[8] Thangaraj, B., & Solomon, P. R. (2019). Immobilization of lipases–A review. Part I: Enzyme immobilization. *ChemBioEng Reviews*, *6*(5), 157-166.

[9] Bashir N, Sood M, Bandral JD. Enzyme immobilization and its applications in food processing: A review. Int. J. Chem. Stud. 2020;8(2):254-61.Doi: 10.22271/chemi.2020.v8.i2d.8779.

[10] Wahab, R. A., Elias, N., Abdullah, F., &Ghoshal, S. K. (2020). On the taught new tricks of enzymes immobilization: An all-inclusive overview. *Reactive and Functional Polymers*, *152*, 104613.

[11] Das R, Dwevedi A, Kayastha AM. Current and future trends on polymer-based enzyme immobilization. In: Dwevedi A, Editor. Polymeric Supports for Enzyme Immobilization: Opportunities and Applications.India.: Academic Press; 2021:1-25. ISBN:0128192070, 9780128192078.Doi: https://doi.org/10.1016/B978-0-12-819206-1.00004-1.

[12] Asaduzzaman F, Salmon S. Enzyme immobilization: polymer–solvent–enzyme compatibility. Molecular Systems Design & Engineering. 2022;7(11):1385-414. Doi: https://doi.org/10.1039/D2ME00140C.

[13] Maghraby YR, El-Shabasy RM, Ibrahim AH, Azzazy HM. Enzyme immobilization technologies and industrial applications. ACS omega. 2023 Jan 31;8(6):5184-96. Doi:10.1021/acsomega.2c07560

[14] Liu, D. M., Chen, J., & Shi, Y. P. (2018). Advances on methods and easy separated support materials for enzyme immobilization. *TrAC Trends in Analytical Chemistry*, *102*, 332-342.

[15] Bilal, M., & Iqbal, H. M. (2019). Chemical, physical, and biological coordination: An interplay between materials and enzymes as potential platforms for immobilization. *Coordination Chemistry Reviews*, *388*, 1-23.

[16] Lyu, X., Gonzalez, R., Horton, A., & Li, T. (2021). Immobilization of enzymes by polymeric materials. *Catalysts*, *11*(10), 1211.

[17] Guisan, J. M., Fernandez-Lorente, G., Rocha-Martin, J., & Moreno-Gamero, D. (2022). Enzyme immobilization strategies for the design of robust and efficient biocatalysts. *Current Opinion in Green and Sustainable Chemistry*, *35*, 100593.

[18] Asmat, S., Husain, Q., & Khan, M. S. (2018). A polypyrrole–methyl anthranilate functionalized worm-like titanium dioxide nanocomposite as an innovative tool for immobilization of lipase: preparation, activity, stability, and molecular docking investigations. *New Journal of Chemistry*, *42*(1), 91-102.

[19] Mokhtar, N. F., Abd. Rahman, R. N. Z. R., Muhd Noor, N. D., MohdShariff, F., &Mohamad Ali, M. S. (2020). The immobilization of lipases on porous support by adsorption and hydrophobic interaction method. *Catalysts*, *10*(7), 744.

[20] Liu, D. M., Chen, J., & Shi, Y. P. (2018). Tyrosinase immobilization on aminated magnetic nanoparticles by physical adsorption combined with covalent crosslinking with improved catalytic activity, reusability, and storage stability. *AnalyticaChimicaActa*, *1006*, 90-98.

[21] Jia, Y., Chen, Y., Luo, J., & Hu, Y. (2019). Immobilization of laccase onto meso-MIL-53 (Al) via physical adsorption for the catalytic conversion of triclosan. *Ecotoxicology and environmental safety*, *184*, 109670.

[22] Zhang, L., & Sun, Y. (2018). Poly (carboxybetaine methacrylate)-grafted silica nanoparticle: A novel carrier for enzyme immobilization. *Biochemical Engineering Journal*, *132*, 122-129.

[23] Rather, A. H., Khan, R. S., Wani, T. U., Beigh, M. A., & Sheikh, F. A. (2022). Overview on immobilization of enzymes on synthetic polymeric nanofibers fabricated by electrospinning. *Biotechnology and Bioengineering*, *119*(1), 9-33.

[24] Imam, H. T., Marr, P. C., & Marr, A. C. (2021). Enzyme entrapment, biocatalyst immobilization without covalent attachment. *Green Chemistry*, *23*(14), 4980-5005.

[25] Wan, D., Tian, L., Li, X., Li, B., & Zhang, Q. (2018). A versatile strategy for enzyme immobilization: Fabricating lipase/inorganic hybrid nanostructures on macroporous resins with enhanced catalytic properties. *Biochemical Engineering Journal*, *139*, 101-108.

[26] Krisch, E., Klimkó, J., Gyarmati, B., László, K., Szilágyi, A., Balogh-Weiser, D., &Poppe, L. (2019). Composite beads of silica gel, alginate and poly (aspartic acid) for the immobilization of a lipase enzyme. *Express Polymer Letters*, *13*(6).

[27] Pauli, O., Ecker, A., Cruz-Izquierdo, A., Basso, A., &Serban, S. (2022). Visualizing hydrophobic and hydrophilic enzyme interactions during immobilization by means of infrared microscopy. *Catalysts*, *12*(9), 989.

[28] Miao, Q., Zhang, C., Zhou, S., Meng, L., Huang, L., Ni, Y., & Chen, L. (2021). Immobilization and characterization of pectinase onto the cationic polystyrene resin. *ACS omega*, *6*(47), 31683-31688.

[29] Semerdzhieva, V., Raykova, R., Marinkova, D., Yaneva, S., Chernev, G., &Iliev, I. (2018). Layer-By-Layer Assembly of Enzymes and Nanoparticles onto Cellulose Support. *J BiosensBioelectron*, *9*(263), 2.

[30] Zhang, S., Xin, P., Demoustier-Champagne, S., & Jonas, A. M. (2021). Tuning the catalytic activity of enzymes embedded in layer-by-layer assembled films. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *631*, 127698.

[31] Zhang, J., Huang, X., Zhang, L., Si, Y., Guo, S., Su, H., & Liu, J. (2020). Layer-by-layer assembly for immobilizing enzymes in enzymatic biofuel cells. *Sustainable Energy & Fuels*, *4*(1), 68-79.

[32] Rouster, P., Dondelinger, M., Galleni, M., Nysten, B., Jonas, A. M., &Glinel, K. (2019). Layer-by-layer assembly of enzyme-loaded halloysite nanotubes for the fabrication of highly active coatings. *Colloids and Surfaces B: Biointerfaces*, *178*, 508-514.

[33] Bilal, M., Zhao, Y., Rasheed, T., & Iqbal, H. M. (2018). Magnetic nanoparticles as versatile carriers for enzymes immobilization: A review. *International journal of biological macromolecules*, *120*, 2530-2544.

[34] Hosseini, S. H., Hosseini, S. A., Zohreh, N., Yaghoubi, M., &Pourjavadi, A. (2018). Covalent immobilization of cellulase using magnetic poly (ionic liquid) support: improvement of the enzyme activity and stability. *Journal of agricultural and food chemistry*, *66*(4), 789-798.

[35] Khoshnevisan, K., Poorakbar, E., Baharifar, H., &Barkhi, M. (2019). Recent advances of cellulase immobilization onto magnetic nanoparticles: an update review. *Magnetochemistry*, *5*(2), 36.

[36] Gennari, A., Führ, A. J., Volpato, G., & de Souza, C. F. V. (2020). Magnetic cellulose: Versatile support for enzyme immobilization-A review. *Carbohydrate polymers*, *246*, 116646.

[37] Tang, W., Ma, T., Zhou, L., Wang, G., Wang, X., Ying, H., & Wang, P. (2019). Polyamine-induced tannic acid co-deposition on magnetic nanoparticles for enzyme immobilization and efficient biodiesel production catalysed by an immobilized enzyme under an alternating magnetic field. *Catalysis Science & Technology*, *9*(21), 6015-6026.

[38] Mariño, M. A., Fulaz, S., &Tasic, L. (2021). Magnetic nanomaterials as biocatalyst carriers for biomass processing: Immobilization strategies, reusability, and applications. *Magnetochemistry*, *7*(10), 133.

[39] Mylkie, K., Nowak, P., Rybczynski, P., & Ziegler-Borowska, M. (2021). Polymer-coated magnetite nanoparticles for protein immobilization. *Materials*, *14*(2), 248.

[40] Bilal, M., Rasheed, T., Zhao, Y., & Iqbal, H. M. (2019). Agarose-chitosan hydrogel-immobilized horseradish peroxidase with sustainable bio-catalytic and dye degradation properties. *International journal of biological macromolecules*, *124*, 742-749.

[41] Grollmisch, A., Kragl, U., &Großeheilmann, J. (2018). Enzyme immobilization in polymerized ionic liquids-based hydrogels for active and reusable biocatalysts. *SynOpen*, *2*(02), 0192-0199.

[42] Hasanah, U., Sani, N. D. M., Heng, L. Y., Idroes, R., &Safitri, E. (2019). Construction of a hydrogel pectin-based triglyceride optical biosensor with immobilized lipase enzymes. *Biosensors*, *9*(4), 135.

[43] Du, H., Shi, S., Liu, W., Teng, H., &Piao, M. (2020). Processing and modification of hydrogel and its application in emerging contaminant adsorption and in catalyst immobilization: a review. *Environmental Science and Pollution Research*, *27*, 12967-12994.

[44] Labus, K., Wolanin, K., &Radosiński, Ł. (2020). Comparative study on enzyme immobilization using natural hydrogel matrices—experimental studies supported by molecular models analysis. *Catalysts*, *10*(5), 489.

[45] Meyer, J., Meyer, L. E., & Kara, S. (2022). Enzyme immobilization in hydrogels: A perfect liaison for efficient and sustainable biocatalysis. *Engineering in Life Sciences*, *22*(3-4), 165-177.

[46] Voběrková, S., Solčány, V., Vršanská, M., & Adam, V. (2018). Immobilization of ligninolytic enzymes from white-rot fungi in cross-linked aggregates. *Chemosphere*, *202*, 694-707.

[47] Frota, E. G., Sartor, K. B., Biduski, B., Margarites, A. C. F., Colla, L. M., &Piccin, J. S. (2020). Co-immobilization of amylases in porous crosslinkedgelatin matrices by different reticulations approaches. *International Journal of Biological Macromolecules*, *165*, 1002-1009.

[48] Morshed, M. N., Behary, N., Bouazizi, N., Guan, J., Chen, G., &Nierstrasz, V. (2019). Surface modification of polyester fabric using plasma-dendrimer for robust immobilization of glucose oxidase enzyme. *Scientific reports*, *9*(1), 15730.

[49] Seenuvasan, M., Kumar, K. S., Kumar, A., &Parthiban, R. (2020). Review on surface modification of nanocarriers to overcome diffusion limitations: An enzyme immobilization aspect. *Biochemical engineering journal*, *158*, 107574.

[50] Rodriguez-Abetxuko, A., Sánchez-deAlcázar, D., Muñumer, P., &Beloqui, A. (2020). Tunable polymeric scaffolds for enzyme immobilization. *Frontiers in Bioengineering and Biotechnology*, *8*, 830.

[51] Sharma, A., Thatai, K. S., Kuthiala, T., Singh, G., & Arya, S. K. (2021). Employment of polysaccharides in enzyme immobilization. *Reactive and Functional Polymers*, *167*, 105005.

[52] Cespugli, M., Lotteria, S., Navarini, L., Lonzarich, V., Del Terra, L., Vita, F., &Gardossi, L. (2018). Rice husk as an inexpensive renewable immobilization carrier for biocatalysts employed in the food, cosmetic and polymer sectors. *Catalysts*, *8*(10), 471.

[53] Sharma, V. K., Binder, T. P., &Allgeier, A. M. (2023). Covalent Immobilization of Yeast Alcohol Dehydrogenase on an Amine-Functionalized Polymeric Resin Enhances Stability for Furfural Hydrogenation to Furfuryl Alcohol Using Ethanol as the Terminal Reductant. *Industrial & Engineering Chemistry Research*.

[54] Ribeiro, E. S., de Farias, B. S., Junior, T. R. S. A. C., de Almeida Pinto, L. A., & Diaz, P. S. (2021). Chitosan–based nanofibers for enzyme immobilization. *International Journal of Biological Macromolecules*, *183*, 1959-1970.

[55] Rafiee, F., &Rezaee, M. (2021). Different strategies for the lipase immobilization on the chitosan based supports and their applications. *International Journal of Biological Macromolecules*, *179*, 170-195.

[56] Yu, Z., Zhang, H., Fu, X., Li, X., Guo, Q., Yang, T., & Li, X. (2020). Immobilization of esterase SulE in cross-linked gelatin/chitosan and its application in remediating soils polluted with tribenuron-methyl and metsulfuron-methyl. *Process Biochemistry*, *98*, 217-223.

[57] Nishida, V. S., de Oliveira, R. F., Brugnari, T., Correa, R. C. G., Peralta, R. A., Castoldi, R.,& Peralta, R. M. (2018). Immobilization of Aspergillusawamori β-glucosidase on commercial gelatin: An inexpensive and efficient process. *International journal of biological macromolecules*, *111*, 1206-1213.

[58] Zahirinejad, S., Hemmati, R., Homaei, A., Dinari, A., Hosseinkhani, S., Mohammadi, S., &Vianello, F. (2021). Nano-organic supports for enzyme immobilization: scopes and perspectives. *Colloids and Surfaces B: Biointerfaces*, *204*, 111774.

[59] Yavaşer, R., &Karagözler, A. A. (2021). Laccase immobilized polyacrylamide-alginate cryogel: A candidate for treatment of effluents. *Process Biochemistry*, *101*, 137-146.

[60] Peng, J., Wu, E., Lou, X., Deng, Q., Hou, X., Lv, C., & Hu, Q. (2021). Anthraquinone removal by a metal-organic framework/polyvinyl alcohol cryogel-immobilized laccase: Effect and mechanism exploration. *Chemical Engineering Journal*, *418*, 129473.

[61] Hideo, H. (2018). 4.1 Grafting of Enzymes with Synthetic Polymers for use in Organic Solvents. In *Synthesis of biocomposite materials* (pp. 183-212). CRC Press.