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Review

Green Synthesis of Biocatalysts Based on Nanocarriers Promises an Effective Role in Pharmaceutical and Biomedical Fields

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Abstract: Nanobiocatalysts (NBCs) are a promising new class of biocatalysts that combine the advantages of enzymes and nanomaterials. Enzymes are biological catalysts that are highly selective and efficient, but they can be unstable in harsh environments. Nanomaterials, on the other hand, are small particles with unique properties that can improve the stability, activity, and selectivity of enzymes. The development of NBCs has been driven by the need for more sustainable and environmentally friendly bioprocessing methods. Enzymes are inherently green catalysts, but they can be expensive and difficult to recover and reuse. NBCs can address these challenges by providing a stable and reusable platform for enzymes. One of the key challenges in the development of NBCs is the immobilization of enzymes on nanomaterials. Enzyme immobilization is a process that attaches enzymes to a solid support, which can protect the enzymes from harsh environments and make them easier to recover and reuse. There are many different methods for immobilizing enzymes, and the choice of method depends on the specific enzyme and nanomaterial being used. This review explores the effective role of NBCs in pharmaceutical and biomedical fields.

Keywords: nanobiocatalysts; nanomaterials; immobilization; drug delivery

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1. Introduction

Enormous academic attention has been devoted to the development of sustainable processes utilizing nanobiocatalysts [1]. Owing to their exceptional selectivity and discriminating characteristics, they offer promising prospects for green bioprocessing methods that require fewer chemicals and minimize hazardous materials. Enzyme immobilization is a popular technique in this domain because of its ability to stabilize biocatalysts in both chemical and environmental contexts. Enzyme immobilization is a commonly employed method in this field owing to its capability to enhance the stability of biocatalysts under diverse chemical and environmental conditions. The use of an immobilized form facilitates effortless retrieval and reuse in sizeable, uninterrupted industrial processing systems, representing one of the merits of this technique [2]. To enhance the power production and lifespan of biofuel cells, innovative biocatalysts have been integrated into biorefineries by certain researchers [3]. Effective coordination between catalysts and novel nanostructured materials is critical for generating NBCs that possess better functionality. Different enzymes can be immobilized using supportive carriers, such as magnetic nanoparticles,

nanotubes, hybrid nanoflowers, nanoporous substrates, and nanocomposites, to manufacture stable NBCs with promising activity levels [2]. Enzyme activity may be diminished in bioprocessing systems when biocatalysts are immobilized because structural changes occur during the immobilization process. Therefore, determining the duration of biocatalyst activity over several cycles is critical when using optimal immobilization protocols. To recover these biocatalysts, it is essential to concentrate on designing enzyme carriers with characteristics such as stability, compatibility with biological systems, and separability. Nanobiotechnology has recently introduced numerous nanoscale carriers suitable for enzyme immobilization [4]. Enzyme immobilization at the nanoscale has been identified as a promising technique to increase enzyme effectiveness. Using functionalized nanosystems, a large surface area can be supplied for effective enzyme loading while minimizing substrate mass transfer resistance, allowing for the creation of supramolecularly organized enzyme complexes [3]. A nanobiocatalyst is a type of functionalized nanocarrier that has the potential to improve enzyme stability, capacity, and engineering performance while also providing an optimized microenvironment surrounding enzymatic catalysts to maximize reaction efficiency [5]. Enzyme immobilization on nanostructured carriers can effectively increase the longevity of NBC, ultimately decreasing bioprocessing costs. Typically, NBCs are created by modifying functional nanomaterial surfaces, such as nanofibers, nanosheets, scaffolds, and nanoparticles, with diverse materials, such as metal oxides, noble metals, or complex compounds, including metal-organic frameworks. Understanding the relationship between nanostructure supports and the improved activity of NBC can provide valuable insights for successful applications in various industries. The aim of this review is to cover the recent advances in enzyme immobilization with a focus on the types of nanomaterials for NBCs and applications of NBCs in the pharmaceutical and biomedical fields.

2. Enzyme Immobilization

Enzymes are large biological molecules that exhibit either intracellular or extracellular activities and possess catalytic capabilities. Their primary function involves accelerating the rate of biochemical reactions by reducing the activation energy required for these processes while maintaining reaction equilibrium [6,7]. The use of soluble enzymes in industrial procedures is subject to certain constraints, including elevated expenses, susceptibility to enzyme instability under moderate or extreme modifications in operating conditions (such as pH alterations, temperature fluctuations, salts, and surfactants), and increased loading prerequisites. Therefore, the use of soluble enzymes is restricted by these limitations [8]. There are several methods for attaining enzyme stability; however, enzyme immobilization has been particularly successful. By reducing the effects of reaction, increasing specificity towards non-natural substrates, and enhancing functional properties, enzyme immobilization stands out among the other techniques. Additionally, this approach offers benefits, such as facile separation of the enzyme from the surrounding medium and reusability, leading to improved economic feasibility of the process [8,9].

2.1. Advantages of Enzyme Immobilization

Enzyme immobilization establishes a microenvironment that safeguards against fluctuations in operating conditions, thus eliminating the need for laborious and expensive removal and purification procedures. Moreover, elevated substrate concentrations or by-products can hinder enzyme function; however, this limitation can be overcome through immobilization, resulting in enhanced enzymatic activity. Additionally, the immobilization process may affect the precision of enzymes possessing more adaptable active centers [10].

2.2. Enzyme Immobilization Techniques

Various methods are available for reducing or eradicating enzyme inhibition through immobilization [11]. These techniques involve fortifying the structure of enzymes by establishing several covalent bonds, obstructing the access and exit of substrates and products through steric hindrance, partially impeding/blocking certain inhibition sites via distortion

or obstruction, and modifying physicochemical attributes within the vicinity of an enzyme through medium partitioning [12–14]. In certain instances, enzyme purification can be achieved through immobilization in a solitary operation. Techniques for immobilizing enzymes can be used independently or in conjunction with one another. Additionally, it is plausible to create hetero-functional support structures that possess functional groups capable of adsorbing and purifying enzymes, along with other nucleophilic functional groups that engage in covalent bonding with enzymes during irreversible immobilization [15–17]. Enzyme immobilization techniques can be classified into three types, as shown in Figure 1.

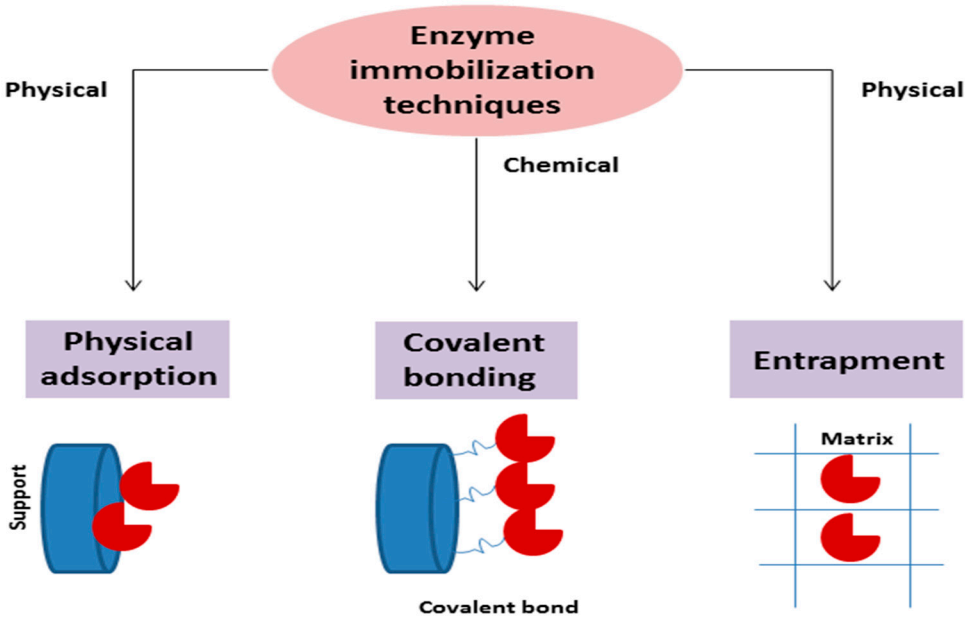

Figure 1. Different techniques of enzyme immobilization.

2.2.1. Physical Adsorption

An immobilization method can be used to attach an enzyme to a nonreactive material. This involves fixing enzymes to surfaces such as glass or alginate beads. Physical adsorption is used during this process, which can obstruct the active site of the enzyme and lead to reduced activity when an immobilizing agent is used. For example, laccase can be physically adsorbed onto nanoporous gold particles for immobilization. A more advanced version of physical adsorption is the entrapment approach, which has demonstrated greater efficiency than surface attachment methods. Although physical adsorption does not affect enzyme conformation and allows for simple regeneration processes, it exhibits a lower conversion rate than other NBCs [18].

2.2.2. Covalent Bonding

Enzymes can be immobilized by covalent attachment to a support matrix or by joining them with cross-linking agents. This approach is highly efficient because it allows for multipoint enzyme attachment, which improves enzyme stability and efficiency. The concave forms of nanopores that cover the enzyme may also improve enzyme stability by allowing for numerous covalent bonds between the internal surfaces of the nanopores and enzyme molecules. The steric barrier prevents more enzymes from passing through the previously blocked nanopores behind the input, where the enzymes are pre-attached. Recently, "ship-in-a-bottle" technology has been employed to encapsulate enzymes in a nanoporous medium, potentially reducing enzyme leaching and enhancing enzyme activity and loading [5]. For example, nanoporous carbon with pores as small as 31 nm can be interconnected by smaller window holes with diameters up to 21 nm, successfully avoiding enzyme leakage via the "ship-in-a-bottle" technique. To stabilize enzyme performance, an enhanced enzyme immobilization technique known as the "nanoscale enzyme reactor

technique" (NER) was created by merging the "ship-in-a-bottle" approach with enzyme cross-linking. The former inhibits enzyme leaching from the nanoporous medium, whereas the latter prevents enzyme denaturation by chemically cross-linking numerous covalent connections [19].

2.2.3. Entrapment

Enzyme entrapment is a low-cost and efficient adsorption technique that involves binding an enzyme to support the utilization of different forces such as hydrogen bonds, ionic interactions, and van der Waals forces. This method is often regarded as the most successful immobilization technique. Enzyme entrapment can be utilized with both hydrophilic and hydrophobic nanoparticles; however, the latter are favored because of their ability to capture more enzymes, resulting in higher degrees of immobilization while retaining greater activity levels. Insoluble calcium alginate beads and hydrophobic silane gels are common materials used [20,21]. Magnetic nanoparticles were effectively immobilized within cross-linked enzyme aggregates on a nanoporous medium using a 'double-ship-in-a-bottle' approach. This method builds on a previously established Nanoparticle Enzyme Reactor (NER) methodology. These highly stable nanobiocatalysts can easily be recycled for repeated use via simple magnetic separation. Furthermore, lipase NERs encapsulated in magnetic nanoparticles showed a significant proteolytic digestion reaction, which is relevant for active NBC applications in the presence of proteases. Multiple covalent bonds between NBCs can improve proteolytic digestion resistance. Recently, it was shown that chymotrypsin NERs respond to proteolytic digestion. These systems were magnetically separable, highly enzyme-loaded, stable under severe shaking, resistant to proteolytic digestion, and recyclable. Because these favorable features can be applied to a wide range of enzymes, NBCs are used in a variety of sectors [22].

3. Characteristics of Nanomaterials Used for NBCs

Support materials for enzyme immobilization should ideally have certain characteristics, such as a large surface area, robust physical structure, and numerous active sites, as well as being non-toxic to enzymes and allowing for efficient mass transfer. Additionally, they should be inert to enzymes and remain stable during catalysis while being non-toxic, environmentally friendly, and safe. Economic feasibility, including availability, low cost, and high cost-effectiveness, is also important [23]. Nanoscale materials have multiple advantages, including ease of preparation, a large surface-area-to-mass ratio, and the possibility of personalizing surfaces with different functionalities. They exhibit attractive electrical, optical, magnetic, and catalytic characteristics [24–27]. Nanoparticles are a popular choice for enzyme immobilization. When nanomaterials are combined with enzymes, they occasionally provide not only natural catalytic capabilities but also enhanced properties owing to the nanoparticles themselves. The catalytic activity and selectivity of enzymes can be efficiently altered, and their stability, separability, and reusability can be significantly improved [28]. In this study, we summarize and examine some of these additional critical characteristics [29].

3.1. Nanomaterial-Mediated Charge Transport

Because the protein shell insulates the active sites, redox enzymes face difficulties in establishing direct electrical contact with electrode surfaces, limiting their utility in amperometric biosensors and biofuel cells [30]. Extensive research has been conducted to explore ways to connect these enzymes to the electrodes [29]. Several approaches for creating integrated enzyme electrodes with electrical contacts have been developed, including the use of diffusional electron mediators and the inclusion of enzymes into redox-active polymers. In addition, advances in nanotechnology have led to the utilization of conductive nanomaterials, such as metal- and carbon-based nanomaterials, to facilitate charge transport between redox enzymes and electrode supports [31,32].

3.2. Using Nanomaterials to Deliver Membrane-Impermeable Enzymes

Both therapeutic and research applications rely on the effective transport of active enzymes into cells [30]. Nevertheless, the low membrane permeability and stability of enzymes often impede their delivery into cells, which poses a challenge. To overcome this obstacle, nanoparticles have been employed as nanocarriers or transporters for enzyme delivery, which has proven to be an effective approach.

3.3. Nanomaterial-Mediated Remote Control of Enzyme Activity

The precise control of enzyme activity has become a significant technique in biochemical research. Trigger signals may be used to temporarily modify protein function, which is critical for understanding biochemical systems and controlling biomolecular processes. Nanomaterials with distinct energy absorption characteristics, such as radio and optical frequencies, and other distinct physical features have emerged as potential materials for controlling biocatalytic processes [33,34].

3.4. Creation of Catalytic Systems with Synergistic Functions

Numerous nanomaterials have shown promise as efficient nanocatalysts that offer durability and simplicity in terms of retrieval and reuse. The integration of diverse biocatalysts with nanocatalysts enables the development of catalytic systems with cooperative and supplementary capabilities [35].

4. Types of Nanomaterials Used for NBCs

The rapid development of nanotechnology and biotechnology has opened up numerous possibilities for combining natural enzymes with various types of nanomaterials, as shown in Table 1. This review article discusses current advances in the synthesis of enzyme-nanomaterial composites that combine the unique electrical, optical, magnetic, and catalytic capabilities of nanomaterials with particular enzyme recognition and biocatalytic activities [29].

Table 1. Examples of some enzymes used for nanobiocatalysis and their application.

Types of Nanomaterials	Enzyme	Nanomaterial Preparation Method	Nanomaterial Characterization Techniques	Application	Ref.
Fe_3O_4	Lipase	Chemical	XRD, SEM and FTIR	Biodiesel production from algal biomass	[36]
Magnetic graphene oxide	Candida rugosa lipase	Chemical	Autosorb-iQ automatic specific surface, pore size distribution Analyzer, XRD, vibrating sample magnetometer, Malvern potentiometer (NANO ZS90), SEM, TEM, and FTIR	-----	[37]
Ni^{2+}-NTA-boosted magnetic porous silica nanoparticles	Bi-functional enzyme (MLG), which consists of 3-Quinuclidinone reductase and glucose dehydrogenase.	Chemical	SEM, TEM, XRD, FTIR, and Thermal Gravimetric analysis	Biotransformation of 3-quinuclidinone to (R)-3-quinuclidinol	[38]
Fe_3O_4 ferromagnetic	Laccase	Chemical	TEM, FTIR, and XRD	-----	[39]

Table 1. *Cont.*

Types of Nanomaterials	Enzyme	Nanomaterial Preparation Method	Nanomaterial Characterization Techniques	Application	Ref.
Glutathione capped CdTe quantum dots (GSH-CdTeQD)	Glucose oxidase	Chemical	CV, EIS, SECM, SEM, FTIR, TEM, XRD, UV-Vis, and ZS	Developing fuel cells	[40]
Polyacrylonitrile (PAN) nanofibers	Carbonic anhydrase	Electro spun nanofibers	SEM, FTIR, and thermal stability measurements	Interconversion between carbon dioxide and water and the dissociated ions of carbonic acid	[41]

4.1. Metal Nanomaterials

Metal nanomaterials exhibit various catalytic activities [42]. Recently, attempts have been made to develop catalytic systems that combine the features of biocatalysts and metallic nanomaterials [35,43]. Because of their small size, large surface area [44], and quantum size effects, metal-based nanomaterials, particularly noble metals such as gold, platinum, and palladium, are well suited for this purpose. These nanomaterials can facilitate charge transport, enzyme delivery, and the control of enzymatic activity.

4.2. Carbon-Based Nanomaterials

Carbon-based nanomaterials have distinct characteristics such as a large surface area, remarkable chemical stability, and electrical and thermal properties, making them suitable supporting materials for biocatalysis modification. Carbon materials have been used to mediate charge transport, control enzyme activity through photothermal effects, and modulate enzyme activity by using photosensitive compounds. Functionalized nanomaterials have also been developed to regulate enzyme activity [29].

4.3. Porous Silica

The stability and selectivity of enzymes can be improved, and their separation and reuse can be facilitated by confining them within the pore channels of porous silica structures. Additionally, porous silica materials have demonstrated potential as delivery vehicles for enzymes [29].

4.4. Magnetite Nanoparticles

The magnetic properties of magnetite nanoparticles (MNPs) enable fast separation in a magnetic field, which is why they are incorporated into enzymes. MNPs with attached enzymes exhibited improved stability and activity.

4.5. Quantum Dots

Semiconductor quantum dots (QDs) have unique electrical and photonic properties that make them attractive for various optoelectronic applications. CdS QDs can create excitons (consisting of e– and h^+) through photoinduction, which can then generate hydroxyl and superoxide radicals in aqueous environments [45].

4.6. Nanofiber

Nanostructured fibers (NFs) have been investigated as promising materials for NBC assembly owing to their remarkable properties, including high enzyme loading and uniform dispersion in the liquid phase. Additionally, their high porosity and interconnectivity allow for low mass transfer resistance. The unique surface characteristics, discrete nanostructures, and self-assembling behavior of NFs offer exciting possibilities for developing NBCs for bioprocesses using bioreactor systems. By integrating enzymes with NFs, a hybrid

assembly was created that combined the biocatalytic capabilities of enzymes with the unique functions of NFs within the nanostructure network [5].

5. Synthesis of Nanomaterials

Top-down and bottom-up approaches are two basic methods for producing nanocatalysts. The bulk material is broken down into smaller nanosized particles using a top-down technique. Top-down processes such as etching, sputtering, and laser ablation can be used to create a variety of metallic nanoparticles [46,47]. In contrast, the bottom-up technique involves constructing a material atom-by-atom or molecule-by-molecule to produce complex nanoscale structures [48]. Supercritical fluid synthesis, laser pyrolysis, plasma or flame spraying synthesis, molecular condensation, sol-gel processes, chemical reduction, and green synthesis are examples of bottom-up processes. The physicochemical interactions that occur in this method can have a considerable impact on the properties of the nanoparticles built from smaller pieces. Kinetic processes determine the final size and shape of the resulting nanoparticles in both the top-down and bottom-up approaches.

5.1. Green Synthesis of Nanomaterials

Owing to its eco-friendliness, low energy usage, and green status, the biological technique of nanoparticle synthesis is increasingly favored over conventional top-down and bottom-up methods [49,50]. The use of biological sources for nanoparticle synthesis offers several advantages, such as increased specific surface area, improved metal salt and enzyme properties, and increased catalytic activity [51]. The primary aim of using biological methods for synthesizing nanoparticles is to use inexpensive resources and enable continuous production while ensuring a uniform particle size. Microbes such as bacteria, fungi, and algae are the most common biological sources employed for nanoparticle fabrication, as shown in Figure 2. Bacteria are the most abundant organisms in the biosphere and can produce a variety of nanoparticles under appropriate parameters, such as pH, temperature, and pressure [52]. Bacterial cells are highly suited to nanoparticle production because of their ability to survive and reproduce under severe conditions, including the presence of high metal concentrations, which might be attributed to their unique resistance mechanisms. Bacterial strains that are not natively resistant to high metal concentrations may still be used to create nanoparticles. Nanoparticles generated by microbes have various applications in bioremediation, bioleaching, biocorrosion, and biomineralization. Apart from bacteria, fungi, and algae are two other sources that can synthesize nanoparticles in an eco-friendly manner. Fungi, with their ability to produce various bioactive compounds, are often employed as reducing and stabilizing agents for large-scale nanoparticle synthesis with controlled shapes and sizes [53]. On the other hand, algae can produce various bioactive compounds, pigments, and proteins that act as capping agents in the synthesis process, aiding the reduction of salts [54] as shown in Table 2.

Table 2. Different biological sources for green synthesis method of nanomaterials.

Green Synthesis Method	Nanomaterial	Organism	Ref.
Bacterial Synthesis	Gold	*Rhodococcus* species	[55]
	Gold	*Rhodopseudomonas casulata*	[56]
	Silver	*Morganella* sp.	[57]
	Silver and gold	*Bacillus subtilis*	[58]
	CdS fluorescent	*Halobacillus* sp.	[59]

Table 2. *Cont.*

Green Synthesis Method	Nanomaterial	Organism	Ref.
Fungal Synthesis	Gold	*Verticillium* sp.	[60]
	ZnO@SPION@Ag	*Fusarium oxysporum*	[61]
	SPION@Ag@Cs	*Fusarium oxysporum*	[62]
	Silver	*Aspergillus fumigatus*	[63]
	Silver	*Trichoderma reesei*	[64]
Algal Synthesis	Gold	*Fucus vesiculosus*	[65]
	Gold	*Tetraselmis suecica*	[66]
	Silver	*Chlamydomonas reinhardtii*	[67]
	Zinc oxide	*Sargassum muticum*	[68]
	Silver	*Portieria hornemannii*	[69]

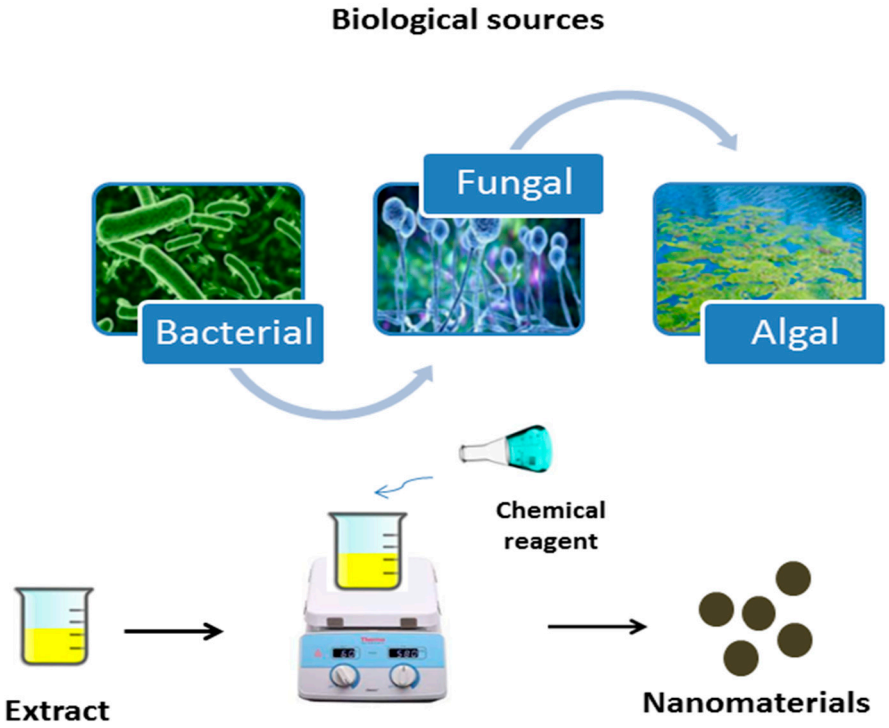

Figure 2. Green synthesis of nanomaterials in an eco-friendly method using biological sources with low cost.

5.1.1. Bacterial Synthesis

Compared to other living organisms, prokaryotic bacteria are preferred for metallic nanoparticle synthesis because of their relative ease of manipulation, resulting in their extensive study. Bacteria have demonstrated the ability to reduce metal ions to synthesize nanoparticles through various studies. Bacterial synthesis is advantageous because of its high reproductive rate and minimal use of toxic chemicals. However, there are some drawbacks to bacterial synthesis, such as lengthy culturing processes and difficulties in controlling the shape, size, and distribution of nanoparticles. A study using buttermilk *Lactobacillus* strains revealed a high concentration of metallic ions and the formation of multiple well-structured gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs). *Lactobacillus* can produce nanoparticles within its plasma membrane while remaining alive [70].

5.1.2. Fungal Synthesis

Owing to their advantages over bacteria, fungi have gained popularity as biological agents for the synthesis of metal nanoparticles. Fungi are an appealing choice for nanoparticle synthesis because of the presence of mycelia, which increases the surface area of the fungi, as well as the economic feasibility and simplicity of scale-up and downstream processing [71]. Fungi can also produce various enzymes that aid in the synthesis of nanoparticles with different shapes and sizes. Owing to their larger biomass than bacteria, fungi can produce higher quantities of nanoparticles. Because of benefits such as greater surface area of mycelia and ease of scale-up, fungal species such as *Fusarium oxysporum*, *Aspergillus oryzae*, *Verticillium luteoalbum*, *Alternata alternata*, and *Colletotrichum* sp. have been used for nanoparticle production. However, there are some challenges associated with using fungi, such as the high cost and labor-intensive nature of the downstream processes. Fungal cultures can produce nanoparticles either intracellularly or extracellularly, with intracellular synthesis involving the addition of a metal precursor to the fungal culture and its internalization by the biomass. Subsequently, nanoparticles can be recovered by breaking down cells using techniques such as chemical treatment, centrifugation, and filtering [72]. Extracellular synthesis is the most commonly used method for fungal nanoparticles. A metal precursor was combined with an aqueous filtrate containing fungal bioactive chemicals using this approach, which allowed for the simple creation of nanoparticles [73].

5.1.3. Algal Synthesis

Algae are a collection of organisms of significant ecological and economic importance. They can be unicellular or multicellular and are found in both marine and freshwater habitats. Algae are divided into two types: macroalgae (large, multicellular seaweeds) and microalgae (unicellular or colonial, often tiny seaweeds). Owing to their unique properties and biochemical composition, algae have numerous commercial applications in industries, such as food and beverages, pharmaceuticals, cosmetics, and biofuels. Algae have several benefits, such as low toxicity and the ability to be synthesized at low temperatures. Typically, three basic processes are involved in the production of nanoparticles using algae. First, an algal extract is prepared by boiling or heating algae in water or an organic solvent for a set period. Second, ionic metal complex molar solutions are produced. Finally, molar solutions of ionic metal compounds are blended and incubated under regulated conditions with or without continuous mixing with the algal extract solution [74]. The types of algae used and their concentrations largely determine the production of metallic nanoparticles. Metal ions can be reduced by various biomolecules, including peptides, polysaccharides, and pigments. Cysteine residues and amino groups in various proteins, as well as sulfur-containing polysaccharides, can cap and stabilize aqueous solutions of metal nanoparticles [75]. Nanoparticle production is faster when using algae compared to other biological agents.

6. Modification of Nanomaterials for NBCs

6.1. Silica Nanoparticle Modification

There are several methods for functionalizing nanoparticles to improve enzyme immobilization, including the use of functionalized silica nanoparticles, as shown in Figure 3 [76]. Surface modification techniques such as covalent bonding or adsorption can be used to functionalize silica nanoparticles with amino, carboxyl, or thiol groups. These functionalized silica nanoparticles can then immobilize enzymes via covalent bonding, electrostatic interactions, or physical adsorption. The surface of silica gel contains silanol groups, which makes it easy to modify with functional group-containing silanes. 3-Glycidyloxypropyl trimethoxysilane (3-GPTMS), which has an epoxy group at the end of its chain, is the most commonly used silane. Owing to its strong reactivity, this epoxy group can form direct linkages with enzyme molecules by interacting with functional groups such as $–NH_2$ and –HSa [77].

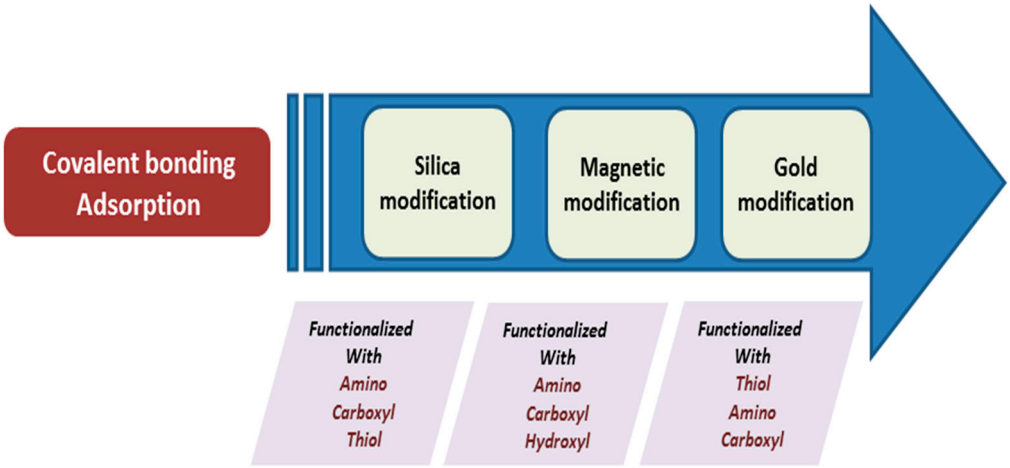

Figure 3. Covalent bonding and adsorption are used in the modification of different types of nanomaterials.

6.2. Magnetic Nanoparticle Modification

Another approach for functionalizing nanoparticles to enhance enzyme immobilization involves the use of magnetic nanoparticles. These nanoparticles can be modified with amino, carboxyl, or hydroxyl groups through surface modification techniques such as covalent bonding or adsorption [78]. Functionalized magnetic nanoparticles can immobilize enzymes via magnetic attraction, covalent bonding, or physical adsorption. One advantage of using magnetic nanoparticles is the ease of separating immobilized enzymes from the reaction mixture using an external magnetic field [79]. (3-aminopropyl) triethoxysilane (APTES), an amino-functional reagent, is commonly used to modify magnetic nanoparticles [80]. Magnetic nanoparticles can be coated with silanes to generate a core–shell structure with an amino-enriched surface via a simple polymerization procedure [81]. Using glutaraldehyde (GA) as a versatile reagent, a magnetic biocatalyst can be produced by covalently bonding lipase to amino groups on the carrier. In a study by Thangaraj et al. [82], Fe_3O_4 particles were enclosed within mesoporous Si and modified with either APTES or 3-mercaptopropyltrimethoxysilane (MPTMS). The lipase was then attached to the modified support using glutaraldehyde as the crosslinking agent. During the conversion of soybean oil to biodiesel, the catalytic activity of lipase immobilized on APTES-modified Fe_3O_4 particles was greater than that of MPTMS, resulting in a yield of more than 90%. Organic polymers, such as synthetic polymers and biopolymers, are often used to modify magnetic nanoparticles. Their surfaces contain numerous functional groups that act as binding sites for the enzymes. One advantage of using polymers is their ability to select monomers and associated functional groups based on immobilized enzymes. Introducing functional groups onto the surface of magnetic nanoparticles using organic polymers can enhance the interaction between the support and enzyme, as well as between the substrate and enzyme, leading to improved enzymatic activity. This approach is commonly employed to alter different inorganic nanomaterials such as magnetic nanoparticles [83]. Chitosan and polydopamine are biopolymers commonly used for material modification. Wan et al. [84] used a quick interface-directed co-assembly method to insert a mesoporous polydopamine layer onto the outer surface of empty magnetic nanoparticles. These hollow magnetic nanoparticles display better magnetism, low density, high specific surface area, and outstanding uniformity compared to ordinary Fe_3O_4 nanoparticles. By altering the number of templates in the emulsion system, the shell structure and pore size (ranging from 11.53 to 49.53 nm) were changed.

6.3. Gold Nanoparticles Modification

Gold nanoparticles are frequently used to immobilize enzymes. They can be modified with thiol, amino, or carboxyl groups through surface modification techniques such as covalent bonding or adsorption [85]. Functionalized AuNPs can immobilize enzymes via covalent bonding, electrostatic interactions, or physical adsorption. One advantage of using AuNPs is the ease of detecting and quantifying immobilized enzymes using techniques such as UV-visible spectroscopy [86].

7. Applications of Nanobiocatalysis

Owing to its stability, bioprocessing efficiency, engineering potential, and ease of downstream recovery, nanobiocatalysis is a promising and safe type of nanomaterial with a wide variety of applications in fields such as biomedicine and pharmaceuticals, as shown in Figure 4. Because enzymes are involved in many of these applications, their physicochemical qualities might vary in ways that are either advantageous or disadvantageous. For instance, nonspecific adsorption of enzymes onto single-walled carbon nanotubes (SWCNTs) may reduce the fundamental physical properties of SWCNTs [87]. A nanoparticle–protein corona, which gives the particles a biological identity, can be formed when proteins or enzymes bond to the surface of the particles [88]. Understanding how nanomaterials affect the structure and function of enzymes is crucial. Enzymatic adsorption onto nanomaterials generally results in conformational changes and either increases or decreases enzyme activity. It has been shown that a variety of nanomaterials, such as metal nanoparticles, graphene, CNTs, and fullerene derivatives, have distinct effects on the structures or activity of enzymes. The various impacts are contingent upon the enzyme type and orientation, the physical characteristics (such as size and shape) of the nanomaterials, the chemical groups that are attached to them, and the surrounding conditions [88]. Because of their varying 3D structures and amino acid compositions, various enzymes interact with nanomaterials in different ways. The way that enzymes are oriented with respect to nanomaterials is important since the wrong orientations can block the enzymatic active sites [89]. The binding orientations and interactions of nanomaterials with enzymes, as well as the stability of enzymes and substrate accessibility, are influenced by their physicochemical qualities and the surrounding environmental variables, such as pH and temperature. The adsorption of lysozyme onto three functionalized multi-walled carbon nanotubes (MWCNTs) that were carboxylated, hydroxylated, or graphitized was studied by Du et al. [90]. The highest adsorption capacity was exhibited by hydroxylated MWCNTs, which was succeeded by carboxylated and graphitized MWCNTs. Significant activity reduction was discovered when Pan et al. [91] investigated the structural basis and adsorption of T4 lysozyme onto silica nanoparticles. Another enzyme that is frequently employed to examine how nanoparticles affect enzymatic activity is α-chymotrypsin. Carboxylated SWCNTs have the ability to control the activity of α-chymotrypsin.

Immobilized enzymes have been used in large-scale commercial processes, such as lipases for food oil transesterification, glucose isomerases for corn syrup fructose processing, penicillin G acylase for antibiotic modification, and laccases for pollutant degradation. However, most laboratory-scale biodevice studies are still in their early phases of development. Nonetheless, recent developments in these areas have shown the possibility of building NBC-based systems in various industries [18]. Table 3 compares different case studies of nanobiocatalysts.

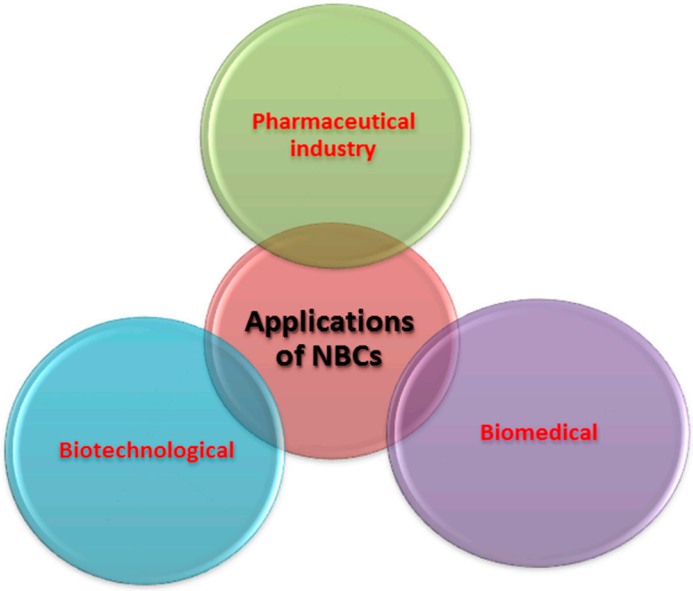

Figure 4. Nanobiocatalysis has many applications in different fields.

Table 3. Comparison of Different case studies of NBCs.

Application	Nanomaterial	Enzyme	Characterization	Ref.
Pharmaceutical industry	Dendrimer-grafted flower-like Fe_3O_4@SiO_2/PAMAM microcarriers	Penicillin G acylase	SEM, TEM, VSM and XRD	[22,92]
	Glutaraldehyde-activated magnetic microspheres	Purine nucleoside 2′-deoxyribosyltransferase from Trypanosoma brucei	SEM and DLS	[93]
	Glutaraldehyde-activated MagReSyn® Amine magnetic iron oxide porous microparticles	Adenine phosphoribo-syltransferase 2 from Thermus thermophilus HB8	-----	[94]
Biomedical	Magnetic, mesoporous, polymeric and liposomes	tPA, streptokinase, and uPA	-----	[95]
	Protective antioxidant carriers	Catalase and superoxide dismutase	Zeta potential and hydrodynamic diameter	[96]
	Co-polymers of polyethylene glycol and poly-lactic/poly-glycolic acid	Catalase, peroxidase, and xanthine oxidase	-----	[97]
	Mesoporous silica	Superoxide dismutase	TEM and zeta potential	[98]
	Peptide-Based Biomaterials	Protease	-----	[99]
	Liposome	Phospholipase	Zeta potential	[100]
Biotechnological	Magnetic Fe_3O_4 nanoparticles modified graphene oxide nanocomposites	Trypsin reactor	TEM and SEM	[101]

7.1. Pharmaceutical Industry

Enzyme immobilization is a promising technique in the pharmaceutical sector, and NBCs have gained importance owing to their ease of recovery, biocompatibility, and environmental safety. The immobilization of penicillin G acylase (PGA) is crucial for the cost-effective large-scale cleavage of penicillin G. covalent immobilization of PGA using various magnetic porous nanoparticles, such as the difunctional 'hierarchical petal' (nanoflower) as has been reported in recent studies. Bilal and Iqbal [22] and Li et al. [92] have demonstrated successful hydrolysis of penicillin G using immobilized PGA with positive results. The use of these supports allows for a decrease in diffusion barriers, resulting in greater access to catalytically active areas and, eventually, an increase in antibiotic production. A comparative study conducted by Illanes et al. [102] revealed that PGA showed enhanced operational efficiency and stability when encapsulated within magnetic sol-gel silica microparticles compared with commercial glyoxal agarose-immobilized PGA and PGA-450 when used for controlled cephalexin production. Additionally, a novel approach using click chemistry was employed to develop magnetically switchable bioelectrocatalysts through ferrocene-grafted Fe_2O_3 onto magnetic mesocellular carbon foam via manipulation of the external magnet positions. The integration of protein engineering technologies with enzyme immobilization methods can optimize the performance of NBCs, making them suitable for use in various industrial settings [103]. To enhance its catalytic activity towards non-natural nucleosides, Trypanosoma brucei purine 2′-deoxyribosyltransferase was modified through hierarchical engineering, as described by P'erez et al. [93]. Arco et al. [94] demonstrated that successfully His-tagged enzymes were effectively adsorbed onto commercial Ni^{2+}-chelated magnetic metal-oxide porous microspheres. In a separate study, Arco et al. [94] covalently immobilized adenine phosphoribosyl transferase in two ways: by attaching it to the N-terminal residue and by multipoint binding with surface-exposed lysine residues.

7.2. Biomedical

The potential therapeutic applications of enzymes in various heart, oncological, viral, and hereditary diseases have been widely studied [95,104–106]. In practice, the application of these enzymes is limited because of their short lifespan, rapid degradation, and potential for immune reactions in the human body [104]. The introduction of nanomaterials into biological fluids leads to protein-shell formation, known as the "protein corona", which has uncontrollable effects. The activation and clearance of nanomaterials by the immune system are attributed to this protein corona. Nonetheless, coupling target enzymes with specific nanocarriers could help overcome these issues by enabling their precise distribution at desired locations. This targeted approach can also regulate enzyme-nanocarrier ratios to minimize immunogenicity risks [107].

7.2.1. Nano-Enzymes for Thrombolytic Therapy

Tissue plasminogen activator (tPA), streptokinase, and urokinase-type plasminogen activator (uPA) are medicinal enzymes used to prevent blood coagulation induced by acute myocardial infarction or cerebral microthrombosis. However, there is a risk of non-specific activation leading to unwanted heavy bleeding. To address these limitations, magnetic nanoparticles, liposomes, and polymeric NPs have been utilized in combination with thrombolytic enzymes to achieve targeted action at the site of blood clotting and minimize the associated risk, as shown in Figure 5 [95]. Magnetic nanoparticles have been proposed as a promising approach for targeted therapies that deliver thrombolytic enzymes to specific sites of blood clotting to reduce the risk of adverse effects. The effectiveness of magnetic nanocarriers in delivering streptokinase to the site of canine carotid artery thrombosis, using an external magnetic field, has been demonstrated. Mesoporous NPs are particularly successful in increasing thrombolytic activity, making them a suitable material for increasing the urokinase capacity for loading up to 30 times. Echogenic liposomes immobilized with tPA have been used to facilitate ultrasound-based thrombolysis. Further-

more, polystyrene latex nanoparticles with a diameter of 40 nm were used to covalently link tPA and anti-fibrin antibodies, enabling them to be delivered directly to the coagulation site, lowering the possibility of systemic toxicity.

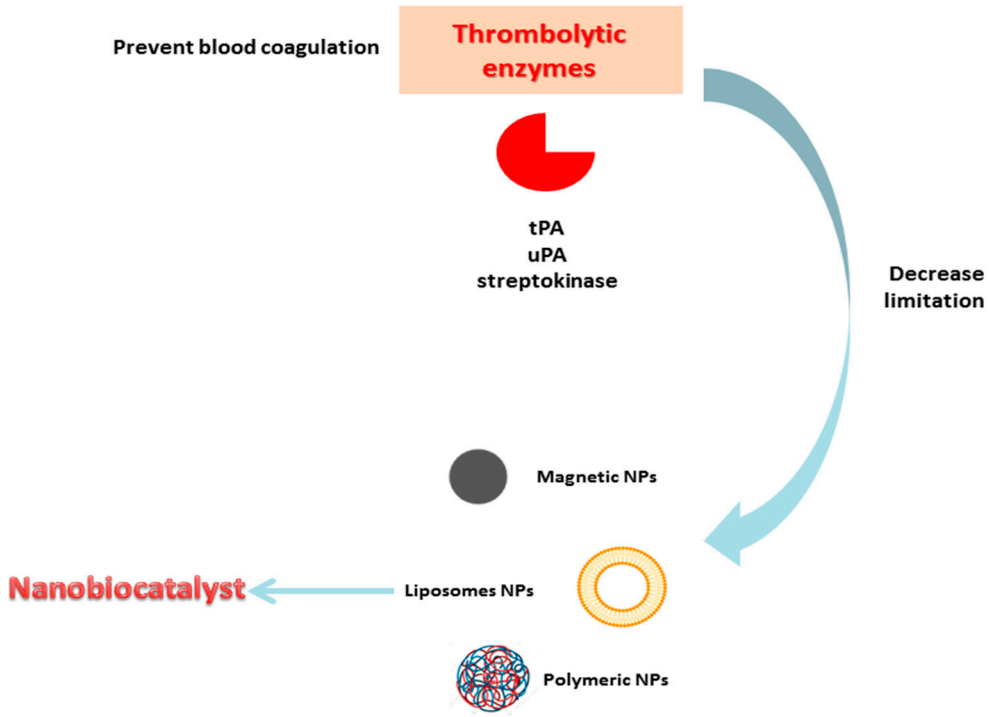

Figure 5. Nanomaterial as a carrier for the thrombolytic enzyme to decrease its limitation.

7.2.2. Nano-Enzymes for Treatment of Oxidative Stress and Inflammation

Endogenous ROS neutralization in cells frequently includes catalytic processes involving enzymes such as superoxide dismutase (SOD), peroxidases, and catalase [96,97]. When there is excess ROS generation, exogenous SOD and catalase can be administered at the site of inflammation. Because of the limited stability of these enzymes, nanocarriers that can protect them from degradation during injection, such as polyethylene glycol (PEG) or poly(lactic-co-glycolic acid) (PLGA) copolymers and oleate-coated magnetite NPs, are used. It has been demonstrated that encapsulating catalase or peroxidase-based compounds into polymeric NPs protects cell cultures from vascular oxidative stress [97]. Polymeric nanoparticles carrying catalase or SOD have entered the pulmonary vasculature at a rate of 33% within 30 min of intravenous infusion. Additionally, these particles have demonstrated protective effects against acute inflammatory responses induced by endotoxin exposure in mice [96]. It is vital to note that prolonged usage of PEGylated materials may result in the development of anti-PEG antibodies, resulting in the "accelerated blood clearance" (ABC) effect. Nanoparticles loaded with SOD, which are aimed specifically at the central nervous system (CNS), have been studied as a possible anti-inflammatory and anti-apoptotic agent. Poly-butyl cyanoacrylate (PBCA) nanoparticles, which include SOD, can efficiently cross the blood–brain barrier while maintaining their enzymatic activity and receptor binding capacity [108]. Recently, mesoporous silica nanoparticles containing a cell-penetrating peptide generated from the HIV-1 transactivator of a transcription protein combined with the enzyme were employed to enable the effective intracellular delivery of SOD [98].

7.2.3. Nano-Enzymes for Antibacterial Treatment

Numerous studies have been performed on the use of enzyme-conjugated nanoparticles to fight a range of human pathogens, including *Mycobacterium tuberculosis*, *Enterococcus faecium*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* [108]. The majority of research has focused on the use of hen egg-white lysozyme as an N-acetylmuramic hydrolase.

This monomeric protein has disulfide bridges that are stable in its polypeptide chain and may break β-(1,4)-glycosidic bonds between bacterial cell wall components, such as N-acetylmuramic acid and N-acetylglucosamine, both of which have substantial antibacterial activity against Gram-positive bacteria. In this regard, co-immobilization onto polystyrene (PS) nanoparticles was performed by coupling lysozyme with anti-Listeria monocytogenes antibodies onto PS-NPs, leading to increased catalytic activity compared with both native lysozyme and antibody-free modification. In addition, the efficacy of AgNPs as potent antimicrobial agents has been confirmed in various bacterial strains that develop resistance to Ag^{+} ions. This was confirmed using lysozyme [109].

7.2.4. Drug Delivery Systems

Endogenous enzymes play an important role in the microenvironment of certain tissues for targeted and regulated drug release, resulting in the successful treatment of many diseases. In this section, we present an overview of diverse techniques that employ the biocatalytic effects of enzymes for drug delivery, therapy, and diagnostics. Enzymes mostly interact with nanocarriers to catalyze reactions within the host system, as shown in Figure 6. The hydrolase class of enzymes, including lipases, proteases, and glycosidases, is extensively used for drug delivery [110]. Bioactive moieties are commonly linked to biocompatible nano-assemblies through cleavable units, enabling nonspecific hydrolases to break down bonds that anchor drug molecules. This process leads to successful and controlled drug release and is referred to as a hydrolase-responsive nanomaterial [111]. Enzyme-triggered drug transporters facilitate the exposure of medicinal molecules to specific target tissues and their internalization into specific cell types. Drug release from different nanocarriers is achieved via enzymatic cleavage at specific sites. Various nanomaterials, such as crosslinked matrices, self-assembled systems, and caged porous structures, can incorporate drugs via physical encapsulation or covalent binding [110,111].

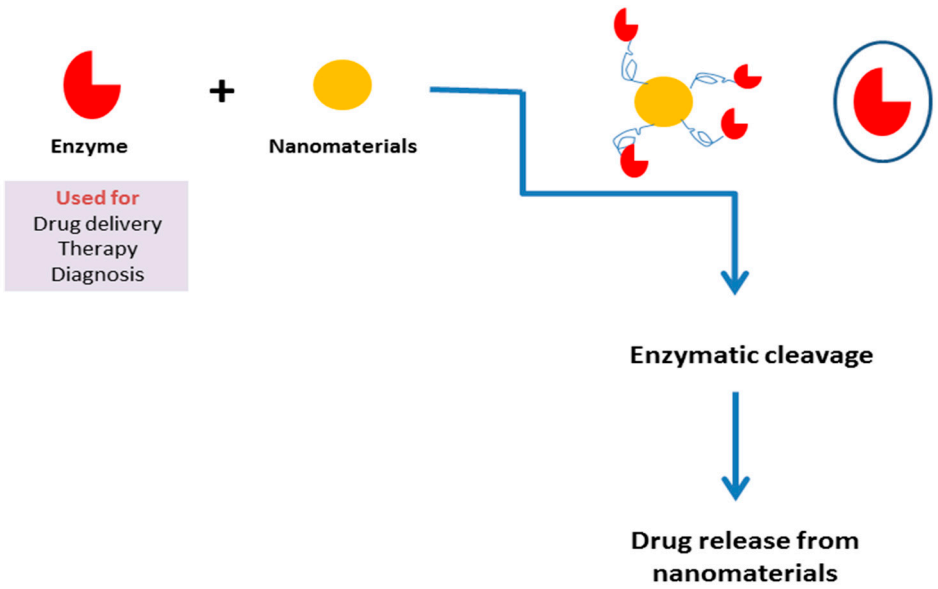

Figure 6. Drug delivery system using nanobiocatalysis.

Diseased tissues exhibit an imbalanced gene expression of specific proteases, which affects their expression and activity. Proteases have potential applications as advanced drug delivery platforms owing to their selective activation abilities. Enzymes play critical roles in a variety of physiological processes, including tissue remodeling, wound healing, and cancer proliferation [110]. For instance, Kang et al. [112] synthesized peptide-conjugated polymer nanoparticles that can activate target genes upon protein kinase or protease stimulation. Law et al. [99] developed self-assembled peptide nanocarriers that engage with infection-associated proteases to release therapeutic medications and destroy

peptide fragments. Because of their role in cancer and other degenerative diseases, matrix metalloproteinases (MMPs) are a family of proteases that have been frequently employed for regulated drug delivery. These enzymes play crucial roles in protein degradation and regulate various cell behaviors that are involved in disease physiology. In contrast to normal physiological conditions, MMPs are excessively expressed in pathological states such as cancers [113]. Jiang et al. showed that proteolytically activated cell-penetrating peptide-modified nanoparticle drug delivery systems induced by MMP-2 and MMP-9 are effective in cancer therapy. As cancer-associated MMP-2 overexpression catalyzes proteolysis inside tumor tissues, these protease-mediated platforms are promising for the targeted delivery of drugs to treat malignant growth [114]. Therefore, the exploitation of proteases is an advantageous strategy for biomolecule design.

Overexpression of lipolytic enzymes, especially phospholipase, is a known pathological indication of various illnesses, including cancer, myocardial infarction, neurological disorders, delayed wound healing, inflammation, and infectious diseases [100,115]. Phospholipase A2 (PLA2), which is commonly observed in cancerous tumors, are among these enzymes that are upregulated [106]. According to previous research, patients with prostate cancer have considerably greater levels of PLA2 expression than disease-free paired controls [116]. Enhanced secretion of PLA2 within the tumor microenvironment facilitates carcinogenesis by releasing arachidonic acid-derived metabolites that promote malignancy and lysophosphatidic acid-induced cell growth by liberating lysophospholipids into circulation [116]. PLA2 enzymes, particularly upregulated PLA2, have been investigated for their potential use in facilitating targeted drug release from tumors. Aili et al. [100] studied the potential use of elevated levels of PLA2 enzymes within tumor microenvironments. Andresen and colleagues [117] created an enzymatically activated liposome-based drug delivery system in which anticancer ether lipids were disguised as prodrugs. PLA2-activated pro-AELs have been shown to be capable of converting water-soluble pro-drugs into effective chemotherapeutic agents by breaking cell membranes and enhancing the cellular absorption of medicines contained inside liposomes.

The hydrolase category, comprising glycosidases, phosphatases, ureases, and amidases, is crucial for initiating the targeted release of drugs at the infection site [118–120]. When released inside the microenvironment surrounding the target tissue, biocatalysts, such as glycosidases, may hydrolyze carbohydrates into smaller sugar molecules, making them essential for target-specific drug delivery [121]. Studies have shown that α-amylase is overexpressed up to eighty-five times more in tumor tissues, providing an opportunity for designing polysaccharide-based nanocarriers capable of releasing anti-cancer drugs specific only to these tumors [121]. To confirm this, dextran was covalently linked to succinylation via PLA2. According to Wang et al., PLA2 has been employed in phase I clinical trials for the treatment of bladder, breast, cervical, and lung cancers; however, more than 50% of patients exhibit neurotoxicity as a side effect. Nevertheless, the conjugation of sugars with PLA2 resulted in decreased activity and eliminated systemic toxicity [118]. Nanocarrier functionalization was successfully achieved by adding α-amylase at optimal concentrations, which facilitated the release of PLA2 [118]. Harnoy et al. [122] used the enzyme penicillin G amidase to deconstruct the synthesized micelles by breaking down the phenyl acetamide end groups. As a result, regulated drug administration occurred at the disease site, emphasizing the importance of hydrolases as essential biomarkers in the creation of new drug delivery systems with enhanced characteristics.

Oxidoreductases have the potential to be a target for drug delivery systems, particularly in diseases that generate oxidative environments such as cancer, diabetes, and neurodegenerative disorders [123]. In enzyme-responsive controlled-release systems for therapeutic drugs, enzymes such as glucose oxidase (GOx), catalase, and peroxidase are often utilized [123]. Gu et al. [124] developed a chitosan-coated nanoparticle drug delivery system that contained insulin as the core therapeutic agent, along with glucose oxidase and catalase enzymes. This study aimed to manage blood sugar levels in diabetic individuals undergoing closed-loop insulin treatment. The enzymatic activity of the nanoparticles

allowed for glucose-triggered insulin release, resulting in improved glucose regulation in vivo. Under hyperglycemic conditions, the nanoparticle network disintegrates upon the conversion of glucose to gluconic acid via GOx, leading to the subsequent release of insulin, which is a target for the treatment of diabetes [124]. Azoreductase is an enzyme that has received significant attention for its potential in treating colon diseases due to its presence in gut microflora. A drug delivery system that was responsive to azoreductases was developed by Rao and Khan [125] using nanoplatforms. The system was created through the copolymerization of drugs and azobenzene-linked poly(ethylene glycol)-b-poly(styrene) and demonstrated sensitivity and selectivity toward azoreductase. This method has the potential to treat colonic disorders. Overall, the discovery of oxidoreductase-responsive nanocarriers provides prospects for enhancing drug delivery.

High enzyme loading capacity, immobilization effectiveness, and adequate yield for administration into the host system are critical aspects to consider when developing viable and efficient drug delivery nanocarrier systems. Physical adsorption, covalent attachment, crosslinking trapping, and self-assembly with nanomaterials are strategies for loading enzymes or drugs onto nanocarriers. It is crucial to select an appropriate method based on the nature of the enzyme or drug, the properties of the nanocarrier, and the desired release kinetics. The molecular weight of the enzyme loaded onto the carrier, along with the matrix composition and functional groups, is a significant factor that affects the loading of therapeutic molecules. Other important variables include polymer solubility interactions between enzymes and polymers. Furthermore, incubation time plays an essential role in the assembly process [126]. Calvo et al. [127] showed that the isoelectric point of nanoparticles can aid in the efficient loading of macromolecules such as enzymes and medicines by allowing for drug and matrix components to interact via ionic bonding. This results in an enhanced capacity for enzyme/drug loading onto the carriers, which ultimately leads to a regulated and sustainable release rate of these substances upon reaching their site of action. The primary goal of the dosing and administration strategy for immobilized pharmaceuticals (or enzymes) is to promote absorption by target cells, maintain efficient delivery, limit the adverse effects of free drug components on nontarget organs, and demonstrate therapeutic efficacy [128]. Determining suitable routes for drug application relies entirely on identifying the target tissues or distribution sites. Evaluating bioactive cargo, such as whole cells, antibodies, enzymes, or drugs, based on biological activity, requires proper administration. Consequently, nanocarriers that are site-specific for targeting and have prolonged circulation times require expertise in engineering [129]. Currently, biodegradable polymers are used to coat drug-loaded nanocarriers to ensure that the drugs are released after degradation in specific tissues following extended travel [130]. To achieve effective target-specific delivery, the design of these nanocarriers must account for environmental factors such as pH, concentration, viscosity, and toxicity levels [128]. While targeted delivery through different routes of administration offers various benefits and drawbacks, such as bioavailability, absorption rates, and metabolic processes [117], it is critical to have an in-depth understanding of doses and procedures for the safe development and application of nanoparticles in drug delivery.

7.3. Biotechnological

Recent progress in molecular biotechnology and nanoscale science has led to significant advancements in optimizing protein structures, managing enzyme nanoenvironments, and improving nanomaterial properties [131,132]. Nano-biocatalysts have several advantages over macroscopic carrier-supported immobilized enzymes. Owing to their high surface-to-volume ratio, they exhibit enhanced enzyme loading, providing several tens of m^2 of space per gram of nanomaterial for enzyme binding. This results in greater catalytic activity and stability, owing to the confinement of enzymes in a restricted environment. This also facilitates efficient mass transfer, resulting in improved substrate accessibility for the immobilized enzyme [133]. The emergence of nanobiocatalysis has significantly enhanced numerous biotechnological processes. Mass spectrometry (MS) has revolutionized

proteomic research by enabling the identification and quantification of proteins in complex biological samples. By providing detailed information on protein structure and function, mass spectrometry has led to a better understanding of cellular signaling pathways, disease mechanisms, and drug targets. In drug development, mass spectrometry is used to determine the pharmacokinetic properties of drugs, assess drug-target interactions, and optimize drug formulations. As a result, the use of mass spectrometry has substantially aided in the development of extremely effective drug compositions [134]. A critical step in this strategy is to digest proteins with trypsin, which is readily accessible, simple to use, and highly selective. To increase the efficiency and efficacy of trypsin digestion over traditional bulk procedures [108], researchers have used a nanoreactor composed of nanoporous silica as a carrier for the trypsin digestion of proteins. The use of nanoporous silica as a binding agent for target protein molecules through absorption followed by trypsin incubation with the resulting protein-laden nanomaterial yielded enhanced results in terms of peptide production efficiency and optimized mass spectrometry analysis. This "in-nanopore" method of hydrolyzing proteins demonstrates improved digestion facilitated by trypsin while significantly reducing the working time. The immobilization of proteases on solid supports at the nanoscale level has become increasingly popular because of their ability to employ limited volumes that allow for high enzyme concentrations, thus promoting shorter digestion times, lower autolysis probability, and the reuse of bound enzymes. Graphene oxide (GO), hybrid aerogels, magnetic nanoparticles, nanotubes, and porous reactors have all been proposed as promising possibilities for enzyme immobilization to enhance soluble enzyme outcomes.

8. Research Needs and Future Directions

According to our comprehension, future studies must concentrate on the diverse domains associated with the implementation of NBCs. (i) There is a need for an in-depth study of the interaction processes and aspects related to mass and heat transport between substrates and outcomes when using NBCs. (ii) It is critical to examine the logical evolution of NBCs and confinement techniques that incorporate unique concepts such as hierarchical, precise, and targeted procedures. (iii) Since many NBCs have intrinsic catalytic characteristics similar to real enzymes, studies on synergistic catalysis employing these materials might be an attractive path for future research beyond immobilization approaches. (iv) To enhance bioprocess outcomes and explore novel avenues for the creation of NBC–enzyme composites that are highly efficient and adaptable, it is imperative to develop additional computer modeling techniques. Practical implementation strategies must be implemented in conjunction with these developments. (v) The achievement of complete enzyme immobilization through NBC matrices necessitates further experimentation for industrial application. Despite encountering various challenges, the utilization of NBCs as a pioneering framework for immobilizing enzymes remains advanced and presents substantial possibilities [18].

9. Conclusions

In conclusion, this review highlights the significant potential of nanobiocatalysts in revolutionizing bioprocessing methods, particularly in pharmaceutical and medical applications. NBCs, by combining the advantageous properties of enzymes and nanomaterials, offer a sustainable and environmentally friendly alternative to traditional biocatalysts. Enzymes, known for their selectivity and efficiency, can be rendered more stable and reusable through immobilization on various nanomaterials, ranging from nanoparticles to nanotubes and nanocomposites.

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Abbreviations

NBCs	Nanobiocatalysts
NER	Nanoscale enzyme reactor technique
NER	Nanoparticle Enzyme Reactor
MNPs	Magnetite nanoparticles
QDs	Quantum dots
NFs	Nanostructured fibers
APTES	(3-aminopropyl) triethoxysilane
GA	Glutaraldehyde
AuNPs	Gold nanoparticles
MMPs	Matrix metalloproteinases
Gox	Glucose oxidase
tPA	Tissue plasminogen activator
uPA	Urokinase-type plasminogen activator
SOD	Superoxide dismutase
CNS	Central nervous system
PBCA	Poly-butyl cyanoacrylate
MS	Mass spectrometry
GO	Graphene oxide
GSH-CdTeQD	Glutathione capped CdTe Quantum dots
CV	Cyclic voltammetry
EIS	Electrochemical impedance spectroscopy
SECM	Scanning electrochemical microscopy
ZS	Zetasizer Nano
PAN	Polyacrylonitrile
AgNPs	Silver nanoparticles
PLA2	Phospholipase A2
MPTMS	3-mercaptopropyltrimethoxysilane
PLGA	Poly(lactic-co-glycolic acid)
PEG	Polyethylene glycol
3-GPTMS	3-Glycidyloxypropyl trimethoxysilane
SWCNTs	Single-walled carbon nanotubes
MWCNTs	Multi-walled carbon nanotubes
PGA	Penicillin G acylase
ABC	Accelerated blood clearance
PS	Polystyrene

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