*chemistry*

MDPI

*Review*

# Polymer/Enzyme Composite Materials—Versatile Catalysts with Multiple Applications

**Larisa-Maria Petrila [1], Vasile Robert Grădinaru [2], Florin Bucatariu [1] and Marcela Mihai [1,*]**

[1] Petru Poni Institute of Macromolecular Chemistry, 700487 Iasi, Romania
[2] Department of Chemistry, Alexandru Ioan Cuza University of Iasi, 700506 Iasi, Romania
* Correspondence: marcela.mihai@icmpp.ro; Tel.: +40-332-880-220

**Abstract:** A significant interest was granted lately to enzymes, which are versatile catalysts characterized by natural origin, with high specificity and selectivity for particular substrates. Additionally, some enzymes are involved in the production of high-valuable products, such as antibiotics, while others are known for their ability to transform emerging contaminates, such as dyes and pesticides, to simpler molecules with a lower environmental impact. Nevertheless, the use of enzymes in industrial applications is limited by their reduced stability in extreme conditions and by their difficult recovery and reusability. Rationally, enzyme immobilization on organic or inorganic matrices proved to be one of the most successful innovative approaches to increase the stability of enzymatic catalysts. By the immobilization of enzymes on support materials, composite biocatalysts are obtained that pose an improved stability, preserving the enzymatic activity and some of the support material's properties. Of high interest are the polymer/enzyme composites, which are obtained by the chemical or physical attachment of enzymes on polymer matrices. This review highlights some of the latest findings in the field of polymer/enzyme composites, classified according to the morphology of the resulting materials, following their most important applications.

**Keywords:** enzyme immobilization; biocatalyst; multilayer; cross-linking agents; polymer/enzyme complexes; enzyme activity; environmental protection; biosensors; biomedical applications



**Citation:** Petrila, L.-M.; Grădinaru, V.R.; Bucatariu, F.; Mihai, M. Polymer/Enzyme Composite Materials—Versatile Catalysts with Multiple Applications. *Chemistry* **2022**, *4*, 1312–1338. https://doi.org/10.3390/chemistry4040087

Academic Editor: Peter J. Rutledge

Received: 27 September 2022
Accepted: 17 October 2022
Published: 19 October 2022



## 1. Introduction

Enzymes are mainly proteins that act as catalysts in biological processes, reducing the activation energy required for various chemical transformations. Unlike chemical catalysts, enzymes operate under moderate conditions of temperature and pH. Due to their increased stability and mild conditions of use, low toxicity, and low environmental impact, enzymes have a great industrial potential. The applications of enzymes cover a large range of fields, such as food (winemaking, cheeses, splitting proteins, clarification of juices, etc.), medical and pharmaceutical (classic drugs and systems with controlled release of active compounds, synthesis of biologically active compounds, protecting implants from the action of microorganisms etc.), cosmetics (manufacturing of hair dyes, skin care products), textile industry (cellulose splitting, natural fiber dyeing and processing technologies, fiber bleaching), paper manufacturing industry (bleaching, pulp processing), obtaining cleaning products (stain removal), chemical synthesis (favoring some chemical processes), environmental protection (degradation of some pollutants, discoloration of wastewater), and biosensors [1–4]. The reduced stability of enzymes with industrial process parameters (high temperatures, extreme pH, and the presence of organic solvents) and their difficult separation have led to numerous techniques' development to improve the performance of enzyme-catalyzed reactions. Therefore, enzyme immobilization technologies using different types of support materials have been implemented. The immobilization of enzymes can be achieved by chemical or physical binding on a specific support material and lead to the fabrication of composite catalysts with improved stability over a large range of

conditions. The support material used for enzyme immobilization is of capital interest since it influences the properties of the obtained biocatalyst, determining the stability, enzymatic activity, and level of reusability of the biocatalyst [5]. There are various organic and inorganic support materials that can be employed in the immobilization of enzymes, starting with metal oxides, silica, or natural and synthetic polymers, and culminating with hybrid materials based on metal nanoparticles, graphene, or carbon nanotubes. Among them, polymers are of particular importance because they can provide a large number of functional groups capable of interacting with enzymes, ensuring their binding and, at the same time, maintaining the substrate accessibility to the active site of the enzyme. The use of polymeric materials allows for obtaining composite materials with a large spectrum of properties, such as hydrophilic or hydrophobic character, porosity, and the flexibility of the support matrix. The use of natural polymers ensures a series of additional properties, such as biocompatibility, biodegradability, and lack of toxicity, leading to obtaining polymer/enzyme composite materials that can be used in a large number of applications, including in the medical and food fields, while the use of synthetic polymers is more economically feasible and ensures a better control of the properties of the polymer/enzyme composite materials thus obtained. These materials are characterized by increased stability at high temperatures and extreme pH or, in the presence of organic solvents, can be reused in a large number of reaction cycles without a significant loss of catalytic activity, and offer a plethora of potential applications. Their properties can be easily tuned by selecting a certain polymeric matrix or material morphology. Additionally, secondary treatments, such as chemical cross-linking, can be used to enhance the materials' stability. Here, the methods for obtaining polymer/enzyme composites are classified according to material shape/morphology. Recent examples from the last 10 years' literature on the fabrication of composite materials by enzyme immobilization, as well as their potential applications, are presented, with a special interest in subjects of concern, such as water treatment or chemical catalysis.

## 2. Enzymes—Structure, Activity, and Characteristics

Most enzymes are protein molecules of natural or artificial origin, capable of catalyzing an important number of chemical reactions, such as protein digestion, the metabolic reaction involving fatty acids and glycerides, and the transformation of some chemical compounds into their corresponding isomers [2]. Enzymes can be obtained from different natural sources of animal origin (e.g., pepsin, trypsin) or vegetal origin (e.g., α-amylase, cellulase), or can be produced by wild-type microorganisms or by inserting their gene, encoding the targeting enzyme, into plasmids and later isolated, fractionated, and purified by chromatography. In recent years, microbial cultures have become the favored source for acquiring enzymes due to ethical concerns. In the case of artificial enzymes, these are chemical compounds that are able to recreate enzymatic functions. Such molecules are the nanozymes that can replace enzymes and are used especially in the medical field. Enzymes can be classified according to the type of chemical reaction they catalyze, named according to the nomenclature proposed by the Enzymology Commission [6], divided into seven classes, as presented in Figure 1.

The catalytic activity of the enzymes is a result of the presence of active centers in their structure. The active site of the enzymes represents the structural element belonging to its tertiary structure at which the chemical reaction takes place, by binding the substrate to the active center. The matching of the substrate to the active center is generally characterized by high selectivity. The active centers of the enzyme represent 10–20% of its total volume. The binding of the substrate to the active center is achieved through hydrogen bonds, hydrophobic forces, or electrostatic or van der Waals interactions and determines the formation of the activated substrate–enzyme complex, also known as the Michaelis complex. The catalytic activity of the enzymes is closely related to the structure of the active center and the mode of interaction with the substrate. Although the active sites of enzymes show high specificity, several general assumptions are valid for all types of enzymes: the active

center of the enzyme is small as compared with the dimension of the protein structure; it has a three-dimensional structure and allows the formation of secondary interactions with the substrate. At the same time, the active centers of the enzyme are located in cavities generated by both secondary and tertiary structures so that the interaction with a solvent is considerably reduced [7].

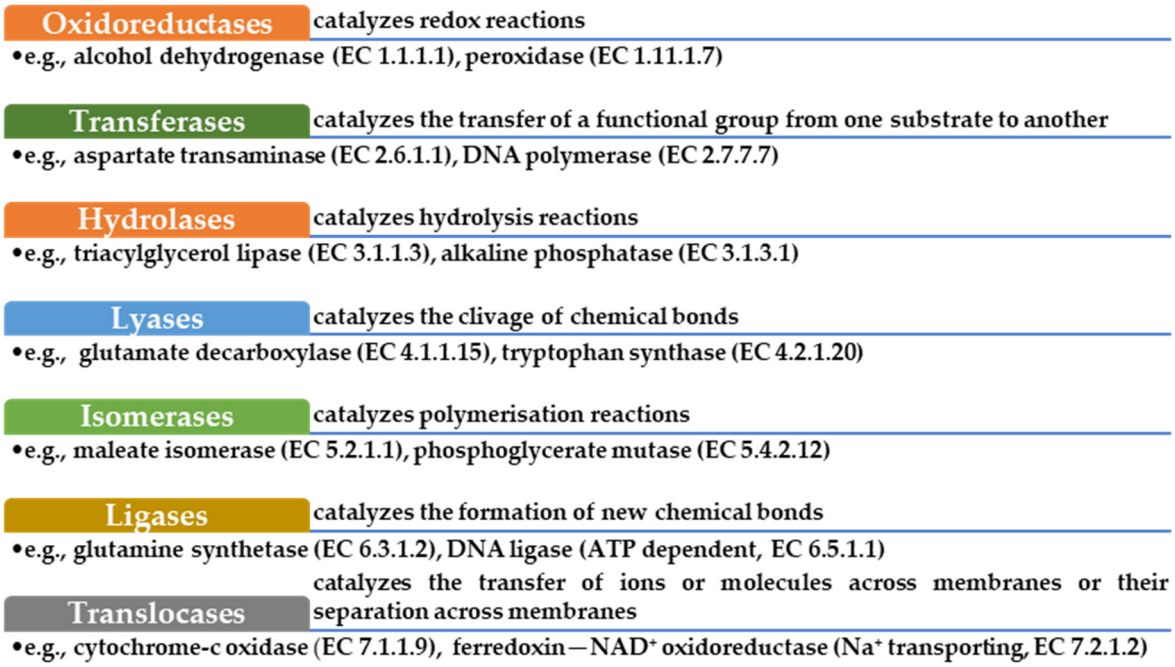


**Figure 1.** Classification of enzymes according to the Enzymology Commission.

## 3. Methods of Enzyme Immobilization on Polymeric Supports

The most important disadvantages of enzymes are related to their instability in several factors, such as temperature, pH, and the presence of organic solvents that can lead to the inhibition of enzyme activity, but also to difficulties in separating enzymes from the reaction medium and the purification of the reaction products. Additionally, enzymes show reduced stability when used in turbulent or continuous flow industrial processes and are difficult to be reused [2]. Therefore, alternative methods have been proposed to improve the characteristics of enzyme catalysis, with a reduction in the costs of the usage of biocatalysts. To limit these disadvantages, as well as to increase the stability of enzymes in conditions of high temperature and extreme pH, or in the presence of solvents, the immobilization of enzymes has been imposed as an effective technique to enhance the enzymatic stability and to increase their reusability. By immobilization, composite materials of different sizes and shapes are obtained, with different properties and characteristics, but with the main advantage of increasing the biocatalyst stability over time. Additionally, a facile separation and the reusability of the immobilized enzymes recommend them as biocatalysts that can be successfully used in industrial processes.

The first studies on enzyme immobilization were carried out in 1916 by Nelson and Griffin [8], who studied the immobilization of invertase using charcoal and aluminum hydroxide. The results suggested that by immobilization, the activity of the enzyme did not decrease considerably, a conclusion that opened new research perspectives in the field of enzyme immobilization. Subsequently, the scientific community's interest in enzyme immobilization grew exponentially, with a large number of immobilization methods being developed, many already being applied in industrial processes.

From the first research study on enzyme immobilization up to now, a large number of immobilization techniques have been proposed (Figure 2). Regarding enzyme immobi-

lization methods, three main techniques have been established: adsorption on a support material, entrapment (encapsulation) in a support matrix, and covalent bonding [9].

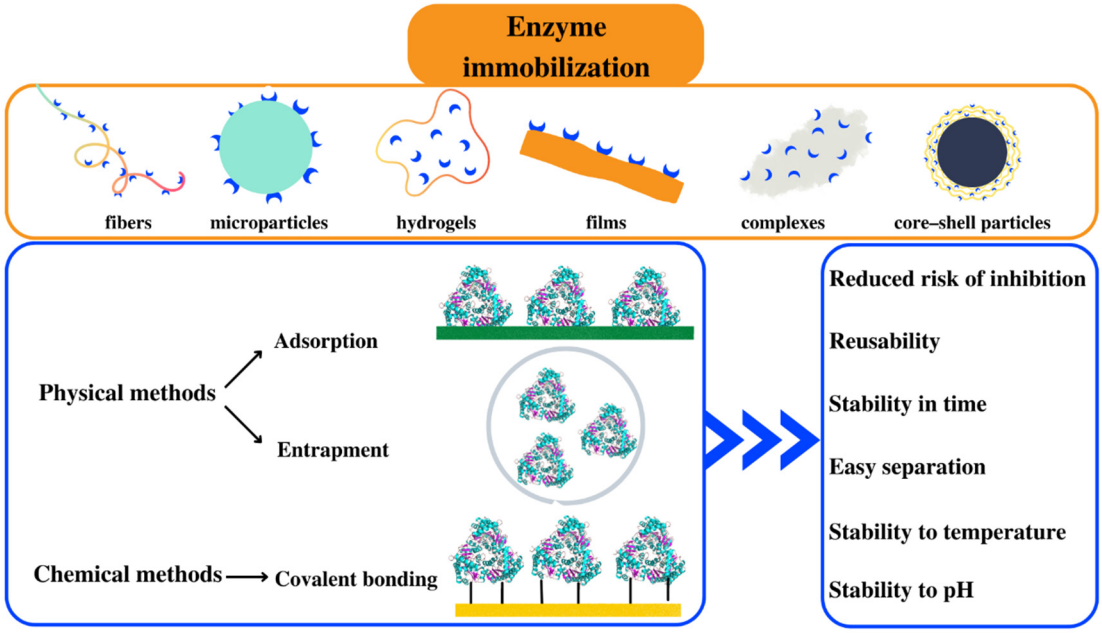


**Figure 2.** The main approaches for enzyme immobilization, common sequestration techniques, and remarkable advantages of immobilized enzymes.

Another method for enzyme immobilization is cross-linking, which is achieved by covalently bonding enzymes with the formation of enzymatic crystals or aggregates. Crystals or enzyme aggregates are formed through the cross-linking of enzymes, but without requiring a support material, the reason why this method of immobilization will not be presented in this paper.

Immobilization methods differ in terms of the chemical and physical interactions that occur between the enzyme and the support, but also by the selection of the support matrix that must ensure the formation of stable enzyme/support composites. In terms of the established interactions, immobilization methods can be classified into chemical methods (covalent bonding and cross-linking) and physical methods (adsorption and entrapment).

### *3.1. Immobilization by Adsorption*

Enzyme adsorption on a support material is the simplest known immobilization method, based on nonspecific physical interactions that occur between the enzyme and the support material, such as hydrogen bonds, van der Waals bonds, and hydrophobic interactions [10]. To avoid the discharge of the enzyme in the reaction medium, it is necessary to ensure certain reaction conditions that prevent desorption, such as the presence of organic solvents or the preservation of certain temperature and pH values [11]. The materials used as a support for enzyme adsorption must ensure the formation of physical interactions with the enzyme, without leading to conformational changes, and can be natural [12,13] and synthetic polymers [14,15], inorganic silica [16], montmorillonite [17], graphene [18], and so on. The composite materials obtained by adsorption are characterized by chemical and thermal stability, affinity for enzymes, the existence of reactive functional groups, reusability, availability, and optimal fabrication costs [19]. Due to the weak and reversible interactions between enzymes and the substrate, the support materials can be easily reused even after the inactivation of the enzyme. Another advantage of immobilization by adsorption is that it does not require the use of additional chemical compounds, such as cross-linking agents. The immobilization conditions are relatively mild and do not lead to enzyme denaturation or loss of catalytic activity during the immobilization, while the physical forces involved

in immobilization are weak and reversible. The main disadvantage of immobilization by adsorption is related to the fact that these materials are susceptible to changes under the action of pH, temperature, or ionic strength, which may lead to enzyme desorption or to its conformational distortion [19].

### *3.2. Immobilization by Entrapment*

Entrapment involves the immobilization of enzymes inside a support matrix, with the formation of composite materials that include the biocatalyst, allowing its interaction with the substrate, but reducing the risk of release of the enzyme from the support matrix. The support material can be represented by micro- or nanoparticles, membranes, films, fibers, or plates. The formation of the support/enzyme composite occurs either by the formation of a solid matrix around the enzyme, by the formation of chemical bonds between the support and the enzyme (by cross-linking or polymerization), or by physical gelation. A similar enzyme immobilization method is encapsulation, which involves the formation of a protective barrier similar to a membrane that "locks" the enzyme inside the support material [20]. Among the materials employed to entrap enzymes, the most popular are chitosan (CHI), calcium alginate (ALGCa), κ-carrageenan, collagen, and gelatin, but also synthetic polymers, such as polyurethanes or polyacrylamide (PAM).

An important parameter of entrapment is represented by the porosity of the support material, which must be high enough to allow the diffusion of the substrate towards the enzyme to favor the catalytic reaction, but low enough to block the desorption of the biocatalyst [10]. Other parameters of interest in entrapment are the dimension and geometry of the support material, the presence of functional groups on its surface, and the hydrophilicity/hydrophobicity of the material [20].

### *3.3. Immobilization by Covalent Bonding*

In immobilization by chemical methods, the most used is the covalent bonding, which involves the formation of chemical bonds between the support and the enzyme under the action of some reactive chemical agents, such as glutaraldehyde (GA) or epichlorohydrin. Genipin, a naturally occurring cross-linking agent, was also used in enzyme immobilization [21]. The most widely used method for covalent bonding is the formation of imide bonds between the enzyme and the support material. The formation of such linkages requires either the existence of carbonyl-type groups on the surface of the support material or the functionalization of the support, introducing reactive functional groups on the surface that allow the formation of bonds with the amino or carboxyl groups of the enzymes [4,11].

Immobilization by covalent bonding involves the formation of strong bonds between the enzyme and the support material, which ensures increased stability of the biocatalyst and reduces the risk of enzyme desorption. However, through the covalent bonding of the enzyme to the support material, conformational changes can occur that hinder or even block the interaction of the substrate with the enzyme active center [11].

Another variant of immobilization is based on affinity between enzymes and particular chemical compounds and involves the formation of specific chemical bonds between the enzyme and the support material, which allows the immobilization of the enzyme while ensuring a maximum number of active centers available for interaction with the substrate. One such example is the use of glycoproteins or proteins as spacers between the enzyme and the support material. Through the occurrence of specific interactions between proteins and enzymes, the attachment of the enzyme can be ensured while maintaining the availability of the active center [11].

## 4. Polymer/Enzyme Composite Materials

By immobilizing enzymes in organic or inorganic support matrices, stable composite materials with good catalytic activity are obtained. Materials used as a support in enzyme immobilization (Figure 2) must possess a series of characteristics, such as physical, chemical, and thermal stability; availability and low cost; large specific surface area; increased

permeability; biocompatibility; and resistance to microbiological contamination [11,22]. Additionally, the support material should ensure an efficient binding to the enzyme and favor the diffusion of the substrate towards the catalytic center [23].

The main types of materials used in enzyme immobilization are inorganic compounds, such as metal oxides, silica, clays, and porous glass, or organic compounds, such as carbon-based materials, natural polymers (CHI, sodium alginate (ALGNa), ALGCa, gelatin, cellulose, etc.), and synthetic polymers (PAM, polyethyleneimine (PEI), etc.). Among them, polymers have prevailed in the enzyme immobilization technology due to their increased availability, low cost, biocompatibility, and high chemical reactivity. It is also worth mentioning that the use of synthetic polymers in enzyme immobilization has an interesting advantage: the starting material can be the monomer itself, giving the composite material an even wider spectrum of properties. Through a careful selection of the monomer, properties such as the solubility of the support matrix, porosity, stability, and satisfactory mechanical properties can be ensured, while also being able to introduce a large number of functional groups, such as carboxyl, carbonyl, epoxy, or amine, which ensure the effective binding of the enzyme to the support material [5]. Hybrid materials obtained based on polymers are of particular interest, especially for biomedical and biotechnological applications, due to the possibility of using natural polymers, characterized by high bioavailability and biocompatibility [23].

By immobilizing enzymes in polymeric materials, different types of polymer/enzyme composites can be obtained, characterized by increased catalytic activity, as well as superior mechanical characteristics, stability in process conditions, and easy recovery and reusability of the biocatalyst [24]. They can be classified according to the nature of the used polymers (natural, artificial, or synthetic), the type of support material used (polymer based only or obtained with hybrid support materials), and the immobilization method used (materials obtained by enzyme adsorption, by entrapment, by covalent bonding), but also according to the morphology of the composite material (fibers, thin films, hydrogels, micro- and nanoparticles, and core–shell particles).

The composite materials, thus, obtained have high stability and increased affinity for biological molecules and preserve the catalytic activity of the immobilized enzyme. Moreover, the introduction of an inorganic component into the matrix leads to improved mechanical properties, as well as a decrease in the chemical reactivity of the material and, implicitly, an increase in its stability in the presence of some toxic chemical agents.

### *4.1. Polymeric Fibers*

Polymeric fibers are usually obtained by electrospinning a polymer solution, and are of high interest for enzyme immobilization because they display a large specific surface area and allow the immobilization of enzymes through the formation of physical or chemical bonds, obtaining composite materials with good catalytic properties [25]. Added to these advantages is the fact that supports in the form of fibers have high porosity, allowing an efficient mass transfer and favoring the transformation of the substrate under the action of the enzyme. Additionally, their surface can be easily modified to ensure good enzyme immobilization [26]. The immobilization of enzymes on the surface or inside of the polymer fibers can be ensured by their adsorption, entrapment, covalent bonding, and electrospinning of a mixture of polymer and enzyme or by retaining the enzyme as aggregates or crystals on the surface of the fibers [10,26].

In recent years, the interest of the scientific community in obtaining polymer/enzyme composite fibers has increased considerably, with a large number of studies being published in this direction, such as the selected examples in Table 1.

**Table 1.** Polymer/enzyme composite fibers.

| Enzyme | Polymer | Immobilization Method | Observations | Reference |
|---|---|---|---|---|
| Alcohol dehydrogenase | polystyrene<br>poly(D,L-lactide-*co*-glycolide) | Covalent bonding | Stable for 7 catalytic cycles, maintaining ~20% of the enzymatic activity | [5] |
| | Poly(vinyl alcohol) (PVA) | Covalent bonding | Stable for 8 cycles of reaction, maintaining 60% of the initial enzymatic activity | [27] |
| α-Amylase | PVA | Entrapment | stable at 80 °C, maintaining 80% of the enzymatic activity at pH = 8 | [28] |
| | Ethyl cellulose | entrapment | stable for 15 catalytic cycles, maintaining 50% of the enzymatic activity | [29] |
| Cyclodextrin—glucan transferase | PVA | Covalent bonding | A 31% increase in the enzymatic activity compared with the control sample | [30] |
| Horseradish peroxidase | PVA<br>PAM | Entrapment | Stable for 25 catalytic cycles, maintaining 54% of the enzymatic activity | [31] |
| | Polyamide | Adsorption<br>covalent bonding | Capable of degrading 70% of the targeted dyes (Reactive Black 5 and malachite green) | [32] |
| Keratinolytic protease | PVA | Covalent bonding | 88% efficiency in the degradation of chicken feathers | [33] |
| Laccase | Zein | Covalent bonding | Used as a time–temperature indicator for food quality control | [34] |
| | Poly(methyl methacrylate) (PMMA)<br>Polyaniline | Adsorption<br>Covalent bonding | Stable for 10 catalytic cycles maintaining 80% of the enzymatic activity | [35] |
| | CHI<br>PVA | Covalent bonding | Used as a time-temperature indicator for food samples | [36] |
| | Polystyrene<br>Poly(D,L-lactide-*co*-glycolide) | Covalent bonding | Stable for 7 catalytic cycles, maintaining ~20% of the enzymatic activity | [5] |
| Lysozyme | CHI | Covalent bonding<br>Adsorption | Stable for 9 reaction cycles, 70% of the enzyme activity is maintained | [37] |
| Papain | PVA | Covalent bonding | Stable after 14 days of storage, maintaining 40% of the initial activity | [38] |

In a recent study, Bayazidi and collaborators described the absorption of lysozyme in cellulose fibers [37]. The obtained results demonstrate that at least 9 reaction cycles may be performed using the immobilized enzyme maintaining about 70% of its activity. At the same time, the loss of activity of the immobilized enzyme compared with that of the free enzyme is only 12%, being correlated with normal phenomena of hampering of some active centers of lysozyme during the immobilization processes, but also with the difficulty of the substrate diffusion towards the active centers of the enzyme. In another study, Jhuang and collaborators obtained composite biocatalysts by immobilizing laccase in electrospun zein fibers [34]. Composite fibers showed improved enzyme stability, with 81% activity retained after 10 days of storage at 4 °C. A similar strategy was proposed by Jankowska and collaborators, who used PMAA and polyaniline fibers for laccase immobilization, using adsorption and covalent bonding to anchor the enzyme to the support material [35]. The method allowed the retention of about 110 mg/g enzyme by adsorption and of 185 mg/g support material by covalent bonding. Regarding the stability of the obtained composites, both types of fibers kept more than 80% of their activity after 30 days of storage and could be used in at least 10 reaction cycles. The same team of researchers proposed the immobilization of horseradish peroxidase by adsorption and covalent bonding, using as support material polyamide fibers obtained by electrospinning [32]. Following the tests carried out, the optimal immobilization parameters of the enzyme were established for the two methods, obtaining the best immobilization yields at 50 °C and pH = 7 in the case of adsorption and at 60 °C and pH = 7 in the case of covalent bonding. Regarding the stability of the composites, it was observed that fibers with covalently bound enzymes retain about 80% of their activity after 30 days of storage, while those obtained by adsorption and the enzyme in its native state lose considerably their activity after 15 days of storage.

### *4.2. Polymeric Hydrogels*

Hydrogels are chemically or physically cross-linked three-dimensional polymer networks. They are versatile materials that can be successfully used in enzyme immobilization, as suggested by the large number of studies carried out in this field. The use of hydrogels in enzyme immobilization comes with several important advantages, such as the lack of toxicity, the biodegradability of the polymer matrix, and the easy, economical, and environmentally friendly methods of fabricating the matrix [39]. They are easy to manufacture, swell strongly in contact with water, and can be successfully used as matrices for the controlled release of active compounds. The most used methods for obtaining polymer/enzyme composite hydrogels are entrapment and covalent bonding. For the fabrication of hydrogels for enzyme immobilization, different natural polymers were used, with ALGNa, CHI, and gelatin being particularly noted, as shown in Table 2.

**Table 2.** Polymer/enzyme composite hydrogels.

| Enzyme | Polymer | Immobilization Method | Observations | Reference |
|---|---|---|---|---|
| Alkaline phosphatase | CHI | Covalent bonding | 71% immobilization yield | [40] |
| Carboxypeptidase A | CHI | Covalent bonding | 86% immobilization yield | [41] |
| Horseradish peroxidase | CHI | Entrapment | ~93% immobilization yield | [42] |
| | PAM | Entrapment | able to catalyze the degradation of 90% of the dye sample tested | [43] |
| | ALGCa | Entrapment | Successfully used for the oxidation of azo-dye Orange II | [44] |
| Laccase | CHI | Entrapment | Stable for 12 catalytic cycles, maintaining 90% of the enzymatic activity | [45] |
| | PAM | Entrapment | ~73% maximum immobilization yield | [46] |
| | Agar-agar | Entrapment | ~80% maximum immobilization yield | [46] |
| | Gelatin | Entrapment | ~64% maximum immobilization yield | [46] |
| | Pectin | Entrapment | Removed ~60% of azo dye Amido Black 10B after 10 catalytic cycles | [47] |
| Lipase | CHI | Covalent bonding | Best results registered for the use of glycidol + ethylenediamine as cross-linking agents | [48] |
| | CHI | Adsorption Covalent bonding | 99% immobilization yield for the covalent bonding | [49] |

An example of composite enzyme/polymer hydrogels was proposed by de Oliveira and collaborators, who used ALGCa spheres for pectinase immobilization [50]. The biocatalyst obtained by immobilizing the enzyme showed increased thermal stability compared with the free enzyme. Deng and collaborators immobilized polygalacturonase in ALGNa spheres, obtaining stable composite particles at different temperatures and pH, while the biocatalyst kept 60% of its activity after 3 cycles of use [51]. A series of hydrogels used for enzyme encapsulation was proposed by Rehman and collaborators, who immobilized polygalacturonase in ALGNa, agar-agar, and PAM spheres [52]. Analyzing the results obtained after immobilization, the following aspects were noticed: the largest amount of enzyme was immobilized in PAM spheres (89% of the enzyme was successfully entrapped in the polymer matrix), followed by agar-agar hydrogels (80% of immobilized enzyme) and by ALGNa spheres (46% immobilized enzyme). Regarding the thermal stability and reusability of the biocatalyst, an increase in the optimal temperature could be observed in the case of the enzyme immobilized in ALGNa and agar spheres, as well as the maintenance of more than 80% of the activity after 2 cycles of use. Additionally, the use of the biocatalyst in a larger number of reaction cycles was observed; the enzyme immobilized in ALGNa spheres lost its activity after 7 cycles of use, while the enzymes immobilized in agar-agar and PAM could be used in 10 reaction cycles, preserving about 20% of the catalytic activity. Awad and collaborators used κ-carrageenan hydrogels as a support for the immobilization of proteases obtained from *Aspergillus welwitschiae* by bonding with polyamidoamine and GA, studying the influence of polyamidoamine concentration on the amount of immobilized enzyme [53]. The best results were obtained when using polyamidoamine in the concentration range 0.8–2.4% at pH = 2.1 and 9.3. The studies carried out on the stability

over time of the composites revealed that they maintain about 90% of their activity after 8 weeks of storage.

Dhiman and collaborators obtained composite materials by entrapping β-mannase in ALGNa and β-cyclodextrin spheres, with an immobilization yield of 91.5% [54], the biocatalyst exhibiting an increase in pH and temperature stability of about one pH unit and 5 °C. The authors also studied the enzymatic activity of the biocatalyst by quantifying the amount of hydrolyzed sugars in carob gum. The obtained results showed that the composite material can be reused up to 15 times with the maintenance of about 70% of the enzyme activity, observing that after 30 days of storage, it maintains about 60% of its catalytic activity.

### *4.3. Polymeric Micro- and Nanoparticles*

Composite materials with nano/micrometric dimensions represent stable and efficient supports for enzyme immobilization. This category of materials includes particles obtained from natural or synthetic polymers (CHI, ALGNa, PEI, etc.), but also composite particles obtained using mixtures of polymers or different types of resins. The stability of this type of composite material is generally ensured by cross-linking [55,56]. Table 3 shows some examples of micro- and nanoparticles obtained from enzymes and polymers.

**Table 3.** Polymer/enzyme composites as micro- and nanoparticles.

| Enzyme | Polymer | Immobilization Method | Observations | Reference |
|---|---|---|---|---|
| Acetylcholinesterase | CHI | Covalent bonding | 81% enzymatic activity after 60 days of storage | [57] |
| Alginate lyase | CHI | Covalent bonding | Antibacterial activity against *P. aeruginosa* | [58] |
| Catalase<br>Diamine oxidase | CHI | Covalent bonding | 100% enzymatic activity after storage for 5 months at −20 °C | [59] |
| Cellulase | ALGNa | Covalent bonding | 67% enzymatic activity after 10 reaction cycles | [60] |
| Cyclodextrin glycosyltransferase | ALGNa<br>gelatin | Entrapment | Can be used for the synthesis of β-cyclodextrin (highest yield of 8.6 g/L) | [61] |
| Glycerol dehydrogenase | ALGNa<br>gelatin | Entrapment | Enzymatic activity was halved after 21 days of storage | [62] |
| Horseradish peroxidase | CHI<br>PEG | Entrapment | Immobilization yield of 65.8% for CHI and 51.7% for CHI/PEG | [63] |
| Inulinase | CHI | Covalent bonding | Suitable to hydrolyze inulin (84.5% at 125 rpm after 4 h) | [64] |
| Laccase | Polyurea | Covalent bonding | Able to degrade Congo red and RBBR dyes | [65] |
| Lactase | ALGNa<br>Guar gum | Covalent bonding | ALG/guar gum/ trehalose exhibiting the highest stability at storage, freezing and freeze/thaw cycles | [66] |
| Lipase | ALGCa | Covalent bonding | 50% enzymatic activity after 4 cycles of reaction | [67] |
| | CHI | Adsorption | 33% yield in the synthesis of 1,3-dicaproyl-2-palmitoyl glycerol | [68] |
| | CHI<br>ALG | Entrapment | 100% enzymatic activity after 5 reaction cycles | [69] |

In a 2020 study, Işik and collaborators immobilized acetylcholinesterase by covalent bonding on CHI microspheres [57]. The polymer/enzyme microparticles thus obtained showed increased storage stability (maintenance of about 80% of the activity after 60 days) and the possibility of using the biocatalyst up to 30 times without an important loss of catalytic activity. Li and collaborators obtained CHI nanoparticles with low molar mass that they were subsequently used for the immobilization of alginate lyase, observing that the enzyme maintains 68.7% of its activity after immobilization, and also that the immobilized enzyme is more stable with temperature variations and pH than the native enzyme [58]. Regarding the reusability of the composite, the authors observed that the CHI and alginate-lyase-based material could be successfully used in 6 reaction cycles, maintaining more than 60% of its activity.

Another option for obtaining polymer/enzyme composites is represented by the use of a mixture of biopolymers. In a 2019 study, Ozaltin and collaborators embedded trypsin and protease of microbial origin into a polymer matrix obtained by blending CHI with

ALGNa [70]. The obtained composites showed an enzyme encapsulation efficiency of about 54% for trypsin and 65% for protease, with reasonable stability to environmental factors. A similar method was proposed by Traffano-Schiffo and collaborators, who immobilized laccase in guar gum and ALGNa microparticles [66].

### *4.4. Core–Shell Particles*

Core–shell particles are a distinct type of composite material obtained by depositing a thin film of an organic material (usually a polyelectrolyte) on a support, generally of inorganic nature. The deposition of the organic film on the support material can be performed in a single step, by precipitation, adsorption, or covalent bonding, obtaining a core–shell material with a single layer, or in a multistep process, obtaining multilayers' core–shell composites. Core–shell composite materials are characterized by high stability, a very good capability to interact with charged chemical species, and satisfactory mechanical, swelling, and permeability properties. Additionally, core–shell composites obtained with natural polymers are generally biocompatible. These properties facilitate the use of core–shell composites as matrices for enzyme immobilization. Depending on the number of layers of organic material deposited on the support, core–shell microparticles can be classified into single-layer or multilayer composite materials [71].

#### 4.4.1. Single-Layer Core–Shell Composite Materials

Single-layer core–shell composite materials are generally obtained by depositing a polyelectrolyte on the surface of a support material, which results in the formation of an outer layer rich in functional groups. These functional groups can interact with the functional groups of the enzymes by forming covalent bonds or electrostatic or hydrogen bonds, leading to their binding to the support material. Materials obtained by depositing a polymer layer on a metallic core with magnetic properties can also be included in the category of core–shell composite materials. Magnetic particles can be modified with polymers, by either in situ treatment, to stabilize the particles during synthesis, or ex situ, by forming polymer films covering the magnetic particles [19]. Their use in the fabrication of composites with enzymes is due to the special properties of these support materials, namely, large specific surface area, good ability to incorporate enzymes, and their effective immobilization. Additionally, magnetic particles have low toxicity and facilitate the separation of the biocatalyst from the reaction medium based on the magnetic properties they possess [11,72]. Table 4 shows some examples of single-layer core–shell composites used in enzyme immobilization.

**Table 4.** Single-layer core–shell polymer/enzyme composite materials.

| Enzyme | Polymer | Immobilization Method | Observations | Reference |
|---|---|---|---|---|
| Acrylamidase | ALG<br>CHI | Covalent bonding | Optimal enzymatic activity at pH = 8.5 and 65 °C | [73] |
| Carboxylesterase | ALG<br>CHI | Entrapment | 80% enzymatic activity after storage at 4 °C for 40 days | [74] |
| Glucoamylase | CHI | Covalent bonding | 80% enzymatic activity after 10 cycles of reaction | [75] |
| β-galactosidase | Bacterial cellulose | Covalent bonding | 80% enzymatic activity after 12 cycles of use | [76] |
| Laccase | CHI | Entrapment | 78% decolorization of textile effluent samples | [77] |
| | CHI | Covalent bonding<br>adsorption | 84% immobilization yield, 78% decolorization of textile effluent sample | [78] |
| | CHI | Entrapment | More than 70% enzymatic activity after 10 cycles of use | [79] |
| Lipase | CHI | Covalent bonding | 67% enantioselectivity in the acetylation of racemic atenolol | [80] |
| | CHI | Covalent bonding | 95% enzymatic activity after 7 days of storage at 25 °C | [81] |
| Oleate hydratase | CHI | Covalent bonding | 75% enzymatic activity after 5 cycles of reaction | [82] |

An example is proposed by Bedade and collaborators, who obtained CHI-coated ALGCa microparticles activated with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide

hydrochloride (EDH) and with N-hydroxysuccinimide (NHS) in which they immobilized acrylamidase by covalent bonding with citric acid, obtaining an immobilization yield of about 75% under optimal immobilization conditions [73]. The biocatalyst thus obtained was distinguished by increased stability at temperature and pH. The catalytic activity of the composite biocatalyst was maintained at approximately 80% upon storage for 10 days at 4 °C and pH = 7, as compared with only 40% of activity maintained in the case of free acrylamidase, stored under the same conditions. Another example was proposed by Girelli and collaborators, who immobilized laccase isolated from *Tinea versicolor* species in a core–shell material obtained from silica and CHI, obtaining a composite with good catalytic properties [76]. The obtained biocatalyst showed about 40% activity after 7 months of storage and an optimal activity at pH = 3 and 50 °C.

Wang and Jiang obtained polymer/enzyme composite materials by covalently binding glucoamylase onto two types of particles: CHI nanoparticles and CHI-coated magnetic microparticles [75]. Both types of composite materials showed satisfactory catalytic activity. In terms of storage stability, the nanocomposites presented better properties; they could be stored for about 2 months, preserving more than 80% of the activity. About the reuse of the biocatalyst, the magnetic microparticles showed superior performance, being reused up to 10 times without a significant loss of catalytic activity. Monteiro and collaborators obtained CHI-functionalized $Fe_3O_4$ nanoparticles that they used for lipase immobilization by cross-linking with GA and by adsorption [78]. By determining the yield of the enzyme immobilization by cross-linking with GA or by adsorption, the authors observed a strong influence of the immobilization method on the amount of enzyme retained in the composite materials. In the case of cross-linked nanoparticles, the immobilization yield was about 84%, while for non-cross-linked nanoparticles, the immobilization yield was only 44%.

A similar method for obtaining composite materials was proposed by Magro and collaborators, who used magnetic particles coated with CHI to immobilize pectinase, obtaining micro- and nanoparticles, but also macroparticles [83]. The tests performed on the three types of particles showed that the macroparticles covered with CHI have the greatest stability and reusability; maintaining about 85% of their activity after 25 cycles of use. Another interesting example was proposed by Tizchang and collaborators, who obtained core–shell composite materials by covering silicon dioxide nanoparticles with covalently bonded β-galactosidase entrapped in bacterial cellulose nanocrystals [76]. The composites created in this manner exhibited a high stability at temperature and pH and were used in 12 reaction cycles maintaining 80% of the enzymatic activity.

#### 4.4.2. Multilayer Core–Shell Composite Materials

Multilayer composite materials are obtained by layer-by-layer deposition of ionic polymers of opposite charge, with the formation of multilayered shells. Their self-assembly occurs as a result of the physical interactions between the support and the polyelectrolyte layers or between the polyelectrolyte layers. Additionally, weak physical forces (van der Waals, hydrogen bonds, hydrophobic interactions) can occur between the functional groups of the component materials, but strong bindings can also be established (electrostatic, covalent, coordinative bonds) [84]. Core–shell materials with polyelectrolyte multilayers can be obtained by immersion, spray deposition, centrifugation, electrodeposition, or microfluidic assembly, on both flat and spherical support materials [85].

The use of multilayered materials for the immobilization of enzymes is a subject intensely debated by the scientific community. This deposition method has several important advantages, such as easy manufacturing; the possibility of using a large number of polyelectrolytes, including those of natural origin; and the versatility of the obtained structures. By layer-by-layer deposition, various types of materials can be obtained, including spherical microparticles, capsules, and thin films, but also hybrid materials [86]. Among them, the most representative are the spherical particles that have the advantage of providing a large specific surface and that can be used in a large number of applications in the biomedical, pharmaceutical, cosmetic fields, as sensors or in the retention and degradation of some

pollutants. The immobilization of enzymes on layer-by-layer support materials can be achieved by different methods, as shown in Figure 3. The enzymes can be adsorbed or cross-linked inside/on the surface of layer-by-layer microparticles, can be entrapped inside layer-by-layer capsules, or can be layer-by-layer assembled on core materials, together with an oppositely charged polymer.

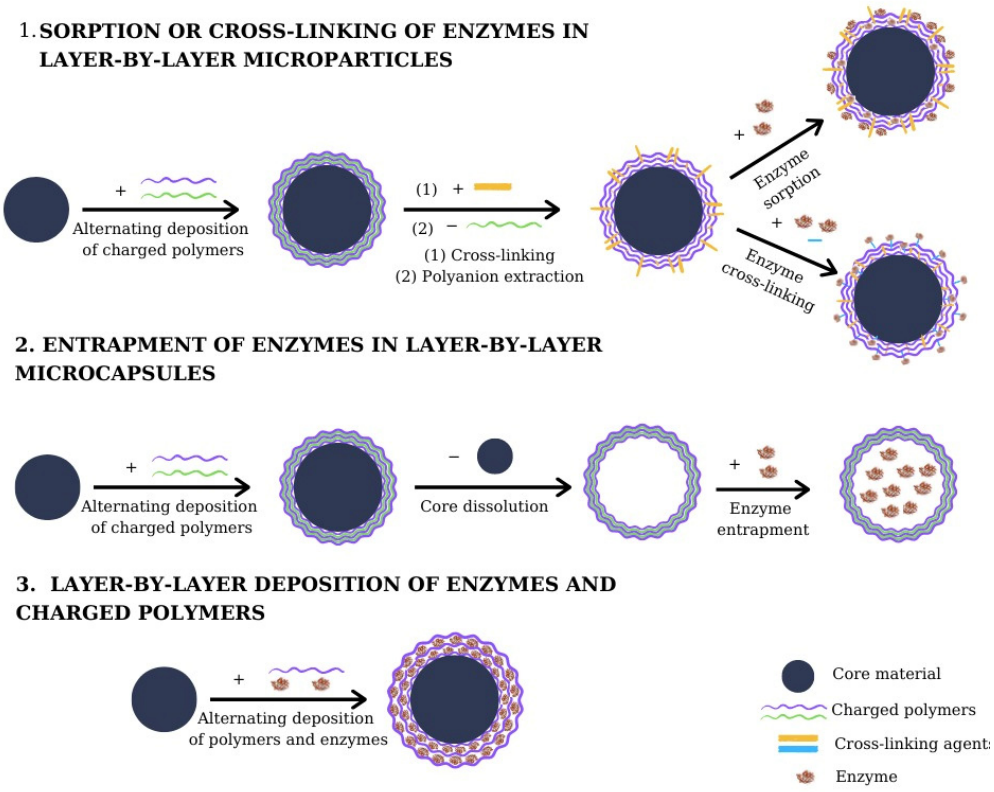


**Figure 3.** Methods for the immobilization of enzymes on layer-by-layer particles.

The immobilization of enzymes on layer-by-layer particles gives rise to versatile composite materials, which can incorporate high amounts of biocatalyst while preserving their activity and increasing enzyme stability. Table 5 shows some examples of layer-by-layer polymer/enzyme composite materials.

**Table 5.** Multilayer core–shell polymer/enzyme composite materials.

| Enzyme | Polymer | Immobilization Method | Observations | Reference |
|---|---|---|---|---|
| Glucosidase | Polystyrene sulfonate (PSS)<br>Polyallylamine hydrochloride (PAH) | Entrapment | 60% enzymatic activity after 15 reaction cycles | [86] |
| | CHI<br>PSS | Adsorption | Used as a biosensor, exhibited high sensitivity and operational stability | [87] |
| Horseradish peroxidase | PSS<br>PAH | Covalent bonding | 90% enzymatic activity after 10 cycles of reaction | [88] |
| | PAH<br>PSS | Entrapment | 90% enzymatic activity after 30 days of storage at 4 °C | [89] |
| Lipase | PEI | Covalent bonding | The enzymatic activity of the multilayer embedding different types of lipases was around 190 U/g | [90] |
| Lysozyme | PDMAEM<br>ALGNa | Adsorption | The immobilization yield was around 5.5% | [91] |
| | PEI | Covalent bonding | The immobilization of lysozyme did not strongly influenced the surface charge of the material | [92] |
| Pepsin | PEI<br>PMMA | Adsorption | The amount of pepsin immobilized was around 200 mg/g | [93] |
| | PEI | Covalent bonding | The amount of pepsin immobilized was around 200 mg/g | [92] |
| | PDMAEMA<br>ALGNa | Adsorption | The amount of pepsin immobilized was around 200 mg/g | [91] |

An example is presented by Perazzini and collaborators, who immobilized horseradish peroxidase on aluminum microparticles, followed by layer-by-layer deposition of PSS and PAH [88]. The immobilization of the enzyme was achieved with a high yield (95%), the enzyme retaining 68% of the initial activity. The authors also analyzed the possibility of reusing the biocatalyst, noting that the immobilized enzyme maintains about 82% of its activity after 10 reuse cycles, compared with only 20% of the activity in the case of the enzyme in its native state.

Zhang and collaborators obtained polymer/enzyme composite microparticles based on polyelectrolytes and glucosidase [86]. $CaCO_3$ microparticles with glucosidase and $CaCO_3$ microparticles with glucosidase and polydopamine (PDA) were used as support, on which PSS and PAH were deposited layer by layer. After polyelectrolyte deposition, the $CaCO_3$ core was dissolved by treatment with ethylenediaminetetraacetic acid, resulting in PSS/PAH capsules entrapping the enzyme. The composite materials thus obtained were used to catalyze the conversion of maltose into isomaltose, observing increased stability and the possibility of reusing this biocatalyst. Thus, the authors demonstrated that the biocatalyst with PDA maintains more than 50% of its activity after 15 reuse cycles and about 35% of its activity when stored for 20 days. The biocatalyst without PDA lost its activity after 5 cycles of reuse and maintained about 25% of its activity after 20 days, while the enzyme in the native state could not be reused and was deactivated after only 12 days of storage. The increased stability of the biocatalysts is the result of the polymer coating that protects the enzyme from the action of environmental factors, which acts as a physical barrier against the desorption of the enzyme from the capsule and favors the optimal folding of the glucosidase molecules as a result of the electrostatic interactions that are established between the PAH chains and the negatively charged groups localized on the enzyme surface. Additionally, the use of PDA allowed the deposition of a denser protective layer, which increased the stability of the biocatalyst.

Arana-Peña and collaborators obtained composite microparticles by layer-by-layer deposition of different types of lipases (lipase A, lipase B extracted from *Candida antarctica* species, lipases extracted from *Rhizomucor miehei* and *Thermomyces lanuginosus*, and a commercially available phospholipase) and PEI on octyl-agarose microspheres [90]. The stability of the microparticles was ensured by cross-linking with GA, the obtained biocatalyst showing a satisfactory enzymatic activity.

### *4.5. Thin Polymeric Films*

Another type of polymer/enzyme composite material is represented by those deposited on flat surfaces. These can be represented by thin films or composite membranes, obtained either by the superficial immobilization of some enzymes or by the layer-by-layer deposition of the component materials (Table 6).

**Table 6.** Polymer/enzyme composite thin films.

| Enzyme | Polymer | Immobilization Method | Observations | Reference |
|---|---|---|---|---|
| Alkaline phosphatase | PSS<br>PAH | Adsorption | The manufactured films were able to prevent the enzyme leaching | [94] |
| | PSS<br>Poly(diallyldimethylammonium chloride) (PDADMAC) | Adsorption | | [94] |
| Bromelain | CHI | Covalent bonding | The films exhibited improved mechanical characteristics | [95] |
| Catalase | Collagen | Covalent bonding | ~50% enzymatic activity after 22 cycles of use | [96] |
| Glucose oxidase | Poly(aniline-*co*-anthranilic acid) | Adsorption | The film was used as a sensor for glucose with a limit of detection of 14 ± 2 µM | [97] |
| Glucosidase | Polyaniline<br>PAA | Covalent bonding | The film was used as a glucose sensor | [98] |
| Horseradish peroxidase | Poly(aniline-*co*-anthranilic acid) | Adsorption | The film was used as a sensor for glucose with a limit of detection of 14 ± 2 µM | [97] |
| Laccase | Poly(vinylidene fluoride) | Covalent bonding | The composite film was able to retain 97.1% of Congo red | [99] |
| Lipase | Polylactic acid<br>Polyethylene glycol (PEG) | Covalent bonding | 70% enzymatic activity after 30 days of storage | [100] |
| Lysozyme | CHI | Entrapment | The amount of enzyme immobilized was dependent on the immobilization time | [101] |
| | PAH<br>PAA<br>Poly(2-hydroxyethyl methacrylate)-*g*-poly(acrylic acid) | Adsorption | The immobilization of the enzyme leads to a 400% increase in the film thickness | [15] |
| | Polycaprolactone<br>PEG | Entrapment | The film exhibited antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Staphylococcus aureus* strains | [102] |

Homma and collaborators obtained polymer/enzyme composites in the form of thin films using polyaniline and PAA as well as glucose oxidase [98]. The polymer films were obtained by the electrochemical polymerization of aniline in the presence of PAA and subsequently used for the covalent immobilization of glucose oxidase. The obtained materials were used as amperometric sensors for glucose detection, demonstrating the feasibility of using these composites as sensors. He and collaborators obtained collagen-based composite films by adsorption and covalent binding of catalase; the materials thus obtained showed increased stability of enzyme activity [96]. Meridor and Gedanken obtained composite materials by adsorbing α-amylase on a PEI film, previously activated with $HNO_3$ [14]. The biocatalyst thus obtained was used for the hydrolysis of starch to maltose, observing increased stability of the material and, at the same time, maintaining the catalytic activity within optimal limits, the determined kinetic parameters being close to the values obtained for the free enzyme. In a recent study, Li and collaborators obtained composite films based on PEG and polylactic acid in which they immobilized lipase by covalent binding with GA and 1,6-hexamethylene diamine [100]. The authors observed that the immobilization of the enzyme facilitated the increase in optimal temperature of the biocatalyst by about 5 °C and in pH by one unit, at the same time demonstrating that the immobilized enzyme is more stable at temperature and storage time. The activity of the biocatalyst was maintained at about 70% upon storage for 30 days and could be reused for 6 reaction cycles with more than 80% of activity maintained.

### *4.6. Polymer/Enzyme Complexes*

Coacervation is a process that involves the formation of complex structures through electrostatic interactions between polymers with opposite charges, which determines them to self-assemble in an aqueous medium, with the elimination of counterions in the medium. This can be explained by the coexistence of two phenomena: one of an entropic nature, due to the loss of counterions, and one of an enthalpic nature, generated by the coulombic attractions between the two polyions of opposite charges [103]. Increasing the polymer concentration causes a strong phase separation, obtaining a phase rich in polymer (the interpolyelectrolyte complex) and one rich in solvent [104]. The association of polyelectrolytes determines the formation of different types of complexes: stoichiometric (insoluble) interpolyelectrolyte complexes, nonstoichiometric complexes (in the form of stable dispersions), and interpolymeric coacervates (soluble structures in aqueous medium) [103]. The process is influenced by pH and ionic strength, which determines the assembly of the polymers. In addition to electrostatic interactions, other types of secondary interactions contribute to the formation of complexes: hydrogen bonds, van der Waals forces, or hydrophobic interactions [105]. The structure and properties of the obtained complexes depend on a large number of factors, both intrinsic (polymer molar mass, charge density, etc.) and extrinsic (mixing speed, pH, ionic strength, etc.) [106]. The presence of salts favors the complexation of polyelectrolytes until reaching a maximum, after which their destabilization and dissociation occur. Instead, low salt concentrations reduce electrostatic interactions, favoring the rearrangement of polymer chains with complex formation [107]. Enzyme/polymer complexes are easy to obtain and have satisfactory stability. Table 7 shows some examples of polyelectrolyte/enzyme complexes.

**Table 7.** Polymer/enzyme complexes.

| Enzyme | Polymer | Immobilization Method | Observations | Reference |
|---|---|---|---|---|
| Glucose oxidase | CHI<br>carrageenan | Adsorption | 80.2% enzymatic activity in pH = 1.2 solution, 73.3% in chitosanase solution and 66.4% in pepsin solution | [13] |
| Horseradish peroxidase | CHI<br>β-cyclodextrin | Adsorption | Able to completely degrade textile dyes after 15 days of operation | [108] |
| Lysozyme | β-conglycinin | Adsorption | The amount of enzyme immobilized was strongly influenced by the presence of NaCl | [109] |
| | κ-carrageenan | Adsorption | The microparticles were able to entrap 70% curcumin | [110] |
| | Sodium caseinate | Adsorption | Stable complexes at pH~11 | [111] |
| | κ-carrageenan | Adsorption | Stable complexes at pH~11 | [112] |
| A-amylase | λ-carrageenan | Adsorption | 70% of enzyme activity after exposure to pH = 3 for 1 h | [12] |
| | Pectin | Adsorption | Unstable at pH < 3 | [12] |
| | PEI<br>PAA | Adsorption | The enzymatic activity after immobilization was around 80% | [113] |
| β-galactosidase | ALGNa | Adsorption | Maximum enzymatic activity at pH = 5 | [114] |
| | ALGNa<br>ι-carrageenan | Adsorption | Maximum enzymatic activity at pH = 7 | [115] |

Zheng and collaborators obtained complexes based on lysozyme and β-conglycinin, by their self-assembly in the presence of NaCl [109]. The authors observed that the formation of the composites is strongly influenced by the concentration of salt, that of lysozyme, but also by pH, the variation of these parameters determining different self-assembly behaviors and obtaining more or less soluble complexes. In a recent study, Jin and collaborators obtained polymer/enzyme composites by self-assembling α-amylase with λ-carrageenan and pectin [12]. The composite obtained with λ-carrageenan showed better catalytic properties, maintaining about 70% of its activity at pH = 3, while the complexes obtained with pectin showed lower stability. At the same time, the α-amylase/λ-carrageenan composite showed better storage stability. Briones and Sato tested the immobilization capacity of glucose oxidase in complexes based on CHI and different types of carrageenan (κ-, λ-, ι-) at charge ratios of 3 and 5 [13]. The highest immobilization yield was obtained for

the CHI/κ-carrageenan complex with a charge ratio of 3 (79% of immobilized enzyme), followed by the same complex at a charge ratio of 5 (62.5% of immobilized enzyme). The stability of the polymer/enzyme complexes was tested by treatment with chitosanase and pepsin, noting that the best stability was registered for the CHI/κ-carrageenan complex, which maintained 80.2% of its activity in a strongly acidic medium, 73.3% of the activity in the presence of chitosanase, and 66.4% of the activity in the presence of pepsin.

## 5. Applications of Polymer/Enzyme Composite Materials

Polymer/enzyme composite materials are distinguished by high stability, specificity, and selectivity, as well as easier separation and reusability. These are biocatalysts with industrial applicability, ensuring the sustainability and efficiency of industrial processes due to the advantages they offer. Unlike chemical catalysts, biocatalysts operate at mild temperature and pH conditions, have higher selectivity, are nontoxic, and can be used in a greater number of applications (Figure 4).

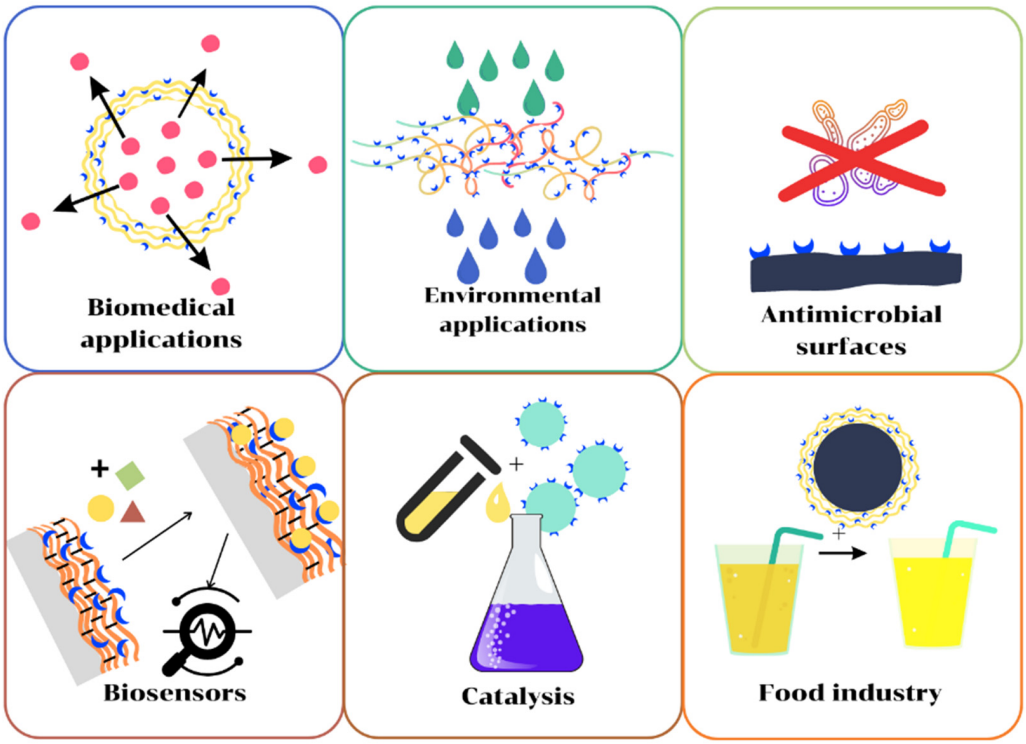


**Figure 4.** Applications of enzyme/polymer composites.

Although limited by technological barriers, the use of polymer/enzyme materials on an industrial scale is beginning to increase; these biocatalysts are successfully used in applications, such as juice clarification, paper bleaching, and the degradation of some polluting agents. A critical examination is necessary to determine the type of composite material suitable for a particular application, but examples in the literature show that polymer/enzyme composite materials find applications in areas of interest, such as the pharmaceutical industry, food industry, chemical synthesis, and environment protection.

### *5.1. Applications in Environmental Protection*

One of the most important problems our society is facing is the increasing level of water, air, and soil pollution. While pollution is generally hard to mitigate, novel techniques for the efficient removal of pollutants are deeply investigated. A special interest is raised by water pollution since water is a very good solvent for a lot of dangerous chemicals, such as dyes, pesticides, and metal ions, which can pass from water sources to plants, animals, and even humans and raise important health concerns due to their toxicity,

bioaccumulation, and carcinogenic effects [116]. The use of polymer/enzyme composite materials in environmental applications has attracted increased interest in recent years due to their versatility and stability, satisfactory catalytic activity, and the possibility of reusing the material without an important loss of catalytic activity [117]. Several studies have reported the use of composite polymer/enzyme systems for the removal of organic compounds found in wastewater, such as phenols, pesticides, and dyes.

Bilal and collaborators immobilized manganese peroxidase in composite microspheres obtained from PVA and ALGNa, which they subsequently used in sorption studies of some dyes from simulated and real polluted water samples [118]. Studies in simulated polluted water revealed that composite particles with immobilized peroxidase were able to discolor water samples by 78–92%, depending on the type of dye dissolved in the water, as compared with only 57–74% discoloration when treating water samples with the free enzyme. The materials were later used in the treatment of polluted water samples taken from textile factories and printing houses, containing mixtures of dyes, obtaining satisfactory results. In another study, Aricov and collaborators used laccase/CHI composite microspheres for the removal of indigo carmine, attaining the full discoloration of the sample after 14 min [119].

Enzymes immobilized in polymer fibers can also be successfully used in the retention and degradation of some pollutants in wastewater. An example in this regard is proposed by Marysková and collaborators, who obtained composite fibers based on polyamide and PEI in which they immobilized laccase and subsequently used it to treat polluted water samples with different emerging contaminants [120]. The authors used three types of water samples: deionized water, polluted river water treated by ultrafiltration, and polluted water to which citrate buffer solution was added. A mixture of pollutants consisting of bisphenol A, 17 α-ethynyl estradiol, triclosan, and diclofenac was added to these samples at an initial concentration of 10 mg/L. Following the treatment with the composite fibers with laccase, the retention of all four contaminants was noticed in a proportion of at least 10–20%. The best results were recorded when using deionized water, with the percentage of destroyed pollutants varying between 73.6% for triclosan and 17.5% for diclofenac.

Leonties and collaborators studied the discoloration of water samples employing laccase/CHI/PAA composite microparticles [121]. The degradation efficiency of these composites was 90% for both naphthol green and indigo carmine, achieved in about 9 and 10 min, respectively. Vera and collaborators used composite materials based on laccase and poly(glycidyl methacrylate) for the enzymatic degradation of diazinon, an insecticidal compound from the class of organophosphorus compounds [122]. The use of the biocatalyst in the presence of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic) acid allowed the total degradation of the pollutant after 48 h of contact and about 88% of the amount of the pollutant, in the absence of the mediator. A study by Lassouane and collaborators used composite materials based on CHI and laccase for the degradation of bisphenol A [123]. After optimizing the degradation conditions, the authors achieved a pollutant degradation efficiency of over 99%, demonstrating at the same time that the biocatalyst can be successfully used in at least 10 reaction cycles, with about 70% of the activity maintained.

### *5.2. Catalysts for Chemical Industry*

Enzymes are biological catalysts distinguished by increased specificity. Due to their versatility, they can catalyze a large number of chemical transformations of interest, such as the digestion of proteins or carbohydrates, the degradation of organic pollutants to nontoxic small molecular compounds, or the transformation of substrates into products of interest, such as drug intermediates, antibiotics, or organic acids. The increased use of polymer/enzyme composites as catalysts is the result of their stability, easy separation, and reusability, making them suitable candidates for industrial processes.

In a recent study, Pereira Cipolatti and collaborators used composite microparticles based on polyurethane and lipase, coated with trehalose to obtain two organic acids (eicosapentaenoic acid and docosahexaenoic acid) that are used in the food industry and some

mandelic acid derivatives with pharmaceutical applications [124]. The authors demonstrated that lipase-based composite microparticles can be successfully used to obtain compounds of interest, showing increased catalytic activity, as well as stability at temperature, in a wide pH range, and storage time. In another study, Pan and collaborators used composite biocatalysts obtained from CHI-coated magnetic microparticles covalently decorated with lipase [125]. The biocatalysts thus obtained were used for the enantioselective acylation of a racemic mixture of L-phenylethylamine. The authors observed that the use of the composite biocatalyst allows the acylation reaction to take place at an elevated temperature without denaturation of the enzyme. Additionally, the biocatalyst could be reused in up to 7 reaction cycles, maintaining about 60% of the activity.

Polymer/enzyme composites also find applications in the cosmetic and perfume industry, especially as catalysts for obtaining chemical compounds of interest. An example in this sense was proposed by Melo and collaborators, who tested the catalytic activity of some composite materials obtained by encapsulating lipase B isolated from *Candida antarctica* in a composite matrix consisting of CHI and sodium polyphosphate [126]. The biocatalyst was used to obtain benzyl acetate (synthetic jasmine flavor) under different reaction conditions. The authors demonstrated that the obtained composite biocatalyst shows increased stability under the tested process conditions, being stable in a wide pH range (pH = 4–10), at high temperatures (maximum activity at around 55–60 °C and total inactivation at temperatures higher than 80 °C), but also in the presence of different organic solvents, the best catalytic activity being recorded when using a mixture of dimethyl sulfoxide and phosphate buffer solution (pH = 7). Additionally, this biocatalyst was successfully reused in 5 reaction cycles with the maintenance of about 86% of the enzyme activity. Tudorache and collaborators reported the fabrication of lipase/ALGCa and lipase/κ-carrageenan composite beads that can be used as catalysts for the conversion of α-pinene to oxy-derivatives, followed by isomerization to campholenic aldehyde and trans-carenol, compounds of interest for the cosmetic and fragrance industry [127].

### *5.3. Biosensors*

Biosensors find applications in the detection of molecules of interest in various fields, including the medicine, cosmetic, and food industries, but also pollution control and environmental protection. Biosensors based on immobilized enzymes are characterized by a high specificity for the molecule of interest, have the ability to mediate a chemical reaction between the receptor and the analyte, and allow the conversion of the chemical response into an electrical response [128]. Additionally, biosensors have increased stability and low environmental impact, in recent years, the use of polymer/enzyme composites being established as one of the most versatile and economical detection methods [128].

In a recent study, Tutunaru and collaborators developed a composite biosensor based on acetylcholinesterase grafted on carboxyl-modified single-walled carbon nanotubes and poly(3,4-ethylenedioxythiophene), which was subsequently used for the detection of organophosphate insecticides in apple juice [129]. The biosensor thus obtained was able to successfully detect and recover dichlorvos, with detection limits of 0.447 and 5.54 ppb, depending on the detection method.

Recently, Cano-Raya and collaborators reported the fabrication of a biosensor for pyrocatechol by immobilizing laccase in polyamide 6 microparticles subsequently deposited on a semipermeable support material allowing substrate diffusion [130]. To determine the selectivity of the biosensor, the authors tested three substrates: pyrocatechol and two of its isomers, resorcinol and hydroquinone, observing an extremely good selectivity of the biosensor for the target analyte. In addition to high selectivity and satisfactory analytical response under controlled conditions, the authors also observed that the obtained composite biosensor can be successfully used in the detection of pyrocatechol from natural water samples. Thus, the authors tested river water samples collected from various areas and in different seasons, to which they added well-determined amounts of pyrocatechol. The authors obtained pyrocatechol recovery rates of over 97% for all samples tested. A

recent example was proposed by Aldea and collaborators, who fabricated a biosensor based on glucose oxidase and PMMA electrospun fibers, coated with a gold layer and deposited on a poly(ethylene terephthalate) film [131]. The sensor exhibited a sensitivity of 3.1 $\mu A \cdot cm^{-2} \cdot mM^{-1}$ and a detection limit of 0.22 mM.

### *5.4. Antimicrobial Applications*

Based on the antimicrobial activity of some natural polymers, such as CHI, but also of some enzymes, polymer/enzyme composites can be used to inhibit the development of some microorganism strains. Among the most significant enzymes with antimicrobial activity, lysozyme exhibits a growing interest based on its ability to lyse a cell wall peptidoglycan layer of Gram-positive bacteria.

In a recent study, Bayazidi and collaborators showed that cellulose fibers with immobilized lysozyme show antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Listeria monocytogenes*, *Yersinia enterocolitica*, *Aspergillus niger*, and *Saccharomyces cerevisiae* [37]. Li and collaborators obtained composite nanoparticles based on CHI and alginate lyase and tested their antimicrobial activity on a *Pseudomonas aeruginosa* strain, observing that the immobilized enzyme showed higher antimicrobial activity than the free enzyme [58]. Thus, the biofilm formed by *Pseudomonas aeruginosa* in the presence of the CHI/alginate-lyase composite was only 21 µm thick, as compared with ~49 µm observed in the presence of the free enzyme and ~87 µm in the absence of any inhibitory agent. In a recent study, Yuan and collaborators demonstrated the potential biomedical applications of composite membranes obtained by layer-by-layer deposition of lysozyme and collagen on a fibroin and nylon 6 support material [132]. The antimicrobial activity of the composite membranes was demonstrated on a *Staphylococcus aureus* strain. The authors observed a decrease of about 98% in the proliferation capacity of the bacterial culture compared with the unmodified membrane. Moreover, these composite membranes with embedded lysozyme showed increased biocompatibility, allowing the development of fibroblast cultures. Baidamshina and collaborators recently reported the fabrication of composite materials with antimicrobial properties by immobilizing papain in a CHI matrix [133]. About 90% of papain's enzymatic activity was maintained in the microparticles, with an extended stability at higher temperature and pH range. The authors demonstrated that the obtained materials can act efficiently on a bacterial biofilm formed by *Staphylococcus aureus* and *Staphylococcus epidermidis* species.

### *5.5. Biomedical Applications*

Another area of interest for the use of polymer/enzyme composites is represented by the fabrication of materials with biomedical applications. This field can include materials for tissue engineering, implants, systems with controlled release of active substances, and biosensors of medical interest, but also catalysts to obtain biologically active substances. In a 2020 research study, Filho Moreira and collaborators reported the fabrication of a composite material with applications in tissue engineering by immobilizing papain in alginate microparticles [134]. The use of this composite is based on the biomedical properties of the two-component materials: alginate, recognized for its properties of stimulating cell regeneration, and papain, involved in the depletion of necrotic tissues. In brief, the enzyme is released through a controlled diffusion, about 64% released for 24 h.

Another potential biomedical application of polymer/enzyme composites is related to the prevention of microbial contamination of some implants. Recently, Teske and collaborators reported novel composite films based on papain and poly(L-lactic acid) with antimicrobial properties [135]. The composite material was customized by cross-linking papain, under the action of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, on the polymer surface. The antimicrobial activity was tested against a *Clostridioides difficile* strain, one of the common pathogens responsible for the occurrence of nosocomial infections, observing a reduction of about 20% of the biofilm formed on the surface of a cardiovascular implant. Another example was proposed by Visan and collaborators, who used a composite coating based on lysozyme, polycaprolactone, and PEG to prevent the

microbial contamination of some Ti implants [102]. The composite film exhibited increased antibacterial activity against both Gram-positive and Gram-negative microbial strains. The results highlighted that the composite film inhibited the growth of *Escherichia coli*, *Bacillus subtilis*, *Enterococcus faecalis*, and *Staphylococcus aureus* strains.

Numerous enzymes successfully catalyzed the transformation of some compounds into pharmacological species, this category including antibiotics [136], analgesics [137], and some anti-inflammatory drugs [138]. An example is presented by Gilani and collaborators, who studied the production of (S)-naproxen under the action of three biocatalysts: CHI/lipase composite microparticles, CHI/lipase microparticles cross-linked with GA, and microparticles obtained by lipase immobilization in a commercial resin of Amberlite XAD7 type [138]. The authors studied the influence of various parameters on the hydrolysis process of the naproxen methyl ester racemic mixture, determining the optimal conditions for obtaining (S)-naproxen. The optimal parameters were a reaction time of 24 h, in the presence of isooctane and 2-ethoxyethanol as solvent/cosolvent, at 35 °C and pH = 7, successfully reused in about 10 reaction cycles.

### *5.6. Applications for Food Industry*

Composite materials with immobilized enzymes are finding increasing use in the food industry. One of their most important applications is in the clarification and discoloration of fruit juices, in order to increase their appearance, stability, and shelf life. The most used types of enzymes for juice clarification are peroxidases and laccases that are able to oxidize phenolic compounds in juices, responsible for sediment formation and changes in their appearance, taste, and smell [139].

Irshad and collaborators used composite materials obtained by immobilizing pectinase in CHI microspheres for the clarification of some fruit juices (apple, mango, peach, and apricot), the use of the biocatalyst leading to a considerable reduction of turbidity, viscosity, and discoloration of fruit juice samples [140]. Bilal and collaborators used gelatin spheres with manganese peroxidase for the clarification of some fruit juices [139]. Following the experiments, a 36.6% decrease in juice turbidity was observed, as well as a 42.7% reduction in juice color intensity. The proposed method has a number of important economic advantages, such as the possibility of reusing the composite material, easy separation, and increased stability over time and with environmental factors.

Benucci and coworkers reported papain immobilization on CHI-clay nanocomposite films. One of these films was efficiently used to reduce haze potential and the protein content of white wines [141]. Fernandez-Pacheco and collaborators immobilized a commercially available β-glucosidase on ALGCa beads in order to improve white wine aroma by the depletion of flavorless glycosides. The immobilized enzyme displays a superior hydrolytic activity, their activity maintained at 96.5% after reutilization for seven times [142].

Another area of interest for the food industry is represented by the prevention of microbiological contamination of some food products. In this regard, Wang and collaborators tested the antimicrobial activity of some composites obtained by immobilizing lysozyme and ALGNa on cellulose acetate nanofibers [143]. The composite materials showed increased stability and reusability, with the maintenance of about 70% of the enzyme activity after 4 cycles of use. At the same time, the authors showed the antibacterial activity of these composites against a *Staphylococcus aureus* strain from UTH milk, at two temperature values, 4 and 25 °C, respectively. Additionally, it was observed that the inhibitory action of composite fibers depends on the number of layers of lysozyme and ALGNa deposited on the support material, the best results being recorded for composites with nine composite layers.

Another application for the food industry is represented by the decontamination of some food products. In this sense, Bedade and collaborators studied the degradation of acrylamide from coffee samples, under the action of a composite biocatalyst obtained by immobilizing acrylamidase on ALGNa microparticles coated with CHI [73]. The authors studied the retention of the contaminant under the action of the enzyme under static and dynamic conditions, demonstrating the retention of 100% of acrylamide in the studies

performed using a batch column under optimal conditions. At the same time, the biocatalyst could be used in 4 reaction cycles with the maintenance of about 80% of the activity.

## 6. Conclusions

Composite materials obtained by enzyme immobilization on different types of solid supports are efficient biocatalysts that find applications in numerous fields. Their use, on both a laboratory and industrial scale, is ensured by their increased stability, as well as by the specificity for different substrates and the possibility of recovery and reuse of the enzyme catalyst. Additionally, the use of polymer/enzyme composite materials can considerably reduce the contamination of the reaction product, as well as of the biocatalyst, thus reducing the costs associated with long-term enzyme usage.

In recent years, numerous studies have been carried out in this direction, with many studies reporting polymer/enzyme composite materials with various functionalities and applications. Additionally, a significant number of scientific reports/reviews have focused on presenting enzyme immobilization methods and the advances made in the field of polymer/enzyme composite materials. As for the directions of use of polymer/enzyme composite materials, they find applications in fields of interest for both the scientific community and the industry, including here the retention and degradation of pollutants, the synthesis of chemical compounds of high value, biosensing, the fabrication of materials with antimicrobial properties, and food processing.

Although there is no method that is generally accepted as the most suitable to produce an ideal biocatalyst, each immobilization method presents a number of noteworthy advantages, among which the most significant are closely related to stability and reusability of the biocatalyst. The choice of a method to fabricate the composite material and, especially, the choice of the support matrix for immobilization depend on the structure and properties of the enzyme of interest, as well as on the intended applications. Thus, a large number of customizations are possible, with numerous studies reporting particular combinations of enzymes, polymers, and support materials of organic or inorganic nature. Additionally, there are differences between the methods of immobilization of the enzyme (physical or chemical, inside the matrix or on its surface), as well as in the final form of presentation of the materials, several different morphologies being available. Nevertheless, intensive research in the field on enzyme/polymer composite material is needed in order to establish suitable biocatalysts for every particular application.

**Author Contributions:** L.-M.P.: conceptualization, methodology, writing—original draft preparation, writing—review and editing; F.B.: conceptualization, writing—review and editing, supervision, funding acquisition; V.R.G.: conceptualization, writing—original draft preparation, writing—review and editing; M.M.: conceptualization, methodology, writing—original draft preparation, writing—review and editing, supervision, project administration, funding acquisition. All authors have read and agreed to the published version of the manuscript.

**Funding:** This work was supported by several grants from the Ministry of Research and Innovation, CNCS-UEFSCDI, within PNCDI III, project numbers: PN-III-P4-ID-PCE-2020-1541, PN-III-P4-ID-PCE-2020-1199, and PN-III-P2-2.1-PED-2019-1996.

**Acknowledgments:** This work was supported by the research infrastructure developed through the European Social Fund for Regional Development, Competitiveness Operational Programme 2014–2020, Axis 1, Action: 1.1.3, Project "Infra SupraChem Lab-Center for Advanced Research in Supramolecular Chemistry" (Contract 339/390015/25.02.2021, cod MySMIS: 108983).

**Conflicts of Interest:** The authors declare no conflict of interest.

## Abbreviations

ALGCa—calcium alginate; ALGNa—sodium alginate; CHI—chitosan; EDH—1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; GA—glutaraldehyde; NHS—N-hydroxysuccinimide; PAA—poly(acrylic acid); PAH—polyallylamine hydrochloride; PAM—polyacrylamide; PAN—polyacrylonitrile; PDA—polydopamine; PDADMAC—poly(diallyldimethylammonium chlo-

ride); PDMAEMA—poly(N,N-dimethylamino ethyl methacrylate); PEG—poly(ethylene glycol); PEI—polyethyleneimine; PMMA—poly(methyl methacrylate); PSS—polystyrene sulfonate; PVA—poly(vinyl alcohol).

## References


1. Li, Y.; Wang, X.; Sun, J. Layer-by-layer assembly for rapid fabrication of thick polymeric films. *Chem. Soc. Rev.* **2012**, *41*, 5998–6009. [CrossRef] [PubMed]
2. Chapman, J.; Ismail, A.E.; Zoica Dinu, C. Industrial applications of enzymes: Recent advances, techniques, and outlooks. *Catalysts* **2018**, *8*, 238. [CrossRef]
3. Bilal, M.; Dourado, C.; Mehmood, T.; Nadeem, F.; Tabassam, Q.; Fernando, L.; Ferreira, R. Immobilized lipases-based nano-biocatalytic systems—A versatile platform with incredible biotechnological potential. *Int. J. Biol. Macromol.* **2021**, *175*, 108–122. [CrossRef]
4. Aggarwal, S.; Chakravarty, A.; Ikram, S. A comprehensive review on incredible renewable carriers as promising platforms for enzyme immobilization & thereof strategies. *Int. J. Biol. Macromol.* **2021**, *167*, 962–986. [CrossRef] [PubMed]
5. Jankowska, K.; Su, Z.; Bj, S. Tailor-made novel electrospun polystyrene/poly (D,L-lactide-co-glycolide) for oxidoreductases immobilization: Improvement of catalytic properties under extreme reaction conditions. *Bioorg. Chem.* **2021**, *114*, 105036. [CrossRef]
6. BRENDA Enzyme Database. Available online: www.brenda-enzymes.org (accessed on 4 June 2022).
7. Copeland, R.A. *Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis*, 2nd ed.; Wiley-VCH: New York, NY, USA, 2000.
8. Nelson, J.M.; Griffin, E.G. Adsorption of invertase. *J. Am. Chem. Soc.* **1916**, *38*, 1109–1115. [CrossRef]
9. Sheldon, R.A.; van Pelt, S. Enzyme immobilization in biocatalysis: Why, what and how. *Chem. Soc. Rev.* **2013**, *42*, 6223–6235. [CrossRef]
10. Tran, D.N.; Balkus, K.J. Enzyme immobilization via electrospinning. *Top. Catal.* **2012**, *55*, 1057–1069. [CrossRef]
11. Zahirinejad, S.; Hemmati, R.; Homaei, A.; Dinari, A.; Hosseinkhani, S.; Mohammadi, S.; Vianello, F. Nano-organic supports for enzyme immobilization: Scopes and perspectives. *Colloids Surf. B* **2021**, *204*, 111774. [CrossRef]
12. Jin, W.; Wang, Z.; Peng, D.; Shen, W.; Zhu, Z.; Cheng, S.; Li, B.; Huang, Q. Effect of linear charge density of polysaccharides on interactions with α-amylase: Self-Assembling behavior and application in enzyme immobilization. *Food Chem.* **2020**, *331*, 127320. [CrossRef]
13. Briones, A.V.; Sato, T. Encapsulation of glucose oxidase (GOD) in polyelectrolyte complexes of chitosan–carrageenan. *React. Funct. Polym.* **2010**, *70*, 19–27. [CrossRef]
14. Meridor, D.; Gedanken, A. Preparation of enzyme nanoparticles and studying the catalytic activity of the immobilized nanoparticles on polyethylene films. *Ultrason. Sonochem.* **2013**, *20*, 425–431. [CrossRef] [PubMed]
15. Ma, Y.; Dong, J.; Bhattacharjee, S.; Wijeratne, S.; Bruening, M.L.; Baker, G.L. Increased protein sorption in poly(acrylic acid)-containing films through incorporation of comb-like polymers and film adsorption at low pH and high ionic strength. *Langmuir* **2013**, *29*, 2946–2954. [CrossRef] [PubMed]
16. Cui, C.; Ming, H.; Li, L.; Li, M.; Gao, J.; Han, T.; Wang, Y. Fabrication of an *in-situ* co-immobilized enzyme in mesoporous silica for synthesizing GSH with ATP regeneration. *Mol. Catal.* **2020**, *486*, 110870. [CrossRef]
17. Gopinath, S.; Sugunan, S. Enzymes immobilized on montmorillonite K10: Effect of adsorption and grafting on the surface properties and the enzyme activity. *Appl. Clay Sci.* **2010**, *35*, 67–75. [CrossRef]
18. Zhou, W.; Zhang, W.; Cai, Y. Enzyme-enhanced adsorption of laccase immobilized graphene oxide for micro-pollutant removal. *Sep. Purif. Technol.* **2022**, *294*, 121178. [CrossRef]
19. Liu, D.; Chen, J.; Shi, Y. Advances on methods and easy separated support materials for enzymes immobilization. *Trends Anal. Chem.* **2018**, *102*, 332–342. [CrossRef]
20. Cao, L. *Carrier-Bound Immobilized Enzymes: Principles, Application and Design*; Wiley-VCH: New York, NY, USA, 2005.
21. Tacias-Pascacio, V.G.; García-Parra, E.; Vela-Gutiérrez, G.; Virgen-Ortiz, J.J.; Berenguer-Murcia, Á.; Alcántara, A.R.; Fernandez-Lafuente, R. Genipin as an emergent tool in the design of biocatalysts: Mechanism of reaction and applications. *Catalysts* **2019**, *9*, 1035. [CrossRef]
22. Ashkan, Z.; Hemmati, R.; Homaei, A.; Dinari, A.; Jamlidoost, M.; Tashakor, A. Immobilization of enzymes on nanoinorganic support materials: An update. *Int. J. Biol. Macromol.* **2021**, *168*, 708–721. [CrossRef]
23. Zhang, S.; Bilal, M.; Zdarta, J.; Cui, J.; Kumar, A.; Franco, M.; Fernando, L.; Ferreira, R.; Iqbal, H.M.N. Biopolymers and nanostructured materials to develop pectinases-based immobilized nano-biocatalytic systems for biotechnological applications. *Food Res. Int.* **2021**, *140*, 109979. [CrossRef]
24. Soares, A.M.B.F.; Gonçalves, L.M.O.; Ferreira, R.D.S.; de Souza, J.M.; Fangueiro, R.; Alves, M.M.M.; Carvalho, F.A.A.; Mendes, A.N.; Cantanhêde, W. Immobilization of papain enzyme on a hybrid support containing zinc oxide nanoparticles and chitosan for clinical applications. *Carbohydr. Polym.* **2020**, *243*, 116498. [CrossRef] [PubMed]
25. Sun, Y.; Cheng, S.; Lu, W.; Wang, Y.; Zhang, P.; Yao, Q. Electrospun fibers and their application in drug controlled release, biological dressings, tissue repair, and enzyme immobilization. *RSC Adv.* **2019**, *9*, 25712–25729. [CrossRef] [PubMed]

26. Li, D.; Wang, Q.; Huang, F.; Wei, Q. Chapter 26—Electrospun Nanofibers for Enzyme Immobilization. In *Electrospinning: Nanofabrication and Applications*; Ding, B., Wang, X., Yu, J., Eds.; Elsevier Inc.: Amsterdam, The Netherlands, 2019; pp. 765–781. [CrossRef]
27. Shinde, P.; Musameh, M.; Gao, Y.; Robinson, A.J.; Louis, I. Immobilization and stabilization of alcohol dehydrogenase on polyvinyl alcohol fibre. *Biotechnol. Rep.* **2018**, *19*, e00260. [CrossRef] [PubMed]
28. Porto, M.D.A.; dos Santos, J.P.; Hackbart, H.; Bruni, G.P.; Fonseca, L.M.; da Rosa Zavareze, E.; Dias, A.R.G. Immobilization of α-amylase in ultrafine polyvinyl alcohol (PVA) fibers via electrospinning and their stability on different substrates. *Int. J. Biol. Macromol.* **2019**, *126*, 834–841. [CrossRef]
29. Hosseini, A.; Ramezani, S.; Tabibiazar, M.; Mohammadi, M. Immobilization of α-amylase in ethylcellulose electrospun fibers using emulsion-electrospinning method. *Carbohydr. Polym.* **2021**, *278*, 118919. [CrossRef]
30. Saallah, S.; Naim, M.N.; Lenggoro, I.W.; Noriznan, M.; Fitrah, N.; Bakar, A.; Gen, M. Immobilisation of cyclodextrin glucanotransferase into polyvinyl alcohol (PVA) nanofibres via electrospinning. *Biotechnol. Rep.* **2016**, *10*, 44–48. [CrossRef]
31. Temocin, Z.; Inal, M.; Gokgoz, M.; Yigitoglu, M. Immobilization of horseradish peroxidase on electrospun poly(vinyl alcohol)–polyacrylamide blend nanofiber membrane and its use in the conversion. *Polym. Bull.* **2018**, *75*, 1843–1865. [CrossRef]
32. Jankowska, K.; Zdarta, J.; Grzywaczyk, A.; Pinelo, M.; Jesionowski, T. Horseradish peroxidase immobilized onto electrospun fibres and its application in decolourisation of dyes from model sea water. *Process Biochem.* **2021**, *102*, 10–21. [CrossRef]
33. Sarathi, M.; Doraiswamy, N.; Pennathur, G. Enhanced stability of immobilized keratinolytic protease on electrospun nanofibers. *Prep. Biochem. Biotechnol.* **2019**, *49*, 695–703. [CrossRef]
34. Jhuang, J.; Lin, S.; Chen, L.; Lou, S.; Chen, S.; Chen, H. Development of immobilized laccase-based time temperature indicator by electrospinning zein fiber. *Food Packag. Shelf Life* **2020**, *23*, 100436. [CrossRef]
35. Jankowska, K.; Zdarta, J.; Grzywaczyk, A.; Kije, E.; Biadasz, A. Electrospun poly(methyl methacrylate)/polyaniline fibers as a support for laccase immobilisation and use in dye decolourisation. *Environ. Res.* **2020**, *184*, 109332. [CrossRef]
36. Jhuang, J.; Lou, S.; Lin, S.; Hsin, S.; Chen, L.; Chen, H. Immobilizing laccase on electrospun chitosan fiber to prepare time-temperature indicator for food quality monitoring. *Innov. Food Sci. Emerg. Technol.* **2020**, *63*, 102370. [CrossRef]
37. Bayazidi, P.; Almasi, H.; Asl, A.K. Immobilization of lysozyme on bacterial cellulose nanofibers: Characteristics, antimicrobial activity and morphological properties. *Int. J. Biol. Macromol.* **2017**, *107*, 2544–2551. [CrossRef]
38. Moreno-Cortez, I.E.; Romero-García, J.; González-González, V.; Garcia-Gutierrez, D.I.; Garza-Navarro, M.A.; Cruz-Silva, R. Encapsulation and immobilization of papain in electrospun nanofibrous membranes of PVA cross-linked with glutaraldehyde vapor. *Mater. Sci. Eng. C* **2015**, *52*, 306–314. [CrossRef]
39. Labus, K.; Wolanin, K.; Radosinski, L. Comparative study on enzyme immobilization using natural hydrogel matrices—experimental studies. *Catalysts* **2020**, *10*, 489. [CrossRef]
40. Jafary, F.; Panjehpour, M.; Varshosaz, J.; Yaghmaei, P. Stability improvement of immobilized alkaline phosphatase using chitosan. *Braz. J. Chem. Eng.* **2016**, *33*, 243–250. [CrossRef]
41. Manzo, R.M.; Ceruti, R.J.; Bonazza, H.L.; Adriano, W.S.; Sihufe, G.A.; Mammarella, E.J. Immobilization of carboxypeptidase A into modified chitosan matrixes by covalent attachment. *Appl. Biochem. Biotechnol.* **2018**, *185*, 1029–1043. [CrossRef]
42. Bilal, M.; Iqbal, M.N.; Hu, H.; Wang, W.; Zhang, X. Enhanced bio-catalytic performance and dye degradation potential of chitosan-encapsulated horseradish peroxidase in a packed bed reactor system. *Sci. Total Environ.* **2017**, *575*, 1352–1360. [CrossRef]
43. Bilal, M.; Rasheed, T.; Iqbal, H.M.N.; Hu, H.; Wang, W.; Zhang, X. Horseradish peroxidase immobilization by copolymerization into cross-linked polyacrylamide gel and its dye degradation and detoxification potential. *Int. J. Biol. Macromol.* **2018**, *113*, 983–990. [CrossRef]
44. Morales Urrea, D.A.; Fernández Gimenez, A.V.; Rodriguez, Y.E.; Contreras, E.M. Immobilization of horseradish peroxidase in Ca-alginate beads: Evaluation of the enzyme leakage on the overall removal of an azo-dye and mathematical modeling. *Process Saf. Environ. Prot.* **2021**, *156*, 134–143. [CrossRef]
45. Vassiliadi, E.; Xenakis, A.; Zoumpanioti, M. Chitosan hydrogels: A new and simple matrix for lipase catalysed biosyntheses. *Mol. Catal.* **2018**, *445*, 206–212. [CrossRef]
46. Asgher, M.; Noreen, S.; Bilal, M. Enhancement of catalytic, reusability, and long-term stability features of *Trametes versicolor* IBL-04 laccase immobilized on different polymers. *Int. J. Biol. Macromol.* **2017**, *95*, 54–62. [CrossRef] [PubMed]
47. Popović, N.; Stanišić, M.; Ilić Đurđić, K.; Prodanović, O.; Polović, N.; Prodanović, R. Dopamine-modified pectin for a *Streptomyces cyaneus* laccase induced microbeads formation, immobilization, and textile dyes decolorization. *Environ. Technol. Innov.* **2021**, *22*, 101399. [CrossRef]
48. Bonazza, H.L.; Manzo, R.M.; Santos, J.C.S.; Mammarella, E.J. Operational and thermal stability analysis of *Thermomyces lanuginosus* lipase covalently immobilized onto modified chitosan supports. *Appl. Biochem. Biotechnol.* **2017**, *184*, 182–196. [CrossRef] [PubMed]
49. Helal, S.E.; Abdelhady, H.M.; Abou-Taleb, K.A.; Hassan, M.G.; Amer, M.M. Lipase from *Rhizopus oryzae* R1: In-depth characterization, immobilization, and evaluation in biodiesel production. *J. Genet. Eng. Biotechnol.* **2021**, *19*. [CrossRef] [PubMed]
50. de Oliveira, R.L.; da Silva, O.S.; Converti, A.; Porto, T.S. Thermodynamic and kinetic studies on pectinase extracted from *Aspergillus aculeatus*: Free and immobilized enzyme entrapped in alginate beads. *Int. J. Biol. Macromol.* **2018**, *115*, 1088–1093. [CrossRef]

51. Deng, Z.; Wang, F.; Zhou, B.; Li, J.; Li, B.; Liang, H. Immobilization of pectinases into calcium alginate microspheres for fruit juice application. *Food Hydrocoll.* **2019**, *89*, 691–699. [CrossRef]
52. Rehman, H.U.; Aman, A.; Nawaz, M.A.; Karim, A.; Ghani, M.; Baloch, A.H.; Qader, S.A.U. Immobilization of pectin depolymerising polygalacturonase using different polymers. *Int. J. Biol. Macromol.* **2016**, *82*, 127–133. [CrossRef]
53. Awad, G.E.A.; Ghanem, A.F.; Abdel, W.A.; Wahba, M.I. Functionalized κ -carrageenan/hyperbranched poly(amidoamine) for protease immobilization: Thermodynamics and stability studies. *Int. J. Biol. Macromol.* **2020**, *148*, 1140–1155. [CrossRef]
54. Dhiman, S.; Srivastava, B.; Singh, G.; Khatri, M.; Arya, S.K. Immobilization of mannase on sodium alginate-grafted-β-cyclodextrin: An easy and cost effective approach for the improvement of enzyme properties. *Int. J. Biol. Macromol.* **2019**, *156*, 1347–1358. [CrossRef]
55. Ghiorghita, C.A.; Bucatariu, F.; Dragan, E.S. Influence of cross-linking in loading/release applications of polyelectrolyte multilayer assemblies. A review. *Mater. Sci. Eng. C* **2019**, *105*, 110050. [CrossRef]
56. Bucatariu, F.; Petrila, L.-M.; Teodosiu, C.; Mihai, M. Versatile nanostructured $SiO_2$/cross-linked polyelectrolyte composites for emerging pollutants removal from aqueous media. *C. R. Chim.* **2021**, *25*, 95–108. [CrossRef]
57. Işık, M. High Stability of immobilized acetylcholinesterase on chitosan beads. *ChemistrySelect* **2020**, *5*, 4623–4627. [CrossRef]
58. Li, S.; Wang, Y.; Li, X.; Lee, B.S.; Jung, S.; Lee, M. Enhancing the thermo-stability and anti-biofilm activity of alginate lyase by immobilization on low molecular weight chitosan nanoparticles. *Int. J. Mol. Sci.* **2019**, *20*, 4565. [CrossRef]
59. Leonida, M.; Belbekhouche, S.; Adams, F.; Kiran, U.; Choudhary, D.; Kumar, I. Enzyme nanovehicles: Histaminase and catalase delivered in nanoparticulate chitosan. *Int. J. Pharm.* **2019**, *557*, 145–153. [CrossRef]
60. Nguyen, L.T.; Lau, Y.S.; Yang, K. Entrapment of cross-linked cellulase colloids in alginate beads for hydrolysis of cellulose. *Colloids Surf. B* **2016**, *145*, 862–869. [CrossRef]
61. Rakmai, J.; Cheirsilp, B.; Prasertsan, P. Enhanced thermal stability of cyclodextrin glycosyltransferase in alginate–gelatin mixed gel beads and the application for β-cyclodextrin production. *Biocatal. Agric. Biotechnol.* **2015**, *4*, 717–726. [CrossRef]
62. Li, J.; Feng, T.; Han, L.; Zhang, M.; Jiang, T. Fabricating biopolymer-inorganic hybrid microspheres for enzyme immobilization: Connect membrane emulsification with biomimetic mineralization. *Particuology* **2021**, *64*, 171–177. [CrossRef]
63. Melo, M.N.; Pereira, F.M.; Rocha, M.A.; Ribeiro, J.G.; Diz, F.M.; Monteiro, W.F.; Ligabue, R.A.; Severino, P.; Fricks, A.T. Immobilization and characterization of horseradish peroxidase into chitosan and chitosan/PEG nanoparticles: A comparative study. *Process Biochem.* **2020**, *98*, 160–171. [CrossRef]
64. Singh, R.S.; Singh, R.P.; Kennedy, J.F. Immobilization of yeast inulinase on chitosan beads for the hydrolysis of inulin in a batch system. *Int. J. Biol. Macromol.* **2016**, *95*, 87–93. [CrossRef]
65. Sun, H.; Wei, Y.; Zheng, X.; Jiang, X. Preparation of uniform polyurea microspheres at high yield by precipitation polymerization and their use for laccase immobilization. *Polymer* **2021**, *216*, 123432. [CrossRef]
66. Traffano Schiffo, M.V.V.; Aguirre Calvo, T.R.; Castro-Giraldez, M.; Fito, P.J.; Santagapita, P.R. Alginate beads containing lactase: Stability and microstructure. *Biomacromolecules* **2017**, *18*, 1785–1792. [CrossRef] [PubMed]
67. Ferraz, L.I.R.; Possebom, G.; Alvez Valandro, E.; Cansian, R.L.; Paroul, N.; de Oliveira, D.; Treichel, H. Application of home-made lipase in the production of geranyl propionate by esterification of geraniol and propionic acid in solvent-free system. *Biocatal. Agric. Biotechnol.* **2015**, *4*, 44–48. [CrossRef]
68. Sanchez, D.A.; Tonetto, G.M.; Ferreira, M.L. Screening of lipases with unusual high activity in the sn-2 esterification of 1,3-dicaprin under mild operating conditions. *J. Agric. Food Chem.* **2017**, *65*, 5010–5017. [CrossRef] [PubMed]
69. Akil, E.; Pereira, A.S.; El-Bacha, T.; Amaral, F.F.; Torres, A.G. Efficient production of bioactive structured lipids by fast acidolysis catalyzed by *Yarrowia lipolytica* lipase, free and immobilized in chitosan-alginate beads, in solvent-free medium. *Int. J. Biol. Macromol.* **2020**, *163*, 910–918. [CrossRef]
70. Ozaltin, K.; Postnikov, P.S.; Trusova, M.E.; Sedlarik, V.; Di, A. Polysaccharides based microspheres for multiple encapsulations and simultaneous release of proteases. *Int. J. Biol. Macromol.* **2019**, *132*, 24–31. [CrossRef]
71. Petrila, L.-M.; Bucatariu, F.; Mihai, M.; Teodosiu, C. Polyelectrolyte multilayers: An overview on fabrication, properties, and biomedical and environmental applications. *Materials* **2021**, *14*, 4152. [CrossRef]
72. Bilal, M.; Zhao, Y.; Rasheed, T.; Iqbal, H.M.N. Magnetic nanoparticles as versatile carriers for enzymes immobilization: A review. *Int. J. Biol. Macromol.* **2018**, *120*, 2530–2544. [CrossRef]
73. Bedade, D.K.; Sutar, Y.B.; Singhal, R.S. Chitosan coated calcium alginate beads for covalent immobilization of acrylamidase: Process parameters and removal of acrylamide from coffee. *Food Chem.* **2019**, *275*, 95–104. [CrossRef]
74. Raghu, S.; Pennathur, G. Enhancing the stability of a carboxylesterase by entrapment in chitosan coated alginate beads. *Turk. J. Biol.* **2018**, *42*, 307–318. [CrossRef]
75. Wang, D.; Jiang, W. Preparation of chitosan-based nanoparticles for enzyme immobilization. *Int. J. Biol. Macromol.* **2019**, *126*, 1125–1132. [CrossRef] [PubMed]
76. Tizchang, S.; Khiabani, M.S.; Mokarram, R.R.; Hamishehkar, H. Bacterial cellulose nano crystal as hydrocolloid matrix in immobilized β-galactosidase onto silicon dioxide nanoparticles. *LWT* **2020**, *123*, 109091. [CrossRef]
77. Mehandia, S.; Ahmad, S.; Sharma, S.C.; Arya, S.K. Decolorization and detoxification of textile effluent by immobilized laccase-ACS into chitosan-clay composite beads using a packed bed reactor system: An ecofriendly approach. *J. Water Process Eng.* **2022**, *47*, 102662. [CrossRef]

78. Monteiro, R.R.C.; Lima, P.J.M.; Pinheiro, B.B.; Freire, T.M.; Dutra, L.M.U.; Fechine, P.B.A.; Goncalves, L.R.B.; de Souza, M.C.M.; dos Santos, J.C.S.; Fernandez-Lafuente, R. Immobilization of lipase A from *Candida antarctica* onto chitosan-coated magnetic nanoparticles. *Int. J. Mol. Sci.* **2019**, *20*, 4018. [CrossRef]
79. Hou, C.; Wang, Y.; Zhu, H.; Wei, H. Construction of enzyme immobilization system through metal-polyphenol assisted $Fe_3O_4$ chitosan hybrid microcapsules. *Chem. Eng. J.* **2016**, *283*, 397–403. [CrossRef]
80. Sikora, A.; Chełminiak-Dudkiewicz, D.; Siódmiak, T.; Tarczykowska, A.; Dariusz, W.; Ziegler-Borowska, M.; Piotr, M. Enantioselective acetylation of (R,S)-atenolol: The use of *Candida rugosa* lipases immobilized onto magnetic chitosan nanoparticles in enzyme-catalyzed biotransformation. *J. Molec. Catal. B Enzym.* **2016**, *134*, 43–50. [CrossRef]
81. Liu, Y.; Zhou, H.; Wang, L.; Wang, S. Stability and catalytic properties of lipase immobilized on chitosan encapsulated magnetic nanoparticles cross-linked with genipin and glutaraldehyde. *J. Chem. Technol. Biotechnol.* **2015**, *76*, 991–996. [CrossRef]
82. Todea, A.; Hiseni, A.; Otten, L.G.; Arends, I.W.C.E.; Peter, F.; Carmen, G.; Boeriu, C.G. Increase of stability of oleate hydratase by appropriate immobilization technique and conditions. *J. Mol. Catal. B Enzym.* **2015**, *119*, 40–47. [CrossRef]
83. Magro, L.D.; de Moura, K.S.; Backes, B.E.; de Menezes, E.W.; Benvenutti, E.V.; Nicolodi, S.; Klein, M.P.; Fernandez-Lafuente, R.; Rodrigues, R.C. Immobilization of pectinase on chitosan-magnetic particles: Influence of particle preparation protocol on enzyme properties for fruit juice clarification. *Biotechnol. Rep.* **2019**, *24*, e00373. [CrossRef]
84. De Villiers, M.M.; Otto, D.P.; Strydom, S.J.; Lvov, Y.M. Introduction to nanocoatings produced by layer-by-layer (LbL) self-assembly. *Adv. Drug Deliv. Rev.* **2011**, *63*, 701–715. [CrossRef]
85. Zhang, L.; Wang, Y.; Tang, N.; Cheng, P.; Xiang, J.; Du, W.; Wang, X. Bioinspired stability improvement of layer-by-layer microcapsules using a bioadhesive for enzyme encapsulation. *React. Funct. Polym.* **2016**, *99*, 73–79. [CrossRef]
86. Sakr, O.S.; Borchard, G. Encapsulation of enzymes in layer-by-layer (LbL) structures: Latest advances and applications. *Biomacromolecules* **2013**, *14*, 2117–2135. [CrossRef] [PubMed]
87. Barsan, M.M.; David, M.; Florescu, M.; Ţugulea, L.; Brett, C.M.A. A new self-assembled layer-by-layer glucose biosensor based on chitosan biopolymer entrapped enzyme with nitrogen doped graphene. *Bioelectrochemistry* **2014**, *99*, 46–52. [CrossRef]
88. Perazzini, R.; Saladino, R.; Guazzaroni, M.; Crestini, C. A novel and efficient oxidative functionalization of lignin by layer-by-layer immobilised horseradish peroxidase. *Bioorg. Med. Chem.* **2011**, *19*, 440–447. [CrossRef]
89. Zhao, Z.X.; Wang, X.S.; Qin, X.; Chen, Q.; Anzai, J. Enzyme microcapsules with substrate selective permeability constructed via layer-by-layer polyelectrolyte self-assembly. *Mater. Sci. Eng. C* **2012**, *32*, 569–573. [CrossRef]
90. Arana-Peña, S.; Rios, N.S.; Mendez-Sanchez, C.; Lokha, Y.; Carballares, D.; Gonçalves, L.R.B.; Fernandez-Lafuente, R. Coimmobilization of different lipases: Simple layer by layer enzyme spatial ordering. *Int. J. Biol. Macromol.* **2020**, *145*, 856–864. [CrossRef]
91. Ghiorghita, C.A.; Bucatariu, F.; Dragan, E.S. Poly(*N*,*N*-dimethylamino) ethyl methacrylate/sodium alginate multilayers and their interaction with proteins/enzymes. *Int. J. Biol. Macromol.* **2018**, *107*, 1584–1590. [CrossRef]
92. Bucatariu, F.; Ghiorghita, C.A.; Simon, F.; Bellmann, C.; Dragan, E.S. Poly(ethyleneimine) cross-linked multilayers deposited onto solid surfaces and enzyme immobilization as a function of the film properties. *Appl. Surf. Sci.* **2013**, *280*, 812–819. [CrossRef]
93. Bucatariu, F.; Ghiorghita, C.A.; Cocarta, A.I.; Dragan, E.S. Cross-linked multilayer-dye films deposited onto silica surfaces with high affinity for pepsin. *Appl. Surf. Sci.* **2016**, *390*, 320–327. [CrossRef]
94. Amorosi, C.; Michel, M.; Avérous, L.; Toniazzo, V.; Ruch, D.; Ball, V. Plasma polymer films as an alternative to $(PSS\text{-}PAH)_n$ or $(PSS\text{-}PDADMAC)_n$ films to retain active enzymes in exponentially growing polyelectrolyte multilayers. *Colloids Surf. B* **2012**, *97*, 124–131. [CrossRef]
95. Benucci, I.; Liburdi, K.; Cacciotti, I.; Lombardelli, C.; Zappino, M.; Nanni, F.; Esti, M. Chitosan/clay nanocomposite films as supports for enzyme immobilization: An innovative green approach for winemaking applications. *Food Hydrocoll.* **2018**, *74*, 124–131. [CrossRef]
96. He, L.; Lan, W.; Cen, L.; Chen, S.; Liu, S.; Liu, Y.; Ao, X.; Yang, Y. Improving catalase stability by its immobilization on grass carp (*Ctenopharyngodon idella*) scale collagen self-assembly films. *Mater. Sci. Eng. C* **2019**, *105*, 110024. [CrossRef] [PubMed]
97. Hosu, O.; Lettieri, M.; Papara, N.; Ravalli, A.; Sandulescu, R.; Cristea, C.; Marrazza, G. Colorimetric multienzymatic smart sensors for hydrogen peroxide, glucose and catechol screening analysis. *Talanta* **2019**, *204*, 525–532. [CrossRef]
98. Homma, T.; Kondo, M.; Kuwahara, T.; Shimomura, M. Polyaniline/poly(acrylic acid) composite film: A promising material for enzyme-aided electrochemical sensors. *Eur. Polym. J.* **2015**, *62*, 139–144. [CrossRef]
99. Zhu, Y.; Qiu, F.; Rong, J.; Zhang, T.; Mao, K.; Yang, D. Covalent laccase immobilization on the surface of poly(vinylidene fluoride) polymer membrane for enhanced biocatalytic removal of dyes pollutants from aqueous environment. *Colloids Surf. B* **2020**, *191*, 111025. [CrossRef]
100. Li, S.; Zhao, S.; Hou, Y.; Chen, G.; Chen, Y.; Zhang, Z. Polylactic acid (PLA) modified by polyethylene glycol (PEG) for the immobilization of lipase. *Appl. Biochem. Biotechnol.* **2020**, *190*, 982–996. [CrossRef]
101. Benbow, N.L.; Sebben, D.A.; Karpiniec, S.; Stringer, D.; Krasowska, M.; Beattie, D.A. Lysozyme uptake into pharmaceutical grade fucoidan/chitosan polyelectrolyte multilayers under physiological conditions. *J. Colloid Interface Sci.* **2020**, *565*, 555–566. [CrossRef]
102. Visan, A.; Cristescu, R.; Stefan, N.; Miroiu, M.; Nita, C.; Socol, M.; Florica, C.; Rasoga, O.; Zgura, I.; Sima, L.E.; et al. Antimicrobial polycaprolactone/polyethylene glycol embedded lysozyme coatings of Ti implants for osteoblast functional properties in tissue engineering. *Appl. Surf. Sci.* **2017**, *417*, 234–243. [CrossRef]

103. Fu, J.; Schlenoff, J.B. Driving forces for oppositely charged polyion association in aqueous solutions: Enthalpic, entropic, but not electrostatic. *J. Am. Chem. Soc.* **2015**, *138*, 980–990. [CrossRef]
104. Salehi, A.; Desai, P.S.; Li, J.; Steele, C.A.; Larson, R.G. Relationship between polyelectrolyte bulk complexation and kinetics of their layer-by-layer assembly. *Macromolecules* **2014**, *48*, 400–409. [CrossRef]
105. Moschakis, T.; Biliaderis, C.G. Biopolymer-based coacervates: Structures, functionality and applications in food products. *Curr. Opin. Colloid Interface Sci.* **2017**, *28*, 96–109. [CrossRef]
106. Wu, D.; Zhu, L.; Li, Y.; Zhang, X.; Xu, S.; Yang, G.; Delair, T. Chitosan-based colloidal polyelectrolyte complexes for drug delivery: A review. *Carbohydr. Polym.* **2020**, *238*, 116126. [CrossRef] [PubMed]
107. Perry, S.L.; Li, Y.; Priftis, D.; Leon, L.; Tirrell, M. The effect of salt on the complex coacervation of vinyl polyelectrolytes. *Polymers* **2014**, *6*, 1756–1772. [CrossRef]
108. Karim, Z.; Adnan, R.; Husain, Q. β-cyclodextrine chitosan complex as the immobilization matrix for horseradish peroxidase and its application for the removal of azo dyes from textile effluent. *Int. Biodeterior. Biodegrad.* **2012**, *72*, 10–17. [CrossRef]
109. Zheng, J.; Gao, Q.; Ge, G.; Wu, J.; Tang, C.; Zhao, M. Dynamic equilibrium of β-conglycinin/lysozyme heteroprotein complex coacervates. *Food Hydrocoll.* **2022**, *124*, 107339. [CrossRef]
110. Xu, W.; Jin, W.; Zhang, C.; Li, Z.; Lin, L.; Huang, Q.; Ye, S.; Li, B. Curcumin loaded and protective system based on complex of κ-carrageenan and lysozyme. *Food Res. Int.* **2014**, *59*, 61–66. [CrossRef]
111. Antonov, Y.A.; Moldenaers, P.; Cardinaels, R. Complexation of lysozyme with sodium caseinate and micellar casein in aqueous buffered solutions. *Food Hydrocoll.* **2017**, *62*, 102–118. [CrossRef]
112. Antonov, Y.A.; Zhuravleva, I.L.; Cardinaels, R.; Moldenaers, P. Macromolecular complexes of lysozyme with kappa carrageenan. *Food Hydrocoll.* **2018**, *74*, 227–238. [CrossRef]
113. Kubelbeck, S.; Mikhael, J.; Schoof, S.; Andrieu-Brunsen, A.; Baier, G. Immobilization of α-amylase in polyelectrolyte complexes. *J. Appl. Polym. Sci.* **2017**, *15*, 45036. [CrossRef]
114. Souza, C.J.F.; Garcia-Rojas, E.E.; Souza, C.S.F.; Vriesmann, L.C.; Vicente, J.; De Carvalho, M.G.; Petkowicz, C.L.O.; Favaro-Trindade, C.S. Immobilization of β-galactosidase by complexation: Effect of interaction on the properties of the enzyme. *Int. J. Biol. Macromol.* **2019**, *122*, 594–602. [CrossRef]
115. Souza, C.J.F.; Garcia-Rojas, E.E.; Favaro-Trindade, C.S. Lactase (β -galactosidase) immobilization by complex formation: Impact of biopolymers on enzyme activity. *Food Hydrocoll.* **2018**, *83*, 88–96. [CrossRef]
116. Bucatariu, F.; Teodosiu, C.; Morosanu, I.; Fighir, D.; Ciobanu, R.; Petrila, L.-M.; Mihai, M. An overview on composite sorbents based on polyelectrolytes used in advanced wastewater treatment. *Polymers* **2021**, *13*, 3963. [CrossRef]
117. Zdarta, J.; Meyer, A.S.; Pinelo, M. Developments in support materials for immobilization of oxidoreductases: A comprehensive review. *Adv. Colloid Interface Sci.* **2018**, *258*, 1–20. [CrossRef]
118. Bilal, M.; Asgher, M. Sandal reactive dyes decolorization and cytotoxicity reduction using manganese peroxidase immobilized onto polyvinyl alcohol–alginate beads. *Chem. Cent. J.* **2015**, *9*, 47. [CrossRef]
119. Aricov, L.; Leonties, A.R.; Gîfu, I.C.; Preda, D.; Raducan, A.; Anghel, D.-F. Enhancement of laccase immobilization onto wet chitosan microspheres using an iterative protocol and its potential to remove micropollutants. *J. Environ. Manag.* **2020**, *276*, 111326. [CrossRef]
120. Maryskova, M.; Schaabova, M.; Tomankova, H.; Novotny, V.; Rysova, M. Wastewater treatment by novel polyamide/polyethylenimine nanofibers with immobilized laccase. *Water* **2020**, *12*, 588. [CrossRef]
121. Leontieș, A.R.; Răducan, A.; Culiță, D.C.; Alexandrescu, E.; Moroșan, A.; Mihaiescu, D.E.; Aricov, L. Laccase immobilized on chitosan-polyacrylic acid microspheres as highly efficient biocatalyst for naphthol green B and indigo carmine degradation. *Chem. Eng. J.* **2022**, *439*, 135654. [CrossRef]
122. Vera, M.; Nyanhongo, G.S.; Guebitz, G.M. Polymeric microspheres as support to co-immobilized *Agaricus bisporus* and *Trametes versicolor* laccases and their application in diazinon degradation. *Arab. J. Chem.* **2019**, *13*, 4218–4227. [CrossRef]
123. Lassouane, F.; Aït-Amar, H.; Amrani, S.; Rodriguez-Couto, S. A promising laccase immobilization approach for Bisphenol A removal from aqueous solutions. *Bioresour. Technol.* **2018**, *271*, 360–367. [CrossRef]
124. Pereira Cipolatti, E.; Valério, A.; Henriques, R.O.; Pinto, M.C.C.; Fernandez Lorente, G.; Manoel, E.A.; Guisan, J.M.; Ninow, J.L.; de Oliveira, D.; Costa Pessela, B. Production of new nanobiocatalysts via immobilization of lipase B from *C. antarctica* on polyurethane nanosupports for application on food and pharmaceutical industries. *Int. J. Biol. Macromol.* **2020**, *165*, 2957–2963. [CrossRef]
125. Pan, J.; Ou, Z.; Tang, L.; Shi, H. Enhancement of catalytic activity of lipase-immobilized $Fe_3O_4$ -chitosan microsphere for enantioselective acetylation of racemic 1-phenylethylamine. *Korean J. Chem. Eng.* **2019**, *36*, 729–739. [CrossRef]
126. Melo, A.D.Q.; Silva, F.F.M.; dos Santos, J.C.S.; Fernandez-Lafuente, R.; Lemos, T.L.G.; Dias Filho, F.A. Synthesis of benzyl acetate catalyzed by lipase immobilized in nontoxic chitosan-polyphosphate beads. *Molecules* **2017**, *22*, 2165. [CrossRef]
127. Tudorache, M.; Gheorghe, A.; Negoi, A.; Enache, M.; Maria, G.-M.; Parvulescu, V. Bifunctional carbohydrate biopolymers entrapped lipase as catalyst for the two consecutive conversions of α-pinene to oxy-derivative. *Carbohydr. Polym.* **2016**, *152*, 726–733. [CrossRef] [PubMed]
128. Nemiwal, M.; Zhang, T.C.; Kumar, D. Enzyme immobilized nanomaterials as electrochemical biosensors for detection of biomolecules. *Enzyme Microb. Technol.* **2022**, *156*, 110006. [CrossRef] [PubMed]

129. Tutunaru, O.; Mihailescu, C.M.; Savin, M.; Tincu, B.C.; Stoian, M.C.; Muscalu, G.S.; Firtat, B.; Dinulescu, S.; Craciun, G.; Moldovan, C.A.; et al. Acetylcholinesterase entrapment onto carboxyl-modified single-walled carbon nanotubes and poly(3,4-ethylenedioxythiophene) nanocomposite film, electrosynthesis characterization, and sensor application for dichlorvos detection in apple juice. *Microchem. J.* **2021**, *169*, 106573. [CrossRef]
130. Cano-Raya, C.; Dencheva, N.V.; Braz, J.F.; Malfois, M.; Denchev, Z.Z. Optical biosensor for catechol determination based on laccase-immobilized anionic polyamide 6 microparticles. *J. Appl. Polym. Sci.* **2020**, *137*, e49131. [CrossRef]
131. Aldea, A.; Leote, R.J.B.; Matei, E.; Evanghelidis, A.; Enculescu, I.; Diculescu, V.C. Gold coated electrospun polymeric fibers as new electrode platform for glucose oxidase immobilization. *Microchem. J.* **2021**, *165*, 106108. [CrossRef]
132. Yuan, M.; Dai, F.; Li, D.; Fan, Y.; Xiang, W.; Tao, F.; Cheng, Y.; Deng, H. Lysozyme/collagen multilayers layer-by-layer deposited nanofibers with enhanced biocompatibility and antibacterial activity. *Mater. Sci. Eng. C* **2020**, *112*, 110868. [CrossRef]
133. Baidamshina, D.R.; Koroleva, V.A.; Olshannikova, S.S.; Trizna, E.Y.; Bogachev, M.I.; Artyukhov, V.G.; Holyavka, M.G.; Kayumov, A.R. Biochemical properties and anti-biofilm activity of chitosan-immobilized papain. *Mar. Drugs* **2021**, *19*, 197. [CrossRef]
134. Filho Moreira, R.N.F.; Vasconcelos, N.F.; Andrade, F.K.; de Freitas Rosa, M.; Silveira Vieira, R. Papain immobilized on alginate membrane for wound dressing application. *Colloids Surf. B* **2020**, *194*, 111222. [CrossRef]
135. Teske, M.; Kießlich, T.; Fischer, J.; Bahl, H.; Wulf, K. Immobilizing hydrolytic active papain on biodegradable PLLA for biofilm inhibition in cardiovascular applications. *Curr. Dir. Biomed. Eng.* **2020**, *6*, 6–9. [CrossRef]
136. Sklyarenko, A.V.; El, M.A.; Kurochkina, V.B.; Yarotsky, S.V. Enzymatic synthesis of β-lactam acids (Review). *Appl. Biochem. Microbiol.* **2015**, *51*, 627–640. [CrossRef]
137. Mohammadi, M.; Habibi, Z.; Gandomkar, S.; Yousefi, M. A novel approach for bioconjugation of *Rhizomucor miehei* lipase (RML) onto amine-functionalized supports; Application for enantioselective resolution of rac-ibuprofen. *Int. J. Biol. Macromol.* **2018**, *117*, 523–531. [CrossRef]
138. Gilani, S.L.; Najafpour, G.D.; Heydarzadeh, H.D.; Moghadamnia, A. Enantioselective synthesis of (S)-naproxen using immobilized lipase on chitosan beads. *Chirality* **2017**, *29*, 304–314. [CrossRef]
139. Bilal, M.; Asgher, M.; Iqbal, H.M.N.; Hu, H. Gelatin-immobilized manganese peroxidase with novel catalytic characteristics and its industrial exploitation for fruit juice clarification purposes. *Catal. Lett.* **2016**, *146*, 2221–2228. [CrossRef]
140. Irshad, M.; Murtza, A.; Zafar, M.; Bhatti, K.H.; Rehman, A.; Anwar, Z. Chitosan-immobilized pectinolytics with novel catalytic features and fruit juice clarification potentialities. *Int. J. Biol. Macromol.* **2017**, *104*, 242–250. [CrossRef]
141. Benucci, I.; Lombardelli, C.; Cacciotti, I.; Esti, M. Papain covalently immobilized on chitosan–clay nanocomposite films: Application in synthetic and real white wine. *Nanomaterials* **2020**, *10*, 1622. [CrossRef]
142. Fernández-Pacheco, P.; García-Béjar, B.; Briones Pérez, A.; Arévalo-Villena, M. Free and immobilised β-glucosidases in oenology: Biotechnological characterisation and its effect on enhancement of wine aroma. *Front. Microbiol.* **2021**, *12*, 723815. [CrossRef]
143. Wang, P.; Zhang, C.; Zou, Y.; Li, Y.; Zhang, H. Immobilization of lysozyme on layer-by-layer self-assembled electrospun films: Characterization and antibacterial activity in milk. *Food Hydrocoll.* **2021**, *113*, 106468. [CrossRef]