



Functionalized Materials as a Versatile Platform for Enzyme Immobilization in Wastewater Treatment

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Abstract

Purpose of Review Untreated wastewater discharge can significantly and negatively impact the state of the environment. Rapid industrialization and economic development have directly contributed to land and water pollution resulting from the application of many chemicals such as organic dyes, pharmaceuticals, and industrial reagents. The removal of these chemicals before effluent discharge is crucial for environmental protection. This review aims to explore the importance of functionalized materials in the preparation of biocatalytic systems and consider their application in eliminating water pollutants.

Recent Findings Wastewater treatment methods can be classified into three groups: (i) chemical (e.g., chemical oxidation and ozonation), (ii) physical (e.g., membrane separation and ion exchange), and (iii) biological processes. Biological treatment is the most widely used method due to its cost-effectiveness and eco-friendliness. In particular, the use of immobilized enzymes has recently become more attractive as a result of scientific progress in advanced material synthesis. The selection of an appropriate support plays an important role in the preparation of such biologically active systems. Recent studies have demonstrated the use of various materials for enzyme immobilization in the purification of water.

Summary This review identifies and discusses different biocatalytic systems used in the enzymatic degradation of various water pollutants. Materials functionalized by specific groups can serve as good support matrices for enzyme immobilization, providing chemical and thermal stability to support catalytic reactions. Enzymatic biocatalysis converts the pollutants into simpler products, which are usually less toxic than their parents. Due to immobilization, the enzyme can be used over multiple cycles to reduce the cost of wastewater treatment. Future studies in this field should focus on developing new platforms for enzyme immobilization in order to improve degradation efficiency.

Keywords Enzyme immobilization · Functionalized materials · Biodegradation · Organic pollutants · Wastewater treatment

Introduction

Functionalized materials in organic, inorganic, hybrid, and composite forms can be modified to have desirable properties for industrial application. Amongst these properties, particle size and size distribution, shape and purity, and crystallographic and porous structure play important roles for surface functionalization [1•, 2, 3]. Through functionalization, it is possible to introduce organic and inorganic moieties into these materials [4]. Functionalized materials have been widely used in many industries such as the food processing (e.g., beer and wine production, nutritive and nonnutritive food additives, and food protection), pharmaceutical (e.g., drug delivery and drug synthesis), cosmetics (fragrance, film formers, and cosmetic ingredients), electronics (molecular electronics, organic photovoltaics, and biosensors), and textile (in the production of antimicrobial, self-cleaning, temperature regulating,

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moisture-wicking, flame-retardant, and self-healing textiles) industries [5, 6]. Enzyme immobilization is an important research topic in the application of functionalized materials to improve performance and potentially reduce the cost of enzymatic treatment. Considering the multifunctionality of the above-mentioned materials, this review describes their use in the process of enzyme immobilization.

A natural enzyme is usually only stable within a narrow pH and temperature range due to the water solubility of free enzymes. They can be hydrolyzed over time. Furthermore, water soluble enzymes are difficult to isolate from the reaction medium. Hence, the cost of enzymatic treatment can be very high [7]. Enzyme immobilization is an effective strategy to overcome the disadvantages of a free enzyme system. Enzyme immobilization can be defined as the localization or closing of an enzyme in a defined region of space [8] to obtain a stable biocatalytic system. The immobilization of an enzyme on the surface of a specified material can improve its pH, thermal, and storage stability. As a significant advantage, after immobilization, the enzyme is in a heterogeneous form and can be easily separated from the reaction medium for reuse [9–11]. From this perspective, the review focuses on the immobilization of enzymes on a selected group of materials (functionalized materials) and on their application in environmental remediation.

A wide variety of materials can be used as support matrices for enzyme immobilization. Their properties are of significant importance in determining the interaction between the enzyme and the support. The selection of an appropriate matrix also depends on the properties of the enzyme and the immobilization method [12–14]. A high surface area, porosity, hydrophilicity, biocompatibility, stability in reaction conditions, and availability at low cost are the most desirable properties of a support material [15–18]. Many of these benefits can be achieved using functionalized materials.

There have been several reviews on the role of immobilized enzymes in environmental applications, describing various types of biocatalytic systems based on oxidoreductases (laccase and peroxidase) and their use as catalysts in wastewater treatment. Some of them concern immobilized laccases [19, 20, 21] and peroxidases [22–24] and their potential application to remediate pollutants. Others refer to methods of immobilization, the support matrices used, and the types of pollutants treated [8, 25–27]. In contrast to previous studies, this review will mainly focus on studies of oxidoreductases immobilized on functionalized materials (modified inorganic, organic compounds, and hybrids or composites) and the subsequent use of such systems in water purification processes (especially in the treatment of organic dyes, pharmaceuticals, phenol compounds, and polycyclic aromatic hydrocarbons).

Functionalized Materials as Supports for Enzymes

A diverse range of materials (e.g., natural, synthetic, inorganic, organic, and composite or hybrid) can be used for enzyme immobilization. Firstly, surface modification is conducted to improve the properties of these materials for a specific application. Different types of compounds are considered for this purpose, depending on the field of use. One key aspect of functionalization is the introduction of various types of functional groups to the material surface. Metal oxides (oxide systems), polymers (including biopolymers), and hybrids or composites can all serve as the base for functionalized materials, and many compounds are used to modify their surface [2, 6]. Amino, carboxylic, hydroxyl, and sulfhydryl groups are commonly introduced. The modification process is of great importance when a material is intended for use as a support for enzyme immobilization. In some cases, support materials are inert and require activation. Functionalization allows the formation of stable covalent bonds between the functional groups found in the enzyme and the functional groups on the surface of the support material [4, 8].

Enzymes on the surface of functionalized materials can be immobilized by both physical (adsorption and entrapment) and chemical (covalent and cross-linking) methods. This is related to the physicochemical properties, the structure of the material, and the types of functional groups introduced to their surface. Physical immobilization is an attractive method because it does not change the conformation of the enzyme. Additionally, many functionalized materials have a large surface area allowing enzyme adsorption. The adsorption method is also simple and enables mobility of the enzyme. Conversely, chemical immobilization contributes to a high catalytic efficiency, stability, and a low mass transfer resistance of the immobilized enzyme. In the chemical method, a reaction occurs between the amino groups on the protein surface and the functional groups (e.g., carboxylic, epoxy, thiol) on the functionalized materials [28]. Figure 1 provides an overview of the functionalized materials, active functional groups for enzyme immobilization, immobilizing mechanisms, and the water pollutants that can be removed by an immobilized enzymatic system.

Inorganic materials, both natural and synthetic (oxides, zeolites, silicates, active carbons, etc.), are commonly used in immobilization processes. These substances are characterized by high resistance, stability, and availability. Moreover, their synthesis can proceed via simple and fast methods, making them relatively cheap. The high value of these materials as carriers in protein immobilization results from their defined porous structure and significant sorption capacity. It should also be noted that on the surface of the most commonly used inorganic materials (inorganic oxides), specific chemical groups can be introduced to increase their affinity to enzymes.

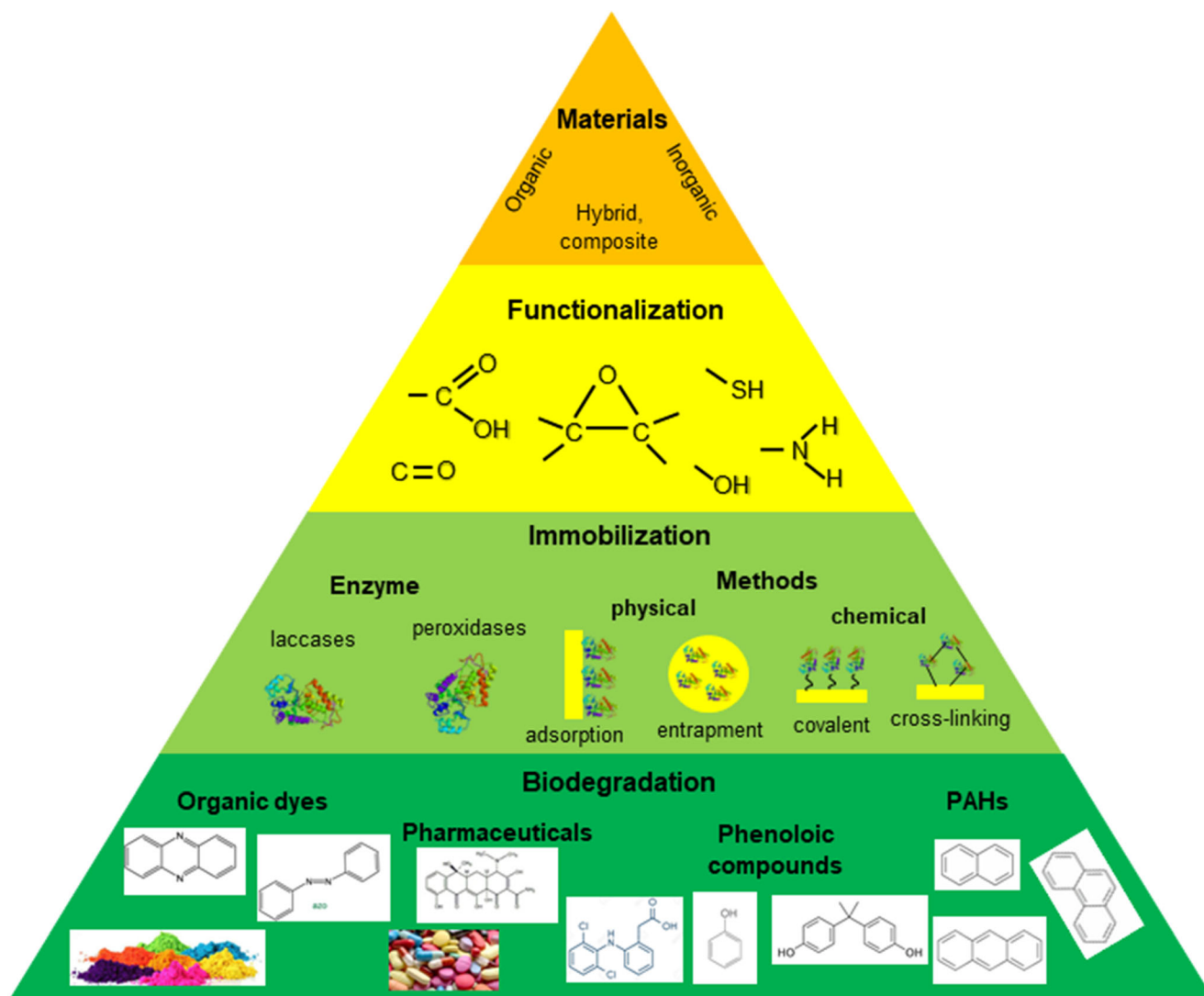


Fig. 1 Overview of preparation of biocatalytic systems and their application in pollutant degradation

This is especially important in the case of immobilization with the formation of covalent bonds. The high activity of the immobilized enzyme prevents any diffusion resistance in the transport of the components of the reaction mixture and enables the use of the immobilized system over several reaction cycles [12].

Polymers are another large group of organic materials utilized in enzyme immobilization. These materials can be obtained from natural sources (biopolymers) or synthesized with high precision in laboratory conditions. The properties and enzymatic capacity of synthetic polymers can be adjusted to the specific enzyme and the intended application. The polymeric matrix can be functionalized by building monomers into its structure. The monomers contain functional groups capable of forming chemical bonds with the enzyme moieties. Besides, synthetic polymers are characterized by high thermal and mechanical stability. These materials can act as supports for enzymes from various catalytic groups with applicability in many industrial processes. Biopolymers are omnipresent.

They have a high affinity to enzymes, offer biocompatibility, and do not cause toxicity of the enzyme [29]. Many of them are chemically resistant and stable under the conditions of catalyzed reactions. The greatest advantage of biopolymers is the presence of many groups in their structure which are responsible for binding with the enzymes or enabling the effective modification of their surface [13].

Apart from inorganic and organic substrates, hybrids and composites also constitute an interesting group of materials for enzyme immobilization, including such systems as inorganic–inorganic (different oxide systems, e.g., SiO_2/MgO and $\text{ZrO}_2/\text{SiO}_2$), organic–organic (e.g., chitosan/alginate and polyvinyl alcohol/chitosan), and inorganic–organic ($\text{SiO}_2/\text{chitosan}$ and $\text{Fe}_3\text{O}_4/\text{polyethyleneimine}$). The properties of these materials can be precisely designed for the specific enzyme and the target application. Additionally, they combine the properties of both precursors, increasing the resulting functionality and extending the range of applicability [26].

Applications of Immobilized Enzymes for Removing Water Pollutants

Many countries around the world have set targets for sustainable socioeconomic development with a focus on minimizing pollution. Highly efficient neutralization technologies are used to detect and degrade pollutants. Adsorption on active materials, chemical oxidation, ozonation, ultrafiltration, nanofiltration, and ion exchange are commonly used to purify wastewater. These technologies require significant energy consumption and are also characterized by low efficiency and stability, high cost, and the formation of harmful by-products [19, 30, 31]. Biological methods are an alternative for treating industrial wastewaters due to their availability, cost-effectiveness, and eco-friendliness. These methods are based on three main processes: biosorption, bioaccumulation, and biodegradation, or a combination of these. Regarding biodegradation, an enzyme participates in the conversion of the pollutants to simpler compounds usually with less toxicity than the initial ones [21, 32, 33]. The use of native enzymes to degrade pollutants is subject to certain limitations, such as low stability and difficulty of recovery, which impair their reusability. For this reason, immobilized enzymes are often utilized in the degradation of wastewaters. During immobilization, the structure is stabilized, while the thermal and pH stability and repeatability are improved; it may thus become a good candidate for industrial application [25, 34].

This review highlights systems containing laccase and peroxidase immobilized on functionalized materials and their application to remove organic dyes, pharmaceuticals, and phenol compounds from wastewaters. A subsequent part of the review provides examples of industrial applications of immobilized enzymes.

Organic Dyes

Organic dyes are widely used in the textile industry. Water pollution by organic dyes is a significant and challenging environmental problem. Some organic dyes are toxic and persistent in the environment. They also discolor bodies of water giving them an unpleasant visual appearance. Despite the low content of dyes in wastewater, the color is a characteristic indicator of their presence. The decolorization and degradation of organic dyes can be effectively achieved with immobilized enzymes.

Previous studies have confirmed the effectiveness of oxidoreductases in the degradation of dyes. Laccases are most commonly used enzyme for this wastewater treatment application [35–37]. The immobilization of these enzymes mainly focuses on silica and its composites [38, 39]. Lopez-Barbosa et al. [40] performed experiments with laccase immobilized on functionalized silica for decolorizing Congo Red. The silica was modified

with APTES (γ -aminopropyltriethoxysilane), and the NH_2 groups were subsequently activated by adding glutaraldehyde (GA). Afterward, the laccase was conjugated by forming the imine bonds. The laccase/silanized silica was introduced into textile filters to extend the activity of the enzyme molecules when incorporated in a continuous remediation process. Functionalized silica was also used by Salami et al. to prepare a biocatalyst [41]. In this case, the silica was modified with 3-glycidylxypropyltrimethoxysilane (3-GPTMS). The laccase/epoxy-functionalized silica was used for the decolorization of five dyes (Acid Orange 156, Acid Red 52, Coomassie Brilliant Blue, Methyl Violet, and Malachite Green). The proposed biocatalyst demonstrated good stability in terms of temperature, pH, and organic solvents. The results showed a correlation between the dye structure and the decolorization rate [41].

Functionalized Fe_3O_4 -based materials are another large group of compounds used as carriers for laccase [42–45]. For example, Liu et al. [46] synthesized a magnetic poly(*p*-phenylenediamine) nanocomposite (PpPD/ Fe_3O_4). Laccase was then immobilized on the PpPD/ Fe_3O_4 system and used as a catalyst to remove Reactive Blue 19 dye. The proposed biocatalytic system (Lac/PpPD/ Fe_3O_4) exhibited high stability, easy recovery, reuse capabilities, and high removal efficiency for Reactive Blue 19 dye. Fe_3O_4 functionalized with amino groups was employed by Gao et al. to support laccase [47]. The results confirmed the effective decolorization of Acid Fuchsin using the obtained biocatalysts (laccase/amino-functionalized Fe_3O_4). In another interesting study by Ulu et al. [48], laccase was immobilized on iron oxide modified with thiolated chitosan (TCS). The results showed the long-term activity of the system in the decolorization of two dyes (Reactive Blue 171 and Acid Blue 74).

Titanium dioxide-based photocatalysis is useful because of its low cost, non-toxicity, high photocatalytic activity, and stability. However, degradation by photocatalysis is only adequate for wastewater with low concentrations of dye. In this case, the aqueous solution should be transparent to allow light penetration [49]. Khakshoor et al. [50] described a two-step decolorization by co-entrapped spore laccase and TiO_2 nanoparticles. The first stage of this process involved the pretreatment of the dye with laccase in dark conditions. This was followed by treatment with TiO_2 (photocatalysis under UV radiation). Titania modified with glycidylacrylate (GA) [51••] and titanium(IV) bis(ammonium lactato)dihydroxide (Ti-BALDH) [52] were also employed as laccase supports and applied in the degradation of different organic dyes (Rhodamine B and Malachite Green). The results showed that a biocatalytic system based on titania and laccase can be successfully utilized to remediate organic pollutants.

Natural and synthetic polymers are attractive materials as enzyme supports. The functionalization of their surface enables their multifunctional application for enzyme

immobilization and wastewater treatment [53]. Gioia et al. [54] described the immobilization of laccase on thiosulfinate–agarose and the ability of the system to degrade azo dyes. Chitosan is another biopolymer commonly used for enzyme immobilization. The practical operability and efficiency of immobilization on chitosan are improved by incorporating certain inorganic materials such as clays. A catalyst based on functionalized chitosan and laccase can be successfully used for dye treatment [55]. Synthetic polymers, especially their modified forms, are frequently employed as enzyme carriers. In the literature, poly(methyl methacrylate)/polyaniline electrospun fibers [56] and a polydopamine-coated poly(vinylidene fluoride) membrane (PDA@PVDF) [57] have also been used to immobilize laccase and subsequently used in the removal of organic dyes from wastewaters.

Peroxidases are another group of enzymes that can catalyze oxidation reactions. Peroxidases immobilized on various kinds of supports have been successfully utilized for the decolorization and detoxification of waste dyes [23, 58–60]. Many researchers have also used horseradish peroxidase (HRP) in the degradation of organic dyes. For example, Sun et al. [61] researched the immobilization of horseradish peroxidase on silica coated with zinc oxide. The results indicated that the proposed catalyst HRP/ZnO/SiO₂ exhibited a high decolorization efficiency and good reusability for anthraquinone dyes. The same enzyme (HRP) was immobilized on a functionalized hybrid system (graphene oxide–silica). The surface of this material was modified with APTS (3-aminopropyltriethoxysilane) [62]. The authors reported that the prepared biocatalytic system achieved 100% efficiency in the decolorization of dyes. In many cases, horseradish peroxidase or other enzymes are co-immobilized with chloroperoxidase (CPO) and immobilized on various supports [63, 64]. A noteworthy study is that of Cheng et al. [65], who proposed a polydopamine-tethered CPO/HRP-TiO₂ nanocomposite as an enzyme-photo bifunctional synergistic catalyst in water treatment. In this case, the TiO₂ played the roles of both a solid and a photocatalyst in the decolorization of organic dyes. Furthermore, Kiran et al. [57] immobilized lignin peroxidase on functionalized MnFe₂O₄ and utilized it to decolorize dyes. Graphene oxide was employed for modification, changing the surface properties of MnFe₂O₄ to make them more hydrophilic for the binding of biomolecules.

In summary, functionalized materials play an important role in the immobilization of enzymes. The selection of an appropriate modifier makes it possible to change the character of the carrier surface in a way that increases the efficiency of the immobilization process. Additionally, in this case, the bonds between the enzyme and support are more stable, which is important when the discussed system is used repeatedly for industrial applications. The examples presented above indicate that biocatalytic systems based on functionalized materials can effectively decolorize and degrade toxic dyes in a

water environment. Moreover, the research shows that purified solutions (both model and real) are less toxic after degradation using functionalized material/enzyme systems compared with the solutions before degradation.

Pharmaceutically Active Compounds

Pharmaceutical pollution has become an increasingly serious problem due to the increased consumption of pharmaceuticals and growth of the pharmaceutical industry. In addition, the prevalence of chronic diseases, the development of science and technology, and the growing world population have contributed to the increasing presence of pharmaceutically active compounds in the aquatic environment [66, 67]. These pollutants are discharged by various antibiotics and medicines, including those available without prescription [68, 69]. As in the case of dyes, pharmaceutical pollutants can be degraded by biological methods (especially using immobilized oxidoreductases) into simpler and safer derivatives [70, 71].

One pharmaceutical frequently used to evaluate the efficiency of enzymatic treatment is diclofenac, which is a non-steroidal anti-inflammatory drug. In water, diclofenac causes genetic and systemic damage to various living organisms [70, 72]. Xu et al. [73] employed functionalized carbon nanotubes as the support matrix for laccase to prepare a polyvinyl alcohol (PVA)/chitosan (CS)/multi-walled carbon nanotubes (MWNTs) composite nanofibrous membrane. It was reported that after immobilization on PVA/CS/MWNTs, the laccase retained high activity and demonstrated a high rate of removal of diclofenac from the wastewater. A study by Teheran et al. [74] presented biochar activated by polyacrylonitrile (PAN) and entrapped in nanofibers by an electrospinning method. This process provided adsorption capability in the membrane and increased the contact time between the immobilized laccase and the pharmaceutical pollutants. Experiments using biochar as a carrier for laccase in order to biodegrade diclofenac were also performed by Lionappan et al. [75]. In this report, the biochar was modified with citric acid, which allowed the introduction of carbonyl groups to the biochar surface. This increased the reaction between the enzyme and the support. Diclofenac was also biotransformed with great efficiency by laccase immobilized on amino-functionalized TiO₂ [76]. The biocatalyst presented by Garcia-Morales et al. is a good alternative for removing pharmaceutical compounds from water environments. Immobilized laccase was also used to transform or degrade diclofenac from industrial effluents in a study by Neelkant et al. [77]. A polyvinyl alginate–silicon dioxide matrix was utilized. Liquid chromatography–mass spectrometry (LC-MS) analysis showed that diclofenac was transformed through a ring-opening reaction and mineralization after hydroxylation.

Tetracycline (TC) and carbamazepine (CBZ) are other pharmaceutical compounds that have also been used to

demonstrate the efficiency of immobilized enzymes. A wide range of sexually transmitted diseases, as well as gastritis, peptic ulcers, and lower respiratory tract infections, are treated with tetracycline and its derivatives. In turn, carbamazepine is used as an anticonvulsant and mood-stabilizing drug to treat epilepsy and bipolar disorder. Interesting research concerning the removal of tetracycline was conducted by Teheran et al. [78]. Laccase was immobilized on a polyacrylonitrile–biochar composite nanofibrous membrane. The resulting biocatalytic system, used in continuous mode, achieved a chlortetracycline removal efficiency of around 50%. The same pharmaceutical compound was biodegraded by Garcia-Delgado et al. [79••]. In this case, amino-functionalized stevensite and biochar were employed to immobilize laccase. The results showed that the immobilized laccase system could degrade 100% of the tetracycline. In other studies, carbamazepine was removed from wastewater by laccase immobilized on oxygen-functionalized nanobiochars [80] and polyimide aerogels [81]. The nanobiochar was treated with acid, thus introducing carboxylic groups to the surface, which led to the greatest immobilization efficiency. At the same time, a high degradation of CBZ was achieved. Similar results were obtained with laccase immobilized on polyimide aerogels.

Based on the above-mentioned reports, it may be concluded that enzymes immobilized on functionalized materials can be used successfully to degrade pharmaceutical pollutants. Different types of materials functionalized by various compounds are used as supports for oxidoreductase immobilization. The results presented demonstrate the great efficiency of biocatalytic systems based on these materials in the degradation of pharmaceutical pollutants. Oxidoreductases supported on functionalized materials exhibit higher activity and better performance in the removal of pharmaceutical compounds. Moreover, they enable the reusability of the supported enzyme over several degradation cycles and can lead to a lower toxicity of the solutions after treatment.

Phenol Compounds and Polycyclic Aromatic Hydrocarbons

Expanding industrial activity can lead to an increased occurrence of toxic chemicals such as phenolic compounds in wastewater. These compounds may derive from the metallurgic, petrochemical, and pharmaceutical industries and organic chemical synthesis. Phenolic compounds pose an enormous hazard to organisms and the environment and should therefore be quickly removed. Enzymatic methods are commonly used to degrade these industrial chemicals [82, 83].

Horseradish peroxidase (HRP) and laccase (Lac) are effective enzymes for converting phenolic compounds in aqueous solutions to polymeric phenolic aggregates [83]. They have been immobilized on different functionalized inorganic oxides to improve their stability in the degradation of phenolic compounds.

Silica and its functionalized forms have also been used as support matrices for immobilizing enzymes in order to remove phenolic pollutants. Vishu et al. [84] reported a biocatalytic system consisting of a co-immobilized lignolytic enzyme cascade on multifunctionalized magnetic silica microspheres (MSMS). A facile method for the co-immobilization of laccase and peroxidase on MSMS was evaluated, the optimization of the operational parameters was carried out, and the reaction kinetics were determined. The prepared biocatalyst was used in the transformation of phenolic contaminants in the wastewater from a biorefinery. The results demonstrated the effective removal of different classes of phenolic compounds (phenol, chloro-, trichloro- and dichlorophenol, cresols, dimethyl-, 2-methyl-4,6-dinitro-, 4-nitro-, tetrachloro- and pentachlorophenol). Silica-based material was also been studied by Mohammadi et al. [85]. In this case, the silica was modified with epoxy groups, and the laccase was covalently immobilized using nucleophilic attack by the amino groups of the laccase on the epoxy groups of the support. The performance of the biocatalytic system was evaluated in the removal of phenolic constituents, including phenol, *p*-chlorophenol, and catechol. The degradation of the catechol was highly efficient (about 95%). A nanocomposite based on reduced graphene oxide (GO) and silica was also used to immobilize HRP [86]. GO and its reduced form (RGO), due to the presence of carboxylic, hydroxyl, and epoxide groups on the surface, are suitable matrices for enzyme immobilization. Moreover, composites with RGO and silica offer good surface area, surface roughness, and functionalization. Vineh et al. [86] evaluated the kinetic parameters, reusability, and stability of the enzyme for catalytic activity. Additionally, the obtained biocatalyst (GO/SiO₂/HRP) was utilized in the removal of high concentrations of phenol compounds from the water solution. The same research group also reported results concerning the immobilization of HRP on amino-functionalized RGO which confirmed the conclusions from the previous study [87•].

Many materials based on Fe₃O₄ are applied in the immobilization of enzymes because of their unique properties. Due to its ferromagnetic features, the magnetite support allows the prepared biocatalytic system to be easily recovered from the reaction [15]. The literature shows that Fe₃O₄ can be modified with biopolymers, polymers, and functional groups. Chitosan was used by Zhang et al. [88] to coat Fe₃O₄ nanoparticles. Furthermore, the product was used as a support for laccase immobilization, and the resulting biocatalysts were highly efficient in the degradation of the chlorophenol in the water solution. Interesting studies were also reported by Wu et al. [89], who modified Fe₃O₄ with amino groups. Then laccase was covalently adsorbed onto the amino-functionalized Fe₃O₄ and used to remove the phenolic compounds, achieving approximately 85% efficiency. After the reaction, the biocatalytic system was separated from the solution using a magnet.

Other papers report on composites or hybrids containing magnetite particles. For example, Fe_3O_4 was coated with MoS_2 during a synthesis process and then modified with polyethyleneimine, which created an electrostatic interaction between the core shell composite (like $\text{Fe}_3\text{O}_4@ \text{MoS}_2$) and laccase and improved the dispersion of the magnetic nanoparticles [90]. In this way the efficiency of the laccase immobilization increased. Moreover, the biocatalytic system constructed was used in the degradation of persistent organic pollutants (e.g., bisphenol) in a water environment. Significant investigations involving the biodegradation of phenol were reported by Zhang et al. [24] and Li et al. [91]. They used Fe_3O_4 -based composites with carbon nanotubes and nanodiamond, respectively, upon which horseradish peroxidase was immobilized. In both cases, the degradation of the phenol was highly efficient.

Polymers and biopolymers represent interesting groups of materials that are utilized as supports for oxidoreductase immobilization. In recent studies, polymers have been functionalized or linked with other compounds to improve their properties before immobilization is performed. In the research by Mohamed et al. [92], an acrylic fiber polymer was employed as a matrix for HRP. Before the immobilization process, the polymer was pretreated with hydroxylamine hydrochloride to obtain an amidoximated polymer. This material was additionally activated with acrylic chloride. Then HRP was immobilized on the cyanuric chloride–amidoxime acrylic polymer and was used as an efficient catalyst in the degradation of aromatic pollutants from wastewater. Similar research was presented by Almulaiky et al. [93]. However, in this case, the polymer was treated with hydrazine to introduce amidrazone groups, which act as nucleophilic sites suitable for cyanuric chloride activation and consequently improve the immobilization of the enzyme. Other investigations concern the enzymatic oxidative polymerization of phenol and bisphenol catalyzed by laccase enzyme immobilized on poly(-acrylonitrile-co-styrene/pyrrole) nanofibers (AN-co-ST/Py) [94]. Interesting studies are reported by Liu et al. [95], who proposed a novel immobilization technology in which laccase was immobilized on a 3D bioprinted matrix hydrogel. The hydrogel contained different monomers: acrylamide (AM), hydroxyapatite (HA), and sodium alginate (SA) in various compositions. The prepared biocatalyst was used in the degradation of phenolic compounds, achieving a high yield.

In addition to synthesized polymers, biopolymers are also useful materials for constructing biocatalytic systems to treat phenolic pollutants. For example, hybrids based on polyamide/chitosan [96] or polyvinyl alcohol/chitosan [97] were used to immobilize laccase and chloroperoxidase, respectively. The proposed matrix was characterized by a high specific surface area, numerous binding sites, and good mechanical properties. This facilitated the immobilization of laccase on its surface, which allowed repeated application in

the degradation of bisphenol and its derivatives. Chitosan chelated with Cu ions and supporting immobilized laccase was successfully used to remove phenol from an aqueous solution [98]. Bisphenol has also been successfully degraded by biocatalysts based on other polysaccharides, such as agarose. Before the immobilization of laccase, the surface of the saccharose was functionalized with monoaminoethyl-*N*-aminoethyl (MANAE) [99]. Similar studies were reported by Brungari et al. [100], who used a different kind of laccase. In both cases, the proposed biocatalytic systems displayed high enzymatic and biodegradation activity.

In addition to studies on traditional materials, recent research by Zu et al. is worth mentioning [101]. A Y-type zeolite was employed as a matrix for the laccase. Zeolites are characterized by wide availability, low price, and high thermal and acidic stability. Furthermore, their surfaces can be modified to improve their mesoporous structure. These properties enable zeolites to be used as supports for biomolecules. Zu et al. prepared sodium zeolite (NaY) and its modified desilicated (DSY) and dealuminated (DAY) forms. The modification led to significant improvement in immobilization performance and enabled the highly efficient biodegradation of bisphenol S and other phenolic compounds.

Polycyclic aromatic hydrocarbons (PAHs) have also been degraded using immobilized enzymes. As with organic dyes, pharmaceuticals and phenol compounds, PAHs are another significant group of environmental pollutants. PAHs have two or more fused benzene rings originating from natural or anthropogenic sources. They have harmful biological, toxic, mutagenic, and carcinogenic effects on the environment [102, 103]. For example, Bautista et al. immobilized laccase on amino-functionalized SBA 15 (a silica material) [104] and used it to remove naphthalene, phenanthrene, and anthracene from wastewater. The designed biocatalyst exhibited high incorporation yields of laccase and excellent biodegradation of various PAHs. Nonionic surfactant-modified clay with immobilized fungal laccase has also been reported as effective in removing PAH from groundwater. Naphthalene and phenanthrene were successfully biodegraded in the presence of this biocatalytic system [105]. The bioconversion of polycyclic aromatic hydrocarbons was also achieved by Apriceno et al. [106], who reported the covalent immobilization of laccase on glutaraldehyde-activated chitosan. Anthracene was efficiently removed by the obtained immobilized laccase with a high catalytic yield. Interesting research was reported by Chen et al. [107], who applied laccase to the surface of calcium-modified chitosan–alginate (CA-Ca-SBE). Their results showed that the immobilized laccase was a promising biocatalyst for application in wastewater treatment due to its high performance in the degradation of phenanthrene in water.

In summary, the high degradation efficiency of phenol compounds and polycyclic aromatic hydrocarbons can be achieved using biocatalysts based on functionalized materials

Table 1 Summary of applications of biocatalytic systems in wastewater treatment

Functionalized materials	Enzyme	Pollutant removed	% degradation or decolorization	Ref.
Organic dyes APTES/silica 3-GPTMS/silica	Laccase	Congo Red	39	[40]
	Laccase	Acid Orange 156 Acid Red 52	43 49	[41]
		Coomassie Brilliant Blue Methyl Violet Malachite Green Reactive Blue 19	34 36 57 90	[46]
PpPD/Fe ₃ O ₄ APTES/Fe ₃ O ₄ TSC-Fe ₃ O ₄	Laccase	Acid Fuchsin	20	[47]
	Laccase	Reactive Blue 171	95	[48]
	Laccase	Acid Blue 74 Rhodamine B	85 95	[51]
Epoxy-functionalized polymers/TiO ₂ Ti-BALDH/TiO ₂ PA/halloysite	Laccase	Malachite Green	90	[52]
	Laccase	Reactive Blue	80	[54]
	Laccase	Methyl Red Remazol Brilliant Blue R Reactive Black 5	73 73 55	[55]
Poly(methyl methacrylate)/polyaniline electrospun fibers PDA/PVDF membrane APTES/RGO-SiO ₂	Laccase	Remazol Brilliant Blue R	60	[56]
	Laccase	Congo Red	90	[57]
	Horse radish peroxidase	Coomassie Brilliant Blue Methyl Orange Aniline Blue	100 100 100	[62]
PDA/TiO ₂ GO-functionalized MnFe ₂ O ₄	Horse radish peroxidase/chloroperoxidase			[65]
	Lignin peroxidase	Nigrosine Bromophenol Blue	57 52	[108]
		Coomassie Brilliant Blue Methylene blue	51 88	
Pharmaceutically active compounds PVA/CS/MWNTs PAN/biochar	Laccase	Diclofenac	100	[73]
	Laccase	Chlortetracycline Carbamazepine	63 49	[74]
		Diclofenac Diclofenac Diclofenac	73 95 68	[75]
Citric acid/biochar APTES/TiO ₂	Laccase	Acetaminophen	90	[76]
	Laccase	Diclofenac	80	[77]
	Laccase	Chlorotetracycline	50	[78]
Polyvinyl-alginate/silicon dioxide PAN/biochar APTES/stevensite	Laccase	Tetracycline	100	[79]
	Laccase	Chlorotetracycline	59	[80]
	Laccase	Carbamazepine	74	[81]
Polyacrylonitrile-biochar	Laccase			
Ethylenediamine/polyimide aerogel	Laccase			

Table 1 (continued)

Functionalized materials	Enzyme	Pollutant removed	% degradation or decolorization	Ref.
Phenolic and polycyclic aromatic hydrocarbons				
MSMS	Laccase/versatile peroxidase	Phenolic contaminants	85	[84]
RGO/SiO ₂	Horseradish peroxidase	Phenol	96	[86]
Amino-functionalized RGO-SiO ₂	Horseradish peroxidase	Phenol	100	[87•]
Amino-functionalized Fe ₃ O ₄ /carbon nanotubes	Horseradish peroxidase	Phenol	80	[24]
Polyglycerol-functionalized magnetic Fe ₃ O ₄ /nanodiamond	Horseradish peroxidase	Phenol	65	[91]
Amidrazone-modified polyacrylonitrile	Horseradish peroxidase	Phenol	70	[93]
Epoxy-functionalized silica	Laccase	Phenol	99	[85]
		<i>p</i> -chlorophenol	85	
		Catechol	40	
Chitosan-coated Fe ₃ O ₄	Laccase	2,4-dichlorophenol	91	[88]
		Chlorophenol	75	
Amino-functionalized Fe ₃ O ₄	Laccase	2,4-dichlorophenol	87	[89]
Polyethyleneimine/Fe ₃ O ₄ @MoS ₂	Laccase	Bisphenol A	88	[90]
Poly(acrylonitrile-co-styrene/pyrrole) nanofibers	Laccase	Bisphenol F	71	[94]
		Phenol	70	
		Bisphenol A	60	
		Bisphenol A	90	[96]
Polyamide 6/chitosan	Laccase	Phenol	96	[98]
Cu(II)-chelated chitosan nanoparticles	Laccase	Bisphenol A	90	[99]
Monoaminoethyl- <i>N</i> -aminoethyl/agarose	Laccase	Bisphenol A	15	[101]
Desilicated/zeolite dealuminated/zeolite	Laccase	Bisphenol A	55	[97]
PVA/CS	Chloroperoxidase	Tetrabromobisphenol	90	[103]
Beads grafted with glycidyl methacrylate and dopamine	Laccase	Acridine	82	[104]
Amino-functionalized SBA-15	Laccase	Naphthalene	73	
		Phenanthrene	55	
		Anthracene	95	[105]
Nonionic surfactant-modified clay	Laccase	Naphthalene	90	
		Phenanthrene	75	[106]
GA/CS	Laccase	Phenanthrene	87	[107]
CA-Ca-SBE	Laccase	Phenanthrene		

and enzymes. The support matrices considered protect the enzyme from the toxic effects of the pollutants and increase the effectiveness of their removal. The utilization of catalytic systems based on enzymes and functionalized materials ensures the rapid, easy, and low-cost degradation of phenolic compounds in wastewater.

The most common functionalized materials employed for enzymatic immobilization and their performance in removing organic dyes, pharmaceuticals, phenolic compounds, and polyacrylic aromatic hydrocarbons from wastewaters are summarized in Table 1.

Conclusions

This review has identified and presented information concerning different biocatalytic systems based on functionalized materials and enzyme immobilization. It aimed to review the applications of such biocatalytic systems for removing chemical pollutants (e.g., organic dyes, pharmaceuticals, phenol compounds, and polycyclic aromatic hydrocarbons) from wastewater. Materials in organic, inorganic, hybrid, and composite forms can be functionalized to increase their affinity to enzymes. In general, using the materials considered here, the immobilized enzymes exhibit high performance in the degradation of pollutants. In most cases, the immobilized enzymes are oxidoreductases, such as laccase and peroxidase. A further search for new enzymes and materials is recommended and is expected to improve the range and practicality of immobilized enzymes for wastewater treatment. This review also demonstrated that immobilized enzymes effectively contribute to the removal of pollutants from aqueous solutions (primarily organics, dyes, pharmaceuticals, and phenolic compounds). This work is expected to be useful for further developing new materials for enzyme immobilization with improved stability and effectiveness in the removal of water pollutants. Additionally, the information from this review may have practical significance in the selection of materials and immobilization methods, and the identification of suitable pollutants for treatment.

Abbreviations 3-GPTMS, 3-glycidylpropyltrimethoxysilane; AN-co-ST/Py, Poly(acrylonitrile-co-styrene/pyrrole) nanofibers; AM, Acrylamide; APTES, Aminopropyltriethoxysilane; CA-Ca-SBE, Calcium-modified chitosan–alginate; CBZ, Carbamazepine; CS, Chitosan; CPO, Chloroperoxidase; DAY, Dealuminated zeolite; DSU, Desilicated zeolite; GA, Glutaraldehyde; GO, Graphene oxide; HA, Hydroxyapatite; HRP, Horseradish peroxidase; Lac, Laccase; LC-MS, Liquid chromatography–mass spectrometry; MANAE, Monoaminoethyl-*N*-aminoethyl; MSMS, Multifunctionalized magnetic silica microspheres; MWNTs, Multi-walled carbon nanotubes; NaY, Sodium zeolite; PAHs, Polycyclic aromatic hydrocarbons; PAN, Polyacrylonitrile; PDA, Polydopamine; PpPD, Poly(*p*-phenylenediamine); PVA, Polyvinyl alcohol; PVDF, Poly(vinylidene fluoride); RGO, Reduced graphene oxide; SA, Sodium alginate; TC, Tetracycline; TCS, Thiolated chitosan; Ti-BALDH, Titanium(IV) bis(ammonium lactato)dihydroxide

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Declarations

Conflict of Interest The authors declare that they have no conflicts of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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