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Developments in support materials for immobilization of oxidoreductases:

A comprehensive review

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Abstract

Bioremediation, a biologically mediated transformation or degradation of persistent chemicals into nonhazardous or less-hazardous substances, has been recognized as a key strategy to control levels of pollutants in water and soils. The use of enzymes, notably oxidoreductases such as laccases, tyrosinase, various oxygenases, aromatic dioxygenases, and different peroxidases (all of EC class 1) is receiving significant research attention in this regard. It should be stated that immobilization is emphasized as a powerful tool for enhancement of enzyme activity and stability as well as for protection of the enzyme proteins against negative effects of harsh reaction conditions. As proper selection of support materials for immobilization and their performance is overlooked when it comes to comparing performance of immobilized enzyme in academic studies, this review summarizes the current state of knowledge regarding the materials used for enzyme immobilization of these oxidoreductase enzymes for environmental applications. In the presented study, thorough physicochemical characteristics of the support materials was presented. Moreover, various types of reactions and notably operational modes of enzymatic processes for biodegradation of harmful pollutants are summarized, and future trends in use of immobilized oxidoreductases for environmental applications are discussed. Our goal is to provide an improved foundation on which new technological advancements can be made to achieve efficient enzyme-assisted bioremediation.

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1. Introduction

Over recent years the production and wide application of synthetic chemical compounds has become essential in many branches of industry. There are, however, some serious drawbacks related to the use of these compounds because they are poorly biodegradable [1]. Interest therefore continues to grow in remediation of endocrine disrupting chemicals (EDCs), hormones, pesticides, synthetic dyes and pharmaceuticals because these substances may alter the functions of hormonal and nervous systems, cause diseases of the male and female reproductive system, disorders and alterations of neurological and metabolism and cause adverse effects in intact organisms [2-4]. These synthetic chemicals exhibit genotoxic activity, can cause diabetes, obesity, cardiovascular diseases, reproductive disorder or even cancer [5,6]. Another cause for serious environmental concern is pollution of communal wastewater and effluents released by the textile or paper industries, agriculture or houses. Nowadays, there are a plenty of both physical and chemical methods for removal of persistent compounds. One of the most effective and eco-friendly of these methods is application of enzymes for biodegradation of hazardous pollutants.

Enzymes have for years been known as extremely efficient and highly effective biocatalysts that additionally are characterized by high chemo-, regio- and stereoselectivity. Furthermore, enzymes through decreasing the amount of toxic solvents and reducing the number of synthesis steps and activation energy, make the catalytic process more cost-effective and environmentally friendly [7]. In the current review, attention is given to laccases, tyrosinases, manganese and horseradish, lignin and phenol peroxidases, however also other oxidoreductases such as monooxygenases, oxygenases and even oxidases might be employed as highly efficient green biocatalysts for remediation of environmental pollutions [8], but literature references about efficient immobilization of monooxygenases and oxygenases for environmental protection are strongly limited. Above-mentioned types of enzymes are able to catalyze redox-transformations and degradation of a large number of organic compounds, in particular phenolic and non-phenolic aromatic compounds such as phenol and its derivatives, EDCs, synthetic and natural dyes, pesticides and pharmaceuticals [9,10]. The catalytic action of these enzymes might range from simple transformation of contaminants to less toxic derivatives to manipulation of contaminated environments or even to bioaccumulation of pollutants [11].

Enzyme immobilization can be accomplished in various ways, but usually involves attachment of the enzyme molecules to a solid carrier, which is usually insoluble in the reaction environment [12]. The result is immobilized biocatalyst where the form of the enzyme has been changed from homogenous (free enzyme) to heterogeneous (immobilized enzyme). Enzyme immobilization allows maximal reuse or recycling of the enzyme in continuous processes where the substrate is fed continuously into the reaction to increase the biocatalytic productivity (amount of substrate molecules converted per amount of enzyme). Immobilized enzymes are usually characterized by enhanced stability against harsh conditions of pH, temperature and pressure [13]. Moreover, storage stability and reusability of the immobilized biocatalyst increases significantly compared with free enzyme [14]. So it could be summarized that immobilization represent simple and effective routes for improvement of enzyme properties as compared to application of the native biocatalysts [15]. The five main immobilization techniques are covalent binding, adsorption, encapsulation, entrapment and cross-linking. However, it should be emphasized that there is no universal method for any particular enzyme [16]. Selection and optimization of the most suitable support material and technique of immobilization is dependent on the type of the enzyme and biocatalytic process. It should also be mentioned that proper selection of support for enzymes such as laccases or tyrosinases for environmental applications not only ensured highly efficient biodegradation of toxic compounds but also protected enzyme molecules from denaturation and allowed their reusability [17]. Many different materials can potentially be used as support materials for enzyme immobilization. Nevertheless, the material has to fulfil some requirements in order to be used as a carrier. First, physicochemical features like the presence of chemical moieties, large surface area or good sorption properties are crucial for establishing of strong and stable interactions between the enzyme and a support material [18]. It is crucial that for environmental use, support materials should also be biocompatible, non-toxic, and environmentally friendly/acceptable and cannot negatively affect solution after biodegradation. Also, hydrophilicity of the carrier, its structure and mechanical or operational stability stronglfy affect the properties of the produced biocatalytic system and ultimately determine successful immobilization [19]. It should be stressed that for attachment of a biocatalyst for environmental applications, materials of organic, inorganic as well as hybrid or composite origin have been frequently studied [20]. Their selection is dictated by type of enzyme, type of biocatalytic conversion, character of the process and required form and shape of biocatalytic beads, all of which influence the efficiency of the whole bioremediation process [21].

The present review briefly describes various immobilization protocols used for binding of oxidoreductases. The main purpose of the literature study was to summarize and compare the current state of knowledge about support materials for enzyme immobilization with particular reference to immobilization of laccases, tyrosinases and phenyloxidases and use of the resulting biocatalytic systems in the removal of hazardous pollutants. The properties of inorganic, organic and hybrid support materials required for effective enzyme binding are specified and presented. Various types and configurations of degradation processes, and types of reactors are highlighted. Furthermore, the effect of operational parameters such as time, pH and temperature on the removal efficiencies of undesirable compounds are compared, as well as storage stability and reusability of the immobilized biocatalysts. We also discuss present and future perspectives for application of immobilized oxidoreductases for environmental protection with emphasis on pollutants removal.

2. Enzymes for environmental applications

As previously mentioned, enzymes belonging to the oxidoreductases (EC 1), such as laccases, tyrosinases, manganese, lignin and horseradish peroxidases, and phenoloxidases, are the most frequently employed for environmental applications [22]. These enzymes are being investigated due to their applicability as "green catalysts" in the bioremediation of various dangerous chemicals such as phenols and derivatives, bisphenols, organic dyes or pharmaceuticals [23]. They have been found to be efficient biocatalysts for remediation of toxic compounds, but the structures, cofactors and mechanisms of action differ for each type of catalyst. Selected examples of the enzymes most frequently used for environmental protection are discussed in detail below.

2.1. Laccases

Laccases of various origin are the most commonly used environmental protection. Laccases, which are extracellular enzymes also known as *p*-diphenol: dioxygen oxidoreductases(EC 1.10.3.2),are oxidoreductases omnipresent in plants and bacteria, however, laccases from fungi like *Trametes versicolor*, *Trametes vilosa* or *Cerrena unicolor* are of the greatest interest due to their high catalytic activity, availability and low price [24]. Laccases, due to their low substrate specificity, are enzymes that can catalyze wide range of reactions, mainly one-electron oxidation of monophenols, diphenols and polyphenols as well as diamines, aromatic amines and related substances such as N-heterocycles, phenothiazines and many others. These oxidation processes generate reactive phenoxy radicals with a simultaneous

reduction of oxygen to water, without the need for hydrogen peroxide [25]. These laccases constitute a wide group of multi-copper oxidase enzymes that contain four copper ions in their structure that exhibit different properties. At a type-1 copper atom (T1) is responsible for the blue color of the laccase and together with the type-2 atom (T2) and two atoms of type 3 (T3) take part in oxidation-reductions reactions catalyzed by laccases [26]. In detailed, the bioconversion acts are initiated at the type-1 copper atom where electrons from substrate molecules are extracted and transferred via His-Cys-His triade to the T2/T3 group. Further, accumulated electrons enable the oxidation reaction and simultaneously allow the reduction of molecular oxygen to water [27]. It also should be added that laccases are employed in various applications such as in the food, textile, fuel and medical industries [28]. Due to their wide substrate specificity and ubiquitous properties, they are able to act on a wide range of phenolic compounds and are increasingly used for bioremediation of environmental pollutants from soils and water [29,30].The example of the catalytic pathways of conversion of bisphenol A and tetracycline by laccase are presented in Fig. 1. Most laccases are extracellular proteins with isoelectric points ranging from 3 to 7 for fungal laccases and around 9 for plant laccases. Moreover, differences in optimal pH for both sources of laccases have been found: fungal laccases exhibit maximal catalytic properties at pH between 3.5 and 5 as laccases from plants have pH optima around pH 7. Differences in the temperature optima between various laccases have also been found [31].

Figure 1

2.2. Tyrosinases

Tyrosinases, *o*-diphenol: oxygen oxidoreductases (EC 1.14.18.1), also contain copper atoms in their active site. These copper atoms are known as CuA and CuB and are coordinated by histidine residues [34].Tyrosinases are frequently abundant in nature and are found in plants, fungi, bacteria, insects and in mammalian issues [35]. They play a key role in the synthesis of melanin – the dye that is responsible for the color of human skin. Tyrosinases differ essentially from laccases in the mechanism of oxidation of phenol and its derivatives. Although tyrosinases also use oxygen as a cofactor, they generate quinones instead of free radicals and water molecules as a by-product [36]. The mechanism of catalytic action of tyrosinases has been defined as a two-step consecutive reaction of monophenol hydroxylation to corresponding ortho-diphenols and their further oxidation to ortho-quinones using O_2 molecules in both steps. Later release of water molecules terminates catalytic oxidation and

leads to a polymerization reaction of *o*-quinones to macromolecular compounds [37].Due to the fact that tyrosinases can occur in various molecular forms, different intermediates may be formed during catalysis of phenolic compounds [9].It should be clearly stated that due to their wide specificity, tyrosinases similarly to laccases are able to catalyze transformation of various compounds such as phenol, monophenols and its multisubstituted derivatives, including chloro- and nitrophenols and bisphenols [38].The effect of temperature and pH on the stability and activity of various tyrosinases has been thoroughly analyzed. A previous study reported pH ranging from 5.5 to 8 as the most suitable for the highest activity of free tyrosinases [39].The effect of temperature on the activity of tyrosinases has also been studied. Published data showed that free tyrosinases exhibit the highest activity at temperatures ranging from 30 to 40°C and that activity significantly decreases above 60°C.It should be added that most of tyrosinases under optimal conditions exhibit relatively good stability and retain high activity (80–100%) even for 8 h [40].

Figure 2

2.3. Lignin peroxidases

Besides laccases and tyrosinases, lignin peroxidases are also used for remediation of hazardous phenolic compounds from water and soil. Lignin peroxidase, 1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol:hydrogen peroxide oxidoreductase (EC 1.11.1.14), also known as LiP, contains 1 mole of iron protoporphyrin (heme) as its cofactor for 1 mole of protein [41]. Lignin peroxidases catalyze the oxidative depolymerization of lignin in the presence of H_2O_2. Exemplary reaction, which characterizes catalytic action of LiP (oxidation of 1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol) could be presented as [42]:

$$1,2-bis(3,4-dimethoxyphenyl)propane-1,3-diol + H_2O_2 \leftrightarrow$$
$$3,4-dimethoxybenzaldehyde + 1-(3,4-dimethoxyphenyl)ethane-1,2-diol + H_2O.$$
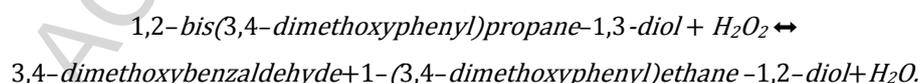
It should be added that LiP is characterized by low substrate specificity with high, non-specific oxidation-reduction potential and is known for its ability to oxidize aromatic phenolic and non-phenolic compounds as well as a wide range of organic chemical compounds, such as xenobiotics with a redox potential of up to 1.4 V [43]. LiP was extracted for the first time from *Phanerochaete chrysosporium* but these enzymes are also known to be found in many microorganisms and white-rot fungi [44]. It should be mentioned further that lignin

peroxidase is known from its low optimum pH value, as these enzymes reach their maximum catalytic activity at a pH near 3. The temperature optimum for LiP is similar to other peroxidases and is within a range of 40 to 50°C [45].

2.4. Manganese peroxidases

Another enzyme with high biotechnology potential that belongs to the oxidoreductase group is manganese peroxidase (EC 1.11.1.13), also known as Mn(II):hydrogen-peroxide oxidoreductase orMnP. These glycosylated heme-containing enzymes can oxidize a wide variety of phenolic compounds, such as dyes and various monomeric and dimeric phenols as well as oxidize Mn(II) ions to Mn(III) using hydrogen peroxide [46]. The catalytic cycle of MnP is initiated by binding of H_2O_2 and formation of an iron-peroxide complex and subsequent transfer of 2 electrons from the heme resulting in formation of MnP Compound I. Later, one water molecule is formed by subsequent cleaving of the dioxygen bond and reduction proceeds through MnP Compound II. Afterwards, monochelated Mn^{2+} ion acts as electron donor for this porphyrin intermediate and is oxidized to Mn^{3+}. Another Mn^{3+} is formed by reduction of Compounds II leading to regeneration of native enzyme and releasing of the second water molecule [47].Manganese peroxidase is a protein found in multiple forms with a typical molecular weight ranging from 40 to 50 kDa [47]. MnPs exhibit their maximal activity at Mn(II) concentrations above 100μM and calcium cations enhance their catalytic properties [48]. Manganese peroxidase was discovered for the first time in *P. chrysosporium* but in later years the enzyme has also been found in bacteria and other white-rot fungi [49]. The optimum temperature for the highest catalytic activity of manganese peroxidases depends on its source, but in most cases lies the range of 30 to 40°C. These enzymes exhibit their highest catalytic properties at slightly acidic pH levels of around 4 [50].

2.5. Horseradish peroxidases

Enzymes extracted from the roots of horseradish have also been assessed in environmental applications. A number of distinctive peroxidase isoenzymes have been found in nature, however, the most abundant is horseradish peroxidase C [51]. Horseradish peroxidase (EC 1.11.1.7) or HRP is also a heme-containing enzyme that includes in its structure iron(III) protoporphyrin and two atoms of calcium. The presence of calcium atoms is important, because loss of calcium results in significant decrease of enzyme activity and thermal stability [52]. Horseradish peroxidase catalyzes an oxidation reaction of phenolic acids, aromatic phenols such as pyrogallol and their derivatives (i.e. various bisphenols), non-aromatic

amines such as 4-aminoantipyrine, indoles, etc. in the presence of hydrogen peroxide, producing two molecules of water. The second products of the catalytic reaction are radicals that can result in formation of polymeric compounds as the final products of oxidation [53,54]. The main practical applications of HRPs includes treatment of the wastewaters contain phenolic compounds, environmental remediation, elimination of toxic compounds such as dyes from drinking water and industrial [55-57]. HRP, particularly its isoenzyme C, exhibits its greatest catalytic activity at temperatures of 25–40°C and at neutral pH, close to 7 [58].

In recent years, lignolytic enzymes, such as laccase, tyrosinase, manganese, lignin and horseradish peroxidases have been used in numerous industrial processes, such biomass conversion and chemical synthesis. However their great oxidizing potential caused that they have found an application in environmental protection to degrade xenobiotics, as dyes, pharmaceuticals and hazardous pollutants that are usually resistant to microbial biodegradation [59]. In our opinion, further study leading to the development of the techniques that improve practical features of the lignolytic enzymes is highly required as might facilitate use of these important biomolecules for various biotechnology applications and environmental protection.

3. Immobilization of enzymes

Many advanced research studies have been carried out in connection with the low stability and reusability of enzymes. Immobilization, a process through which the enzyme is bound to a solid support, changes the form of the catalyst from homogenous (free enzyme) to heterogenous (immobilized enzyme) [60]. Creation of interactions between the enzyme and a matrix (immobilization) stabilizes the peptide structure of the biocatalyst and results in improvement of enzyme stability towards strong pH, high temperature or the presence of organic solvents [61].The possibility of separation of the biocatalytic system from the reaction mixture is strongly enhanced and thus contamination of products by the enzyme particles is minimalized. Additionally, after the immobilization, the biocatalysts can easily be removed by simple mechanical separation or centrifugation without using sophisticated analytical techniques. However, the greatest advantage of immobilization is the production of an enzymatic system that could be reused in many catalytic cycles without significant loss of its unique properties [62,63].

According to the previously published reviews, methods of immobilization have been classified in many different ways [64-66]. The techniques differ between each other by the

types of the created interactions and by type and form of the solid support. In each case, for selection of the immobilization technique, a compromise has been made between retention of high catalytic activity and operational benefits. For the purpose of the current discussion, five different immobilization techniques are distinguished, namely: (i) non-covalent (adsorption) immobilization; (ii) covalent immobilization on a carrier; (iii) encapsulation; (iv) entrapment and (v) cross-linking of the enzyme particles by creation of the cross-linked enzyme aggregates (CLEA) and cross-linked enzyme crystals (CLEC), as summarized in Table 1.

Table 1

Non-covalent immobilization, also known as adsorption immobilization, is based on the creation of non-specific interactions mainly via hydrogen bonds and ionic and hydrophobic interactions. In effect, conformational changes in the enzyme particle are limited and retention of high catalytic activities by the immobilized enzymes is usually observed [67,68]. Immobilization by covalent binding is based on the reaction of functional groups of the support material with functional groups of the enzyme (mainly $-NH_2$, $-SH$ and $-OH$). Creation of strong chemical bonds between the biocatalyst and matrix significantly reduces leakage of the enzyme and enhances the reusability of the enzyme [69,70]. When the biomolecule is immobilized by encapsulation or entrapment it is physically placed in the pores of the support material but its structure remains unaltered. Single enzymes as well as complex biocatalytic systems built from different types of enzymes may by immobilized by the use of these methods. The disadvantage of these techniques is that due to the location of the enzyme within the porous system of the matrix, transfer of the reaction mixture ingredients is more difficult [71]. Enzymes in the form of single crystals (CLEC) or as aggregates (CLEA) are cross-linked within bifunctional compounds such as glutaraldehyde (GA) or carbodiimides. Establishing covalent bonds through the use of cross-linking agents allows formation of a stable structure without the use of a solid support [72,73].

Different techniques could be applied for immobilization of the same enzyme, however, changes in the enzyme structure and its properties are usually not equal with improvements of the biocatalysts properties. Thus, mild process conditions are highly required to retain good catalytic properties of the immobilized enzymes. Moreover, the more simple is the immobilization method, such as adsorption immobilization, the more cost effective is the process. As every enzyme differ from each other, it could be concluded that

proper selection of the immobilization protocol is a key step to obtain biocatalytic systems which might be applied for practical applications.

4. Materials for immobilization of enzymes used for environmental protection

As was mentioned earlier, the main purpose of the current review is to present and characterize various enzyme support materials that can be applied in processes related to environmental protection. Free oxidoreductases have been applied for removal of micropollutants from environments, however, large-scale use of these enzymes in biodegradation requires their immobilization to increase stability and prolong activity [74]. These biocatalysts can be immobilized through the use of various materials of different origin, from inorganic through organic to hybrids and composite supports. The most important properties of selected examples of these support materials are presented in Fig. 3.The great variety of the possible carriers with regard to shapes, sizes and forms means that enzymes can be immobilized via various methodologies. In this section the effect of different features of support material on enzyme activity and biodegradation efficiency is presented and discussed.

Figure 3

4.1. Materials of organic origin

Many different materials of organic origin, both natural and synthetic, have been employed for immobilization of enzymes for environmental protection using different immobilization techniques (Table 2).

Table 2

4.1.1. Biopolymers
4.1.1.1. Chitosan

As was previously stated, chitosan is one of the most frequently used support materials for immobilization due to its easy availability, low cost, biocompatibility and hydrophilicity. The presence of many hydroxyl and amine groups in the structure of this biopolymer facilitates effective binding of laccases and other peroxidases without involving any modifying or cross-linking agents [111]. The ability of chitosan to create various forms, such as fibers, beads, microspheres or membranes, enhances its application as a support material for enzymes like laccase or lignin peroxidase and makes immobilized biomolecules more stable. For instance,

Zhang et al. have covalently attached laccase from *Coriolus versicolor* via glutaraldehyde to the surface of chitosan. The obtained biocatalytic system was used for biodegradation of mono- and dichlorophenols and brought about the degradation of over 90% of 2,4-dichlorophenol in water solution. Moreover, immobilized enzyme was characterized by higher stability in comparison to free enzyme and exhibited good reusability [75]. In another study, chitosan, chitosan microspheres with high specific surface area and chitosan beads were used for immobilization of laccase, manganese peroxidase and lignin peroxidase by entrapment, covalent binding and adsorption, respectively. The produced biocatalysts, due to their wide specificity, were used for degradation of synthetic and natural azo-dyes in model and real water solutions. Enzymatic systems are characterized by various thermal and chemical stabilities, but under optimal conditions (temperature range 30–50 °C, pH range 5–8) they usually enable degradation of over 70% of the pollutants. Lack of electron mediator and low transfer of the electrons as well as complicated structure of the dyes are the main factors, which limit biodegradation efficiency [78]. However, the presence of the mediator agent is not necessary for total degradation of the contaminating dye. As Jaiswal et al. have shown, after 8 h at 37 °C laccase from papaya entrapped in chitosan beads was able to remove Indigo carmine completely from water solution due among other factors to low diffusional limitation [79].

4.1.1.2. Alginates

Other extensively used support materials for environmental applications of enzymes are various alginate salts derived from brown algae, *Phaeophyceae,* under alkaline conditions. After gelation under mild conditions after addition of mono- or divalent cations like Na^+, Ca^{2+}, Cu^{2+}, Zn^{2+} and Mn^{2+} [112], alginates are characterized by viscosity and stiffness, which can easily be controlled by manipulation of the pH and molecular weight of the alginate solution. For enzyme immobilization, alginates are used in the form of beads, hydrogels and capsules. And as a consequence of the features and diversity of form of alginates, immobilization of laccases, tyrosinases and other peroxidases by alginates mainly occurs through entrapment and encapsulation. Usually, after biomolecules have been immobilized in alginates, there is a lack of covalent bonds between the enzyme and a support, and thus functional groups of the matrix and the enzyme interact based on relatively weak, ionic or adsorption forces [113]. Because interference in the structure of the enzyme is strongly limited, immobilized enzymes usually retain most of their catalytic properties. On the other hand, creation of relatively weak interactions might lead to leakage of the enzyme from the

matrix and result in a decrease in biocatalytic activity of the produced system. Additionally, lower catalytic properties may be related to diffusional limitations in transport of the substrates and the products through the alginates layer. Sodium and calcium ions are the most commonly used ions for gelation of alginates. For instance, calcium alginate was used for immobilization of manganese peroxidase from *G. lucidum* and tyrosinase from *Agaricus bisporus* [80,81], and tyrosinase from *Streptomyces espinosus* and laccase from *Bacillus subtilis* were entrapped in sodium alginate [82,83]. Irrespective of metal cation used for gelation, immobilized tyrosinases were used for removal of phenol from water solutions. Immobilized enzyme exhibited high catalytic activity and under optimal conditions was able to totally degrade phenol from solution after 1 h of the process [81]. Silica ions have also been used to increase the mechanical stability of the alginate. Silica alginates were further used for encapsulation and entrapment of tyrosinase and the obtained biocatalytic system then applied for biodegradation of bisphenol A (BPA). This biocatalytic system not only allowed highly efficient removal of BPA in a relatively short time, but most of all was characterized by much higher pH and temperature resistance in comparison to free enzyme. However, fast drop of the catalytic properties over consecutive catalytic cycles related to the enzyme leakage should be presented as the weak point of this study [85]. Special attention should also be paid to copper alginates as supports for environmentally enzymes. Due to the fact that laccase contains three different copper ions in its structure, utilization of supports containing copper ions increases the bioactivity of the immobilized biomolecules. Laccases or polyphenol oxidases were entrapped and encapsulated in materials prepared in this way and used for degradation of natural and synthetic dyes and phenol derivatives with removal efficiencies that reached over 80%. However, the significant effect of copper ions on the immobilized enzyme should be emphasized. Phetsom et al. encapsulated laccase from *Lentinus polychrous* in Cu-alginate beads. The immobilized biomolecule exhibited more than two times the catalytic activity of free enzyme after 7 days of storage at pH 5.5 due to the presence of Cu^{2+} ions [91]. Alginates are also used in the form of beads for immobilization mainly by entrapment of laccases, tyrosinases and various peroxidases. Due to the mechanical stability of the capsules with biomolecules that form beads, these products are commonly employed in bioreactors of different configurations used for degradation of synthetic textile dyes as well as phenol and its derivatives in water solutions. As reported by Daassi et al., laccase from *Coriolopsis gallica* immobilized in alginate beads exhibited over 80% of its catalytic activity over a wide pH range from 5 to 9, but the free enzyme was most active only at pH 7. Furthermore, immobilized biocatalyst was characterized by high thermal stability. After

incubation for 3 h at 55 °C, immobilized laccase still exhibited over 80% of initial activity, meanwhile free laccase under the same conditions completely lost its properties after 2 h of heating [88].

4.1.1.3. Cellulose

Another organic material used as support for environmental enzymes is cellulose and its modified forms. Cellulose is known from its great sorption capacity and the presence of many hydroxyl groups in its structure. This facts allows effective attachment of enzyme molecules and enhances opportunities for modification of cellulose surfaces by glutaraldehyde, diethylaminoethanol or introduction of epoxy groups [114]. Cellulose is also relatively easy to obtain, is nontoxic and biocompatible. The abovementioned features taken together mean that cellulose and its derivatives are suitable support materials for adsorption or covalent immobilization of oxidoreductase enzymes. Application of cellulose-based materials is therefore growing quickly as a response to the importance of sustainable and cost-effective immobilization. As a result, many new cellulosic materials with desired properties and stability are under development for attachment of biomolecules. Sathishkumar et al. used cellulose nanofibers modified by glutaraldehyde for covalent immobilization of laccase. Thermal and pH stability as well as storage stability of the immobilized enzyme was considerably improved compared to the free enzyme. Laccase-cellulose nanofibers also exhibited great reusability and retained over 80% of the initial properties after five catalytic cycles. The biocatalyst thus obtained was applied for decolorization of simulated dye effluent (SDE) consisting of Remazol Black 5, Remazol Brilliant Blue R, Remazol Brilliant Violet 5R, Reactive Orange 16 and Reactive Red 120. The authors demonstrated that in the presence of the mediator, 1-hydroxybenzotriazole, decolorization of SDE reached around 40%. Although the stability of the immobilized enzyme was improved, changes in the structure of biomolecules due to covalent binding, caused that relatively low biodegradation efficiency was achieved. Also multiply of the dyes in the solution decreases process effectivity [94]. In another study, cellulose modified by diethylaminoethanol formed diethylaminoethyl-cellulose (DEAE-cellulose)was used by Cienska et al. for immobilization of tyrosinase. The authors report that tyrosinase immobilized on modified cellulose exhibited good storage stability and great reusability, and could be used in five subsequent catalytic cycles without loss of the initial activity. Moreover, after binding to the polysaccharide support, the negative effect of the toxic mixture component was strongly limited and the catalytic properties of the enzyme were retained at a high level [96]. Firooz and his team have also modified cellulose surfaces

for covalent immobilization of tyrosinase. They used commercially available sheets of Whatman paper and immersed the sheets in a solution of ethylenediamine or propylenediamine to introduce amine groups. Laccase immobilized on amine-functionalized cellulose paper exhibited even higher catalytic activity – over 150% of that of free enzyme – under optimal pH (7.0) and temperature (35 °C) conditions. Although immobilized laccase is characterized by lower substrate affinity (higher value of Michaelis-Menten constant) than free enzyme, it still seems to be a very promising biocatalyst for degradation of dihydroxyphenylalanine (L-DOPA) [93]. It should be stated that other forms of cellulose such as cellulose beads or cellulose beads modified by carboxyl groups (carboxymethyl cellulose) may also be used for immobilization of polyphenol oxidases. The resulting systems could be further used for degradation of phenolic compounds and synthetic dyes, with efficiencies of over 90% [95].

4.1.1.4. Other biopolymers

Other biopolymers, mainly polysaccharides, may also be used for immobilization of laccases. A broad range of materials, including agarose, κ-carrageenan, gum Arabic and other natural products such as coconut fibers or wood sawdust, can be used as support for oxidoreductase immobilization [97,98,115]. These materials are known for their biocompatibility and high affinity for peptides, which makes them useful for many practical applications, including industrial processes with immobilized enzymes. Gioia and co-workers applied thiolsulfinate-agarose for reversible covalent immobilization of laccase from *Trametes villosa*. The great advantage of this support material is that after enzyme inactivation using a reducing agent like dithiothreitol (DTT), the biomolecules can be easily removed from the support surface and the matrix can be used for the next cycle of immobilization. Presented results indicated that after 24 h of the process at 22 °C and pH 4.8, it would likely be possible to remove over 80% of Remazol Brilliant Blue R from textile industry effluents [97]. In another study, Cristovao et al. used green coconut fiber modified by 3-glycidoxypropyl-trimethoxysilane for immobilization of laccase at pH 7 for one-point and at pH 10 for multipoint covalent immobilization. Comparison of the systems so obtained showed that conducting the process under neutral pH resulted in production of biocatalyst with higher activity and affinity to the substrates. Both biocatalytic products were used for decolorization of reactive textile dyes such as Reactive Black 5 and Reactive Blue 114. Over 90% of the dyes could be degraded in the solution through use of the laccase-coconut fiber system [99].

4.1.2. Synthetic polymers

Besides many different organic materials of natural origin, synthetic polymers of different sizes and shapes are also used for immobilization of laccases, tyrosinases and peroxidases. Due to the presence of many functional moieties in the structure of synthetic polymers, the creation of relatively strong interactions has been observed between the enzymes and the support. Polymers can be formed into various shapes that are tailored to enzymes and products according to the particular immobilization application. Additionally, due to their structure and high stability, synthetic polymers usually protect biomolecules against degradation and enhance their stability. One of the most frequently used polymeric supports for environmental enzymes is polyvinyl alcohol (PVA). PVA is rich in hydroxyl groups, and is a strongly hydrophilic material which may be easily modified to achieve a suitable enzymatic matrix. For instance, PVA was used as fibers, beads or microspheres for covalent immobilization of horseradish peroxidase and for entrapment and encapsulation of laccase [100-102]. Chhabra and his team entrapped laccase from *Cyathus bulleri* in polyvinyl alcohol beads for degradation of azo-dyes in a continuous packed column. The biocatalytic system they thus obtained, in addition to retaining high activity (over 90%), was characterized by good storage stability (70% after 5 months of storage at 4 °C) and reusability (over 90% of initial activity after 5 days of the process). Continuous batch decolorization in a packed bed bioreactor under optimal conditions led to nearly 90% decolorization of Acid Violet 17, Basic Green 4 and Acid Red 27 [102]. Other common synthetic polymers might also be useful for enzyme immobilization. Laccases and tyrosinases of different origin as well as lignin or manganese peroxidases could be immobilized mainly by encapsulation or covalent immobilization through use of polyacrylamide hydrogel, polyacrylonitrile beads, poly-N-vinylpyrrolidone or epoxy activated polyethersulfone beads. The produced biocatalytic systems have been utilized for various environmental applications, but these systems are most commonly applied for degradation of phenol and its derivatives and to allow selected removal of environmental contaminants at relatively high efficiencies. For example, Nicolucci et al. applied tyrosinase immobilized on polyacrylonitrile beads to eliminate bisphenol A, B, F and tetracholorobisphenol A. In a degradation process carried out in bed reactor, a degradation of over 90% was obtained for each of the tested endocrine disruptor [104]. In study by Shesterenko and co-workers, tyrosinase from *Agaricus bisporus* was immobilized on poly-N-vinylpyrrolidone and used for removal of phenol. After 1 h of the process at 25 °C, total degradation of phenol was observed [105]. Polyamide in the form of a nylon membrane has also been applied for immobilization of polyphenol oxidases. This solution not only increases

stability of the immobilized enzyme and permits continuous use of the immobilized oxidase, but also limits inhibition of the biocatalyst due to easy separation of the product from the reaction mixture. Nylon membrane with loaded enzyme was used for continuous biodegradation of phenol and its derivatives carried out in a bioreactor; the system has potential as a biocatalyst for biotransformation of phenols or bioremediation of phenol-polluted water [108]. Supports based on polymeric resins have been used to achieve multisubunit or multipoint immobilization of enzymes. Application of multipoint immobilization is required as its usually prevents biomolecule structure from denaturation, inactivation and structural damage and therefore helps to maintain catalytic activity at a high level. However, on the other hand might also leads to creation of diffusional limitations due to the blocking of enzymes active sites. Thus, Satoh et al. used a commercially available anion-exchange resin for covalent immobilization of mushroom tyrosinase. The immobilized enzyme exhibited high catalytic properties and stability under reaction conditions as well as reusability, as indicated by almost unaltered enzyme activity after five repeated catalytic cycles. Furthermore, a column-packed reactor with immobilized tyrosinase was used for effective degradation of *p*-cresol and allowed its removal at an efficiency of around 80%. To achieve higher effectivity of reactor, additional hydrogen peroxide supply must be ensured, that is the main limitation of the presented concept [110].

According to the references presented above it can be concluded that application of organic support materials for enzymes used for environmental decontamination is quite popular. The great advantage of these materials is that they can be used in different shapes (particles fibers), forms (membranes, beads and sizes). For instance, nylon membrane, fibers obtained from green coconut and chitosan microspheres was applied for covalent immobilization of polyphenol oxidase, laccase from *Aspergillus* and lignin peroxidase from *Coriolus versicolor*, respectively and used for removal of various pollutants, like *p*-cresol, Reactive Black 5 or even waste waters after molasses conversion [77,99,108]. Biocatalytic systems obtained, irrespectively of the applied form of support material, allowed degradation of over 80% of hazardous compounds. As it was also presented above, alginate-based materials were employed for entrapment of i.e. tyrosinase from *Streptomyces espinosus*, manganese peroxidase from *G. lucidum* BL05 or horseradish peroxidase and used for degradation of natural reactive dyes, phenol or pyrogallol [80,82,87]. Obtained removal efficiencies that exceed 75% proved versatility of these materials as the support could be used for immobilization of different enzymes and applied for removal of various contaminants. On the one hand the great variety of the enzymes could be immobilized, but on the other hand high

diffusional limitations and leakage of the biomolecules from the support limit higher biodegradation efficiencies.

It could be concluded that using organic support for immobilization of oxidoreductases, there is some freedom in selection of attachment technique, because all above-mentioned immobilization protocols, that could be applied results in production of immobilized enzymes characterized by improved thermal, chemical and operational stability. Biocatalytic systems based on the organic matrices are employed for degradation of a very large number of environmental pollutants, ranging from synthetic and natural dyes, through phenol and its derivatives, to more complex compounds like pharmaceuticals. However, compared to the synthetic polymers, application of biopolymers have received wider consideration over the recent years. Several factors can justify this fact. Among others, the presence of many functional groups, ability to form various geometrical structures that increase protection of enzymes against the reaction conditions, biocompatibility, abundance in nature and fact that they are renewable should be enumerated. We believe that even though biopolymers, such as chitosan, alginates or cellulose are commonly used as a carriers for enzyme immobilization, these materials still have a great potential which can be used to develop a multidisciplinary approach covering the areas of biocatalysis, environmental protection and bioprocess engineering.

4.2. Inorganic materials

Besides the many organic materials presented above, inorganic supports are also widely used for immobilization of enzymes for environmental applications. Inorganic materials are suitable for enzyme immobilization due to their exceptional mechanical and chemical stability and electrical properties and are known for their large specific surface area, which may even be up to 1000 m^2/g for silica SBA 15 [116], porous structure, and controllable nanometer sizes similar to that of enzyme molecules [117,118]. The great advantage of inorganic supports in comparison to other materials is that they can be obtained relatively cheaply and simply due to their abundant presence and usually non-complicated synthesis procedure.Moreover, the presence of many hydroxyl, carbonyl and carboxyl groups on the surface of the inorganic carriers causes easier enzyme attachment and facilitates support functionalization through the use of surface modifying agents like glutaraldehyde or 3-aminpropyltriethoxysilane (APTES) [119]. Thus, inorganic materials are commonly used for immobilization of laccases, tyrosinase and phenoloxidases. Selected examples of the various inorganic materials of different origins and their application for immobilization of laccases,

tyrosinases and lignin, manganese and phenol oxidases to biodegrade environmental pollutants are presented in Table 3, followed by discussion.

Table 3

4.2.1. Silicas

Silica in various forms and sizes is the most commonly used inorganic support material for immobilization of oxidoreductases for environmental protection. It should be stressed that the hydrophilic character of the silica surface as well as presence of many hydroxyl groups on its surface leads to immobilization of biomolecules not only via adsorption [125] but also by creation of covalent bonds [123,127] and even by encapsulation [131]. Special attention should be paid to multiplicity of forms of silica which could be exploited for immobilization. For example, mesoporous, ordered silica at pore sizes of 14.1 nm was used for immobilization of lignin peroxidase from *Phanerochaete chrysosporium*. The resulting product was used for degradation of Acid Orange II and removed over 75% of the dye from water solution [122]. In another study, Nair and co-workers used mesoporous silica spheres with a high surface area for covalent attachment of laccase from *Coriolopsis gallica* using glutaraldehyde as cross-linking agent. The biocatalytic system thus produced was characterized by high catalytic activity (over 380 U/g) and allowed for effective biodegradation of bisphenol A, diclofenac and 17-α-ethinylestradiol at efficiencies of over 70% for each compound [124]. Laccase from *Cerrena unicolor* has been covalently immobilized via glutaraldehyde on the surface of silica beads at control porosity with a pore size at around 375 Å. Immobilized enzyme was more active than its free form over a wide pH range from 4 to 9 and at temperatures of up to 70 °C. The resulting systems were used for elimination of 80% of bisphenol A, 40% of nonylphenol, and 60% of triclosan from water solution [129]. An interesting example of use of a silica matrix for immobilization of enzymes was presented by Sani et al. In their study, they used semi-transparent and fluffy powder of silica aerogel with a surface area of around 600 m^2/g for encapsulation of tyrosinase. The structure of the matrix strongly reduced diffusional limitation in transport of the substrates and as a result helped to retain high catalytic activity by the immobilized biomolecules. Furthermore, leakage of tyrosinase from the carrier was reduced by its encapsulation in the silica matrix which also protected tyrosinase from harsh reaction conditions. This effects resulted in reusability of the produced system, which after 10 repeated degradation cycles still retained over 70% of the initial catalytic properties. The biocatalytic system was used for degradation of phenol in water solution. After 3 h of

biodegradation under optimal conditions (pH 7, 30 °C), over 90% of the pollutant was removed [131]. In summary, oxidoreductases have thus been immobilized on various types of silica supports and applied for biodegradation of a several organic compounds ranging from phenol and its derivatives, and endocrine disrupting chemicals, to synthetic and natural dyes, with high efficiencies, usually over 80%. However, it also should be noticed that the limitation of the silica-based materials is fact that whit out surface modification, usually adsorption immobilization occurred. This results in enzyme leakage and decrease in catalytic properties.

4.2.2. Inorganic oxides

Besides silica, other inorganic oxides such as titania, zinc oxide, alumina and magnetic iron (II,III) oxide are also used for immobilization of enzymes for environmental applications due to the presence of many functional groups, mainly hydroxyl and carbonyl groups. These materials are known from their high surface area and defined porosity that enhance attachment of a large amount of enzyme and increase catalytic activity of the produced systems. The abovementioned materials are characterized by thermal, chemical and mechanical resistance, which in comparison with free enzyme significantly extends the tolerated pH and temperature ranges over which immobilized enzyme exhibits high catalytic activity. For instance, laccase covalently immobilized on alumina pellets exhibited over 80% of its catalytic activity at pH 4 to 6 and from at temperature from 35 to 60 °C. By contrast, free laccase exhibited its maximum activity only at pH 4 and temperatures of 35–45 °C [126]. Another advantage of the inorganic oxides is that they can be used in various morphological forms. Hou and co-workers used titania nanoparticles for adsorption immobilization of laccase from *Trametes versicolor* and degradation of 2,4-dichlorophenol and 2,6-dimethoxyphenol. The produced biocatalytic systems, besides increases in thermal and chemical stability, seemed to be effective tools for biodegradation of disubstituted phenol derivatives. At pH 3.5 and 25 °C, over 85% of 2,4-dichlorophenol and over 60% of 2,6-dimethoxyphenol were removed from water solution [133]. In this study higher degradation rates were achievable due to the sorption of the phenols on the nanoparticles. In consequence, actives sites are overloaded by the substrate molecules and catalytic efficiency decreases. In recent years magnetite has become a very promising support material for enzymes due to its magnetic properties and easy separation of the produced biocatalytic systems from the reaction mixture using external magnetic fields. For instance, Balaji et al. used this material for covalent binding of laccase using glutaraldehyde and further for decolourization of water

solution by removal of Reactive Green 19A dye. According to the presented results, over 75% of the dye was removed by using the described biocatalytic system. Moreover, use of magnetic particles for laccase immobilization significantly increased reusability of the biocatalytic system and facilitated control of the technological process [138].

4.2.3. Minerals

Inorganic materials such as minerals are also used for immobilization of oxidoreductases. The biggest advantage of these materials is that they are abundant in nature and inexpensive. They offer high surface areas, the presence of various functional moieties, high sorption capacity and stiffness as well as high thermal stability and resistance against mechanical destruction [151]. Various minerals, for example halloysite, kaolin, clays or bentonite could might be used for immobilization of laccases and peroxidases. Due to good resistance of these supports, irrespectively of the material used, the produced biocatalytic systems are characterized by increase in their stability in comparison to free enzyme and are known for their great reusability. For instance, Chao and co-workers used halloysite nanotubes modified by dopamine for effective covalent immobilization of laccase from *Trametes versicolor*. The support material exhibited high loading ability to enzyme binding which facilitated the attachment of almost 170 mg of the peptide on 1 g of the support. This biocatalytic system was used for biodegradation of 2,4-dichlorophenolin waste water. Under optimal operational conditions, laccase bound to halloysite nanotubes was able to remove over 90% of the pollutants in 10 h [139]. In another study, horseradish peroxidase was immobilized by adsorption on the surface of aluminum-pillared interlayered clay for the treatment of wastewater polluted with phenolic compounds. After immobilization, the system exerted a perfect removal of phenol products over a broader pH range of 4.5 to 9.3 than the free biocatalyst was able to achieve. Furthermore, addition of polyethylene glycol to the wastewater was shown to significantly enhance phenol removal efficiency while reducing the amount of immobilized enzyme required to achieve a removal efficiency of over 90% [142].

4.2.4. Carbon-based materials

Special attention should be paid to carbon-based materials from among the very wide range of inorganic materials used for immobilization of enzymes for environmental applications. Carbon-based materials are considered as a valuable support for enzyme immobilization because they have well-developed porous structures with pores of various size or volume and high specific surface areas (up to 1000 m^2/g), which means that these materials have a large

number of contact sites on their surface for enzyme immobilization [152]. Carbon-based materials are also characterized by the presence of many functional groups on their surface [153]. Single-walled and multi-walled carbon nanotubes are those mostly used for covalent immobilization of laccases and tyrosinases. Carbon nanotubes could significantly enhance the electron transfer rate between substrates and laccase and as a consequence increase the catalytic activity of the immobilized biomolecules. Additionally, through the creation of relatively strong chemical interactions between enzyme and support, carbon nanotubes increase the stability of the immobilized biomolecule towards harsh reaction conditions and enhance its reusability. In addition to carbon nanotubes, other materials such as carbon nanospheres or mesoporous carbon materials may also be used for immobilization of horseradish peroxidase or laccase [146,147]. Subrizi and his team used multi-walled carbon nanotubes functionalized by poly(diallyldimethylammonium chloride) for covalent immobilization of tyrosinase from *Agaricus bisporus*. The obtained biocatalytic systems were used for degradation of phenol in aqueous media and achieved removed of over 85% of phenol after 24 h of the process. It should be noted that immobilized tyrosinase was also able to catalyse removal of phenol derivatives of complicated chemical structure that react only to a small extent with the native enzyme [145]. Another example of carbon-based materials for laccase immobilization is fullerene C_{60}. It was used by Pang et al. for covalent immobilization of laccase from *Trametes versicolor* and applied for degradation of bisphenol A and catechol in water solution. Though immobilized laccase, due to creation of diffusional limitations, exhibited lower affinity to the substrate (higher value of Michaelis-Menten constant), it could still be used for efficient degradation of the pollutants under mild reaction conditions [148]. As the above-mentioned materials exhibited a great variety of advantages, it should be considered that their synthesis and production is usually complex and expensive.

4.2.5. Other inorganic materials

It should be added that other inorganic materials such as porous glass, calcium carbonate and noble metals can also be used for oxidoreductase immobilization. Due to inertness, large surface area and electronic properties which provide good electron transfer between the enzyme and a substrate, noble metals such as gold can be used for enzyme immobilization for environmental protection. Moreover, through the use of glass nanoparticles, biomolecules can be attached to the support homogeneously and retain their rigidity and catalytic activity [154]. Qiu et al. used nanoporous gold particles with pores of diameter around 40 nm for adsorption immobilization of lignin peroxidase from *Phanerochaete chrysosporium*. Gold-bounded

enzyme retained over two times higher catalytic activity than free enzyme after incubation for 2 h at pH 5 and 45 °C. The resulting biocatalytic system was used for degradation of fuchsine, rhodamine B and pyrogallol red and removed over 75% of each compound [150]. Highly viscous liquids such as porous glass was used as beads for adsorption immobilization of laccase from *Trametes versicolor*. The obtained biocatalytic system, which was characterized by high thermal stability, was used in a recirculating packed or fluidized bed reactor for decolorization of textile dyes like Reactive Blue 19, Dispersed Blue 3, Acid Blue 74, Acid Red 27 and Reactive Black 5. After 30 min treatment in a fluidized bed reactor at pH 5 and 23 °C, over 80% of the Dispersed Blue 3 and Acid Blue 74 were removed from textile industry wastewater [149]. The limitation in this this study was the adsorption binding of the enzyme, as in the recirculation mode of the reactor it caused leakage of the laccase from the support.

As has been clearly shown, various inorganic materials including inorganic oxides (mainly silica or titanium dioxide), minerals, noble metals and carbon based materials, such as single- and multi-walled carbon nanotubes, are the most commonly used for immobilization of oxidoreductases for environmental applications. For example, laccase was covalently immobilized onto the surface of alumina pellets and multi-walled carbon nanotubes without any additional cross-linker [137,145]. Produced immobilized enzymes were applied for degradation of Reactive Black 5 and Reactive Blue 19 with removal efficiencies exceed 85% and showed great reusability. These materials are known for their thermal and pH stability, mechanical resistance and good operational stability. Some attention should also be paid to the possibility of reuse of selected inorganic compounds after enzyme deactivation as a result of their high stability. Following immobilization on the surface of inorganic compounds, the range of pH and temperature over which the immobilized enzymes could effectively be used is often extended. For instance, laccase from *Trametes versicolor* or *Coriolopsis polyzona* was immobilized onto the surface of titania nanoparticles, porous glass beads or fumed silica, respectively [127,133,149]. Obtained systems were used for degradation of phenol derivatives and reactive dyes over a broad range pH from 3.5 up to 7.5 that confirmed stabilization of enzyme structure in both, acidic and base conditions. It also should be added that inorganic support materials, due to their presence in nature or easy preparation method are relatively inexpensive, which increases their application possibilities. According to the studies reviewed above, biocatalytic systems for removal of toxic compounds produced on the basis of inorganic supports bring highly efficient biodegradation of endocrine disruptor chemicals or dyes. To support this statement it could be presented, that various fungal laccases, immobilized onto the surface of nanoporous, mesoporous and sol-gel silica were applied for

degradation of 2,4-dinitrophenol, 2,4,6-trichlorophenol, bisphenol A, diclofenac or triclosan [121,123,128]. It should be emphasized that silica is very versatile support material as each of the produced biocatalytic system degraded over 85% of the contaminant.

Factors such as high stability, mechanical resistance, the presence of many functional groups, mainly hydroxyl, and abundance in nature caused that inorganic materials as supports for oxidoreductases are commonly used for environmental applications. Enzymes may be effectively attached to these materials principally mainly via adsorption immobilization due to the good sorption properties of these carriers. However, the creation of covalent bonds due to the presence of many functional groups, should not be excluded, which results in creation of stable and reusable biocatalytic systems. It has been shown that that immobilized oxidoreductases could be applied in large scale processes for effective remediation of wastewater contaminated with toxic pollutants. However, in our opinion some operational parameters still needs an improvement. Even so, due to the enhanced sensitivity and selectivity of the enzymes after immobilization using for instance noble metals or materials that enhance transfer of the electrons, biocatalytic systems containing oxidoreductases could be soon applied not only for environmental protection, but also in biosensors to detect even trace amounts of various compounds in effluents from industry or in human body fluids.

4.3. Hybrid materials

As previously mentioned, composite and hybrid materials as supports for enzymes have attracted researchers' attention over the last two decades. A combination of the properties of their precursors maximizes the benefits of these materials and makes them suitable supports for enzymes for many practical environmental applications. Many components of both organic and inorganic origin have been combined to create new supports for immobilization of oxidoreductases. Increases in stability and reusability of the immobilized enzyme can be achieved by the use of these types of matrices. Hybrid/composite support materials also protect biomolecules against denaturation and loss of the bioactivity under reaction conditions. Moreover, the properties of these support materials are usually designed for selected enzymes and the technological process in which the product will be applied after immobilization. An additional advantage of these materials is that due to their properties they can be applied in all immobilization techniques [155]. Selected examples of the various hybrid and composite materials used for immobilization of enzymes for environmental pollutants degradation are presented in Table 3,followed by comments.

Table 4

4.3.1. Inorganic-inorganic hybrid materials

With regards to inorganic materials, mainly inorganic oxides such as silica or zinc and titanium oxide are used to produce hybrid supports for oxidoreductase immobilization. Other oxides, minerals, carbon-based materials and noble metal ions are also frequently involved in the design of the new supports. Inorganic-inorganic hybrids are usually characterized by inertness, resistance against harsh reaction conditions and good mechanical stability. As a result, the immobilized enzyme also possesses improved thermal and chemical stability and its structure is protected by the composite matrix from denaturation [181]. A great advantage of inorganic-inorganic hybrids is that they may be used in a wide variety of shapes and sizes, such as nanoparticles, nanowires, fibers, tubes and even as membranes [159,160,192]. Sun et al. used macroporous SiO_2/ZnO nanowires modified by diethylene glycol diglycidyl ether for covalent immobilization of horseradish peroxidase. The immobilized enzyme exhibited high activity in decolorization of azo dyes, like Acid Blue 113 and Acid black 10 BX, and removed as much as 95 and 90% of these dyes, respectively. The support-bonded enzyme was able to remove over 90% of the selected dye from a solution containing dye at over 50 mg/L after less than 1h. Furthermore, storage stability and reusability of the immobilized biomolecule was significantly improved in comparison with the free enzyme [156]. Special attention, though, among others inorganic materials should also be paid to magnetic particles representing by i.e. magnetite. Biocatalytic systems based on hybrids incorporating magnetic particles may be easily separated from reaction mixtures by an external magnetic field. Magnetite particles for immobilization of laccases and removal of phenol derivatives have been combined with silica particles or mesoporous carbon material [161,164]. Superparamagnetic nanoparticles were successfully deposited on graphene oxide sheets by ultrasound-assisted co-precipitation by Chang and his team. Synthesized material was then used for immobilization of horseradish peroxidase and applied for batch biodegradation of 2-chlorophenol, 4-chlorophenol and 2,4-dichlorophenol. On the one hand, removal efficiency was strongly affected by the number and position of electron-withdrawing substituents in the phenol ring, but on the other hand also lack of proper hydrogen peroxide supplying limit high removal rates. Nevertheless, the highest removal efficiency, over 80%, was observed for 2-chlorophenol. The results presented by these authors suggest that storage stability and tolerance to changes in temperature and pH of the immobilized biomolecules were better than for the free biomolecules [163].

4.3.2. Inorganic-organic hybrid materials

Though magnetite is often combined with inorganic moieties, it can be also mixed with organic materials such as biopolymers, for example chitosan or alginates [166,175] as well as synthetic polymers like poly(*p*-phenylenediamine) or poly(4-vinyl pyridine) [184,185]. In a bench study magnetite nanoparticles were combined with chitosan to increase the bioaffinity of the hybrid and with clay to increase stability and mechanical resistance of the hybrid. The produced material was applied for covalent immobilization of laccase from *Trametes versicolor*. In comparison to the free enzyme, the immobilized biomolecules exhibited improved storage stability and better tolerance to changes in pH and temperature, and retained over 70% of initial activity after 10 repeated cycles. This biocatalytic system was then used for phenol degradation. After 4 h of treatment under optimal conditions, about 80% of phenol was removed from solution [165]. In another study, highly biocompatible chitosan core alginate capsules were enriched by magnetite nanoparticles and used for encapsulation of mushroom tyrosinase. The biomagnetic capsules thus obtained showed great storage stability, faster removal rate and greater reproducibility. This biocatalytic system could be applied for remediation of phenol and bisphenol A from real environmental water samples, because after 39 h of treatment of this process, 100% and over 85%, respectively, of the two pollutants were degraded [174]. In work by Bayramoglu and co-workers, synthetic polymer-poly(4-vinyl pyridine) was grafted on magnetic nanoparticle beads. Next, the polymer was chelated by Cu(II) ions for adsorption immobilization of laccase from *Trametes versicolor* and enhancement of the catalytic activity of the enzyme. The biocatalyst thus produced was used in an enzyme reactor for degradation of three textile dyes - Reactive Green 19, Reactive Red 2 and Reactive Brown 10 - in a batch system. The described results show that immobilized laccase could successfully remove the three test dyes from water solution at efficiencies of 64, 88 and 91%, respectively. However, as the main factor that limit the decolorization rate was the chemical structure and type of substitute group of the dye molecules [184]. Biopolymers can also be combined with other inorganic materials to create suitable supports for immobilization of enzymes for environmental applications. For instance, chitosan was mixed with silica or cesium oxide, minerals or glass beads [169,170,172], while alginate was combined with silica gel [176]. An interesting example of the creation of inorganic-organic hybrids was presented by Dincer and co-workers. They mixed clay with chitosan and cross-linked hybrid material by glutaraldehyde to form beads that were used for covalent immobilization of tyrosinase. The authors reported high enzyme activity and loading

efficiency due to the presence of many functional groups in the structure of the support. The immobilized tyrosinase was used for degradation of phenol in water solution. After treatment for 6 h at 25 °C and pH 7, all of the phenol was removed. The biocatalytic system was able to retain over 50% of its initial activity after seven repeated tests [171]. In this study achievement of the better reusability was limited by the inhibition and inactivation of the enzymes over consecutive catalytic cycles. Besides biopolymers, synthetic polymers may also be combined with inorganic components to form suitable matrices for immobilization of enzymes for environmental protection. For instance, poly(2-chloroethyl acrylate) was combined with zeolite particles, as latex hydrogel was mixed with graphene oxide to formed nanobeads. Both hybrids were then used for immobilization of laccase and applied for degradation of textile dyes with high efficiencies [180,182]. Xu et al. used mesoporous SiO_2 nanofibers and connected them with poly(acrylic acid). Finally, the hybrid system was modified by vinyl groups to increase its affinity for laccase. The synthesized matrix was characterized by a mesoporous structure (pore size 1.73–3.54 nm) and a high specific surface area (542.91 m^2/g) which allowed covalent bonding of about 420 mg of the enzyme per 1 g of the support. The immobilized biomolecule exhibited better storage stability and higher tolerance to harsh pH and temperature conditions in comparison with free laccase. The biocatalytic system, after 2 h of triclosan treatment, under optimal process conditions (pH 4, 30 °C) removed around 65% of the pollutant from water solution [181]. The factor that limit degradation efficiency might be an enzyme overloading, which blocked active sites of the biocatalysts and decrease their activity.

4.3.3. Organic-organic hybrid materials

When there is a need for materials with more sophisticated features such as special reactive functional groups or unusual shape, polymers can be fixed with biopolymers or other synthetic polymer. Synthesized organic-organic hybrids due to the presence of biopolymers, such as calcium or sodium alginate, are biocompatible and non-toxic, because the presence of the polymers such as poly(acrylamide-crotonic acid) or polyvinyl alcohol ensures stability, mechanical resistance and stiffness of the hybrid support [177,178]. Polyamide 6/chitosan nanofibers modified by using two different spacers (bovine serum albumin and hexamethylenediamine) have been used for immobilization of laccase from *Trametes versicolor* and applied for biodegradation of endocrine disrupting chemicals like bisphenol A and 17-*α*-ethinylestradiol. The resulting biocatalytic systems proved to be efficient not only in removal of a mixture of the pollutants (92% of bisphenol A removal, total degradation of 17-

α-ethinylestradiol) but also showed great reusability. After three treatment cycles, the initial activity of this system was unaltered [179]. It should also be mentioned that hybrid supports built from two synthetic polymers may be used for immobilization of various oxidoreductases. Synthetic polymer hybrids are formed by polymerization using various monomers with desired properties. These materials exhibit chemical and mechanical stability and possess many desirable functional groups and are characterized by a large specific surface area that allows a great number of biomolecules to be attached in a stable way mainly via covalent bonds. For example, horseradish peroxidase was immobilized on composite poly(vinyl alcohol)/4-hydroxybenz-aldehyde cinnamate or poly(styrene-co-methacrylic acid) nanofibers [186,190]. The produced biocatalytic systems were applied for degradation of phenol derivatives and were able to remove over 80% of the pollutants from water solution. But the limitation of the presented biocatalytic system was covalent binding of the molecules and in disruption in the structure of the enzyme active sites. In another study, Uygun and co-workers used poly(methyl methacrylate-co-glycidyl methacrylate) cryogel for immobilization of laccase from *Trametes versicolor* and degradation of textile dyes effluent. The immobilized enzyme degraded up to 80% of the selected dye with degree of degradation dependent on the chemical structure and molecular mass of the pollutants [189]. Sathishkumar et al. applied poly(lactic-co-glycolic acid) nanofibers for covalent immobilization of laccase from *Pleurotus florida*. The obtained biocatalytic system was used for biodegradation of diclofenac from aqueous sources. The results the authors present demonstrated that the environmental pollutant could be completely transformed into non-hazardous compounds. Moreover, their biocatalytic system exhibited great reusability. After three reuse cycles diclofenac was completely removed. Additionally, after addition of syringaldehyde, reusability of the system was extended to six catalytic cycles. The immobilized laccase had better storage, pH and thermal stability than the free biocatalyst [187]. It should be also noted that when synthetic polymer hybrids are used, entrapment also takes place. For this reason poly(acryl-amide) gel was used for entrapment of manganese peroxidase from *Ganoder malucidum* IBL-05. The immobilized enzyme exhibited good storage stability and could retain over 50% of initial activity after storage for two months at 4 °C. The thermal stability of the immobilized peroxidase was also significantly improved. After incubation for 72 h at 50°C, the enzyme retained over 40% of its initial properties while the free biomolecule was completely inactive. The immobilized catalyst was further used for removal of textile dyes from real water solutions [188].

As has been clearly demonstrated various types of hybrids and composite materials can be applied for immobilization of enzymes for remediation of parlous pollutants. It is becoming more and more common to use these materials due to the possibility of designing their properties to fulfill the requirements of the technological process and of the enzyme. Precursor materials may be chosen to protect of the enzyme structure, increase thermal and chemical resistance of the immobilized biomolecules as well as to improve their catalytic properties. For example, carbon nanotubes could enhance transfer of the electron during catalytic reaction as the addition of Cu^{2+} ions in general increases the catalytic activity of the oxidoreductases. For example, copper ions were incorporated into the structure of tetra-aminophthalocyanine/magnetic nanoparticles and poly(4-vinyl pyridine)/magnetic beads. Hybrid supports were further used for covalent and adsorption immobilization of laccase from *Pycnoporuss anguineus* and *Trametes versicolor*, respectively. Produced immobilized enzymes were applied for degradation of 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) and reactive dyes and allowed total removal of pollutants from water solution [184,186]. Presented examples proved that addition of inorganic ions into the structure of polymeric matrix could significantly improve removal efficiency. Other precursor materials are chosen in order to improve operational control of the remediation process. For instance, magnetic nanoparticles make separation of the immobilized enzyme from reaction mixture fast and simple as inorganic oxides ensure high mechanical stability of the biocatalytic system and its reusability. Application of the hybrid supports for enzymes and their use in bioremediation of environmental pollutants, such as phenol and its derivatives, dyes and even estrogens from wastewater, results in high efficiency of the removal process. Pollutants like 2,4-dichlorophenol was efficiently removed by laccase covalently immobilized on silica magnetic particles and Cu^{2+}/silica magnetic particles [159,160] as organic dye Reactive Green 19 was degraded with efficiencies exceed 65% by laccase from *Trametes versicolor* immobilized on poly(4-vinyl pyridine)/Cu(II) magnetic beads or poly(methyl methacrylate-co-glycidyl methacrylate) cryogel [184,189]. Undesirable compounds are effectively transformed into non-hazardous products, under mild conditions, whilst immobilized enzymes retain their high catalytic activity over repeated reaction cycles. Hybrids and composite materials can be formed into the shape that will be most suitable for the process. Therefore all techniques of immobilization may be used for immobilization of laccases of various origins, and for tyrosinases or peroxidases. However, note should be stated that proper selection of the immobilization technique is required to avoid diffusional limitations and enzyme inactivation. Hybrid support of inorganic-inorganic, organic-organic and mixed inorganic-organic origin,

like magnetic nanoparticles/graphene oxide nanocomposite, chitosan/polyacrylamide hydrogel and alginate/SiO$_2$ gel were applied for covalent immobilization of horseradish peroxidase, encapsulation of laccase from *Trametes versicolor* and entrapment of polyphenol oxidase, respectively [163,173,176]. Immobilized enzymes were then used for degradation of phenol and its derivatives and organic dyes with removal efficiencies around 80% that proved versatility of the hybrid materials. However, higher degradation rate is limited by the diffusional limitations of reaction mixture.

Irrespectively of the origin of precursors, properties of the hybrid and composites materials could be tailored to meet the requirements of the enzymes and the process, making these materials particularly interesting as support for immobilization. Therefore, we strongly suggest more advanced research related to the application of the above-mentioned materials as a carriers for oxidoreductases which should result in formation of active biocatalytic systems for removal of hazardous compounds with high efficiencies despite the fact that they are expensive and methodology for their synthesis is usually complicated. We also believe that hybrid and composite materials has continued to grow during recent years, as these materials are also particularly interesting in terms of potential for further exploitation. In the near future, hybrid and composite supports materials could be used for immobilization of enzymes for production of highly sensitive biosensors, as well as for efficient, more specific and "cleaner" catalysts in chemicals synthesis, or might even find application in clinical medicine. It should be mentioned that hybrids materials could be also used as an efficient and stable support for co-immobilization of enzymes and application of the resulting biocatalytic systems in multienzymatic bioconversion processes.

5. Effect of support materials and immobilization technique on substrates accessibility

As it was previously stated, laccases and tyrosinases required molecular oxygen as a substrate that is reduced to water during catalytic pathways of these enzymes [191,192]. Similarly, peroxidases (LiP, MnP, HRP) need hydrogen peroxide, that during reaction is also reduced to water. The catalytic mechanism of laccases, tyrosinases and peroxidases differ from each other, but accessibility of the molecular oxygen and hydrogen peroxide, beside availability of phenolic substrates, is a key factor that limits catalytic efficiency of the immobilized oxidoreductases [193,194].

To ensure high exposition of the enzyme active site for contact with substrates, proper selection of immobilization protocol has to be done. For instance, in case of adsorption immobilization by limited number of formed interactions, interference in the structure of the

enzyme and amino acids rearrangements is reduced, as it facilitates easy binding of O_2 or H_2O_2. In contrast, covalent binding of the biocatalysts usually disrupts the structure of enzyme and its catalytic site, what makes interactions with reducing substrates more difficult. Enzymes immobilized by encapsulation or entrapment are usually physically locked into the matrix that limits structural changes of the biomolecules. But on the other hand, significant diffusional limitations occurs that reduce efficient transfer of substrates and decrease catalytic activity [195]. To support this statement some examples are presented below. For instance, fungal laccases were immobilized by adsorption, covalent binding and entrapment, using supports of organic origin, and applied for degradation of reactive dyes. Acid Orange 7 was almost totally removed from water solution by adsorbed enzyme, as 80% of Reactive Yellow 15 and about 50% of Remazol Brilliant Blue R were degraded by laccase immobilized by covalent binding and entrapment, respectively [76,99,147]. Different values of removal efficiencies might be explained mainly by various immobilization strategies and related to this different diffusional limitations, which are the lowest in adsorption immobilization and the highest in encapsulation due to enzyme surrounding by the support layer. Formed diffusional limitations strongly influence accessibility of the molecular oxygen and hydrogen peroxide to the active site of the enzymes and in consequence affect effectivity of biodegradation [196,197].

When the supply of the oxygen or hydrogen peroxide is hindered, mesoporous materials at highly ordered structure of pores should be use, while they enable ease penetration of the oxygen or hydrogen peroxide molecules. In consequence higher removal efficiencies might be achieved using these materials. That proves, that application of various matrix for immobilization of the same enzyme could results in different degradation values and indicates that structural composition and properties of support material affect accessibility of O_2 or H_2O_2. Although most of the bioremediation processes using immobilized oxidoreductases are carried out without additional supply of reducing substrates there is a room for improvements leading to the higher robustness of the enzymes and cost reduction. For instance, some additional process like bubbling, oxygenation, aeration and even simple shaking could be applied to increase accessibility of the reducing substrates for immobilized enzymes.

6. Summary and comparison of the support materials of various origin

In the presented study it has been clearly shown that many various materials of organic, inorganic and hybrid/composite origin could be efficiently applied for immobilization of

oxidoreductases for environmental application. The most important advantages and disadvantages of each type of the support materials were briefly summarized and are presented in Fig 4.

Figure 4

The multiplicity of the possible supports caused that their proper selection is strongly governed by the type of the enzyme, immobilization technique and undesirable compound that has to be removed. For instance for immobilization of laccase from *Trametes versicolor*, the most commonly applied oxidoreductase for environmental application, materials of different hydrophilicity and functional moieties (–OH, –NH$_2$, C=O) of organic, inorganic and hybrid origin, such as chitosan and polyvinyl alcohol capsules [76,101], mesoporous silica and Fullere C$_{60}$ [121,148] as well as magnetic chitosan/clay beads or poly(4-vinyl pyridine)/Cu(II) magnetic beads [165,184] were applied. Also mushroom tyrosinase was immobilized using wide range of support materials, such as cellulose paper [83], silica gel [130] and chitosan/clay and chitosan/alginate magnetic capsules [171,174]. Obtained biocatalytic systems were applied for degradation of synthetic and natural dyes and phenol and its derivatives with removal efficiencies usually higher than 80%, which proves that immobilized oxidoreductases are stable, irrespectively of the origin of support material. More attention should be paid on immobilization of laccase and tyrosinase on hybrid supports, such as poly(4-vinyl pyridine)/Cu(II) magnetic beads and chitosan/alginate magnetic capsules by adsorption and encapsulation, respectively. Immobilized laccase was used for degradation of Reactive Brown 91 and removed over 90% of the dye [184] as encapsulated tyrosinase degraded about 85% of bisphenol A from water solution [174]. Presented results prove that immobilized enzymes not only retain their robustness but also might be easily separated from reaction mixture by addition of magnetic particles. That suggest that hybrid and composite materials might be interesting for immobilization of laccases and tyrosinases and their practical application on larger scale for bioremediation of pollutants. Although various supports could be used for immobilization of laccases, tyrosinases and peroxidases, during selection of the specific material for practical applications mainly its accessibility and costs of its obtaining, purification and synthesis should be taken into account. Also high stability in harsh process conditions and mechanical resistance are a crucial factors determining selection of the carrier for immobilization of oxidoreductases. As inorganic carriers and biopolymers seems to be particularly interesting from the point of view of economy and stability, hybrid

and composites materials are outstandingly attractive because they might be tailored and produced to enhance catalytic activity of the immobilized enzymes and in consequence to improve efficiencies of the processes carried out.

As it was previously mentioned, immobilization methodology also strongly influence the selection of enzyme support. Highly porous materials, at high specific surface area facilitate adsorption immobilization and enhance attachment of greater amounts of enzyme. As an example, chitosan beads, titania nanoparticles, mesoporous carbon and silica/magnetic/methacrylyol particles should be enumerated [78,133,147,158]. These materials were used for immobilization of laccase, tyrosinase and even lignin peroxidase and applied for removal of mainly dyes and phenol from water solutions with removal efficiencies not exceeding 90%. This might be related to the binding of great amount of enzyme, which might leads to biomolecules overcrowding and in consequence decrease in catalytic activity [196]. On the other hand, immobilization of oxidoreductases on materials with numerous of functional groups facilitate covalent immobilization and multipoint attachment of the enzyme molecules. That is preferred in terms of improving stability of immobilized enzyme, however might also lead to creation of diffusional limitations in transport of substrates. From that point of view, particularly interesting are hybrid materials, such as magnetic chitosan/clay beads and poly(lactic-co-glycolic acid) nanofibers that were used for covalent binding of laccase from *Trametes versicolor* and applied for degradation of phenol and diclofenac, respectively [165,187]. Even taking into account diffusional limitations, stability of the enzyme was significantly improved and allow total removal of phenol and diclofenac from the tested solution. Inorganic materials such as mesoporous silica spheres and halloysite nanotubes [124,139] and organic carriers of natural origin like chitosan or coconut fibers could be applied for covalent immobilization [75,99]. The above-mentioned supports were used for immobilization of all reviewed oxidoreductases and applied for biodegradation of synthetic and natural dyes, pharmaceuticals and phenols usually with efficiencies exceeding 80%. However, it should be emphasized that inorganic and organic materials for covalent attachment of the enzyme usually require intermediate agents, such as glutaraldehyde, that facilitate creation of covalent bonds. For immobilization of oxidoreductases also encapsulation and entrapment are applied. As supports for these techniques of immobilization mainly alginates-based materials are adapted due to their remarkable abilities for gelation under mild conditions and high operational stability [78-91]. Also poly vinyl alcohol beads, silica aerogel and hybrid chitosan/polyacrylamide hydrogel were used for encapsulation of laccases and tyrosinases [102,131,173]. For instance laccase was immobilized by

encapsulation into chitosan beads and by entrapment into copper alginate beads and they were applied for degradation of Indigo carmine [79,91]. Both biocatalytic systems allowed total degradation of the dye, but addition of copper ions into alginate beads increased activity of the laccase and caused that degradation process was four times shorter and was carried out in milder temperature conditions (30 °C) as compared to chitosan beads (37 °C). It is our belief that among the existing immobilization protocols, two approaches are of particular interest. Firstly, immobilization of oxidoreductases in the way where interference in the enzyme structure is highly limited as it results no conformational changes in the biocatalysts structure and retention of high catalytic properties. The second approach is a multipoint immobilization of the enzyme. As a result biomolecule is permanently attached to the support material that results in the improvement of the reusability of the immobilized biocatalysts. However, we would like to emphasize that selection of the most suitable immobilization protocol is governed mainly by the type of the support and practical requirements for the biocatalytic system.

Irrespectively of the origin, support materials could be applied for biodegradation of the same type of the persistent pollutants. For instance, fungal laccases covalently immobilized using mesoporous silica and polyamide/chitosan composite [123,179] as well as mushroom tyrosinase immobilized by entrapment using silica alginate and by covalent binding on polyacrylonitrile beads [85,104]were applied for remediation of bisphenol A. Removal efficiencies ranging from 80% (mesoporous silica) up to 92% (composite support) were noticed for immobilized laccase as for tyrosinase it was 35% (silica alginate) and 90% (hybrid support). On the other hand, synthetic dyes could be efficiently removed by oxidoreductases immobilized using wide range of support materials. For example, for remediation of synthetic dye Remazol Brilliant Blue R, laccase immobilized by covalent binding on gum Arabic and graphene oxide/latex hydrogel and encapsulated into magnetic Cu alginate beads were applied [97,175,182]. The remediation efficiencies varied from 80% (gum Arabic) to 100% (hybrid material) for covalently immobilized enzyme. In case of encapsulated laccase, mainly due to the occurrence of diffusional limitations, biodegradation efficiency reached 76% and even a presence of copper ions in the support material did not improve performance of the process. These data clearly shows that porosity of the support material, type of surface functional groups and immobilization technique strongly affect degradation process of bisphenol A and Remazol Brilliant Blue R.

Many studies from research laboratories show promising early stage proof-of-concept results for application of oxidoreductases for environmental application, however, they suffer because there is a significant lack of work targeting the whole chain including production costs of the immobilized enzymes, any scale-up advantages of manufacturing of matrices and biocatalytic systems, as well as presenting their storage stability and practical implementation. Also broader comparison of several different types of immobilized enzyme systems, for example laccase systems that have broad substrate selectivity, are warranted. Presented studies and their limitations show that the time is now ripe for moving to the next level of multidisciplinary assessment and there is a need to involve the companies and environmental agencies in the research as they should help to overcome various practical limitations. As the results of these studies we should obtain immobilization protocols allowing production of universal biocatalytic systems for degradation and remediation of hazardous pollutants.

7. General summary and remarks

In recent years, environmental pollutants such as synthetic and natural dyes, pharmaceuticals and phenol and its derivatives have become a very serious problem that needs to be solved to minimize the direct threats posed by these compounds to the health and life of living organisms. One possible way to remove hazardous contamination from wastewater and soils is their biodegradation using enzymes. It has been shown that phenyloxidase enzymes, such as laccase, tyrosinase or lignin, manganese and horseradish peroxidases, as a members of oxidoreductase catalytic class, may catalyse oxidation and transformation of numerous persistent compounds into non-toxic derivatives. These processes were usually carried out without addition of cofactors or mediators except molecular oxygen or hydrogen peroxide, which are widely available. The studies reviewed here can lead to a better understanding of enzymatic biodegradation and illustrate the following benefits of oxidoreductase enzymes:

(i) biodegradation processes may be carried out in highly efficient way under mild conditions,

(ii) removal of environmental contaminants is possible without use of toxic solvents or high energy consumption, in accordance with the rules of green chemistry,

(iii) remediation of pollutants may usually be carried out in more cost-effective way, without the need for advanced equipment,

(iv) conversion of hazardous compounds into their non-toxic derivatives may be achieved.

Despite the many promising results, it is evident that more research is needed to clarify the levels of different pollutants and residual pharmaceuticals locally, particular near industrial area and near hospitals. Next, a much better understanding of the competitive kinetics of different substrates on enzymatic removal by oxidoreductases, such as laccases or tyrosinases is clearly missing. Lastly, durability and robustness of different types of enzymes in genuine application environments should be assessed. This review also shows the need for larger comparative application trial among different types of enzymatic options for removal of pharmaceutical residues in particular.

It should further be pointed out that the immobilization process is frequently applied to increase stability of the enzymes and enhance their reusability as well as to create insoluble biocatalyst that may be reused. Here we have summarized and reviewed materials and their properties that could be used as supports in immobilization of enzymes for environmental applications. As we have described, materials of both organic and inorganic origin as well as hybrids and composites supports have been be applied for immobilization of oxidoreductases. We have also reported the key factors that determine selection of the support for immobilization of enzymes for contaminants removal as being the following:

(i) the presence of numerous functional groups for effective enzyme binding,

(ii) good sorption properties,

(iii) good thermal and chemical resistance and mechanical and operational stability.

However, materials with desired properties that increase activity and stability of the immobilized biocatalysts have also been examined. Furthermore, we have summarized the possible ways to perform the biodegradation processes and possibilities for increasing their efficiency.

Thus, our aim has been to review support materials that may be applied for immobilization of enzymes for environmental applications and show how these materials affect catalytic activity and stability of the biocatalysts. We wanted to present criteria for selection of support materials to produce biocatalytic systems which have excellent catalytic properties and good stability for use in modern remediation of undesirable pollutants from domestic and industrial wastewaters. Information presented in this review could form a basis for evaluation of a novel, highly efficient detoxification process conducted under mild conditions, without utilization of a hazardous reagents, in accordance with the rules of green chemistry.

While applications of immobilized laccases have been well studied, only few previous studies have dealt with the efficient use of other oxidoreductases for environmental

protection. The information displayed on this review confirms that tyrosinases, and peroxidases such as horseradish, manganese and lignin peroxidase show promise as efficient treatments for removal of hazardous pollutants after immobilization, even at industrial scale. The main challenge to this respect is to build systems able to operate in continuous mode, so the operational costs of such bioremediation processes can be reduced. Additionally, further studies on the role of these enzymes on the removal of other compounds such as hormones or antibiotics will have to be also addressed in future studies.

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Table 1. Main immobilization techniques, their characteristic and advantages.

Immobilization technique	Main functional groups of the support	Type of interactions	Strength of interactions	Advantages
Non-covalent (adsorption)	$-NH_2$, $-SH$, $-OH$, $C=O$, COOH, epoxy groups	hydrogen bonds, ionic interactions, hydrophobic interactions	weak	no enzyme modification, simple and inexpensive reusability of the support
Covalent binding	$-NH_2$, $-SH$, $-OH$, $C=O$	covalent bonds	strong	strong and stable interactions, multipoint attachment, reducing of enzyme leakage
Encapsulation	$-NH_2$, $-OH$,	ionic interactions, hydrophobic interactions	weak	no enzyme modification, protection of the enzyme
Entrapment	$-NH_2$, $-OH$, $C=O$	ionic interactions, hydrophobic interactions, covalent bonds	weak/strong	no enzyme modification,
Cross-linking	$C=O$, $-NH_2$	covalent bonds	strong	no support needed, high strength of interactions

Table 2. Materials of organic origin used for immobilization of laccases, tyrosinases and lignin, manganese and phenols oxidases for biodegradation of various environmental pollutants.

Support material	Enzyme	Immobilization technique	Contaminants	Process conditions	Removal efficiency	Reference
Chitosan	Laccase from *Coriolus versicolor*	Covalent immobilization	2,4-dichlorophenol, 4-chlorophenol, 2-chlorophenol	pH=5.5, 35 °C, 24 h	94%, 75%, 69%	[75]
Chitosan	Laccase from *Trametes pubescens*	Entrapment	Reactive Brilliant Blue X-BR, Remazol Brilliant Blue R, Congo Red, Acid Black 172, Methylene Blue, Neutral Red, Indigo Blue, Naphthol Green B, Direct Fast Blue FBL, Crystal Violet	pH=5, 50 °C,	55%, 50%, 55%, 70%, 25%, 45%, 45%, 40%, 60%, 20%	[76]
Chitosan microspheres	Lignin peroxidase and manganese peroxidase from *Coriolus versicolor*	Covalent immobilization/ cross-linking	Decolorizing of molasses waste water	pH=8.4, 30 °C, 6 h	80%	[77]
Chitosan beads	Lignin peroxidase from *Schizophyllum commune* IBL-06	Adsorption immobilization	Synthetic textile dyes	30 °C, 4 h	95.5%	[78]
Chitosan beads	Laccase from papaya	Entrapment	Indigo Carmine	pH=10, 37 °C, 8 h	100%	[79]
Calcium alginate	Manganese peroxidase from *G. lucidum* BL05	Entrapment	Sandal tree reactive dyes	pH=4.5, 35 °C, 12 h	up to 95%	[80]
Calcium alginate	Tyrosinase from *Agaricusbisporus*	Entrapment	Phenol	pH=7.2, 25 °C, 1 h	100%	[81]
Sodium alginate	Tyrosinase from *Streptomyces espinosus*	Entrapment	Phenol	4 h	up to 60%	[82]
Sodium alginate	Laccase from *Bacillus subtilis* MTCC 2414	Entrapment	Orange 3R, Yellow GR, T-Blue	40 °C, 120 h	74%, 79%, 71%	[83]
Silica alginate	Tyrosinase from *Agaricusbisporus*	Entrapment	Bisphenol A	20 °C, 24 h	100%	[84]
Silica alginate	Tyrosinase from *Agaricusbisporus*	Encapsulation	Bisphenol A	20 °C, 0.5 h	35%	[85]

Alginate beads	Polyphenol oxidase from *Taraxacumofficinale*	Entrapment	synthetic dyes from industrial effluents	25 °C, 1 h	80%	[86]
Alginate beads	Horseradish peroxidase	Entrapment	Pyrogallol	pH=7, 25 °C	75%	[87]
Alginate beads	Laccase from *Coriolopsisgallica*	Entrapment	Remazol Brilliant Blue R, Reactive Black 5, Bismark Brown R, Lanaset Grey G	pH=5, 30 °C 24 h	90%, 81%, 53%, 87%	[88]
Alginate beads	Laccase	Entrapment	Direct Blue 2	pH=5, 30 °C	86%	[89]
Copper alginate beads	Polyphenol oxidase from *Cynarascolymus* L.	Entrapment	L-DOPA	pH=7, 25 °C	88%	[90]
Copper alginate beads	Laccase from *Lentinuspolychrous*	Encapsulation	Remazol Brilliant Blue R, Methyl red, Indigo carmine, Bromophenol blue	30 °C, 2.5 h	89%, 91% 100%, 86%	[91]
Cellulose	Polyphenol oxidase	Adsorption immobilization	Phenol, 4-chlorophenol, 4-bromophenol	pH=8, 5 h	20%, 30%, 45%	[92]
Cellulose paper	Tyrosinase from mushroom	Covalent immobilization	L-DOPA	pH=7, 22 °C, 2 h	70%	[93]
Cellulose nanofibers	Laccase	Covalent immobilization	Simulated dye effluent consist of Remazol Black 5, Remazol Brilliant Blue R, Remazol Brilliant Violet 5R, Reactive Orange 16 and Reactive Red 120	30 °C, 12 h	39%	[94]
Carboxymethylcellulose beads	Polyphenol oxidase	Covalent immobilization	L-tyrosine	pH=6.5, 25 °C	93%	[95]
Diethylaminoethyl cellulose	Tyrosinase from mushroom	Covalent immobilization	L-DOPA	pH=9, 4 h	60%	[96]
Thiolsulfinate-agarose	Laccase from *Trametes villosa*	Covalent immobilization	Remazol Brilliant Blue R	pH=4.8, 22 °C, 24 h	80%	[97]
Gum Arabic	Laccase	Covalent immobilization	Remazol Brilliant Blue R	pH=5, 40 °C, 2 h	81%	[98]
Green coconut fiber	Laccase from *Aspergillus*	Covalent immobilization	Reactive Black 5, Reactive Blue 114, Reactive Yellow 15, Reactive Yellow 176, Reactive Red 239, Reactive Red 180	pH=7, 35 °C	90%, 90%, 77%, 5%, 33%, 35%	[99]

Polyvinyl alcohol	Horseradish peroxidase	Covalent immobilization	pyrogallol	pH=6, 25 °C, 1 h	80%	[100]
Polyvinyl alcohol capsules	Laccase from *Trametes versicolor*	Encapsulation	Saturn Blue L4G dye	pH=5.5, 25 °C, 24 h	48%	[101]
Polyvinyl alcohol beads	Laccase from *Cyathusbulleri*	Entrapment	Acid Violet 17, Basic Green 4, Acid Red 27	pH=5.5, 30 °C,	90%, 95%, 90%	[102]
Polyacrylamide hydrogel	Lignin peroxidase from *P. chrysosporium*	Encapsulation	Bisphenol A	pH=5, 25 °C, 8 h	90%	[103]
Polyacrylonitrile beads	Tyrosinase	Covalent immobilization	Bisphenol A, Bisphenol B, Bisphenol F, Tetrachlorobisphenol A	25 °C, 1.5 h	92%, 93%, 94%, 91%	[104]
Poly-N-vinylpyrrolidone	Tyrosinase from *Agaricusbisporus*	Covalent immobilization	Phenol	25 °C, 1 h	100%	[105]
Poly(2-hydroxyethyl methacrylate-co-glycidyl methacrylate)	Laccase from *Trametes versicolor*	Covalent immobilization	3,5-dinitro salicylic acid	pH=5, 55 °C, 2 h	75%	[106]
Epoxy activated polyethersulfone beads	Laccase from *Trametes versicolor*	Covalent immobilization	Acid Red 1	pH=5, 65 °C, 10 days	88%	[107]
Nylon membrane	Polyphenol oxidase from *Agaricusbisporus*	Covalent immobilization	Phenol, *p*-cresol, *m*-cresol, 4-chlorophenol, 4-methoxyphenol	25 °C, 8 h	Over 80% for *p*-cresol	[108]
Amberlite IRA 400 resin	Lignin peroxidase from *Phanerochaetechrysosporium*	Adsorption immobilization	Kraft E1 effluent	pH= 3 and 7, 25 °C, 3 h	50%	[109]
Anion-exchange resin	Tyrosinase from mushroom	Covalent immobilization	*p*-cresol	25 °C, 72 h	83%	[110]

Table 3. Materials of inorganic origin used for immobilization of laccases, tyrosinases and lignin, manganese and phenol oxidases for biodegradation of various environmental pollutants.

aterial	Enzyme	Immobilization technique	Contaminants	Process conditions	Removal efficiency	Re
-15	Laccase from *Trametes versicolor*	Adsorption immobilization	Naphthalene	pH 4.5, 5 h	30%	[12
s silica	Laccase from *Trametes versicolor*	Adsorption immobilization	2,4-dinitrophenol	pH 5, 50 °C, 12 h	>90%	[12
s silica	Lignin peroxidase from *Phanerochaetechrysosporium*	Covalent immobilization	Acid Orange II	pH 4, 35 °C	77%	[12
s silica	Laccase from *Cerrena unicolor*	Covalent immobilization	Bisphenol A, 4-nonylphenol, Triclosan	pH 5, 1 h	80%, 40%, 60%	[12
s silica	Laccase from *Coriolopsisgallica*	Covalent immobilization	Bisphenol A, Diclofenac, 17-α-ethinylestradiol	pH 5, 25 °C	90%, 85%, 70%	[12
s 2D silica	Lignin peroxidase	Adsorption immobilization	Phenol	pH 5, 25 °C	60%	[12
us silica	Phenol oxidase from *Mycelia sterilia* IBR 35219/2	Covalent immobilization	Phenolic compounds of the green tea extract	pH 5.2, 30 °C	45%	[12
ca	Laccase from *Coriolopsispolyzona*	Covalent immobilization	Bisphenol A	pH 7.5, 24 °C, 72 h	80%	[12
ca	Rot fungi laccase	Adsorption immobilization	2,4-dichlorophenol, 2,4,6-trichlorophenol	25 °C, 4 h	95%, 100%	[12
s	Laccase from *Cerrena unicolor*	Covalent immobilization	Bisphenol A, Nonylphenol, Triclosan	pH 5, 30 °C	80%, 40%, 60%	[12
	Tyrosinase from mushroom	Adsorption immobilization	Phenol	pH 6.8, 72 h	58%	[13
gel	Tyrosinase	Encapsulation	Phenol	pH 7, 30 °C, 3 h	90%	[13
osilica	Tyrosinase from mushroom	Covalent immobilization	Phenol, *p*-cresol, Phenyl acetate	pH 7, 35 °C, 12 h	84%, 74%, 90%	[13
oparticles	Laccase from *Trametes versicolor*	Adsorption immobilization	2,4-dichlorophenol, 2,6-dimethoxyphenol	pH 3.5, 25 °C	85%, 63%	[13
oparticles	Laccase	Adsorption immobilization	Direct Red 31, Acid Blue 92, Direct Green 6	pH 3, 25 °C, 1 h	87%, 84% 83%	[13
	Horseradish peroxidase	Covalent immobilization	4-aminoantipyrine	pH 4.5, 35 °C,	>95%	[13
ellets	Laccase	Covalent immobilization	Melanoidins	pH 4.5, 28 °C, 6 h	47%	[13
ellets	Laccase from *Trametes hirsuta*	Covalent immobilization	Basic Red 9, Reactive Blue 19, Acid Blue 225	pH 4.5, 30 °C	62%, 85%, 78%	[13
es	Laccase	Covalent immobilization	Reactive Green 19A	pH 5, 25 °C	75%	[13
nanotubes	Laccase from *Trametes versicolor*	Covalent immobilization	2,4-dichlorophen	pH 5, 30 °C, 10 h	93%	[13
	Horseradish peroxidase	Adsorption immobilization	Pyrogallol, Acid Violet 109	pH 5, 24 °C, 1 h	70%, 87%	[14
noclay	Manganese peroxidase from *Anthracophyllum discolor*	Adsorption immobilization	Pyrene, Anthracene, Fluoranthene, Phenanthrene	pH 4.5, 35 °C, 24 h	86%, 65%, 15%, 10%	[14
-pillared	Horseradish peroxidase	Adsorption	Phenol	pH 5, 25 °C, 4 h	>95%	[14

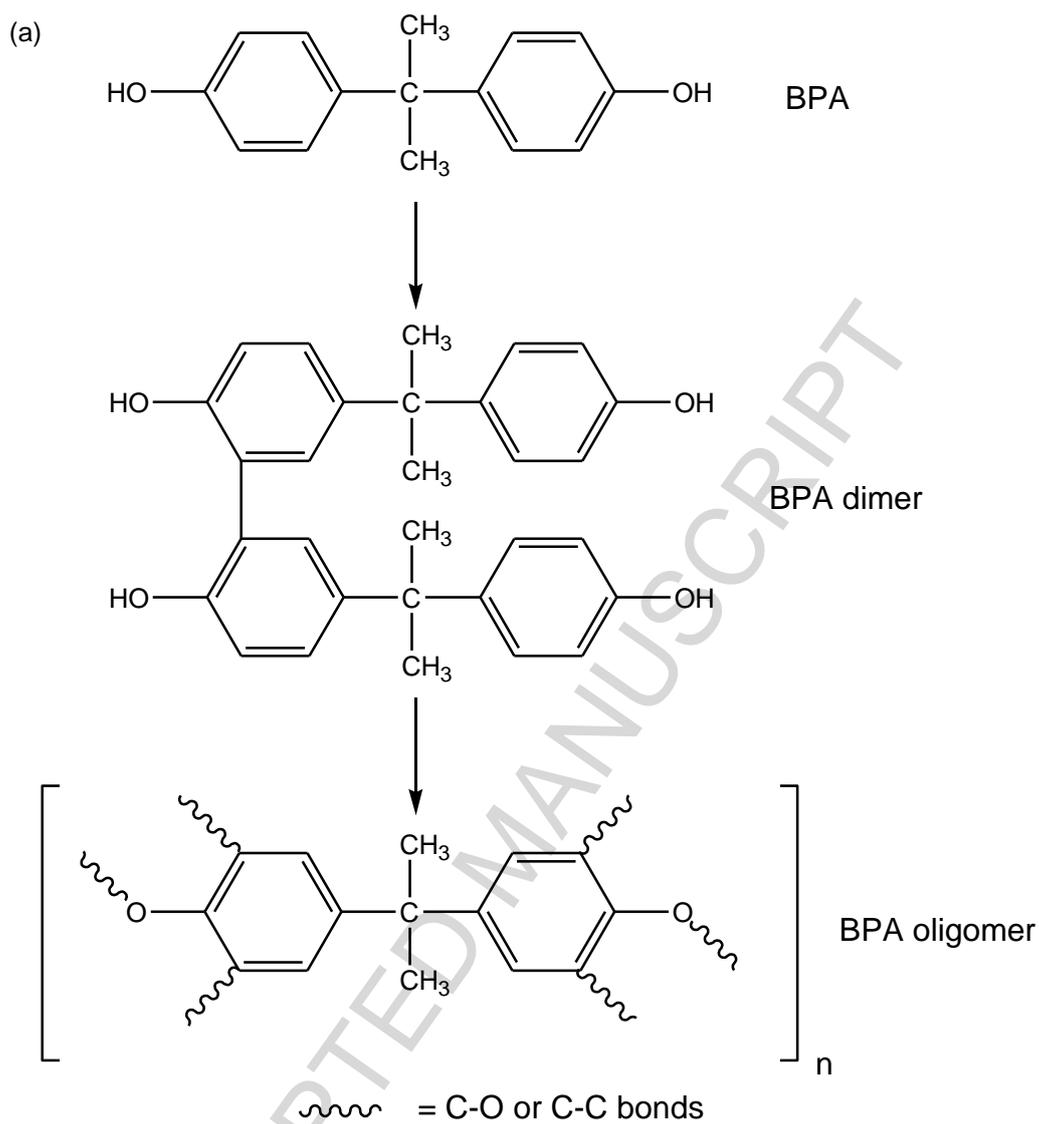|						
d clay		immobilization				
notubes	Lignin peroxidase from *Ganodermalucidum*	Covalent immobilization	Remazol Brilliant Blue R	pH 3.5, 25 °C, 24 h	78%	[14
ed carbon	Laccase from *Myceliophthorahermophile*	Covalent immobilization	Reactive Black 5	pH 5, 25 °C, 24 h	84%	[14
ed carbon	Tyrosinase from *Agaricusbisporus*	Covalent immobilization	Phenol	pH 7, 25 °C, 24 h	85%	[14
nospheres	Horseradish peroxidase	Covalent immobilization	2,4 dichlorophenol, 4-methoxyphenol, bisphenol A	pH 7, 25 °C, 1.5 h	95%, 99%, 52%	[14
us carbon shells	Laccase from *Trametes versicolor*	Adsorption immobilization	Acid Orange 7, Acid Blue 74, Reactive Red 2, Reactive Black 5	pH 6, 30 °C, 72 h	94%, 92%, 48%, 5%	[14
C$_{60}$	Laccase from *Trametes versicolor*	Covalent immobilization	Bisphenol A, Catechol	pH 5, 25 °C, 24 h	23%, 33%	[14
ss beads	Laccase from *Trametes versicolor*	Adsorption immobilization	Reactive Blue 19, Dispersed Blue 3, Acid Blue 74, Acid Red 27, Reactive Black 5	pH 5, 23 °C,	76%, 82% 82%, 27%, 10%	[14
s gold	Lignin peroxidase from *Phanerochaetechrysosporium*	Adsorption immobilization	Fuchsine, Rhodamine B, Pyrogallol Red	pH 7, 25 °C, 10 h	85%, 75%, 87%	[15

Table 4. Hybrid and composite support materials used for immobilization of laccases, tyrosinases and lignin, manganese and phenol oxidases for biodegradation of various environmental pollutants.

Support material	Enzyme	Immobilization technique	Contaminants	Process conditions	Removal efficiency	Reference
macroporous SiO_2/ ZnO nanowires	Horseradish peroxidase	Covalent immobilization	Acid Blue 113, Acid Black 10 BX	pH 7, 25 °C, 35 min	95%, 90%	[156]
Multi-walled carbon nanotube/cordierite	Horseradish peroxidase	Covalent immobilization	4-aminoantipyrine	pH 7, 25 °C, 1 h	96%	[157]
Silica/magnetic/ methacrylyol particles	Laccase from *Trametes versicolor*	Adsorption immobilization	Methyl Red	pH 6, 35 °C, 12 h	90%	[158]
Cu^{2+}/silica magnetic particles	Laccase	Covalent immobilization	2,4-dichlorophenol	pH 5, 25 °C, 12 h	100%	[159]
Silica magnetic particles	Laccase	Covalent immobilization	2,4-dichlorophenol	pH 5, 35 °C, 6 h	85%	[160]
Magnetic mesoporous silica nanoparticles	Laccase from *Trametes versicolor*	Covalent immobilization	Phenol	25 °C, 40 h	90%	[161]
Magnetic tubular mesoporous silica	Laccase	Covalent immobilization	Methoxychlor	pH 4.5, 35 °C, 10 h	69%	[162]
Magnetic nanoparticles/ graphene oxide nanocomposite	Horseradish peroxidase	Covalent immobilization	2-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol	pH 6, 25 °C, 3 h	82%, 52%, 33%	[163]
Carbon mesoporous magnetic composites	Laccase from *Trametes versicolor*	Covalent immobilization	Phenol, *p*-chlorophenol	pH 6, 45 °C, 12 h	74%, 82%	[164]
Magnetic chitosan/ clay beads	Laccase from *Trametes versicolor*	Covalent immobilization	Phenol	pH 5, 25 °C, 4 h	80%	[165]
Superparamagnetic/ chitosan microspheres	Tyrosinase	Covalent immobilization	Phenol	pH 7, 25 °C, 48 h	65%	[166]
Cyanuric chloride/ silica magnetic nanoparticles	Tyrosinase	Covalent immobilization	2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid)	pH 7, 35 °C, 1 h	95%	[167]
Chitosan/SiO_2 gel	Polyphenol oxidase from potato	Covalent immobilization	Phenol	pH 7, 25 °C, 24 h	86%	[168]
Chitosan/biomimetic silica nanoparticles	Manganese peroxidase from *Phanerochaete chrysosporium*	Covalent immobilization	2,6-dimethoxyphenol	pH 4.5, 30 °C, 1 h	95%	[169]
Chitosan/CeO_2microspheres	Laccase from *Trametes versicolor*	Covalent immobilization	Methyl Red, Orange II	pH 6, 25 °C, 9 days	83%, 93%	[170]
Chitosan/clay	Tyrosinase from	Covalent	Phenol	pH 7, 25	100%	[171]

				°C, 6 h		
composite	mushroom	immobilizati on		°C, 6 h		
Chitosan nanoparticles/glass beads	Laccase *Paraconiothyrium variabile*	Covalent immobilizati on	Congo Red	pH 5, 40 °C, 15 min	98%	[172]
Chitosan/ polyacrylamide hydrogel	Laccase from *Trametes versicolor*	Encapsulatio n	Acid Orange 7, Malachite Green	pH 5, 25 °C, 6 h	70%, 97%	[173]
Chitosan/alginate/ magnetic capsules	Tyrosinase from mushroom	Encapsulatio n	Phenol, Bisphenol A	pH 6, 25 °C, 39 h	100%, 85%	[174]
Magnetic Cu alginate beads	Laccase from *Trametes versicolor*	Encapsulatio n	Triclosan, Remazol Brilliant Blue R	pH 5.2, 25 °C, 8 h	89%, 76%	[175]
Alginate/SiO$_2$ gel	Polyphenol oxidase from potato	Entrapment	Phenol	pH 7, 25 °C, 8 h	90%	[176]
Poly(acrylamide-crotonic acid)/ Na alginate	Laccase from *Trametes versicolor*	Covalent immobilizati on	Acid Orange 52	pH 4.5, 30 °C, 6 h	73%	[177]
Polyvinyl alcohol/ Ca alginate beads	Manganese peroxidase from *Ganodermalucidu m* IBL-05	Entrapment	Sandal reactive dyes, Textile wastewater	pH 5, 25 °C	92%, 80%	[178]
Polyamide 6/ chitosan nanofibers	Laccase from *Trametes versicolor*	Covalent immobilizati on	Bisphenol A, 17-α-ethinylestradiol	pH 5, 37 °C, 6 h	92%, 100%	[179]
Poly(2-chloroethyl acrylate)/zeolite particles	Laccase from *Trametes versicolor*	Covalent immobilizati on	Reactive Red 120	pH 6.5, 35 °C, 3 h	100%	[180]
poly(acrylic acid)/ SiO$_2$nanofibrous	Laccase	Covalent immobilizati on	Triclosan	pH 4, 30 °C, 2 h	60%	[181]
Graphene oxide/latex hydrogel	Laccase	Covalent immobilizati on	Remazol Brilliant Blue R	pH 4.5, 25 °C, 17 h	100%	[182]
Cu tetra-aminophthalocyanine /magnetic nanoparticles	Laccase from *Pycnoporussangu ineus*	Covalent immobilizati on	2,2'-azinobis-(3-ethylbenzothiazo line-6-sulphonic acid)	pH 3, 45 °C	100%	[183]
Poly(4-vinyl pyridine)/Cu(II) magnetic beads	Laccase from *Trametes versicolor*	Adsorption immobilizati on	Reactive Green 19, Reactive Red 2, Reactive Brown 10	pH 5.5, 30 °C, 18 h	64%, 88%, 91%	[184]
Poly (*p*-phenylenediamine)/ magnetic nanocomposite	Laccase	Covalent immobilizati on	Reactive Blue 19	pH 4, 25 °C, 2 h	90%	[185]
Poly(styrene-co-methacrylic acid) nanofibers	Horseradish peroxidase	Covalent immobilizati on	*o*-methoxyphenol	pH 5, 2 h	80%	[186]
Poly(lactic-co-glycolic acid) nanofibers	Laccase from *Pleurotusflorida*	Covalent immobilizati on	Diclofenac	pH 4, 30 °C, 5 h	100%	[187]
Poly(acryl-amide) gel	Manganese peroxidase from *Ganodermalucidu m* IBL-05	Entrapment	Textile dyes	pH 5.5, 30 °C	>70%	[188]

| Poly(methyl methacrylate-co-glycidyl methacrylate) cryogel | Laccase from *Trametes versicolor* | Covalent immobilization | Procion Red, Reactive Green 5, Reactive Brown 10, Reactive Green 19, Cibacron Blue F3GA, Alkali Blue 6B, Brilliant Blue 6 | pH 4, 45 °C, 10 h | 81%, 60%, 74%, 66%, 63%, 60%, 62% | [189] |
| Poly(vinyl alcohol)/ 4-hydroxybenz-aldehyde cinnamate | Horseradish peroxidase | Covalent immobilization | 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) | pH 4.5, 25 °C | 98% | [190] |

Figure 1

(a)

BPA

BPA dimer

BPA oligomer

$\sim\!\!\sim\!\!\sim$ = C-O or C-C bonds

(b)

Figure 2

Phenolic compound → Catechol → Quinone → Polymerization

Figure 3

Organic materials

presence of numerous of reactive functional groups,
high affinity for peptides, biocompatibility,
limited negative effect on enzyme structure, abundant in nature

biopolymers: chitosan, alginates, cellulose, carrageenan, collagen, agar
synthetic polymers: ion exchange resins, polystyrene, polyamide, polyacrylonitrile

Inorganic materials

temperature and pH stability, mechanical resistance,
operational stability, good sorption properties,
inertness, easy surface functionalization, relatively cheap

silica, titania, alumina, zirconia, zinc oxide, magnetite, kaolin, hydroxyapatite,
halloysite, active carbons multi-walled carbon nanotubes, porous glass, noble metals

Hybrid and composite materials

reusability of the matrix, strong binding of the enzyme, high
stability, properties of the support material designed for
selected enzyme and catalytic process

silica-magnetite, silica-zinc oxide, chitosan-silica, chitosan-clay, alginate-chitosan,
polyamide-chitosan, polilactic-polyglycolic acid, polyvinyl alcohol-4-hydroxybenzaldehyde

Figure 4

Synthetic polymers	
immobilization of greater amount of enzyme	
strong binding of the enzyme	
diversity of shapes and forms	non-renewable
numerous of functional groups	complicated manufacturing procedure

Biopolymers	
biocompatibility	
high affinity for peptides	
renewable	
numerous of functional groups	necessity of clearing and proper preparation

Inorganic	
aasy surface functionalization	
reusability of the support	possibility of unspecific interactions
high stability	without modifying agents usually weak enzyme-support interactions
good sorption properties	

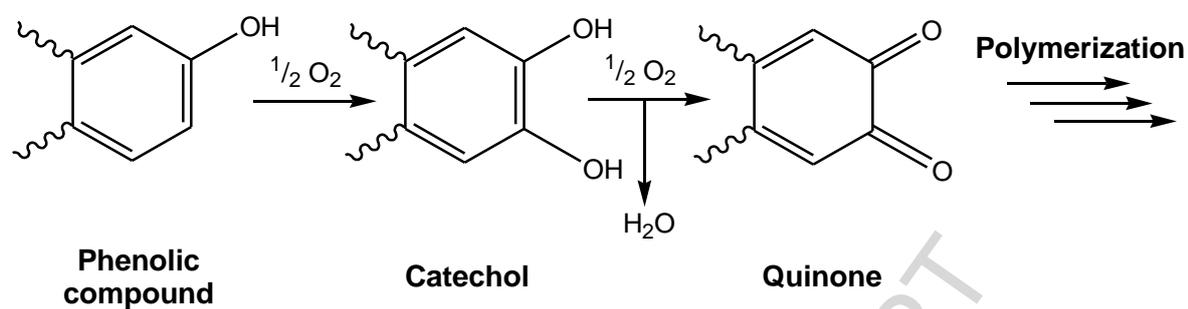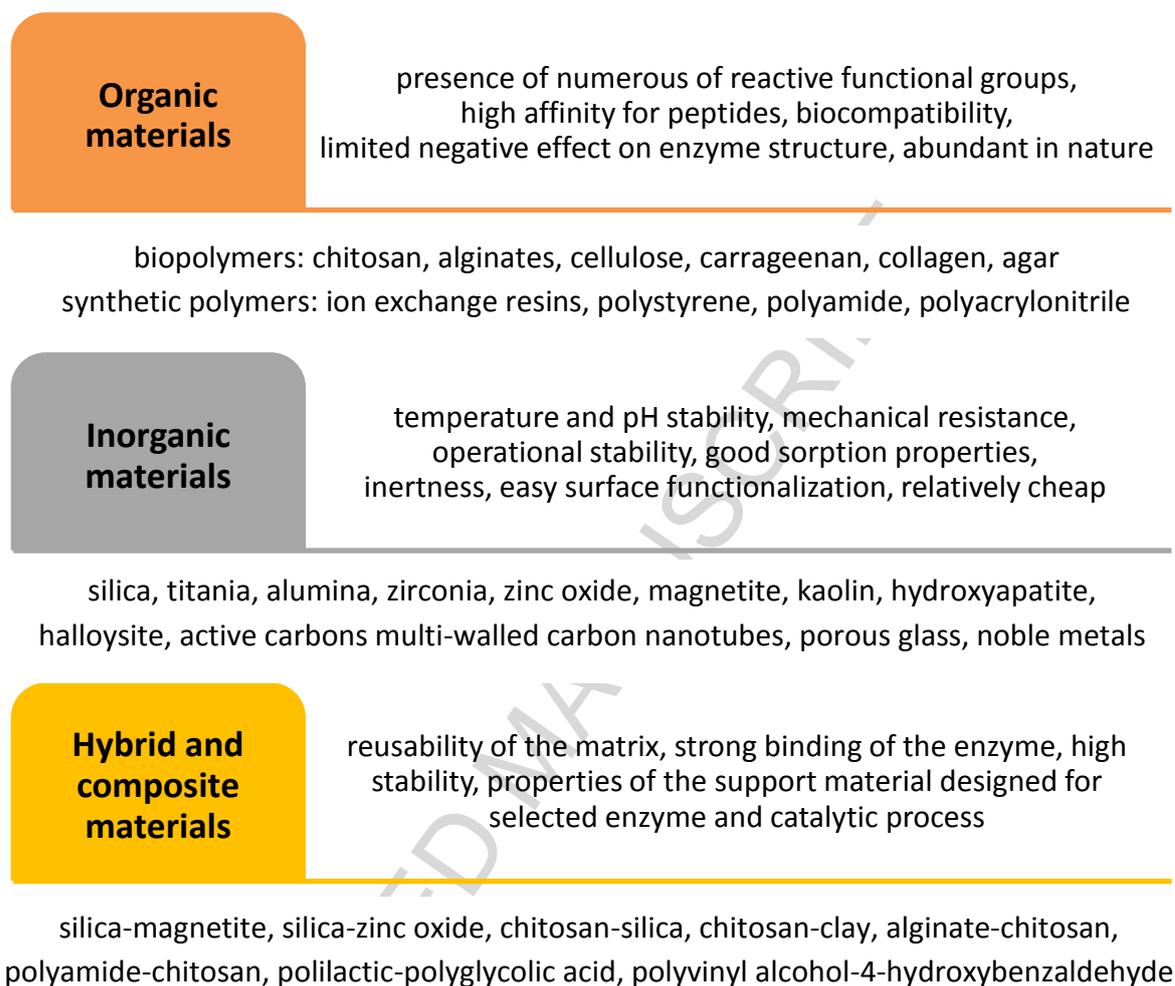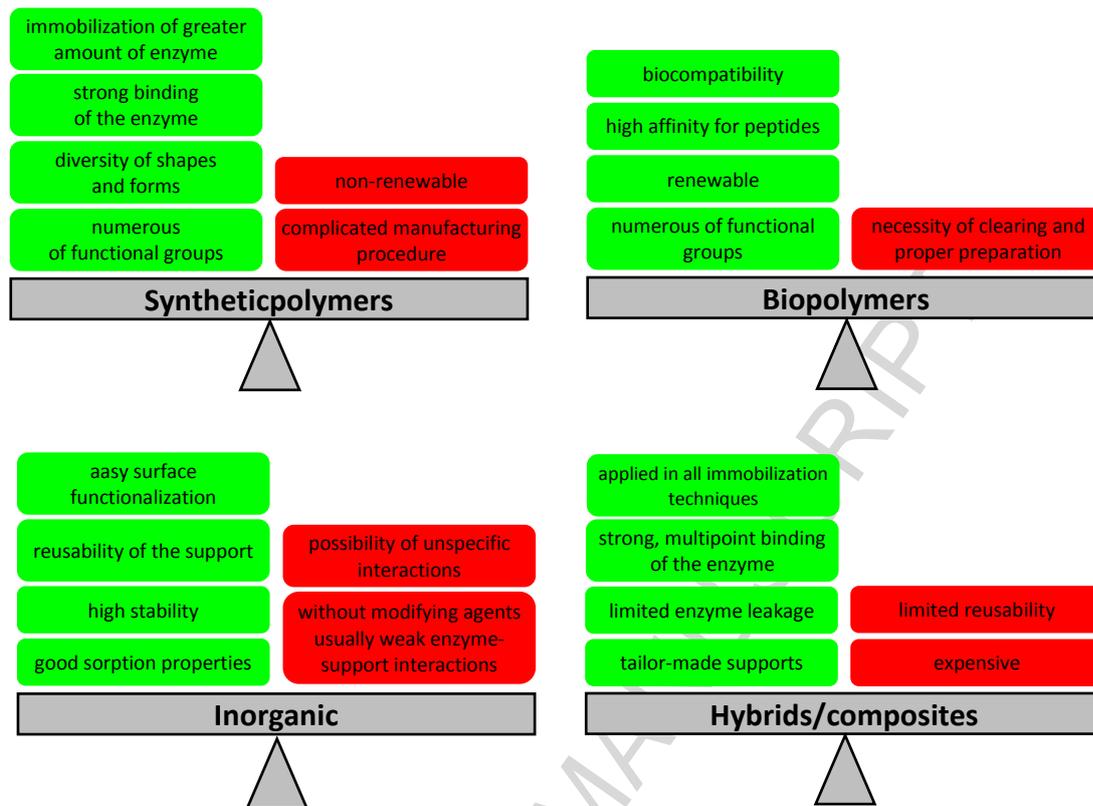| Hybrids/composites	
applied in all immobilization techniques	
strong, multipoint binding of the enzyme	
limited enzyme leakage	limited reusability
tailor-made supports	expensive

Figure captions:

Figure 1.

Degradation pathways of: (a) bishenol A and (b) tetracycline by laccase (adapted from [32] and [33]).

Figure 2.

Catalytic pathway of phenolic substrate by tyrosinase.

Figure 3.

Selected examples and the most important properties of support materials used for immobilization of enzymes for environmental application.

Figure 4.

Summary of advantages and disadvantages of support materials for immobilization of oxidoreductases.

Highlights

- Recent advances of carriers for immobilization of oxidoreductases are presented.

- Features of the various support materials are summarized and discussed.

- Selection of support material depends of enzyme and immobilization protocol.

- Properties and advantages of different oxidoreductases are highlighted.

- Application of immobilized enzymes for removal of hazardous pollutants is reviewed.